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SCIENTIFIC OPINION

Scientific Opinion on Flavouring Group Evaluation 21, Revision 5 (FGE.21Rev5): Thiazoles, thiophenes, thiazoline and thienyl derivatives from chemical groups 29 and 30[1]

EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF)[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

The Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids of the European Food Safety Authority was requested to evaluate 41 flavouring substances in Flavouring Group Evaluation 21, Revision 5, using the Procedure in Commission Regulation (EC) No 1565/2000. This revision was carried out because of the inclusion of the assessment of new toxicity data on the candidate substances, 5-ethylthiophene-2-carbaldehyde [FL-no: 15.074] and 2-pentylthiophene [FL-no: 15.096]. These data on 5-ethylthiophene-2-carbaldehyde [FL-no: 15.074] should cover 2-acetylthiophene [FL-no: 15.040] and 2-propionylthiophene [FL-no: 15.097]. These data on 2-pentylthiophene [FL-no: 15.096] should cover 2-butylthiophene [FL-no: 15.045], 2-hexylthiophene [FL-no: 15.076] and 2-octylthiophene [FL-no: 15.093]. For two of the substances [FL-no: 15.060 and 15.119] the Panel concluded that additional genotoxicity data are required. The remaining 39 substances were evaluated through a stepwise approach (the Procedure) that integrates information on structure–activity relationships, intake from current uses, toxicological threshold of concern and available data on metabolism and toxicity. The Panel concluded that the 39 flavouring substances [FL-no: 15.038, 15.039, 15.040, 15.044, 15.045, 15.050, 15.051, 15.052, 15.054, 15.055, 15.057, 15.058, 15.061, 15.062, 15.063, 15.067, 15.068, 15.069, 15.071, 15.074, 15.076, 15.078, 15.079, 15.080, 15.082, 15.084, 15.085, 15.086, 15.087, 15.089, 15.093, 15.096, 15.097, 15.098, 15.108, 15.115, 15.116, 15.118 and 15.135] do not give rise to safety concerns at their levels of dietary intake, estimated on the basis of the Maximised Survey-derived Daily Intake (MSDI) approach. Besides the safety assessment of these flavouring substances, the specifications for the materials of commerce have also been considered. Adequate specifications, including complete purity criteria and identity for the materials of commerce, have been provided for all 41 candidate substances.

[1] On request from the European Commission, Question Nos EFSA-Q-2013-00861 to -00864, and EFSA-Q-2014-00119 to -00121, adopted on 19 March 2015.

[2] Panel members: Claudia Bolognesi, Laurence Castle, Jean-Pierre Cravedi, Karl-Heinz Engel, Paul Fowler, Konrad Grob, Rainer Gürtler, Trine Husøy, Wim Mennes, Maria Rosaria Milana, André Penninks, Vittorio Silano, Andrew Smith, Maria de Fátima Tavares Poças, Christina Tlustos, Fidel Toldrá, Detlef Wölfle and Holger Zorn. Correspondence: fip@efsa.europa.eu

[3] Acknowledgement: The Panel wishes to thank the members of the Working Group on Flavourings: Ulla Beckman Sundh, Leon Brimer, Karl-Heinz Engel, Rainer Gürtler, Trine Husøy, Wim Mennes, Gerard Mulder and Harriet Wallin for the preparatory work on this scientific opinion and the hearing experts: Vibe Beltoft and Karin Nørby and EFSA staff: Annamaria Rossi, Maria Carfí and Maria Anastassiadou for the support provided to this scientific opinion.

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KEY WORDS

flavourings, safety, thiophene derivatives, thiazole derivatives, thiazoline derivatives, dithiazine derivatives, FGE.21

SUMMARY

Following a request from the European Commission (EC), the European Food Safety Authority (EFSA) Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF Panel) was asked to deliver scientific opinion on the implications for human health of chemically defined flavouring substances used in or on foodstuffs in the Member States. In particular, the Panel was requested to evaluate 41 flavouring substances in the Flavouring Group Evaluation 21, Revision 5 (FGE.21Rev5), using the Procedure as referred to in the Commission Regulation (EC) No 1565/2000. These 41 flavouring substances belong to chemical group 29 and 30, Annex I of the Commission Regulation (EC) No 1565/2000.

The present revision of FGE.21, FGE.21Rev5, includes the assessment of additional toxicity data for 2-pentylthiophene [FL-no (Flavour Information System number): 15.096] supporting 2-butylthiophene [FL-no: 15.045], 2-hexylthiophene [FL-no: 15.076] and 2-octylthiophene [FL-no: 15.093] and also additional toxicity data for 5-ethylthiophene-2-carbaldehyde [FL-no: 15.074] supporting 2-acetylthiophene [FL-no: 15.040] and 2-propionylthiophene [FL-no: 15.097]. Updated information on European production figures has been provided by the European Flavour and Fragrance Association (EFFA) for these seven substances [FL-no: 15.040, 15.045, 15.074, 15.076, 15.093, 15.096 and 15.097] and the information has been included in the evaluation.

All candidate substances are five- or six-member sulphur-containing heterocyclic compounds, some of which also contain nitrogen. They have been divided into two main groups: (A) those with an aromatic ring (33 candidate substances) and (B) those with a non-aromatic ring structure (8 candidate substances). All are ring-substituted with one or more of the substituents alkyl, alkenyl, aryl, alcohol, keto and thio. For assessment purposes, the following further subdivision of groups (A) and (B) has been made:

- Group (A): Aromatic:
  - (Subgroup A-Ia: Thiophene. The substance previously allocated to the group is no longer supported for use as a flavouring substance in Europe by Industry.)
  - Subgroup A-Ib: Thiophene derivatives with non-thiol-containing ring substituents.
  - Subgroup A-Ic: Thiophene derivatives with thiol-containing ring substituents.
  - Subgroup A-II: Thiazole derivatives.
  - (Subgroup A-III: Benzothiazoles. The substance previously allocated to the group is no longer supported for use as flavouring substance in Europe by Industry.)
- Group (B): Non-aromatic:
  - (Subgroup B-I: Dihydrothiophenes. The substance previously allocated to the group is no longer supported for use as a flavouring substance in Europe by Industry.)
  - Subgroup B-II: Thiazolines.
  - (Subgroup B-III: Thiazolidines. The substances previously allocated to the group are no longer supported for use as flavouring substances in Europe by Industry.)
  - Subgroup B-IV: Dithiazine derivatives.
  - (Subgroup B-V: Dihydrothiazines. The substances previously allocated to the group are no longer supported for use as flavouring substances in Europe by Industry.)
  - (Subgroup B-VI: Thiadiazine derivatives. The substance previously allocated to the group is no longer supported for use as a flavouring substance in Europe by Industry.)

Two of the 41 flavouring substances possess one chiral centre [FL-no: 15.060 and 15.119], three substances possess two chiral centres [FL-no: 15.054, 15.057 and 15.135] and two possess three chiral centres [FL-no: 15.055 and 15.079]. The stereoisomeric composition has been specified for all substances.

Thirty-three of the candidate substances belong to structural class (SC) II and eight belong to SC III.

Thirty-one of the candidate substances have been reported to occur naturally in a wide range of foods.

In its evaluation, the Panel used as a default the "Maximised Survey-derived Daily Intake" (MSDI) approach to estimate the *per capita* intakes of the flavouring substances in Europe. However, when the Panel examined the information provided by the European Flavour Industry on the use levels in various foods, it appeared obvious that the MSDI approach in a number of cases would grossly underestimate the intake by regular consumers of products flavoured at the use level reported by the Industry, especially in those cases where the annual production values were reported to be small. In consequence, the Panel had reservations about data on use and use levels provided and the intake estimates obtained by the MSDI approach.

In the absence of more precise information that would enable the Panel to make a more realistic estimate of the intakes of the flavouring substances, the Panel has decided also to perform an estimate of the daily intakes per person using a "modified Theoretical Added Maximum Daily Intake" (mTAMDI) approach based on the normal use levels reported by Industry. In those cases where the mTAMDI approach indicated that the intake of a flavouring substance might exceed its corresponding threshold of concern, the Panel decided not to carry out a formal safety assessment using the Procedure. In these cases, the Panel requires more precise data on use and use levels.

According to the default MSDI approach, the flavouring substances in this FGE.21Rev5 have intakes, in Europe, ranging from 0.0012 to 5.7 µg/capita per day, which are below the thresholds of concern values for SC II (540 µg/person per day) and SC III (90 µg/person per day) substances.

On the basis of the reported annual production volumes of the candidate substances as flavourings in Europe, the combined estimated daily *per capita* intakes per subgroup and SC range from 0.14 to 8.0 µg. For all subgroups the total combined intakes are below the thresholds of concern of 540 µg/person per day and 90 µg/person per day for SCs II and III, respectively.

The candidate substances are structurally related to 28 supporting substances evaluated by the Joint Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO) Expert Committee on Food Additives (JECFA) at its 59th and 68th JECFA meetings. The total combined daily *per capita* intakes (in Europe) of candidate and supporting substances evaluated through the Procedure in each of the five subgroups for which supporting substances were available in the same SC are: subgroup A-Ib (8 substances from SC II) 2.9 µg; subgroup A-Ic (5 substances from SC III) 0.15 µg; subgroup A-II (37 substances from SC II) 500 µg; subgroup B-II (2 substances from SC II) 0.52 µg; subgroup B-IV (4 substances from SC II) 3.6 µg.

For all subgroups, the total combined intakes are below the thresholds of concern of 540 µg/person per day and 90 µg/person per day for SCs II and III, respectively.

It is concluded that genotoxicity data are limited and that genotoxicity could not be assessed adequately for the flavouring substances in FGE.21Rev5. However, except for the two 3-thiazolines, 2,4-dimethyl-3-thiazoline [FL-no: 15.060] and 2-isobutyl-3-thiazoline [FL-no: 15.119], the available genotoxicity data do not preclude the evaluation of the remaining 39 candidate substances using the Procedure.

The available metabolism data were insufficient to allow conclusions about the metabolic fate of the candidate substances and, accordingly, the 39 candidate substances evaluated through the Procedure

could not be anticipated to be metabolised to innocuous products. However, the Panel concluded that the evidence of binding to macromolecules from possible formation of electrophilic metabolites, (e.g. by either ring scission or *S*-oxidation) was not sufficiently strong to preclude the application of the Procedure.

Valid toxicological data were available for candidate substances from subgroup A-Ib (thiophenes with non-thiol-containing ring substituents), subgroup A-Ic (thiophenes with thiol-containing ring substituents), subgroup A-II (thiazoles), subgroup B-II (only 2-thiazolines) and subgroup B-IV (dithiazines). Based on these data, the margin of safety compared with the intakes from use as flavouring substances was considered adequate, based on the MSDI approach.

In order to determine whether the conclusion for the 39 candidate substances, which have been evaluated using the Procedure, can be applied to the materials of commerce, it is necessary to consider the available specifications. Adequate specifications, including purity and identity for the materials of commerce, have been provided for all 39 flavouring substances evaluated through the Procedure.

For the 39 flavouring substances [FL-no: 15.038, 15.039, 15.040, 15.044, 15.045, 15.050, 15.051, 15.052, 15.054, 15.055, 15.057, 15.058, 15.061, 15.062, 15.063, 15.067, 15.068, 15.069, 15.071, 15.074, 15.076, 15.078, 15.079, 15.080, 15.082, 15.084, 15.085, 15.086, 15.087, 15.089, 15.093, 15.096, 15.097, 15.098, 15.108, 15.115, 15.116, 15.118 and 15.135], evaluated using the Procedure, the Panel concluded that they would present no safety concerns at their estimated levels of intake based on the MSDI approach.

The estimated intakes, based on the mTAMDI, for the 34 candidate substances assigned to SC II and evaluated using the Procedure, range from 78 to 220 µg/person per day, which is below the threshold of concern for SC II of 540 µg/person per day. The estimated intakes based on the mTAMDI of the seven candidate substances assigned to SC III and evaluated through the Procedure ranged from 78 to 250 µg/person per day. For the candidate substances [FL-no: 15.055 and 15.135] the estimated intakes are above the threshold of concern for SC III of 90 µg/person per day. For two candidate substances [FL-no: 15.057 and 15.079], assigned to SC III, no use levels were provided.

In conclusion, more reliable exposure data are required for [FL-no: 15.055, 15.057, 15.079 and 15.135]. On the basis of such additional data, these flavouring substances should be re-evaluated using the Procedure.

For two substances [FL-no: 15.060 and 15.119] the Panel concluded that additional genotoxicity data are required.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

The use of flavourings in food is regulated under Regulation (EC) No 1334/2008 of the European Parliament and of the Council of 16 December 2008[4] on flavourings and certain food ingredients with flavouring properties for use in and on foods. On the basis of Article 9(a) of this Regulation, an evaluation and approval are required for flavouring substances.

The Union List of flavourings and source materials was established by Commission Implementing Regulation (EC) No 872/2012[5]. The list includes flavouring substances for which the scientific evaluation should be completed in accordance with Commission Regulation (EC) No 1565/2000[6].

On 24 November 2011, the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) adopted an opinion on Flavouring Group Evaluation 21, Revision 3 (FGE.21Rev3): thiazoles, thiophenes, thiazoline and thienyl derivatives from chemical groups 29 and 30[7].

In its opinion, the Panel stated that for the 3 candidate substances [FL-nos: 15.040, 15.074 and 15.097] evaluated through the Procedure, no appropriate NOAEL was available and that additional toxicity data are required.

The substances are currently listed in the Union List with a Footnote 2.

On 23 January 2014, the applicant submitted additional data of these 3 flavouring substances (candidate chemicals) relevant to the Flavouring Group Evaluation of the Chemical Groups 29 and 30 (Annex I of 1565/2000/EC): consideration of thiazoles, thiophenes, thiazoline and thienyl derivatives as evaluated by EFSA in FGE.21Rev3.

In addition, we would like to draw your attention to the earlier request for re-evaluation of flavouring substances belonging to the same evaluation group FGE.21Rev2. On 28 November 2013, you informed us that you agreed to perform the safety assessment of these substances [FL-nos: 15.045, 15.076, 15.093 and 15.096] by 25 July 2014 (your reference KL/cc(2013)out-8076804, our reference Ares(2013)3602415).

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

The European Commission requests the European Food Safety Authority (EFSA) to evaluate this new information and, depending on the outcome, proceed to the full evaluation on these flavouring substances in accordance with Commission Regulation (EC) No 1565/2000.

If appropriate, EFSA may combine this re-evaluation with the above-mentioned re-evaluation of flavouring substances from FGE.21Rev2 (EFSA-Q-2013-00861 to 864).

SUPPORTING DOCUMENTS

Submission by the European Flavour Association.

[4] Regulation (EC) No 1334/2008 of the European Parliament and of the Council of 16 December 2008 on flavourings and certain food ingredients with flavouring properties for use in and on foods and amending Council Regulation (EEC) No 1601/91, Regulations (EC) No 2232/96 and (EC) No 110/2008 and Directive 2000/13/EC. OJ L 354, 31.12.2008, p. 34–50.

[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. 1–161.

[6] Commission Regulation 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. OJ L 180, 19.7.2000, p. 8–16.

[7] EFSA Journal 2012;10(2)2457

INTERPRETATION OF THE TERMS OF REFERENCE

The above background and terms of reference include also a previous mandate received from the European Commission on 25 October 2013[8]. The present scientific opinion FGE.21Rev5 covers the safety assessment of the following flavouring substances: 2-pentylthiophene [FL-no: 15.096], 2-butylthiophene [FL-no: 15.045], 2-hexylthiophene [FL-no: 15.076] and 2-octylthiophene [FL-no: 15.093], 5-ethylthiophene-2-carbaldehyde [FL-no: 15.074], 2-acetylthiophene [FL-no: 15.040] and 2-propionylthiophene [FL-no: 15.097].

[8] SANCO.E3/MGR/ave (2013) Ares(2013)3318725

ASSESSMENT

1. History of the evaluation of the substances in the present Flavouring Group Evaluation (FGE)

In FGE.21, and the first revision (FGE.21Rev1), the European Food Safety Authority (EFSA) Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF), hereafter referred to as the Panel, considered that additional toxicity data were needed for 23 of the substances (subgroups A-Ia, A-Ib, A-III, B-I, B-IV and B-VI) evaluated through the Procedure, as no adequate toxicity study from which a no-observed-adverse-effect level (NOAEL) could be established was available, neither on the candidate substances nor on supporting substances.

In the second revision of FGE.21 (FGE.21Rev2) additional toxicity data and metabolism data became available (Flavour Industry, 2010a) for two substances, thiophene [FL-no: 15.106] from subgroup A-Ia and 2-pentylthiophene [FL-no (Flavour Information System number): 15.096] from subgroup A-Ib. The Panel concluded that these data were not valid for the purposes of establishing a NOAEL. Further, information on stereoisomeric composition was provided by the European Flavour and Fragrance Association (EFFA) on nine substances [FL-nos 15.042, 15.054, 15.055, 15.060, 15.077, 15.090, 15.099, 15.119 and 15.129] (EFFA, 2010, 2011b).

The third revision of FGE.21 (FGE.21Rev3) included the assessment of three additional substances, 4,6-dimethyl-2-(1-methylethyl)dihydro-1,3,5-dithiazine [FL-no: 15.057], 2-isobutyldihydro-4,6-dimethyl-1,3,5-dithiazine [FL-no: 15.079] and ethyl thialdine [FL-no: 15.135]. For these three candidate substances [FL-no: 15.057, 15.079 and 15.135], a NOAEL could not be derived for the substance or a structurally related substance. Accordingly, additional data were required for these three flavouring substances.

Since the publication of FGE.21Rev3, 18 substances [FL-nos: 15.037, 15.042, 15.043, 15.064, 15.070, 15.072, 15.077, 15.088, 15.090, 15.091, 15.092, 15.094, 15.099, 15.106, 15.107, 15.114, 15.129 and 15.133] of the 59 candidate substances are no longer supported for use as flavouring substances in Europe by Industry (DG SANCO, 2012, 2013) and will therefore not be considered any further. The 18 substances are listed in Table 1.

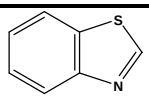
Table 1: Substances no longer supported for use as flavouring substances

FL-no	EU register name
15.037	2-Acetyl-3-methylthiophene
15.042	2-Butyl-4-methyl(4*H*)pyrrolidino[1,2*d*]-1,3,5-dithiazine
15.043	2-Butyl-5-ethylthiophene
15.064	2,5-Dimethylthiophene
15.070	2-Ethyl-5-methylthiophene
15.072	2-Ethylthiophene
15.077	4-Hydroxy-2,5-dimethylthiophen-3(2*H*)-one
15.088	2-Methyl-4,5-benzothiazole
15.090	2-Methylthiazolidine
15.091	2-Methylthiophene
15.092	3-Methylthiophene
15.094	2-Pentanoylthiophene
15.099	2-Propylthiazolidine
15.106	Thiophene
15.107	Thiophene-2-carbaldehyde
15.114	6-Acetyl-2,3-dihydro-1,4-thiazine
15.129	Tetrahydro-2,4,6-trimethyl-1,3,5(2*H*)-thiadiazine
15.133	5-Acetyl-2,3-dihydro-1,4-thiazine

These 18 substances will be excluded in the following text, except in Tables 4 and 10. Information in the text on these substances will be kept only if relevant for the remaining candidate substances.

As a consequence of this, the following supporting substance (Table 2) has been deleted from this revision.

Table 2: Supporting substance deleted from this revision

FL-no/JECFA no	EU register name	Structural formula
15.016/1040	Benzothiazole	

JECFA, the Joint Food and Agriculture Organization (FAO)/World Health Organization (WHO) Expert Committee on Food Additives.

The fourth revision of FGE.21 (FGE.21Rev4) included a re-evaluation of six candidate substances, dihydro-2,4,6-triethyl-1,3,5(4*H*)-dithiazine [FL-no: 15.054], [2*S*-(2*a*,4*a*,8*ab*)]2,4-Dimethyl-(4*H*)pyrrolidino[1,2*e*]-1,3,5-dithiazine [FL-no: 15.055], 4,6-dimethyl-2-(1-methylethyl)-dihydro-1,3,5-dithiazine [FL-no: 15.057], 2-isobutyldihydro-4,6-dimethyl-1,3,5-dithiazine [FL-no: 15.079], 2-methyl-2-thiazoline [FL-no: 15.086] and ethyl thialdine [FL-no: 15.135]. Additional data (a 14-day and 90-day dietary study, a bacterial reverse mutation assay and a micronucleus induction assay) became available for supporting substances and FGE.21Rev4 included the evaluation of these data submitted by Industry (Bauter, 2012a, 2013a; McGarry, 2012; Watters, 2012). The Panel concluded that these six candidate substances are not of safety concern at their estimated levels of intake based on the Maximum Survey-derived Daily Intake (MSDI) approach.

Furthermore, new information on European production figures has been provided for five substances [FL-no: 15.054, 15.055, 15.057, 15.079 and 15.135] (EFFA, 2013b), and new information on missing stereoisomeric composition for [FL-no: 15.057, 15.079 and 15.135] was also included (EFFA, 2013b).

Table 3 gives information on publication dates and links to the published versions.

Table 3: Publication dates and links to the published versions of FGE.21

FGE	Opinion adopted by EFSA	Link	No of candidate substances
FGE.21	8 February 2007	http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178694698331.htm	54
FGE.21Rev1	26 March 2009	http://www.efsa.europa.eu/en/scdocs/scdoc/1023.htm	56
FGE.21Rev2	4 February 2011	http://www.efsa.europa.eu/en/efsajournal/doc/1989.pdf	56
FGE.21Rev3	23 November 2011	http://www.efsa.europa.eu/en/efsajournal/pub/2457.htm	59
FGE.21Rev4	24 October 2013	http://www.efsa.europa.eu/en/efsajournal/pub/3451.htm	41
FGE.21Rev5	19 March 2015		41

The present revision of FGE.21, FGE.21 Revision 5, deals with the following additional toxicity data which have become available:

- A 90-day dietary rat study for 2-pentylthiophene [FL-no: 15.096] (Bauter, 2013b). This substance has been selected as a representative substance for 2-butylthiophene [FL-no: 15.045], 2-hexylthiophene [FL-no: 15.076] and 2-octylthiophene [FL-no: 15.093]. Further information on the stability of 2-pentylthiophene [FL-no: 15.096] in feed has been submitted (EFFA, 2014; Mendes, 2014).

- A 14-day dietary study and a 90-day gavage rat study for 5-ethylthiophene-2-carbaldehyde [FL-no: 15.074] (Bauter, 2012b, 2013c). This substance has been selected as a representative substance for 2-acetylthiophene [FL-no: 15.040] and 2-propionylthiophene [FL-no: 15.097]. A literature search for toxicity data on [FL-no: 15.074 and 15.096] has been performed, but no pertinent data have been found.

Since the publication of FGE.21Rev4, updated information on European production figures has been provided by EFFA for seven substances [FL-no: 15. 040, 15.045, 15.074, 15.076, 15.093, 15.096 and 15.097] (IOFI, 2013a, b) In the Union List, the name of [FL-no: 15.096] has been changed from *sec*-pentylthiophene to 2-pentylthiophene. The name of [FL-no: 15.055] has also been changed from 2,4-Dimethyl(4*H*)pyrrolidino[1,2*e*]-1,3,5-dithiazine to [2*S*-(2*a*,4*a*,8*ab*)] 2,4-Dimethyl(4*H*)-pyrrolidino-[1,2*e*]-1,3,5-dithiazine.

2. Presentation of the substances in Flavouring Group Evaluation 21, Revision 5

2.1. Description

The present FGE.21Rev5, using the Procedure as referred to in the Commission Regulation (EC) No 1565/2000 (the Procedure is shown in schematic form in Appendix A of this FGE), deals with 41 flavouring substances (candidate substances) from chemical groups 29 and 30, Annex I of Commission Regulation (EC) No 1565/2000. The candidate substances in FGE.21Rev5 fall into the chemical groups of thiazoles (*S*- and *N*-containing) and dithiazines (*S*- and *N*-containing) (Table 4, and Table 8 in Section 5).

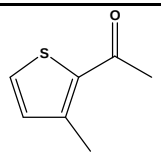
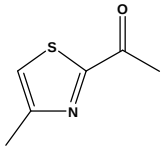
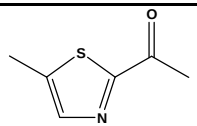
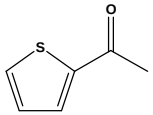
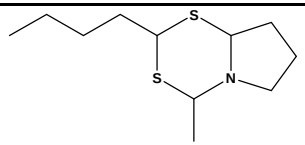
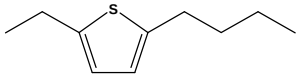
The candidate substances, as well as their chemical register names, Flavour Information System (FLAVIS) (FL-no), Chemical Abstract Service (CAS), Council of Europe (CoE) and Flavor and Extract Manufacturers Association (FEMA) numbers, structure and specifications, are listed in Table 4.

A summary of the outcome of the safety evaluation of the candidate substances is listed in Table 10.

The candidate substances are structurally related to 28 flavouring substances (supporting substances) evaluated at the 59th and 68th JECFA meetings (JECFA, 2002a, 2003, 2007, 2008) in the group of "sulphur-containing heterocyclic compounds". Two of these supporting substances (see Table 11) are mixtures (one of two isomeric isobutyl-substituted and one of two isomeric isopropyl-substituted dimethyldihydrodithiazine derivatives) and are not included in the register.

SUMMARY OF SPECIFICATION DATA

Table 4: Specification Summary of the Substances in the Flavouring Group Evaluation 21, Revision 5

FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Physical form Molecular formula Molecular weight	Solubility [(a)] Solubility in ethanol [(b)]	Boiling point, (°C) [(c)] Melting point, (°C) ID test Assay minimum	Refractive Index [(d)] Specific gravity [(e)]	Specification comments
15.037	2-Acetyl-3-methylthiophene		11590 13679-72-6	Liquid C_7H_8OS 140.20	Practically insoluble or insoluble Freely soluble	216 MS 95 %	1.558-1.564 1.130-1.136	No longer supported by Industry (DG SANCO, 2012).
15.038	2-Acetyl-4-methylthiazole		11589 7533-07-5	Solid C_6H_7NOS 141.19	Practically insoluble or insoluble Freely soluble	95 (16 hPa) 34 MS 95 %	n.a. n.a.	
15.039	2-Acetyl-5-methylthiazole		59303-17-2	Solid C_6H_7NOS 141.19	Practically insoluble or insoluble Freely soluble	93 (16 hPa) 30 MS 95 %	n.a. n.a.	
15.040	2-Acetylthiophene		11728 88-15-3	Solid C_6H_6OS 126.17	Practically insoluble or insoluble Freely soluble	213 34 MS 98 %	1.563-1.569 1.164-1.171	
15.042	2-Butyl-4-methyl(4H)pyrrolidino[1,2d]-1,3,5-dithiazine		132344-97-9	Solid $C_{11}H_{21}NS_2$ 231.42	Practically insoluble or insoluble Freely soluble	304 164 NMR 95 %	n.a. n.a.	No longer supported by Industry (DG SANCO, 2012).
15.043	2-Butyl-5-ethylthiophene		11596 54411-06-2	Solid $C_{10}H_{16}S$ 168.30	Practically insoluble or insoluble Freely	231 54 MS 95 %	n.a. n.a.	No longer supported by Industry (DG SANCO, 2012).

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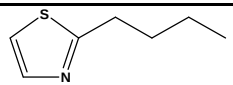
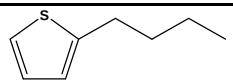
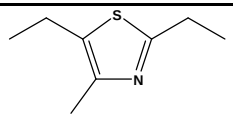
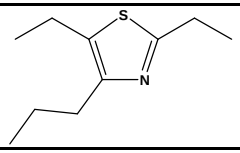
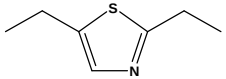
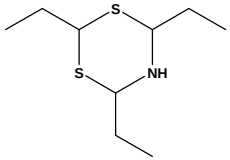
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Physical form Molecular formula Molecular weight	Solubility [a] Solubility in ethanol [b]	Boiling point, (°C) [c] Melting point, (°C) ID test Assay minimum	Refractive Index [d] Specific gravity [e]	Specification comments
					soluble			
15.044	2-Butylthiazole		11597 37645-61-7	Solid $C_7H_{11}NS$ 141.23	Practically insoluble or insoluble Freely soluble	212 79 NMR 95 %	n.a. n.a.	
15.045	2-Butylthiophene		1455-20-5	Liquid $C_8H_{12}S$ 140.24	Practically insoluble or insoluble Freely soluble	182 MS 95 %	1.504-1.510 0.951-0.957	
15.050	2,5-Diethyl-4-methylthiazole		41981-71-9	Solid $C_8H_{13}NS$ 155.26	Practically insoluble or insoluble Freely soluble	85 (20 hPa) 115 MS 95 %	n.a. n.a.	
15.051	2,5-Diethyl-4-propylthiazole		4276-68-0	Solid $C_{10}H_{17}NS$ 183.31	Practically insoluble or insoluble Freely soluble	72 (21 hPa) 139 NMR 95 %	n.a. n.a.	
15.052	2,5-Diethylthiazole		15729-76-7	Solid $C_7H_{11}NS$ 141.23	Practically insoluble or insoluble Freely soluble	187 92 MS 95 %	n.a. n.a.	
15.054	Dihydro-2,4,6-triethyl-1,3,5(4H)-dithiazine		54717-17-8	Solid $C_9H_{19}NS_2$ 205.38	Practically insoluble or insoluble Freely soluble	287 188 MS 95 %	n.a. n.a.	Mixture of diastereoisomers (EFFA, 2010). Mixture of isomers ((R/R), (R/S),

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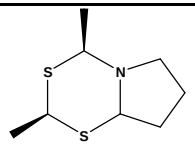
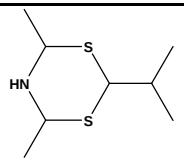
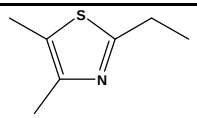
								(S/R) and (S/S) at equal ratio, i.e. 25 % of each) (EFFA, 2011b).
15.055 1763	[2S-(2a,4a,8ab)] 2,4-Dimethyl(4H)pyrrolidino[1,2e]-1,3,5-dithiazine		4321 116505-60-3	Solid $C_8H_{15}NS_2$ 189.34	Practically insoluble or insoluble Freely soluble	235 130 MS 95 %	n.a. n.a.	
15.057	4,6-Dimethyl-2-(1-methylethyl)dihydro-1,3,5-dithiazine		3782 104691-40-9	Liquid $C_8H_{17}NS_2$ 191.36	Slightly soluble Soluble	109 (0.23hPa) MS 71 %	1.496-1.500 0.951-0.959	Mixture of four diastereoisomers (25 % of each) (EFFA, 2013b). At least 44 % 2-Isopropyl-4,6-dimethyl and 27 % 4-Isopropyl-2,6-dimethyl; secondary components at least 24 %: 2,4,6-Trimethyldihydro-; 6-Methyl-2,4-diisopropyl-; 4-Methyl-2,6-diisopropyl-; 2,4,6-Triisopropyl-dihydro-1,3,5-dithiazine (EFFA, 2011c).
15.058	4,5-Dimethyl-2-ethylthiazole		 873-64-3	Solid $C_7H_{11}NS$ 141.23	Practically insoluble or insoluble Freely soluble	185 104 MS 96 %	n.a. n.a.	

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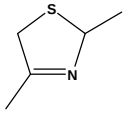
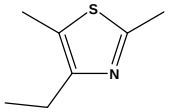
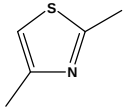
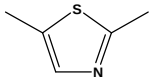
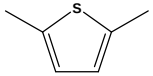
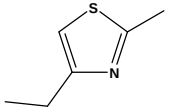
15.060	2,4-Dimethyl-3-thiazoline		60755-05-7	Solid C_5H_9NS 115.19	Practically insoluble or insoluble Freely soluble	52 (15 hPa) 102 MS 95 %	n.a. n.a.	Racemate (EFFA, 2010).
15.061	2,5-Dimethyl-4-ethylthiazole		32272-57-4	Solid $C_7H_{11}NS$ 141.23	Practically insoluble or insoluble Freely soluble	185 104 MS 95 %	n.a. n.a.	
15.062	2,4-Dimethylthiazole		11605 541-58-2	Solid C_5H_7NS 113.18	Practically insoluble or insoluble Freely soluble	145 69 MS 95 %	n.a. n.a.	
15.063 1758	2,5-Dimethylthiazole		4175-66-0	Solid C_5H_7NS 113.18	Practically insoluble or insoluble Freely soluble	152 69 MS 95 %	n.a. n.a.	
15.064	2,5-Dimethylthiophene		11609 638-02-8	Liquid C_6H_8S 112.19	Practically insoluble or insoluble Freely soluble	135 MS 95 %	1.508-1.514 0.982-0.988	No longer supported by Industry (DG SANCO, 2013).
15.067	4-Ethyl-2-methylthiazole		32272-48-3	Solid C_6H_9NS 127.20	Practically insoluble or insoluble Freely soluble	91 (89 hPa) 80 MS 95 %	n.a. n.a.	

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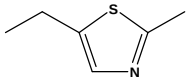
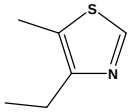
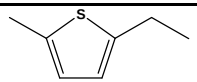
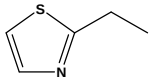
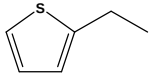
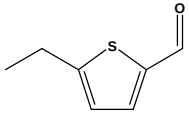
15.068	5-Ethyl-2-methylthiazole		19961-52-5	Solid C_6H_9NS 127.20	Practically insoluble or insoluble Freely soluble	170 80 MS 95 %	n.a. n.a.	
15.069	4-Ethyl-5-methylthiazole		52414-91-2	Solid C_6H_9NS 127.20	Practically insoluble or insoluble Freely soluble	174 80 MS 95 %	n.a. n.a.	
15.070	2-Ethyl-5-methylthiophene		40323-88-4	Liquid $C_7H_{10}S$ 126.22	Practically insoluble or insoluble Freely soluble	158 MS 97 %	1.503-1.509 0.963-0.970	No longer supported by Industry (DG SANCO, 2012).
15.071	2-Ethylthiazole		15679-09-1	Liquid C_5H_7NS 113.18	Practically insoluble or insoluble Freely soluble	148 MS 95 %	1.511-1.517 1.058-1.064	
15.072	2-Ethylthiophene		11614 872-55-9	Liquid C_6H_8S 112.19	Practically insoluble or insoluble Freely soluble	133 MS 95 %	1.510-1.516 0.989-0.995	No longer supported by Industry (DG SANCO, 2013).
15.074	5-Ethylthiophene-2-carbaldehyde		36880-33-8	Solid C_7H_8OS 140.20	Practically insoluble or insoluble Freely soluble	104 (12 hPa) 62 MS 95 %	n.a. n.a.	

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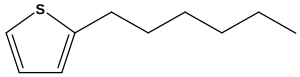
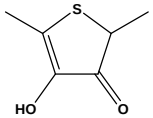
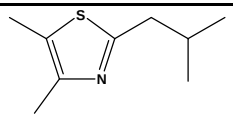
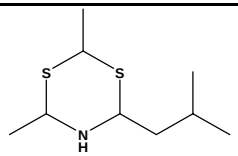
15.076 1764	2-Hexylthiophene		4137 11616 18794-77-9	Solid $C_{10}H_{16}S$ 168.30	Practically insoluble or insoluble Freely soluble	92 (13 hPa) 41 MS 95 %	n.a. n.a.	
15.077	4-Hydroxy-2,5-dimethylthiophen-3(2H)-one		26494-10-0	Solid $C_6H_8O_2S$ 144.19	Practically insoluble or insoluble Freely soluble	296 133 MS 95 %	n.a. n.a.	No longer supported by Industry (DG SANCO, 2012).
15.078	2-Isobutyl-4,5-dimethylthiazole		11617 53498-32-1	Solid $C_9H_{15}NS$ 169.29	Practically insoluble or insoluble Freely soluble	267 112 MS 97 %	n.a. n.a.	
15.079	2-Isobutyldihydro-4,6-dimethyl-1,3,5-dithiazine		3781 101517-87-7	Liquid $C_9H_{19}NS_2$ 205.39	Slightly soluble Soluble	115 (0.33 hPa) MS 82 %	1.488-1.492 0.961-0.967	Mixture of diastereoisomers, each of them racemic (EFFA, 2013b). At least 64 % 2-isobutyl-4,6-dimethyl and 18 % 4-Isobutyl-2,6-dimethyl; secondary components at least 13 %: 2,4,6-trimethyl-; 2,4-diisobutyl-6-methyl-; 2,6-dimethyl-4-butyldihydro-1,3,5-dithiazine; substituted 1,3,5-thiadiazine (EFFA, 2011c).

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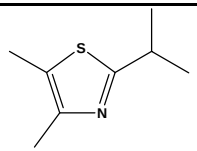
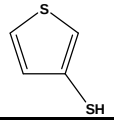
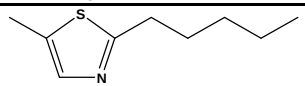
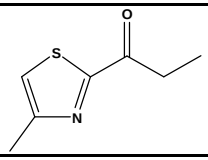
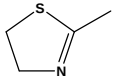
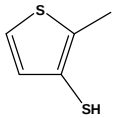
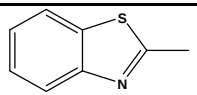
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Physical form Molecular formula Molecular weight	Solubility [a] Solubility in ethanol [b]	Boiling point, (°C) [c] Melting point, (°C) ID test Assay minimum	Refractive Index [d] Specific gravity [e]	Specification comments
15.080	2-Isopropyl-4,5-dimethylthiazole		53498-30-9	Solid $C_8H_{13}NS$ 155.26	Practically insoluble or insoluble Freely soluble	244 101 MS 95 %	n.a. n.a.	
15.082	3-Mercaptothiophene		7774-73-4	Liquid $C_4H_4S_2$ 116.20	Slightly soluble Freely soluble	171 MS 95 %	1.617-1.623 1.248-1.254	
15.084	5-Methyl-2-pentylthiazole		86290-21-3	Solid $C_9H_{15}NS$ 169.26	Practically insoluble or insoluble Freely soluble	262 114 NMR 95 %	n.a. n.a.	
15.085	4-Methyl-2-propionylthiazole		11622 13679-83-9	Solid C_7H_9NOS 155.21	Practically insoluble or insoluble Freely soluble	86 (12 hPa) 142 NMR 95 %	n.a. n.a.	
15.086	2-Methyl-2-thiazoline		2346-00-1	Solid C_4H_7NS 101.17	Slightly soluble Freely soluble	144 62 MS 95 %	n.a. n.a.	
15.087	2-Methyl-3-mercaptothiophene		2527-76-6	Solid $C_5H_6S_2$ 130.22	Slightly soluble Freely soluble	73 (15 hPa) 27 MS 95 %	n.a. n.a.	
15.088	2-Methyl-4,5-benzothiazole		120-75-2	Liquid C_8H_7NS 149.21	Practically insoluble or insoluble	238 MS	1.612-1.618 1.173-1.179	No longer supported by Industry (DG SANCO,

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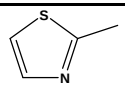
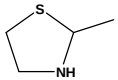
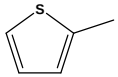
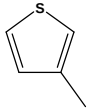
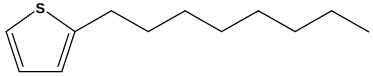
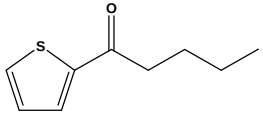
					Freely soluble	95 %		2012).
15.089	2-Methylthiazole		11626 3581-87-1	Liquid C_4H_5NS 99.15	Slightly soluble Freely soluble	128 MS 95 %	1.511-1.517 1.109-1.116	
15.090	2-Methylthiazolidine		24050-16-6	Liquid C_4H_9NS 103.18	Slightly soluble Freely soluble	160 MS 95 %	1.522-1.528 1.519-1.525	No longer supported by Industry (DG SANCO, 2012).
15.091	2-Methylthiophene		11631 554-14-3	Liquid C_5H_6S 98.16	Practically insoluble or insoluble Freely soluble	112 MS 97 %	1.516-1.523 1.013-1.022	No longer supported by Industry (DG SANCO, 2013).
15.092	3-Methylthiophene		11632 616-44-4	Liquid C_5H_6S 98.16	Practically insoluble or insoluble Freely soluble	115 MS 95 %	1.514-1.520 1.018-1.024	No longer supported by Industry (DG SANCO, 2013).
15.093	2-Octylthiophene		880-36-4	Solid $C_{12}H_{20}S$ 196.35	Practically insoluble or insoluble Freely soluble	259 64 MS 95 %	n.a. n.a.	
15.094	2-Pentanoylthiophene		53119-25-8	Solid $C_9H_{12}OS$ 168.26	Practically insoluble or insoluble Freely soluble	257 80 MS 95 %	n.a. n.a.	No longer supported by Industry (DG SANCO, 2012).

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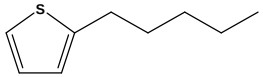
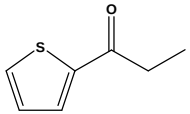
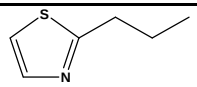
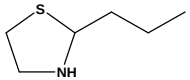
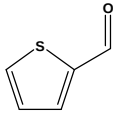
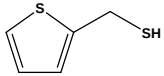
15.096 2106	2-Pentylthiophene		4387 11634 4861-58-9	Liquid $C_9H_{14}S$ 154.27	Practically insoluble or insoluble Freely soluble	201 MS 95 %	1.495-1.501 0.940-0.946	The former incorrect name *sec*-pentylthiophene has been changed.
15.097	2-Propionylthiophene		11635 13679-75-9	Solid C_7H_8OS 140.20	Practically insoluble or insoluble Freely soluble	225 57 MS 95 %	n.a. n.a.	
15.098	2-Propylthiazole		17626-75-4	Solid C_6H_9NS 127.20	Practically insoluble or insoluble Freely soluble	172 78 MS 95 %	n.a. n.a.	
15.099	2-Propylthiazolidine		24050-10-0	Solid $C_6H_{13}NS$ 131.24	Practically insoluble or insoluble Freely soluble	75 (13 hPa) 84 MS 95 %	n.a. n.a.	No longer supported by Industry (DG SANCO, 2012).
15.106	Thiophene		11647 110-02-1	Liquid C_4H_4S 84.14	Practically insoluble or insoluble Freely soluble	84 MS 95 %	1.525-1.531 1.062-1.068	No longer supported by Industry (DG SANCO, 2012).
15.107	Thiophene-2-carbaldehyde		11874 98-03-3	Liquid C_5H_4OS 112.15	Slightly soluble Freely soluble	198 MS 95 %	1.585-1.592 1.216-1.225	No longer supported by Industry (DG SANCO, 2012).
15.108	2-Thiophenemethanethiol			Liquid $C_5H_6S_2$	Slightly soluble	82 (16 hPa)	1.571-1.578 1.165-1.171	

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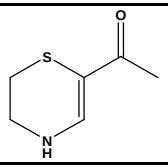
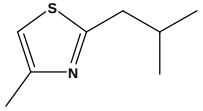
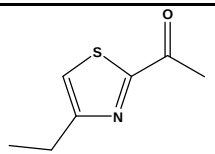
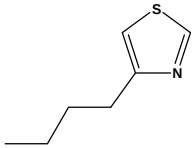
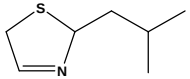
			6258-63-5	130.22	Freely soluble	MS 95 %		
15.114	6-Acetyl-2,3-dihydro-1,4-thiazine		4296 101417-25-8	Solid C_6H_9NOS 143.2	Sparringly soluble Freely soluble	242 126 NMR 95 %	n.a. n.a.	No longer supported by Industry (DG SANCO, 2012).
15.115	2-Isobutyl-4-methyl thiazole		 61323-24-8	Solid $C_8H_{13}NS$ 155.26	Slightly soluble Freely soluble	189 88 MS 95 %	n.a. n.a.	
15.116	2-Acetyl-4-ethylthiazole		 233665-91-3	Solid C_7H_9NOS 155.22	Slightly soluble Freely soluble	270 142 NMR 95 %	n.a. n.a.	
15.118	4-Butylthiazole		 53833-33-3	Solid $C_7H_{11}NS$ 141.23	Practically insoluble or insoluble Freely soluble	212 79 MS 95 %	n.a. n.a.	
15.119	2-Isobutyl-3-thiazoline		 39800-92-5	Solid $C_7H_{13}NS$ 143.25	Very slightly soluble Freely soluble	197 80 MS 95 %	n.a. n.a.	Racemate (EFFA, 2011b).

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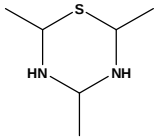
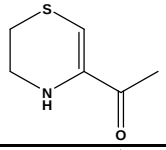
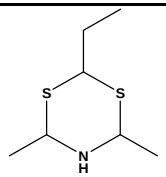
15.129	Tetrahydro-2,4,6-trimethyl-1,3,5(2H)-thiadiazine		53897-63-5	Solid $C_6H_{14}N_2S$ 146	Soluble Soluble	216-266 (1 hPa) 65-70 99 %	n.a. n.a.	No longer supported by Industry (DG SANCO, 2012).
15.133 1766	5-Acetyl-2,3-dihydro-1,4-thiazine		164524-93-0	Solid C_6H_9NOS 143	Soluble Soluble	289+/-30 67-69 98 %	n.a. n.a.	No longer supported by Industry (DG SANCO, 2012).
15.135	Ethyl thialdine		4667 54717-14-5	Liquid $C_7H_{15}NS_2$ 177.33	Poorly soluble Soluble	75 IR NMR MS 90 %	1.5344-1.5544 1.0745-1-0765	Mixture of diastereoisomers, each of them racemic (EFFA, 2013b). Minimum assay value 90 %. Secondary components: 3,5-diethyl-1,2,4-trithiolane (< 5 %); thialdine (< 2 %); others (< 3 %) (Flavour Industry, 2011).

(a): Solubility in water, if not otherwise stated.
(b): Solubility in 95 % ethanol, if not otherwise stated.
(c): At 1013.25 hPa, if not otherwise stated.
(d): At 20°C, if not otherwise stated.
(e): At 25°C, if not otherwise stated.

2.2. Stereoisomers

It is recognised that geometrical and optical isomers of substances may have different properties. Their flavour may be different; they may have different chemical properties resulting in possible variability in their absorption, distribution, metabolism, elimination and toxicity. Thus, information must be provided on the configuration of the flavouring substance, i.e. whether it is one of the geometrical/optical isomers or a defined mixture of stereoisomers. The available specifications of purity will be considered in order to determine whether the safety evaluation carried out for candidate substances for which stereoisomers may exist can be applied to the material of commerce. Flavouring substances with different configurations should have individual chemical names and codes (CAS number, FLAVIS number, etc.)

Two of the 41 candidate substances possess one chiral centre [FL-no: 15.060, and 15.119], three possess two chiral centres [FL-no: 15.054, 15.057 and 15.135] and two possess three chiral centres [FL-no: 15.055 and 15.079]. The stereoisomeric composition has been specified for all substances (EFFA, 2010, 2011b, 2013b) (see Table 4).

2.3. Natural occurrence in food

Thirty-one candidate substances have been reported to occur naturally in one or more of the following food items: shellfish, shrimps, squid, pork, beef, lamb, chicken, vegetables, peanut, wheaten bread, butter, cheese, tea, coffee, cocoa and various types of alcoholic beverages. Quantitative data on the natural occurrence in foods have been reported for nine of these substances (TNO, 2000, 2010, 2011), see Table 5.

Table 5: Candidate substances reported to occur in food

FL-no	Name	Quantitative data reported
15.038	2-Acetyl-4-methylthiazole	0.02 mg/kg in kohlrabi
15.040	2-Acetylthiophene	Up to 1.25 mg/kg in coffee, 0.02 mg/kg in kohlrabi, up to 0.00002 mg/kg in pork (grilled, roasted)
15.061	2,5-Dimethyl-4-	0.00001 mg/kg in pork (grilled, roasted)
15.062	2,4-Dimethylthiazole	Up to 0.00004 mg/kg in pork (grilled, roasted)
15.069	4-Ethyl-5-methylthiazole	Up to 0.00001 mg/kg in pork (grilled, roasted)
15.076	2-Hexylthiophene	Up to 0.002 mg/kg in guinea hen
15.096	2-Pentylthiophene	0.001 mg/kg in chicken (roasted)
15.097	2-Propionylthiophene	0.8 mg/kg in coffee
15.135	Ethyl thialdine	0.02 mg/kg in krill, up to 0.3 mg/kg in shrimps

Ten of the candidate substances have not been reported to occur naturally in any food items according to the Nederlandse Organisatie voor Toegepast Natuurwetenschappelijk Onderzoek (TNO) (TNO, 2000, 2010, 2011, 2014), see Table 6.

Table 6: Candidate substances not reported to occur in food

FL-no	Name
15.044	2-Butylthiazole
15.055	[2S-(2a,4a,8ab)] 2,4-Dimethyl(4H)pyrrolidino[1,2e]-1,3,5-dithiazine
15.074	5-Ethylthiophene-2-carbaldehyde
15.079	2-Isobutyldihydro-4,6-dimethyl-1,3,5-dithiazine
15.082	3-Mercaptothiophene
15.087	2-Methyl-3-mercaptothiophene
15.108	2-Thiophenemethanethiol
15.115	2-Isobutyl-4-methylthiazole
15.116	2-Acetyl-4-ethylthiazole
15.119	2-Isobutyl-3-thiazoline

3. Specifications

Purity criteria for the candidate substances have been provided by the flavouring industry (EFFA, 2004d, 2011a; Flavour Industry, 2010b).

Judged against the requirements in Annex II of Commission Regulation (EC) No 1565/2000, the information is adequate for all 41 substances (see Section 2.2 and Table 4).

4. Intake data

Annual production volumes of the flavouring substances as surveyed by the Industry can be used to calculate the "Maximised Survey-derived Daily Intake" (MSDI) by assuming that the production figure only represents 60 % of the use in food because of underreporting and that 10 % of the total EU population are consumers (SCF, 1999).

However, the Panel noted that because of year-to-year variability in production volumes, to uncertainties in the underreporting correction factor and to uncertainties in the percentage of consumers, the reliability of intake estimates on the basis of the MSDI approach is difficult to assess.

The Panel also noted that in contrast to the generally low *per capita* intake figures estimated on the basis of this MSDI approach, in some cases the regular consumption of products flavoured at use levels reported by the Flavour Industry in the submissions would result in much higher intakes. In such cases, the human exposure thresholds below which exposures are not considered to present a safety concern might be exceeded.

Considering that the MSDI model may underestimate the intake of flavouring substances by certain groups of consumers, the Scientific Committee on Food (SCF) recommended also taking into account the results of other intake assessments (SCF, 1999).

One of the alternatives is the "Theoretical Added Maximum Daily Intake" (TAMDI) approach, which is calculated on the basis of standard portions and upper use levels (SCF, 1995) for flavourable beverages and foods in general, with exceptional levels for particular foods. This method is regarded as a conservative estimate of the actual intake by most consumers because it is based on the assumption that the consumer regularly eats and drinks several food products containing the same flavouring substance at the upper use level.

One option to modify the TAMDI approach is to base the calculation on normal rather than upper use levels of the flavouring substances. This modified approach is less conservative (e.g. it may underestimate the intake of consumers being loyal to products flavoured at the maximum use levels reported). However, it is considered as a suitable tool to screen and prioritise the flavouring substances according to the need for refined intake data (EFSA, 2004).

4.1. Estimated daily *per capita* intake (MSDI approach)

The intake estimation is based on the MSDI approach, which involves the acquisition of data on the quantities used in food as flavourings (SCF, 1999). These data are derived from surveys on annual production volumes in Europe. These surveys were conducted in 1995 by the International Organization of the Flavour Industry (IOFI), in which flavour manufacturers reported the total quantity of each flavouring substance incorporated into food sold in the EU during the previous year (IOFI, 1995). The intake approach does not consider the possible natural occurrence in food.

Average *per capita* intake (MSDI) is estimated on the assumption that the quantity added to food is consumed by 10 % of the population[9] (Eurostat, 1998). This is derived for candidate substances from estimates of annual volume of production provided by Industry and incorporates a correction factor of 0.6 to allow for incomplete reporting (60 %) in the Industry surveys (SCF, 1999).

The total annual volume of production of the candidate substances from use as flavouring substances in Europe has been reported to be approximately 110 kg (EFFA, 2004b, 2011a, 2012a; Flavour Industry, 2010b; IOFI, 2013a, b). 4,6-Dimethyl-2-(1-methylethyl)dihydro-1,3,5-dithiazine [FL-no: 15.057] and 2-isobutyldihydro-4,6-dimethyl-1,3,5-dithiazine [FL-no: 15.079] accounts for 60 kg. For 27 of the 28 supporting substances the annual volume of production is approximately 4 100 kg in Europe (JECFA, 2003; EFFA, 2012a, b, 2013a). Thiamine hydrochloride [FL-no: 16.027] accounts for 2 500 kg and 5-(2-hydroxyethyl)-4-methylthiazole [FL-no: 15.014] for 1 200 kg.

On the basis of the annual volumes of production reported for the candidate substances, the daily *per capita* intakes for each of these flavourings have been estimated (Tables 9 and 10).

The estimated daily *per capita* intake of 2-isobutyldihydro-4,6-dimethyl-1,3,5-dithiazine [FL-no: 15.079], from use as a flavouring substance, is 5.7 µg, of 4,6-dimethyl-2-(1-methylethyl)dihydro-1,3,5-dithiazine [FL-no: 15.057] 1.6 µg and of 4-butylthiazole [FL-no: 15.118] 1.3 µg. For the remaining 37 substances, the estimated daily *per capita* intakes are in the range of 0.0012 to 0.85 µg (Tables 9 and 10).

4.2. Intake estimated on the basis of the modified TAMDI (mTAMDI)

The method for calculation of the modified Theoretical Added Maximum Daily Intake (mTAMDI) values is based on the approach used by SCF up to 1995 (SCF, 1995).

The assumption is that a person may consume a certain quantity of flavourable foods and beverages per day.

For the present evaluation of the candidate substances, information on food categories and normal and maximum use levels[10,11,12] were submitted by the Flavour Industry (EFFA, 2004c, d, 2007; Flavour Industry, 2004–5, 2010b) for 39 substances. No information on use levels have been submitted for [FL-no: 15.057 and 15.079]. The candidate substances are used in flavoured food products, divided into the food categories outlined in Annex III of the Commission Regulation (EC) No 1565/2000, as shown in Table 7. For the present calculation of the mTAMDI, the reported normal use levels were used. In cases where different use levels were reported for different food categories the highest reported normal use level was used.

[9] EU figure, 375 million. This figure relates to EU population at the time for which production data are available, and is consistent (comparable) with evaluations conducted prior to the enlargement of the EU. No production data are available for the enlarged EU.

[10] "Normal use" is defined as the average of reported usages and "maximum use" is defined as the 95th percentile of reported usages (EFFA, 2002).

[11] The normal and maximum use levels in different food categories (EC, 2000) have been extrapolated from figures derived from 12 model flavouring substances (EFFA, 2004a).

[12] The use levels from food category 5 "Confectionery" have been inserted as default values for food category 14.2 "Alcoholic beverages" for substances for which no data have been given for food category 14.2 (EFFA, 2007).

Table 7: Use of the 39 candidate substances for which data on use have been provided in various food categories

Food category	Description	Flavourings used [(a)]
01.0	Dairy products, excluding products of category 2	All
02.0	Fats and oils, and fat emulsions (type water-in-oil)	All
03.0	Edible ices, including sherbet and sorbet	All except one [FL-no: 15.135]
04.1	Processed fruits	All
04.2	Processed vegetables (including mushrooms and fungi, roots and tubers, pulses and legumes), and nuts and seeds	[FL-no: 15.062 and 15.135]
05.0	Confectionery	All except two [FL-no: 15.062 and 15.135]
06.0	Cereals and cereal products, including flours and starches from roots and tubers, pulses and legumes, excluding bakery	All
07.0	Bakery wares	All
08.0	Meat and meat products, including poultry and game	All
09.0	Fish and fish products, including molluscs, crustaceans and echinoderms	All except two [FL-no: 15.039 and 15.135]
10.0	Eggs and egg products	None
11.0	Sweeteners, including honey	None
12.0	Salts, spices, soups, sauces, salads, protein products, etc.	All except one [FL-no: 15.089]
13.0	Foodstuffs intended for particular nutritional uses	All except one [FL-no: 15.135]
14.1	Non-alcoholic ("soft") beverages, excluding dairy products	All except one [FL-no: 15.135]
14.2	Alcoholic beverages, including alcohol-free and low-alcoholic counterparts	All except two [FL-nos: 15.062 and 15.135]
15.0	Ready-to-eat savouries	All except one [FL-no: 15.068]
16.0	Composite foods (e.g. casseroles, meat pies, mincemeat)—foods that could not be placed in categories 1–15	All except one [FL-no: 15.135]

(a): No use levels have been submitted for [FL-nos: 15.057 and 15.079].

According to the Flavour Industry, the normal use levels range from 0.1 to 2 mg/kg food, and the maximum use levels range from 0.2 to 12 mg/kg (EFFA, 2004c, d, 2007; Flavour Industry, 2004–5, 2010b) for 39 of the 41 substances for which use levels have been provided (Section 7 and Appendix B).

The mTAMDI values for the 34 candidate substances from structural class (SC) II (see Section 7) range from 78 to 220 µg/person per day. For the remaining seven candidate substances from SC III, for which data have been provided, the mTAMDI range from 78 to 250 µg/person per day.

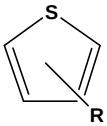
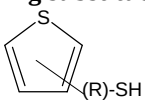
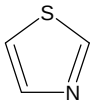
For detailed information on use levels and intake estimations based on the mTAMDI approach, see Section 7 and Appendix B.

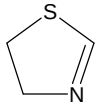
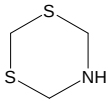
5. Absorption, distribution, metabolism and elimination

The 41 candidate substances are structurally related to 28 supporting substances evaluated by the JECFA in "Sulfur-containing heterocyclic compounds" (JECFA, 2003, 2008). The substances are divided into subgroups based on the nature of the ring; aromatic (clustered in subgroups A-Ib, A-Ic and A-II) vs. non-aromatic (clustered in subgroups B-II and B-IV)), on type and number of ring

heteroatoms (sulphur or sulphur with nitrogen) and the degree of saturation in the non-aromatic rings. The assignment of the individual substances to the different subgroups is presented in Table 8 (the substances previously allocated to subgroup A-Ia, A-III, BI, B-III, B-V and B-VI are no longer supported by Industry). The structures of the substances and a description of the characteristic features of the subgroups are also given in Table C.1 and the accompanying text in Appendix C.

Table 8: Division of candidate substances into structural subgroups

Subgroup and common ring structure	Register name	FL-no
A Aromatic subgroups		
A-Ia: Thiophene	The substance previously allocated to the group is no longer supported for use as a flavouring substance in Europe by Industry	
A-Ib: Thiophenes (with non-thiol-containing ring substituents) 	2-Acetylthiophene 2-Butylthiophene 5-Ethylthiophene-2-carbaldehyde 2-Hexylthiophene 2-Octylthiophene 2-Pentylthiophene 2-Propionylthiophene	15.040 15.045 15.074 15.076 15.093 15.096 15.097
A-Ic: Thiophenes (with thiol-containing ring substituents) 	3-Mercaptothiophene 2-Thiophenemethanethiol 2-Methyl-3-mercaptothiophene	15.082 15.108 15.087
A-II: Thiazoles 	2-Methylthiazole 2-Ethylthiazole 2-Propylthiazole 2-Butylthiazole 4-Butylthiazole 2,4-Dimethylthiazole 2,5-Dimethylthiazole 5-Ethyl-2-methylthiazole 4-Ethyl-2-methylthiazole 4-Ethyl-5-methylthiazole 2,5-Diethylthiazole 5-Methyl-2-pentylthiazole 4,5-Dimethyl-2-ethylthiazole 2,5-Dimethyl-4-ethylthiazole 2,5-Diethyl-4-methylthiazole 2,5-Diethyl-4-propylthiazole 2-Isobutyl-4-methylthiazole 2-Isobutyl-4,5-dimethylthiazole 2-Isopropyl-4,5-dimethylthiazole 2-Acetyl-4-methylthiazole 2-Acetyl-5-methylthiazole 2-Acetyl-4-ethylthiazole 4-Methyl-2-propionylthiazole	15.089 15.071 15.098 15.044 15.118 15.062 15.063 15.068 15.067 15.069 15.052 15.084 15.058 15.061 15.050 15.051 15.115 15.078 15.080 15.038 15.039 15.116 15.085
A-III: Benzothiazoles	The substance previously allocated to the group is no longer supported for use as a flavouring substance in Europe by Industry	
B Non-aromatic subgroups		
B-I: Dihydrothiophenes	The substance previously allocated to the group is no longer supported for use as a flavouring substance in Europe by Industry	

B-II: Thiazolines 	2-Methyl-2-thiazoline	15.086
	2,4-Dimethyl-3-thiazoline	15.060
	2-Isobutyl-3-thiazoline	15.119
B-III: Thiazolidines	The substances previously allocated to the group are no longer supported for use as flavouring substances in Europe by Industry	
B-IV: Dithiazines 	Dihydro-2,4,6-triethyl-1,3,5(4*H*)-dithiazine	15.054
	[2*S*-(2*a*,4*a*,8*ab*)] 2,4-Dimethyl(4*H*)pyrrolidino[1,2*e*]-1,3,5-dithiazine	15.055
	4,6-Dimethyl-2-(1-methylethyl)dihydro-1,3,5-dithiazine	15.057
	2-Isobutyldihydro-4,6-dimethyl-1,3,5-dithiazine	15.079
	Ethyl thialdine	15.135
B-V: Dihydrothiazines	The substances previously allocated to the group are no longer supported for use as flavouring substances in Europe by Industry	
B-VI: Thiadiazine	The substance previously allocated to the group is no longer supported for use as a flavouring substance in Europe by Industry	

From the limited data available on absorption, distribution and elimination of the aromatic candidate substances in this FGE, it is anticipated that they may be absorbed and eliminated after biotransformation. Some volatile substances may also be eliminated unchanged via exhalation. For the non-aromatic substances there are no data available for the candidate or for the supporting substances.

For the evaluation of the metabolism of the candidate substances, only limited data were available. These were confined to a few studies on some thiophene and thiazole derivatives (i.e. only directly relevant for the evaluation of subgroups A (A-Ib, A-Ic and A-II)), the aromatic candidate substances. Other information was found in several review papers. However, virtually no data were found on the metabolism of substances from the B subgroups possessing non-aromatic ring structures.

5.1. Metabolism of substances in subgroup A-I

The structurally related thiophene and the ring-substituted thiophenes (A-Ib and A-Ic) may undergo *S*-oxidation to give sulphoxides. These primary metabolites may react spontaneously or via glutathione transferase with glutathione (GSH), and it is likely that they also exhibit reactivity towards protein thiols. The glutathione conjugates may be further metabolised to the corresponding mercapturic acids and excreted in the urine. *S*-oxide intermediates may also be subject to dimerisation via a Diels-Alder reaction. In addition, 4,5-epoxide formation has been reported for 2-phenylthiophene, followed by subsequent conjugation of the epoxide with glutathione. This may also be anticipated for other ring-substituted thiophenes.

Only very limited information was submitted to show whether side chain oxidation of the ring-substituted thiophenes could also occur. Based on some studies with a substituted thiazole (chlormethiazole) and one (heavily) substituted thiophene (olanzapine), such side chain reactions may be anticipated. For the substances in subgroup A-Ib, metabolites of the side chain oxidation pathways may be expected to be conjugated, e.g. with glucuronic acid. Similar reactions (e.g. omega or omega-1 oxidations) for ring-substituent chains have been discussed for alkylated pyrazines (EFSA CEF Panel, 2011a). The thiophene carbaldehyde candidate substance, 5-ethylthiophene-2-carbaldehyde [FL-no: 15.074] may be expected to be oxidised to the corresponding carboxylic acid. Where applicable, conjugation with amino acids (e.g. glycine) or glucuronic acid may also be expected to occur (see FGE.13Rev2 (EFSA CEF Panel, 2011b); FGE.14Rev1 (EFSA, 2009); FGE.17Rev2 (EFSA CEF Panel, 2011a)). The acyl-substituted thiophenes [FL-no: 15.040 and 15.097] may be subject to keto-reduction, possibly followed by conjugation, similar to acetyl derivatives of furans (EFSA CEF Panel, 2011b) and of pyrazines (EFSA CEF Panel, 2011a).

The mercapto group in the ring-substituent of the substances in subgroup A-Ic may undergo *S*-methylation to produce the corresponding methyl thioether, with further oxidation to the corresponding sulphoxide and sulphone. They may also react with glutathione or other endogenous thiol substances to form mixed disulphides, which may undergo reduction to thiols or oxidative desulphuration. Alternatively, they may undergo enzymatic oxygenation resulting in the formation of the corresponding sulphinic or sulphonic acids. These metabolites are all expected to be excreted in the urine. A more detailed discussion on the metabolism of sulphur compounds can be found in FGE.13Rev2 (EFSA CEF Panel, 2011b).

5.2. Metabolism of substances in subgroup A-II

Thiazoles may be subject to ring *S*- and *N*-oxidation. From the various studies with thiazole derivatives (A-II), it can be seen that their metabolites may react spontaneously with glutathione, and it is likely that they also exhibit reactivity towards biomacromolecules. In addition, for some thiazole derivatives, ring *C*-oxidation may be accompanied by heterocyclic ring cleavage which can result in the formation of alpha-diketone and thioamide intermediates. For the latter, a relationship with nephro- and hepatotoxicity has been established, especially after glutathione depletion, but this is a high-dose phenomenon. Owing to the compound specificity of these reactions, the extent of formation cannot be generalised; however, these thioamide intermediates and the *S*-oxides seem to be quantitatively minor metabolites.

Similar to the situation with the ring-substituted thiophenes, only very limited information was available which could demonstrate whether side chain oxidation of the ring-substituted thiazoles could occur. Based on some studies with a substituted thiazole (chlormethiazole) such side chain reactions may be anticipated. Metabolites of the side chain oxidation pathways may be expected to be conjugated, e.g. with glucuronic acid. Similar reactions (e.g. omega or omega-1 oxidations) for ring-substituent chains have been discussed for alkylated pyrazines (EFSA CEF Panel, 2011a). The acyl-substituted thiazoles [FL-no: 15.038, 15.039, 15.085 and 15.116] may be subject to keto-reduction, possibly followed by conjugation, similar to acetyl derivatives of furans (EFSA CEF Panel, 2011b) and pyrazines (EFSA CEF Panel, 2011a).

5.3. Conclusions for the substances in the subgroups A-I to A-II

From the very limited data available on the aromatic subgroups (A) it is not anticipated that the thiophenes (A-I) and thiazoles (A-II) utilise similar metabolic pathways. These sulphur-containing heteroaromatic derivatives can be expected to participate in metabolic pathways principally involving side chain *C*-oxidation, epoxidation of double bonds, ring *S*-oxidation and (for the thiazoles) ring *N*-oxidation to yield sulphoxides or sulphones and *N*-oxides respectively. The reactive intermediates (e.g. epoxides and *S*-oxides) may be conjugated with glutathione; the glutathione conjugates may be excreted directly in the urine or following further biotransformation to the corresponding mercapturic acids.

In the light of the above considerations, and as a consequence of the expected reactivity of the thiol groups and the possible reactions of the ring side chains, it cannot be anticipated that the substances in the aromatic subgroups (A) are metabolised to innocuous substances. The Panel was aware of the potential bio-activation of candidate substances with heteroaromatic rings to yield intermediates via ring-scission or *S/N*-oxidation which could be reactive to proteins or DNA. However, taking into account available data on structurally related thiazoles and considering the available genotoxicity data on thiophenes and thiazoles in the present revision of FGE.21 (FGE.21Rev5) (negative Ames tests and a chromosome aberration test), the Panel concluded that there are not sufficient indications to preclude the application of the Procedure to the thiophenes and thiazoles (A-I and A-II).

5.4. Metabolism of the substances in subgroups B-II and B-IV

No specific information was available on the metabolism of the thiazoline and dithiazine derivatives or related substances for any of these non-aromatic substances in the B subgroups. It may be

speculated that the substances in these groups are metabolised primarily by ring *S*-oxidation, or, if applicable, via *N*-oxidation. In addition, metabolism of the ring substituents is likely to occur.

The substances exhibiting thioacetal structures could be subject to acid hydrolysis in the stomach, similar to oxygen-containing acetals. However, thioacetals are more resistant to hydrolysis than oxygen acetals. It is thus to be anticipated that these substances may reach the intestinal lumen intact and may also be absorbed as such.

5.5. Conclusions for the substances in the subgroups B-II and B-IV

Owing to the lack of metabolism data it cannot be concluded that the candidate substances in the B subgroups will be metabolised to innocuous products.

A more detailed description of the metabolism is given in Appendix C.

6. Application of the Procedure for the safety evaluation of flavouring substances

The application of the Procedure is based on intakes estimated on the basis of the MSDI approach. Where the mTAMDI approach indicates that the intake of a flavouring substance might exceed its corresponding threshold of concern, a formal safety assessment, using the mTAMDI approach, is not carried out using the Procedure. In these cases, the Panel requires more precise data on use and use levels. For comparison of the intake estimations based on the MSDI approach and the mTAMDI approach see Section 7.

For the three thiazolines in subgroup B-II (2-methyl-2-thiazoline [FL-no: 15.086], 2,4-dimethyl-3-thiazoline [FL-no: 15.060] and 2-isobutyl-3-thiazoline [FL-no: 15.119]), the Panel concluded in FGE.21Rev3 that, in the absence of further genotoxicity data, the Procedure could not be applied to these substances from subgroup B-II. However, new adequate *in vitro* genotoxicity studies (gene mutation test in bacteria and micronucleus assay in human peripheral blood lymphocytes) on 2-acetyl-2-thiazoline [FL-no: 15.010], which is considered as a supporting substance for 2-methyl-2-thiazoline [FL-no: 15.086] (EFSA CEF Panel, 2012a), have become available. Based on these new data, the Panel concluded that 2-methyl-2-thiazoline [FL-no: 15.086] was not of concern with respect to genotoxicity and could be evaluated through the Procedure. For the 3-thiazolines in subgroup B-II, 2,4-dimethyl-3-thiazoline [FL-no: 15.060] and 2-isobutyl-3-thiazoline [FL-no: 15.119], no studies with respect to genotoxicity have become available, nor on the supporting substance (4,5-dimethyl-2-isobutyl-3-thiazoline [FL-no: 15.032]). These two compounds were therefore not evaluated through the Procedure.

Thus, the Panel concluded that, in the absence of further genotoxicity data, the Procedure could not be applied to 2,4-dimethyl-3-thiazoline [FL-no: 15.060] and 2-isobutyl-3-thiazoline [FL-no: 15.119].

For the safety evaluation of the remaining 39 candidate substances from chemical groups 29 and 30 the Procedure as outlined in Appendix A was applied, based on the MSDI approach. The outcome of the evaluations of the 41 substances are summarised in Table 10.

6.1. Step 1

According to the decision tree approach, presented by Cramer et al. (1978), 32 of the candidate substances, evaluated through the Procedure, are classified into SC II and seven into SC III (see Table 10).

6.2. Step 2

None of the 39 candidate substances can be predicted to be metabolised to innocuous products. Therefore, the evaluation of all 39 candidate substances, evaluated through the Procedure, proceeds via the B-side of the Procedure.

6.3. Step B3

The 32 candidate substances in SC II have estimated European daily *per capita* intakes (MSDI) ranging from 0.0012 to 2.2 µg (Table 9). These intakes are below the threshold of concern of 540 µg/person per day for SC II substances.

Similarly, the estimated daily *per capita* intakes of the seven candidate substances in SC III, ranging from 0.0073 to 5.7 µg (Table 9), are below the threshold of concern of 90 µg/person per day for SC III substances.

Accordingly, the evaluation of all 39 candidate substances proceeds to step B4.

6.4. Step B4

6.4.1. Subgroup A-Ib: Thiophenes with non-thiol-containing ring substituents

For candidate substance 5-ethylthiophene-2-carbaldehyde [FL-no: 15.074] an oral gavage 90-day study on rats has been made available. From this study, a NOAEL of 7.5 mg/kg body weight (bw) per day can be derived. This NOAEL covers the acyl-substituted thiophenes in subgroup A-Ib [FL-no: 15.040 and 15.097]. The combined estimated daily *per capita* intake of 0.10 µg for the acyl- or carbaldehyde-substituted thiophene candidate substances of subgroup A-1b [FL-no: 15.040, 15.074 and 15.097] corresponds to 0.0017 µg/kg bw per day for 60 kg body weight. Thus, an adequate margin of safety of 4.4×10^6 can be calculated.

For the candidate substance 2-pentylthiophene [FL-no: 15.096] a 90-day dietary study has been provided from which the Panel derived a NOAEL of 5.5 mg/kg bw per day. This NOAEL covers the remaining 2-alkylthiophenes from subgroup A-Ib [FL-no: 15.045, 15.076, 15.093 and 15.096]. The combined estimated daily *per capita* intake of 2.07 µg for the candidate substances [FL-no: 15.045, 15.076, 15.093 and 15.096] corresponds to 0.034 µg/kg bw per day for 60 kg body weight. Thus, a margin of safety of 1.6×10^5 can be calculated.

On the basis of the application of the Procedure [FL-no: 15.040, 15.045, 15.074, 15.076, 15.093, 15.096 and 15.097] are not expected to be of safety concern at their estimated levels of intake.

6.4.2. Subgroup A-Ic: Thiophenes with thiol-containing ring substituents

A NOAEL of 0.29 mg/kg bw per day was reported for the supporting substance 2-thienyl disulphide [FL-no: 15.008] in a single-dose level 90-day study in rats. The combined estimated daily *per capita* intake of 0.14µg for the three candidate substances 3-mercaptothiophene [FL-no: 15.082], 2-methyl-3-mercaptothiophene [FL-no: 15.087] and 2-thiophenemethanethiol [FL-no: 15.108] in subgroup A-Ic corresponds to 0.0023µg/kg bw per day for 60 kg body weight. Thus, a margin of safety of 1.3×10^5 can be calculated**.**

On the basis of the application of the Procedure, 3-mercaptothiophene [FL-no: 15.082], 2-methyl-3-mercaptothiophene [FL-no: 15.087] and 2-thiophenemethanethiol [FL-no: 15.108] are not expected to be of safety concern at their estimated levels of intake.

6.4.3. Subgroup A-II: Thiazoles

A NOAEL of 25 mg/kg bw per day was reported for the supporting substance 5-acetyl-2, 4-dimethylthiazole [FL-no: 15.011] in a single-dose level 90-day study in rats. The combined estimated daily *per capita* intake of 2.3 µg for the 23 candidate substances in subgroup A-II corresponds to 0.038 µg/kg bw per day for 60 kg body weight. Thus, a margin of safety of 6.6×10^5 can be calculated**.**

On the basis of the application of the Procedure, the 23 candidate substances [FL-no: 15.038, 15.039, 15.044, 15.050, 15.051, 15.052, 15.058, 15.061, 15.062, 15.063, 15.067, 15.068, 15.069, 15.071, 15.078, 15.080, 15.084, 15.085, 15.089, 15.098, 15.115, 15.116 and 15.118] in subgroup A-II are not expected to be of safety concern at their estimated levels of intake.

6.4.4. Subgroup B-II: Thiazolines

No toxicological data were available for 2-methyl-2-thiazoline [FL-no: 15.086]. However, for 2-acetyl-2-thiazoline [FL-no: 15.010], which is considered a supporting substance for 2-methyl-2-thiazoline [FL-no: 15.086], a NOAEL of 1.8 mg/kg bw per day from a 90-day rat study that examined a mixture of flavouring substances (including 2-acetyl-2-thiazoline [FL-no: 15.010] (0.9 %)) was examined (Munday and Kirkby, 1971). The estimated daily *per capita* intake of 0.012 µg (0.0002 µg/kg bw per day) for 2-methyl-2-thiazoline [FL-no: 15.086] provides a margin of safety of 9×10^6, in relation to the estimated levels of exposure from the use of 2-methyl-2-thiazoline [FL-no: 15.086] as flavouring substance. The Panel agrees that this provides a sufficient safety margin and that the flavouring substance 2-acetyl-2-thiazoline [FL-no: 15.010] can be concluded at step B4 in the Procedure to be of no safety concern.

6.4.5. Subgroup B-IV: Dithiazines

There were no toxicological data available on the candidate substances in this subgroup B-IV. However, a new adequate 90-day oral toxicity study in rats on the supporting substance 5,6-dihydro-2,4,6-tris(2-methylpropyl)-4*H*-1,3,5-dithiazine [FL-no: 15.113] from FGE.76Rev1 (EFSA CEF Panel, 2013) provides a NOAEL of 9.3 mg/kg bw per day. The combined estimated daily *per capita* intake of approximately 8.2 µg for the candidate substances in subgroup B-IV corresponds to 0.136 µg/kg bw per day for 60 kg body weight. Thus, a margin of safety of 7×10^4 can be calculated.

On the basis of the application of the Procedure, 2-butyl-4-methyl(4*H*)pyrrolidino[1,2*d*]-1,3,5-dithiazine [FL-no: 15.042], dihydro-2,4,6-triethyl-1,3,5(4*H*)-dithiazine [FL-no: 15.054], [2*S*-(2*a*,4*a*,8*ab*)] 2,4-Dimethyl(4*H*)pyrrolidino[1,2*e*]-1,3,5-dithiazine [FL-no: 15.055], 4,6-dimethyl-2-(1-methylethyl)dihydro-1,3,5-dithiazine [FL-no: 15.057], 2-isobutyldihydro-4,6-dimethyl-1,3,5-dithiazine [FL-no: 15.079] and ethyl thialdine [FL-no: 15.135] are not considered to be of safety concern at their estimated levels of intake. However, 2-butyl-4-methyl(4*H*)pyrrolidino[1,2*d*]-1,3,5-dithiazine [FL-no: 15.042] is no longer supported by the Industry (DG SANCO, 2012).

6.5. Summary of application of the Procedure

None of the 39 candidate substances evaluated through the Procedure is of safety concern at their estimated levels of intake based on the MSDI approach (see Table 10).

7. Comparison of the intake estimations based on the MSDI approach and the mTAMDI approach

The estimated intakes, based on the mTAMDI, for the 32 candidate substances assigned to SC II and evaluated using the Procedure range from 78 to 220 µg/person per day, which is below the threshold of concern for SC II of 540 µg/person per day.

The estimated intakes, based on the mTAMDI, for five candidate substances assigned to SC III and evaluated using the Procedure, range from 78 to 250 µg/person per day. For two of these candidate substances [2*S*-(2*a*,4*a*,8*ab*)] 2,4-Dimethyl(4*H*)pyrrolidino[1,2e]-1,3,5-dithiazine [FL-no: 15.055] and

ethyl thialdine [FL-no: 15.135] the mTAMDI values are above the threshold of concern for SC III substances of 90 µg/person per day. For comparison of the MSDI and mTAMDI values see Table 9. For two candidate substances [FL-no: 15.057 and 15.079] assigned to SC III, no information on use levels has been provided.

Therefore, further information is required for four substances [FL-no: 15.055, 15.057, 15.079 and 15.135]. This would include more reliable intake data. On the basis of such additional data, these flavouring substances should be reconsidered along the steps of the Procedure. Following this procedure, additional toxicological data might become necessary.

Table 9: Estimated intakes based on the MSDI approach and the mTAMDI approach

FL-no	EU register name	MSDI (µg/capita per day)	mTAMDI (µg/person per day)	Structural class	Threshold of concern (µg/person per day)
15.038	2-Acetyl-4-methylthiazole	0.0049	160	Class II	540
15.039	2-Acetyl-5-methylthiazole	0.0024	160	Class II	540
15.040	2-Acetylthiophene	0.049	78	Class II	540
15.044	2-Butylthiazole	0.011	160	Class II	540
15.045	2-Butylthiophene	0.012	78	Class II	540
15.050	2,5-Diethyl-4-methylthiazole	0.012	160	Class II	540
15.051	2,5-Diethyl-4-propylthiazole	0.0012	160	Class II	540
15.052	2,5-Diethylthiazole	0.015	160	Class II	540
15.058	4,5-Dimethyl-2-ethylthiazole	0.015	130	Class II	540
15.061	2,5-Dimethyl-4-ethylthiazole	0.011	160	Class II	540
15.062	2,4-Dimethylthiazole	0.61	140	Class II	540
15.063	2,5-Dimethylthiazole	0.0061	160	Class II	540
15.067	4-Ethyl-2-methylthiazole	0.0037	160	Class II	540
15.068	5-Ethyl-2-methylthiazole	0.0061	220	Class II	540
15.069	4-Ethyl-5-methylthiazole	0.012	160	Class II	540
15.071	2-Ethylthiazole	0.028	160	Class II	540
15.074	5-Ethylthiophene-2-carbaldehyde	0.037	78	Class II	540
15.076	2-Hexylthiophene	0.46	78	Class II	540
15.078	2-Isobutyl-4,5-dimethylthiazole	0.12	160	Class II	540
15.080	2-Isopropyl-4,5-dimethylthiazole	0.012	160	Class II	540
15.084	5-Methyl-2-pentylthiazole	0.0037	160	Class II	540
15.085	4-Methyl-2-propionylthiazole	0.0037	160	Class II	540
15.086	2-Methyl-2-thiazoline	0.012	160	Class II	540
15.089	2-Methylthiazole	0.018	150	Class II	540
15.093	2-Octylthiophene	0.012	160	Class II	540
15.096	2-Pentylthiophene	1.6	160	Class II	540
15.097	2-Propionylthiophene	0.012	160	Class II	540
15.098	2-Propylthiazole	0.085	160	Class II	540
15.115	2-Isobutyl-4-methyl thiazole	0.011	160	Class II	540
15.116	2-Acetyl-4-ethylthiazole	0.024	160	Class II	540
15.118	4-Butylthiazole	1.3	160	Class II	540
15.060	2,4-Dimethyl-3-thiazoline	0.012	160	Class II	540
15.119	2-Isobutyl-3-thiazoline	0.011	160	Class II	540
15.054	Dihydro-2,4,6-triethyl-1,3,5(4*H*)-dithiazine	0.85	160	Class III	90
15.055	[2*S*-(2*a*,4*a*,8*ab*)] 2,4-Dimethyl(4*H*)pyrrolidino[1,2*e*]-1,3,5-dithiazine	0.012	160	Class III	90

FL-no	EU register name	MSDI (µg/capita per day)	mTAMDI (µg/person per day)	Structural class	Threshold of concern (µg/person per day)
15.057	4,6-Dimethyl-2-(1-methylethyl)dihydro-1,3,5-dithiazine	1.6		Class III	90
15.079	2-Isobutyldihydro-4,6-dimethyl-1,3,5-dithiazine	5.7		Class III	90
15.082	3-Mercaptothiophene	0.011	78	Class III	90
15.087	2-Methyl-3-mercaptothiophene	0.12	78	Class III	90
15.108	2-Thiophenemethanethiol	0.0073	78	Class III	90
15.135	Ethyl thialdine	0.012	250	Class III	90

8. Considerations of combined intakes from use as flavouring substances

Because of structural similarities of candidate and supporting substances, it can be anticipated that many of the flavourings are metabolised through the same metabolic pathways and that the metabolites may affect the same target organs. Further, in cases of combined exposure to structurally related flavourings, the pathways could be overloaded. Therefore, combined intake should be considered. As flavourings not included in this FGE may also be metabolised through the same pathways, the combined intake estimates presented here are only preliminary. Currently, the combined intake estimates are based on only MSDI exposure estimates, although it is recognised that this may lead to underestimation of exposure. After completion of all FGEs, this issue should be readdressed.

The total estimated combined daily *per capita* intake of structurally related flavourings is estimated by summing the MSDI for individual substances.

Two of the candidate substances [FL-no: 15.060, and 15.119] have not been evaluated through the Procedure (see Section 6) and are therefore not considered together with the other 39 candidate substances evaluated in FGE.21Rev5 in the combined intake.

On the basis of the reported annual production volumes in Europe (EFFA, 2004b, 2011a, 2012a; Flavour Industry, 2010b; IOFI, 2013a, b), the combined estimated daily *per capita* intakes as flavourings of the candidate substances in subgroups, with more than one substance, evaluated through the Procedure, from a SC, are: subgroup A-Ib (seven substances from SC II) 2.2 µg; subgroup A-Ic (three substances from SC III) 0.14 µg; subgroup A-II (23 substances from SC II) 2.3 µg; subgroup B-IV (four substances from SC III) 8.2 µg.

The 39 candidate substances evaluated through the Procedure, are structurally related to 28 supporting substances evaluated by JECFA at its 59th and 68th meetings (JECFA, 2002a, 2007). The total combined daily *per capita* intakes (in Europe) of candidate and supporting substances in each of the five subgroups for which supporting substances were available in the same SC are: subgroup A-Ib (eight substances from SC II) 2.9 µg; subgroup A-Ic (five substances from SC III) 0.15 µg; subgroup A-II (37 substances from SC II) 500 µg. subgroup B-II (two substances from SC II) 0.52 µg; subgroup B-IV (four substances from SC II) 3.6 µg.

For all subgroups, the total combined intakes are below the thresholds of concern of 540 µg/person per day and 90 µg/person per day for SCs II and III, respectively.

9. Toxicity

9.1. Acute toxicity

Data are available for 16 of the 28 supporting substances. The oral median lethal dose (LD_{50}) values in rats or mice ranged from 400 to 4800 mg/kg bw.

The acute toxicity data are summarised in Table 12.

9.2. Subacute, subchronic, chronic and carcinogenicity studies

Subacute toxicity data were available for the candidate substances 2-pentylthiophene [FL-no: 15.096] and the structurally related thiophene former [FL-no: 15.106] and thiophene-2-carbaldehyde former [FL-no: 15.107] and for 11 supporting substances, of which nine are in the register [FL-no: 15.002, 15.004, 15.005, 15.008, 15.010, 15.011, 15.020, 15.029 and 15.113] and two are mixtures of structurally related substances (one mixture of 2-isobutyl-4,6-dimethyldihydro-1,3,5-dithiazine and 4-isobutyl-2,6-dimethyldihydro-1,3,5-dithiazine, and one mixture of 2-isopropyl-4,6-dimethyldihydro-1,3,5-dithiazine and 4-isopropyl-2,6-dimethyldihydro-1,3,5-dithiazine).

Repeated dose toxicity data are summarised in Table 13.

9.2.1. Subgroup A-Ib: Thiophenes with non-thiol-containing ring substituents

9.2.1.1. 28-Day gavage study on 2-pentylthiophene [FL-no: 15.096]

In a study conducted in accordance with Organisation for Economic Co-operation and Development (OECD) Guideline 407, 2-pentylthiopene [FL-no: 15.096] was administered by gavage to three groups each comprising five male and five female Sprague–Dawley Crl:CD® (SD) IGS BR strain rats, for 28 days, at dose levels of 15, 150 or 500 mg/kg bw per day (Dhinsa et al., 2006). A control group of five males and five females was dosed with vehicle alone (Arachis oil). Clinical signs during treatment included increased salivation in all animals treated with 500 mg/kg bw per day and the majority of animals treated with 150 mg/kg bw per day. A slight reduction in body weight gain was observed in males only treated with 500 mg/kg bw per day, while males and females treated with 500 mg/kg bw per day showed an increase in water consumption. Haematological examination revealed haemolytic anaemia in animals treated with 500 or 150 mg/kg bw per day, as evidenced by reductions in haemoglobin, haematocrit and erythrocyte count and evidence of reticulocytosis. Clinical chemical determinations on day 28 showed increases in bilirubin and alanine aminotransferase for animals of both sexes treated with 500 mg/kg bw per day, with increases in bilirubin also noted in both sexes for animals treated with 150 mg/kg per day, and in females receiving 15 mg/kg bw per day. Absolute and relative liver, kidney and spleen weights were increased in both sexes treated with 500 mg/kg bw per day or 150 mg/kg bw per day. At necropsy, animals in these dose groups had dark and enlarged spleens. Histopathological examination revealed hepatocellular hypertrophy in animals of both sexes treated with 500 or 150 mg/kg bw per day. Histopathological evidence of extramedullary haemopoiesis and haemosiderin accumulation in the spleen of animals of both sexes treated with 500 or 150 mg/kg bw per day, together with observations of haemosiderin deposits in the liver and kidney, were typical of a haemolytic anaemia, as also evidenced by the haematological measurements. There was also some evidence of renal damage in males and females treated with 500 or 150 mg/kg bw per day, as evidenced in only a few animals by tubular hypertrophy, tubular dilatation and tubular basophilia, pyelitis with associated hyperplasia of the renal papillary/pelvic epithelium (females only) and globular accumulations of eosinophilic material in the renal tubules (males only). Epithelial hyperplasia of the urinary bladder and associated epithelial and subepithelial inflammatory cell infiltrates were observed for two females treated with 500 mg/kg bw per day. Hypertrophy of the thyroid was reported in animals of both sexes treated with 500 mg/kg bw per day, and for males treated with 150 mg/kg bw per day.

In a follow-up study to evaluate the hepatic effects and the slight increase in bilirubin seen at the lowest dose of 15 mg/kg bw per day in this study, 2-pentylthiophene was administered by gavage to one group comprising five male and five female Sprague–Dawley Crl:CD® (SD) IGS BR strain rats, for 28 consecutive days, at a dose level of 3 mg/kg per day. A control group of five males and five females was given vehicle alone (Arachis oil BP). Clinical signs, body weight development and food and water consumption monitored during the study revealed no significant differences between test and control animals. Haematology and blood chemistry were evaluated for all animals at the end of the study and showed no significant changes. There were no treatment-related changes in spleen weights

for treated animals in comparison to controls. No macroscopic abnormalities were detected at necropsy (Marr and Watson, 2007).

The authors concluded that oral administration of 2-pentylthiophene to rats for a period of up to 28 days at a dose level of 3 mg/kg bw per day resulted in no toxicologically or haematologically significant effects.

9.2.1.2. 90-Day dietary study on 2-pentylthiophene [FL-no: 15.096]

A 90-day dietary study was performed with 2-pentylthiophene [FL-no: 15.096] (Bauter, 2013b). The study was performed according to OECD Guideline 408. Four groups of rats (10/sex/dietary intake level) of male and female Charles River Laboratories (CRL) Sprague–Dawley CD®IGS rats were fed a diet containing 0 (dietary control), 28, 140 and 700 mg 2-pentylthiophene per kilogram of diet in order to obtain target approximate intake levels of 2, 10 and 50 mg/kg bw per day. The animal diets were renewed weekly. To test the stability of the test substance in the dietary matrix, subsamples of test diets were placed into feeding jars and maintained for up to 10 days unrefrigerated in the animal holding room were the study was conducted. Samples were taken for analysis at days 4, 7 and 10. In seven days the amount of test substance in the diet declined to 53, 59 and 54 % of the respective starting levels. The Panel considered this loss of the candidate substance in the feed and decided that the intake levels of 2-pentylthiophene for the different age groups will be calculated on the basis of the dietary level measured on the seventh day of the study. Thus, the intake levels that have been calculated for the dosage groups are the following: males 1.0, 5.5 and 24.4 mg/kg bw per day, females 1.1, 6.2 and 28.4 mg/kg bw per day.

There were no mortalities and no clinical, ophthalmological, body weight, body weight gain, food consumption or food efficiency changes attributable to the administration of 2-pentylthiophene. General decreases in body weight and food consumption were not statistically or biologically significant. Sporadic changes in body weight gain and food consumption in males were considered incidental and not of toxicological concern.

There were few clinical pathology, macroscopic, microscopic, or organ weight changes associated with the administration of 2-pentylthiophene.The only statistically significant ($p < 0.05$) haematology finding among females was limited to a decrease in mean cell haemoglobin concentration in the highest dose group. There were no statistically significant differences in haematology parameters for males. There were minimal changes in haemoglobin concentration, erythrocyte count and haematocrit in males of the high-dose group, which did not reach statistical significance. For males in the highest dosage group there was a statistically significant ($p < 0.05$) decrease in prothrombin time.

Based on the haematological effects observed in males at the high-dose level, a NOAEL can be derived from the mid-dose group of 5.5 mg/kg bw per day. This NOAEL is supported by the observation that no significant toxicological or haematological effects were observed after oral administration (gavage) of 2-pentylthiophene at a dose level of 3 mg/kg bw per day for a period of 28 days (Dhinsa et al., 2006; Marr and Watson, 2007).

9.2.1.3. 14-Day oral toxicity study with 5-ethylthiophene-2-carbaldehyde [FL-no: 15.074]

A 14-day dietary toxicity study was conducted in Hsd:SD® rats (Bauter, 2012b). Twenty-four healthy rats (12 males and 12 females) were selected for the test and equally distributed into four groups (three males and three females per group). Dietary levels of (Group 1, basal diet control) 0, (Group 2, low dose level) 240, (Group 3, middle dose level) 2400 and (Group 4, high dose level) 12000 mg 5-ethylthiophene-2-carbaldehyde per kg diet were selected for the test. The mean overall (days 0–14) daily intake of 5-ethylthiophene-2-carbaldehyde in male rats was 0, 21.9, 193.6 and 675.5 mg/kg bw per day, respectively. The mean overall daily intake in female rats was 0, 21.5, 195.3, and 686.2 mg/kg bw per day, respectively. The test substance, as received and in the diet, was considered stable over the course of the study.

Body weights were recorded twice during the acclimatisation period, including prior to test product introduction (day 0), and on days 3, 7 and 14 prior to terminal sacrifice. Individual food consumption was also recorded to coincide with body weight measurements. Gross necropsies were performed on all animals.

There were no test substance-related or other mortalities during this study. The substance-related adverse effects observed for the high dose group, included clinical signs of reduced faecal volume and variations in body weight, feed consumption and feed efficiency. One male of the high dose group, was severely affected by multiple clinical signs such as red oral/nasal discharge, red facial and anogenital staining, nose/snout swelling, cuts and eschar, tremors, biting and hyperactivity. Males fed with middle doses of 5-ethylthiophene-2-carbaldehyde also exhibited reduced body weights after exposure.

There were no macroscopic findings at scheduled sacrifice for all dose levels in males and females that were considered attributable to test substance administration.

9.2.1.4. 90-Day oral toxicity study with 5-ethylthiophene-2-carbaldehyde [FL-no: 15.074]

A 90-day gavage study was performed with 5-ethylthiophene-2-carbaldehyde [FL-no: 15.074] (Bauter, 2013c). The study was performed according to OECD Guideline 408. Four groups of rats (10/sex/dietary intake level) of male and female CRL Sprague-Dawley CD®IGS rats were administered 0 (dietary control: maize oil), 2.0, 7.5, 75 mg/kg bw per day.

Urine and blood samples were collected on day 84 from all study animals for urinalysis, haematology and clinical chemistry determinations. Coagulation assessments were performed on day 92 or 93, prior to necropsy. Gross necropsies and histological evaluation of selected organs and tissues were performed on all study animals.

There were no test substance-related mortalities during this study. There were no clinical or ophthalmological signs in either male or female animals attributed to the administration of 5-ethylthiophene-2-carbaldehyde.

There were no changes in mean body weight, mean daily body weight gain, food consumption or food efficiency attributed to the administration of 5-ethylthiophene-2-carbaldehyde. There were no statistically significant relative organ weight findings except for an increase in liver to total body weight in male rats in the high dose group ($p < 0.05$), the same tendency was recorded for female rats although not statistically significant.

There were no statistically significant changes in haematology parameters, except for a decrease ($p < 0.05$) in neutrophil counts between low dose females and control.

Statistically significant findings ($p < 0.05$) in clinical chemistry were increased inorganic phosphorus concentration and decreased urea nitrogen in males in the high dose group, the same trend was found in females of the high dose group but was not significant. Females of the high dose group showed a statistically significant decrease in total protein.

Statistically significant ($p < 0.05$) differences in urine parameters were the increase in urine volume and specific gravity in male rats of low and high dose groups, along with an increased pH and a decreased urine protein in male rats of the low dose group. The decrease of urine protein in males of the high dose group was of the same magnitude as for the low dose one, although not recorded as significant. Female rats in the low dose group showed a significantly decreased urinary pH.

At sacrifice there were no macroscopic observations that were attributed to the test substance.

Microscopic findings that were attributed to the test substance were minimal to moderate submandibular salivary gland atrophy of animals in the high dose group, which, with regard to

severity, was more pronounced in males than in females. The changes included moderate atrophy of the convoluted (granular) glands, which appeared as a decrease in the glands' epithelial cell height and was accompanied with a loss of intra-cytoplasmic zymogen granules in all high dose males and in 9 out of 10 high dose females.

The Panel derived a NOAEL for 5-ethylthiophene-2-carbaldehyde [FL-no: 15.074] of 7.5 mg/kg bw per day, based on histopathological findings of atrophy of submandibular salivary gland and clinical chemistry parameters observed at the highest dose level of 75 mg/kg bw per day.

9.2.2. Subgroup A-Ic: Thiophenes with thiol-containing ring substituents

No toxicity data were available for the three candidate substances in this subgroup [FL-nos: 15.082, 15.087 and 15.108]. The supporting substance 2-thienyl disulphide (2,2′-dithiodithiophene) [FL-no: 15.008] was tested in a 90-day dietary feeding study in male and female rats at a single-dose level of 0.29 mg/kg bw per day (Morgareidge and Oser, 1970). Body weight changes, food consumption, haematological and clinical chemistry parameters were assessed, urinalysis was undertaken and a comprehensive range of tissues were examined histopathologically. No changes were detected in any of the parameters assessed, and accordingly a NOAEL of 0.29 mg/kg bw per day could be established.

9.2.3. Subgroup A-II: Thiazoles

There were no subacute, subchronic or chronic toxicity studies on any of the 23 candidate thiazoles in subgroup A-II.

A 90-day dietary feeding study was carried out on the supporting substance 5-acetyl-2,4-dimethylthiazole [FL-no: 15.011] in male (23) and female (23) rats, at a single-dose level of approximately 25 mg/kg bw per day (Shellenberger, 1971). Body weight, food consumption, haematological and clinical chemistry parameters were assessed, urinalysis was undertaken and a comprehensive range of tissues were examined histopathologically. No changes were detected in any of the parameters assessed, and accordingly a NOAEL of 25 mg/kg bw per day could be established.

In a 90-day study on the supporting substance 2-acetylthiazole [FL-no: 15.020], male rats (10/group) were fed diets containing 0, 100, 1000 or 10 000 mg/kg test substance, reported to be equivalent to an average daily intake of 0, 5, 50 and 500 mg/kg bw per day (Wheldon et al., 1970). The dose level in the highest dose group was increased to 20 000 mg/kg at week 6. Test substance-related changes at 10 000–20 000 mg/kg included reduced body weight gain, increased relative liver, adrenal and thyroid weights and minimal fatty changes in the liver. The authors reported a NOAEL of 50 mg/kg bw per day. However, because of limitations in the study design and reporting, the Panel concluded that this NOAEL could not be used in the Procedure.

Two further 90-day studies were carried out at single-dose levels with the supporting substances 2-methyl-5-methoxythiazole [FL-no: 15.002] and 2,4-dimethyl-5-vinylthiazole [FL-no: 15.005] (Posternak et al., 1975). Owing to limitations in reporting of experimental details and results, the Panel concluded that these studies could not be used to derive a NOAEL to be used in the Procedure.

Oral intakes of up to 500 mg thiamine per day (8 mg/kg bw per day for 60 kg body weight) have been reported to have no adverse effects in humans (SCF, 2001).

9.2.4. Subgroup B-II: Thiazolines

There were no toxicological data available for the three candidate substances in this subgroup. The supporting substance 2-(*sec*-butyl)-4,5-dimethyl-3-thiazoline [FL-no: 15.029] was tested in a 90-day dietary feeding study in male and female rats (15/sex) at one-dose level of approximately 1 mg/kg bw per day (Babish and Re, 1978). Body weight changes, food consumption, limited haematological and clinical chemistry parameters were assessed, urinalysis was undertaken and a comprehensive range of tissues were examined histopathologically. No changes were detected in any of the parameters assessed and accordingly a NOAEL of 1 mg/kg bw per day could be established.

The supporting substance 2-acetyl-2-thiazoline [FL-no: 15.010] was tested in a 90-day dietary feeding study in male and female rats (eight/sex). The diet contained 197, 492, 983, 1966, 2949 and 3932 mg/kg diet of a mixture of flavouring substances (including 2-acetyl-2-thiazoline [FL-no: 15.010] (0.9 %) (Munday and Kirkby, 1971). The parameters assessed during the study were body weight gain, food intake, food utilisation, water intake, urine analysis (pH, protein, blood, glucose, refractive index and a transaminase activity), serum chemistry (various enzymes, urea nitrogen, creatinine, various ions, several proteins), organ weights (only heart, liver, spleen, kidneys, brain, testes, adrenals, thyroid and pituitary), haematology (packed cell volume, haemoglobin level, white blood cell count and a differential leucocyte count) and histopathology of lung, liver, spleen and kidney. Clearly the assessment was much more limited than that of an OECD Guideline 408 study. No changes were detected in any of the parameters assessed and accordingly a NOAEL of 1.8 mg/kg bw per day could be established for 2-acetyl-2-thazoline [FL-no: 15.010].

9.2.5. Subgroup B-IV: Dithiazines

There were no toxicological data available on the five candidate substances in subgroup B-IV [FL-nos: 15.054, 15.055 15.057, 15.079 and 15.135].

Short-term toxicity tests are available on the mixture 2-isobutyl-4,6-dimethyldihydro-1,3,5-dithiazine and 4-isobutyl-2,6-dimethyldihydro-1,3,5-dithiazine (Rush, 1989a) and the mixture 2-isopropyl-4,6-dimethyldihydro-1,3,5-dithiazine and 4-isopropyl-2,6-dimethyldihydro-1,3,5-dithiazine (Rush, 1989b). The mixtures were incorporated in the diet of Sprague–Dawley rats (five/sex/group) intake of approximately 10 mg/kg bw per day over a period of 14 days. No treatment-related effects were seen. The Panel considered that these studies were inadequate for deriving a NOAEL to be used in the Procedure because of the short duration of the studies.

New 14-day and 90-day dietary studies in rats were available on the supporting substance 5,6-dihydro-2,4,6-tris(2-methylpropyl)-4*H*-1,3,5-dithiazine [FL-no: 15.113].

A 14-day range-finding dietary study was performed with 5,6-dihydro-2,4,6-tris(2-methylpropyl)-4*H*-1,3,5-dithiazine [FL-no: 15.113] (Bauter, 2012a). The study was performed according to OECD Guideline 407. Groups (three/sex/dietary intake level) of male and female Hsd:SD® rats were fed a diet containing 0 (dietary control), 120, 1200 and 2400 mg 5,6-dihydro-2,4,6-tris(2-methylpropyl)-4*H*-1,3,5-dithiazine per kilogram diet. These dietary levels were equal to daily intakes of 0, 11.3, 111.2 and 217.0 mg/kg bw for males and 0, 11.2, 107.2 and 206.1 mg/kg bw for females, respectively. Clinical observations were recorded daily and body weights and food consumption observations were made on days 0, 7 and 14. No mortality was observed throughout the course of the study and the general condition of the rats was unremarkable. No gross pathology was related to the test substance.

A 90-day dietary study was performed with 5,6-dihydro-2,4,6-tris(2-methylpropyl)-4*H*-1,3,5-dithiazine [FL-no: 15.113] (Bauter, 2013a). The study was performed according to OECD Guideline 408. Four groups of rats (10/sex/dietary intake level) of male and female CRL Sprague–Dawley CD®IGS rats were fed a diet containing 0 (dietary control), 140, 1050 and 2100 mg 5,6-dihydro-2,4,6-tris(2-methylpropyl)-4*H*-1,3,5-dithiazine per kilogram diet. These dietary levels were equivalent to daily intakes of 0, 9.3, 67.9 and 131.9 mg/kg bw for males and 0, 11.0, 77.1 and 153.7 mg/kg bw for females, respectively (Bauter, 2013a). The animals were observed daily for signs of gross toxicity, viability and behavioural changes. Clinical observations of toxicity were performed on day 0 and weekly until sacrifice. Animals were weighed on day 0 at the start of the study and weekly thereafter. Food consumption and efficiency were measured and calculated weekly. Blood chemistry and haematology were performed on blood drawn via sublingual bleed during week 12 after overnight fast and coagulation assessment was performed prior to necropsy. Urine was collected during the 15 hours prior to the blood draw. Prior to initiation of the study and on day 91 the eyes of all rats were examined by focal illumination and indirect ophthalmoscopy. At termination of the study all animals were sacrificed and subject to full necropsy. The following tissues were weighed wet post dissection: adrenals, brain, epididymides, heart, kidneys, liver, ovaries, testes, spleen,

thymus, uterus with oviducts. Histopathology was performed on a comprehensive number of tissues and organs in accordance with the guidelines.

No mortality was observed in any group throughout the study. There were no toxicologically significant or dose-related differences in animals between treatment and control groups in food consumption or food efficiency, body weight and body weight gain and clinical or ophthalmological parameters. Only a reduction in food consumption in females at the highest dose was reported to be statistically significant, which, however, was not accompanied with body weight changes and was not considered adverse or biologically relevant. No treatment-related differences in clinical or gross pathology or in organ weights were observed. Treatment-related microscopic findings were reported in the urinary bladder of males and females of the two highest dose groups (1050 and 2100 mg/kg diet) and involved a statistically significant increase in the incidence of minimal to slight simple and diffuse hyperplasia of the mucosal epithelium with increased severity in the highest dose group. This finding was not correlated to any other clinical or pathological changes.

The author concluded that under the conditions of the study, the NOAEL for dietary administration of 5,6-dihydro-2,4,6-tri(2-methylpropyl)-4*H*-1,3,5-dithiazine [FL-no: 15.113] was determined to be 140 mg/kg diet, equivalent to an estimated daily intake of 9.3 mg/kg bw per day for males and 11.0 mg/kg bw per day for females, based on the effects noted on the urinary bladder. The Panel agreed to this conclusion and used 9.3 mg/kg bw per day in the margin of safety assessment of the candidate substances in subgroup B-IV using the Procedure.

Repeated dose toxicity data are summarised in Table 13.

9.3. Developmental/reproductive toxicity studies

No reproductive and developmental toxicity studies have been available for the candidate substances or the supporting substances in this revision of FGE.21 (FGE.21Rev5). One study, a combined repeat dose/reproductive and developmental toxicity study, in accordance with OECD Guideline 422, has been carried out with the structurally related thiophene [former FL-no: 15.106], which is no longer supported for use as flavouring substance in Europe by Industry (DG SANCO, 2012).

In the study, males were dosed for 42 days and females from 14 days before mating until day 3 of lactation (Nagao, 2006). The full report of this study was in Japanese; however, a summary of the study design, results and conclusions to be drawn were available to the Panel in English.

Groups of Sprague–Dawley Rats (13/sex/dose) were administered thiophene via gavage at dose levels of 0, 25, 100 or 400 mg/kg bw per day. No adverse effects on copulation, ovulation or fertility in treatment groups were observed when compared with the control groups. However, in each group, abnormal parturition was found. Females in the 100 or 400 mg/kg bw per day group, showing evidence of histopathological change in the cerebellum, exhibited abnormal lactation. Pups born to the 400 mg/kg bw per day group showed reduced birth weights and viability decreased at postnatal day 4. No morphological abnormalities associated with the administration of thiophene were found in any pups. With respect to effects on reproduction, the NOAEL was suggested by the authors to be 400 mg/kg bw per day for males and 25 mg/kg bw per day for females (Nagao, 2006).

It should be noted, however, based on available data on metabolism in the previous versions of FGE.21, the toxicity of thiophene was considered separately from the substituted thiophenes in subgroup A-Ib.

The developmental/reproductive toxicity study is summarised in Table 14.

9.4. Genotoxicity studies

Genotoxicity data were provided for three of the candidate substances, six structurally related substances (no longer supported) and four supporting substances. These 13 substances belong to the following subgroups: Subgroup A-Ia: Thiophene (former [FL-no: 15.106]), subgroup A-Ib: 2-Methylthiophene (former [FL-no: 15.091]), 3-methylthiophene (former [FL-no: 15.092]), 2,5-dimethylthiophene (former [FL-no: 15.064]), 2-acetylthiophene [FL-no: 15.040], 2-acetyl-3-methylthiophene (former [FL-no: 15.037]), thiophene-2-carbaldehyde (former [FL-no: 15.107]), 5-ethylthiophene-2-carbaldehyde [FL-no: 15.074], 5-methylthiophene-2-carbaldehyde [FL-no: 15.004]; subgroup A-II: 2,4-Dimethylthiazole [FL-no: 15.062], 4,5-dimethylthiazole [FL-no: 15.017], 4-methylthiazole [FL-no: 15.035] and subgroup B-II: 3-Acetyl-2- thiazoline [FL-no: 15.010].

9.4.1. Subgroup A-I

The candidate substance 2-acetylthiophene [FL-no: 15.040] was negative in microbial tests, using *Salmonella typhimurium* strains TA98 and TA100, with and without metabolic activation, and in the SOS chromotest with metabolic activation. 2-Acetylthiophene was reported to be positive without metabolic activation in the SOS *Escherichia coli* chromotest (Mosier et al., 2003). In the same study, the structurally related 2-acetyl-3-methylthiophene former [FL-no: 15.037], thiophene-2-carbaldehyde former [FL-no: 15.107] and candidate substance 5-ethylthiophene-2-carbaldehyde [FL-no: 15.074] gave positive results without metabolic activation in the SOS *E. coli* chromotest. The concentrations tested were not reported for any of the substances subjected to the SOS *E. coli* chromotest (Mosier et al., 2003). The Panel considered the endpoint of this test inappropriate for the estimation of genotoxic potential. The supporting substance 5-methyl-2-thiophenecarbaldehyde [FL-no: 15.004] was negative in a microbial mutagenicity assay. The structurally related substances, thiophene former [FL-no: 15.106], 2-methylthiophene former [FL-no: 15.091], 3-methyl-thiophene former [FL-no: 15.092] and 2,5-dimethylthiophene former [FL-no: 15.064] were reported to be negative in microbial mutagenicity assays.

Thiophene (former [FL-no: 15.106]), i.e. no longer supported, was tested in accordance with OECD Guidelines in a bacterial reverse mutation test in strains of *S. typhimurium* and in strain WP2 uvrA of *E. coli*. No evidence of mutagenic response was reported when strains TA100, TA1535, TA98 and TA1537 of *S. typhimurium* were incubated at concentrations of 0, 78.1, 156, 313, 625, 1250, 2500 and 5000 µg/plate with and without S9 metabolic activation. Toxicity was observed at 1250 µg/plate in TA1537 and 2500 µg/plate in strains TA100, TA1535 and TA98, with and without metabolic activation. Toxicity was observed at 5 000 µg/plate in WP2, with and without S9 metabolic activation (Shibuya, 2006).

In a chromosomal aberration test, thiophene was tested on Chinese hamster lung cells in accordance with Japanese Guidelines. No chromosomal aberrations or polyploidy was reported when incubated with concentrations of 0, 210, 420, 840 µg/ml of thiophene, with and without metabolic activity (Tanaka, 2006).

9.4.2. Subgroup A-II

2,4-Dimethylthiazole [FL-no: 15.062] was reported to be negative in microbial assays, using *S. typhimurium*, but only in strain TA100 and only in the absence of metabolic activation (Voogd et al., 1983). Two supporting substances, 4,5-dimethylthiazole [FL-no: 15.017] and 4-methylthiazole [FL-no: 15.035] were negative (with and without S9 metabolic activation) in microbial mutagenicity assays.

9.4.3. Subgroup B-II

No genotoxicity information was available for any candidate or supporting substances in this subgroup. However, considering the structural similarities between the thiazolines in subgroup B-II and the thiazolidines in subgroup B-III, the Panel in FGE.21Rev3 concluded that the thiazolines [FL-

no: 15.060, 15.086 and 15.119] could not be evaluated through the Procedure (see subgroup B-III in FGE.21Rev3 (EFSA CEF Panel, 2012b).

However, new *in vitro* genotoxicity studies (gene mutation test in bacteria and micronucleus assay in human peripheral blood lymphocytes) have become available on 2-acetyl-2-thiazoline [FL-no: 15.010], which is considered to be structurally related to and a supportive substance for the 2-thiazoline included in this FGE, 2-methyl-2-thiazoline [FL-no: 15.086].

9.4.3.1. Ames test

2-Acetyl-2-thiazoline was tested for the induction of gene mutations in *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and TA102, both in the presence and absence of Aroclor 1254-induced rat liver S9 mix. Two independent experiments were carried out (McGarry, 2012).

In the first experiment, 2-acetyl-2-thiazoline [FL-no: 15.010] was tested in all five strains in the absence and presence of S9 mix using the plate incorporation methodology at concentrations of 5, 15.8, 50, 158.1, 500, 1581 and 5000 µg/plate. No evidence of toxicity was observed.

In the second experiment, the concentrations of 2-acetyl-2-thiazoline [FL-no: 15.010] used in all strains in the absence and presence of S9 mix were 156.3, 312.5, 625.0, 1250, 2500 and 5000 µg/plate of 2-acetyl-2-thiazoline, and the pre-incubation method was used for treatments in the presence of S9. No evidence of toxicity was observed.

It is concluded that 2-acetyl-2-thiazoline [FL-no: 15.010] did not induce mutations in five histidine-requiring strains (TA98, TA100, TA1535, TA1537 and TA102) or *S. typhimurium* when tested under the conditions of the study (Table 15). The study was conducted using Good Laboratory Practice (GLP) and the design complied with current recommendations (OECD Guideline 471) and an acceptable top concentration was achieved.

9.4.3.2. Micronucleus assay

2-Acetyl-2-thiazoline [FL-no: 15.010] was tested for the induction of chromosome damage and potential aneugenic effects in an *in vitro* micronucleus assay using duplicate human peripheral blood lymphocytes prepared from pooled blood from two healthy male volunteers. Treatments were performed both in the absence and presence of Aroclor 1254-induced rat liver S9 mix (Watters, 2012).

Cells were stimulated for 48 hours with phytohaemagglutinin to produce exponentially growing cells.

A preliminary toxicity range-finding experiment was carried out using a 3-hour treatment with S9 mix and 3- and 24-hour treatments without S9 mix. Toxicity was evaluated as the effect of treatment on the Replication Index (RI). Twelve concentrations from 4.6 to 1262 µg/ml were tested. The concentrations selected for the main experiment were based on toxicity data from this preliminary test.

In the main experiment, cells were treated for 3 hours, followed a 21-hour recovery period, (3 + 21 hours) with 0, 600, 1000 or 1292 µg/ml of 2-acetyl-2-thiazoline in the absence and in the presence of S9 mix. The levels of toxicity (reduction in RI) at the top concentration were 15 % and 0 % in the absence and presence of S9 mix, respectively. In a parallel assay, cells were treated for 24 hours (24 + 0 hours) with 0, 100, 200, 400 or 600 µg/ml of 2-acetyl-2-thiazoline in the absence of S9 mix with no recovery period. The top concentration induced 55 % toxicity. Relevant positive and negative controls were included in all experiments. There were two replicate cultures per treatment, and 1000 binucleate cells per replicate (i.e. 2000 cells per dose) were scored for micronuclei. Thus, the study was conducted under GLP and the design complies with current recommendations (including draft OECD Guideline 487). No evidence of chromosomal damage or aneuploidy was observed as indicated by the lack of increased levels of micronucleated binucleate cells (MNBN) in the presence or absence of rat liver S9 mix metabolic activation (Table 15).

In conclusion, 2-acetyl-2-thiazoline [FL-no: 15.010] did not induce micronuclei in male human peripheral blood lymphocyte cultures when tested for 3 + 21 hours in the presence of S9 mix and 24 + 0 hours in the absence of S9 mix.

Based on the new studies on the supporting substance 2-acetyl-2-thiazoline [FL-no: 15.010], the Panel concluded that 2-methyl-2-thiazoline [FL-no: 15.086] does not give rise to concern with respect to genotoxicity and can, accordingly, be evaluated using the Procedure.

9.4.4. Subgroup B-IV

No genotoxicity information was available for any candidate or supporting substance in this subgroup.

9.4.4.1. Overall conclusion on genotoxicity

Based on the new studies on the supporting substance 2-acetyl-2-thiazoline [FL-no: 15.010], the Panel concluded that 2-methyl-2-thiazoline [FL-no: 15.086] does not give rise to concern with respect to genotoxicity and can accordingly be evaluated using the Procedure.

For the remaining substances the genotoxicity data are limited and genotoxicity could not be assessed adequately for these substances. However, except for the two 3-thiazolines, 2,4-dimethyl-3-thiazoline [FL-no: 15.060] and 2-isobutyl-3-thiazoline [FL-no: 15.119], the genotoxicity data available do not preclude the evaluation of the remaining 39 candidate substances using the Procedure.

Data on genotoxicity are summarised in Table 15.

CONCLUSIONS

The present revision of FGE.21, Revision 5 includes the assessment of a 90-day study on 2-pentylthiophene [FL-no: 15.096] supporting 2-butylthiophene [FL-no: 15.045], 2-hexylthiophene [FL-no: 15.076] and 2-octylthiophene [FL-no: 15.093]. It also includes a 90-day study on 5-ethylthiophene-2-carbaldehyde [FL-no: 15.074] supporting 2-acetylthiophene [FL-no: 15.040] and 2-propionylthiophene [FL-no: 15.097]. Updated information on European production figures has been provided by EFFA for seven substances [FL-no: 15.040, 15.045, 15.074, 15.076, 15.093, 15.096 and 15.097] and the information has been included in their evaluation.

All candidate substances are five- or six-member sulphur-containing heterocyclic compounds, some of which also contain nitrogen. They have been divided into two main groups: (A) those with an aromatic ring (33 candidate substances) and (B) those with a non-aromatic ring structure (8 candidate substances). All are ring-substituted with one or more of the substituents alkyl, alkenyl, aryl, alcohol, keto, thio. For assessment purposes, the following further subdivision of groups (A) and (B) has been made:

- Group (A): Aromatic:
  - (Subgroup A-Ia: Thiophene. The substance previously allocated to the group is no longer supported for use as a flavouring substance in Europe by Industry.)
  - Subgroup A-Ib: Thiophene derivatives with non-thiol-containing ring substituents.
  - Subgroup A-Ic: Thiophene derivatives with thiol-containing ring substituents.
  - Subgroup A-II: Thiazole derivatives.
  - (Subgroup A-III: Benzothiazoles. The substance previously allocated to the group is no longer supported for use as a flavouring substance in Europe by Industry.)

- Group (B): Non-aromatic:
  - (Subgroup B-I: Dihydrothiophenes. The substance previously allocated to the group is no longer supported for use as a flavouring substance in Europe by Industry.)
  - Subgroup B-II: Thiazolines.
  - (Subgroup B-III: Thiazolidines. The substances previously allocated to the group are no longer supported for use as flavouring substances in Europe by Industry.)
  - Subgroup B-IV: Dithiazine derivatives.
  - (Subgroup B-V: Dihydrothiazines. The substances previously allocated to the group are no longer supported for use as flavouring substances in Europe by Industry.)
  - (Subgroup B-VI: Thiadiazine derivatives. The substance previously allocated to the group is no longer supported for use as a flavouring substance in Europe by Industry.)

Two of the 41 candidate substances possess one chiral centre [FL-no: 15.060 and 15.119], three substances possess two chiral centres [FL-no: 15.054, 15.057 and 15.135] and two possess three chiral centres [FL-no: 15.055 and 15.079]. The stereoisomeric composition has been specified for all substances.

Thirty-three of the candidate substances belong to SC II and eight belong to SC III.

Thirty-one of the candidate substances have been reported to occur naturally in a wide range of foods.

According to the default MSDI approach, the flavouring substances in this FGE.21Rev5 have intakes, in Europe, ranging from 0.0012 to 5.7 µg/*capita* per day, which are below the thresholds of concern values for SC II (540 µg/person per day) and SC III (90 µg/person per day) substances.

On the basis of the reported annual production volumes of the candidate substances as flavourings in Europe, the combined estimated daily *per capita* intakes, per subgroup and SC range from 0.14 to 8.2 µg. For all subgroups, the total combined intakes are below the thresholds of concern of 540 µg/person per day and 90 µg/person per day for SCs II and III, respectively.

The candidate substances are structurally related to 28 supporting substances evaluated by the JECFA at its 59th and 68th meetings. The total combined daily *per capita* intakes (in Europe) of candidate and supporting substances evaluated through the Procedure in each of the five subgroups for which supporting substances were available are: Subgroup A-Ib (8 substances from SC II) 2.9 µg; subgroup A-Ic (5 substances from SC III) 0.15 µg; subgroup A-II (37 substances from SC II) 500 µg; subgroup B-II (2 substances from SC II) 0.52 µg; subgroup B-IV (4 substances from SC II) 3.6 µg.

For all subgroups, the total combined intakes are below the thresholds of concern of 540 µg/person per day and 90 µg/person per day for SCs II and III, respectively.

It is concluded that genotoxicity data are limited and that genotoxicity could not be assessed adequately for the flavouring substances in FGE.21Rev5. However, except for the two 3-thiazolines, 2,4-dimethyl-3-thiazoline [FL-no: 15.060] and 2-isobutyl-3-thiazoline [FL-no: 15.119], the available genotoxicity data do not preclude the evaluation of the remaining 39 candidate substances using the Procedure.

The available metabolism data were insufficient to allow conclusions about the metabolic fate of the candidate substances and, accordingly, the 39 candidate substances evaluated through the Procedure could not be anticipated to be metabolised to innocuous products. However, the Panel concluded that the evidence of binding to macromolecules from possible formation of electrophilic metabolites, (e.g. by either ring scission or *S*-oxidation) was not sufficiently strong to preclude the application of the Procedure.

Valid toxicological data were available for candidate substances from subgroup A-Ib (thiophenes with non-thiol-containing ring substituents), subgroup A-Ic (thiophenes with thiol-containing ring substituents), subgroup A-II (thiazoles), subgroup B-II (only 2-thiazolines) and subgroup B-IV (dithiazines). Based on these data, the margin of safety compared with the intakes from use as flavouring substances was considered adequate.

In order to determine whether the conclusion for the 39 candidate substances, which have been evaluated using the Procedure, can be applied to the materials of commerce, it is necessary to consider the available specifications. Adequate specifications including purity and identity for the materials of commerce have been provided for all 39 flavouring substances evaluated through the Procedure.

For the 39 flavouring substances [FL-no: 15.038, 15.039, 15.040, 15.044, 15.045, 15.050, 15.051, 15.052, 15.054, 15.055, 15.057, 15.058, 15.061, 15.062, 15.063, 15.067, 15.068, 15.069, 15.071, 15.074, 15.076, 15.078, 15.079, 15.080, 15.082, 15.084, 15.085, 15.086, 15.087, 15.089, 15.093, 15.096, 15.097, 15.098, 15.108, 15.115, 15.116, 15.118 and 15.135], evaluated using the Procedure, the Panel concluded that they would present no safety concern at their estimated levels of intake based on the MSDI approach.

The estimated intakes, based on the mTAMDI, for the 33 candidate substances assigned to SC II and evaluated using the Procedure, range from 78 to 220 µg/person per day, which is below the threshold of concern for SC II of 540 µg/person per day. The estimated intakes based on the mTAMDI of the eight candidate substances assigned to SC III and evaluated through the Procedure ranged from 78 to 250 µg/person per day. For the candidate substances [FL-no: 15.055 and 15.135] the estimated intakes are above the threshold of concern for SC III substances of 90 µg/person per day. For two candidate substances [FL-no: 15.057 and 15.079], assigned to SC III, no use levels were provided.

In conclusion, more reliable exposure data are required for [FL-no: 15.055, 15.057, 15.079 and 15.135]. On the basis of such additional data, these flavouring substances should be re-evaluated using the Procedure. Following this procedure additional toxicological data might become necessary.

For two substances [FL-no: 15.060 and 15.119] the Panel concluded that additional genotoxicity data are required.

SUMMARY OF SAFETY EVALUATION

Table 10: Summary of Safety Evaluation Applying the Procedure

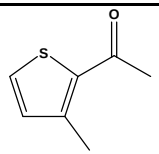
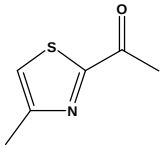
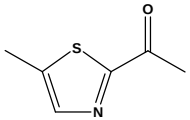
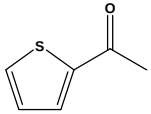
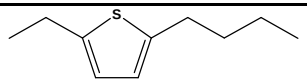
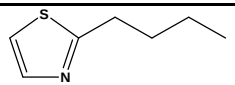
FL-no	EU Register name	Structural formula	MSDI [(a)] (µg/*capita* per day)	Class [(b)] Evaluation procedure path [(c)]	Outcome on the named compound [[(d)] or [(e)]]	Outcome on the material of commerce [[(f)], [(g)] or [(h)]]	Evaluation remarks
15.037	2-Acetyl-3-methylthiophene		0.18	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		No longer supported by Industry (DG SANCO, 2012).
15.038	2-Acetyl-4-methylthiazole		0.0049	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	d	f	
15.039	2-Acetyl-5-methylthiazole		0.0024	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	d	f	
15.040	2-Acetylthiophene		0.049	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	d		
15.043	2-Butyl-5-ethylthiophene		0.0012	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		No longer supported by Industry (DG SANCO, 2012).
15.044	2-Butylthiazole		0.011	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	d	f	

Table 10: Summary of Safety Evaluation Applying the Procedure

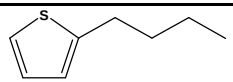
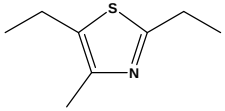
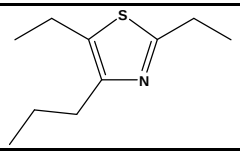
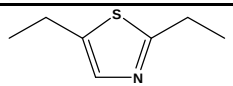
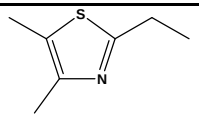
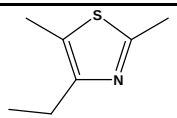
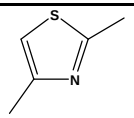
FL-no	EU Register name	Structural formula	MSDI [(a)] (µg/*capita* per day)	Class [(b)] Evaluation procedure path [(c)]	Outcome on the named compound [[(d)] or [(e)]]	Outcome on the material of commerce [[(f)], [(g)] or [(h)]]	Evaluation remarks
15.045	2-Butylthiophene		0.012	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	d		
15.050	2,5-Diethyl-4-methylthiazole		0.012	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	d	f	
15.051	2,5-Diethyl-4-propylthiazole		0.0012	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	d	f	
15.052	2,5-Diethylthiazole		0.015	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	d	f	
15.058	4,5-Dimethyl-2-ethylthiazole		0.015	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	d	f	
15.061	2,5-Dimethyl-4-ethylthiazole		0.011	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	d	f	
15.062	2,4-Dimethylthiazole		0.61	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	d	f	

Table 10: Summary of Safety Evaluation Applying the Procedure

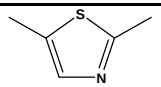
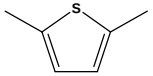
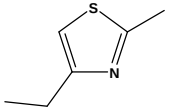
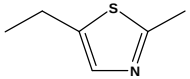
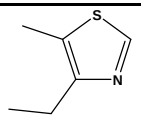
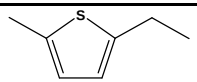
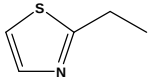
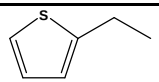
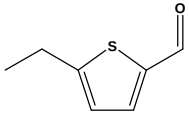
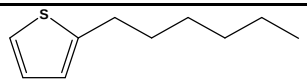
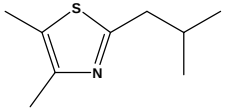
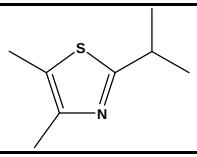
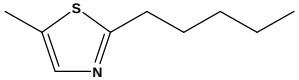
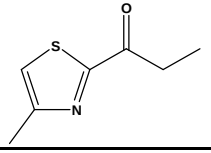
FL-no	EU Register name	Structural formula	MSDI [(a)] (µg/*capita* per day)	Class [(b)] Evaluation procedure path [(c)]	Outcome on the named compound [(d) or (e)]	Outcome on the material of commerce [(f), (g) or (h)]	Evaluation remarks
15.063 1758	2,5-Dimethylthiazole		0.0061	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	d	f	
15.064	2,5-Dimethylthiophene		0.23	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		No longer supported by Industry (DG SANCO, 2013).
15.067	4-Ethyl-2-methylthiazole		0.0037	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	d	f	
15.068	5-Ethyl-2-methylthiazole		0.0061	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	d	f	
15.069	4-Ethyl-5-methylthiazole		0.012	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	d	f	
15.070	2-Ethyl-5-methylthiophene		0.061	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		No longer supported by Industry (DG SANCO, 2012).
15.071	2-Ethylthiazole		0.028	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	d	f	

Table 10: Summary of Safety Evaluation Applying the Procedure

FL-no	EU Register name	Structural formula	MSDI [(a)] (µg/*capita* per day)	Class [(b)] Evaluation procedure path [(c)]	Outcome on the named compound [[(d)] or [(e)]]	Outcome on the material of commerce [[(f)], [(g)] or [(h)]]	Evaluation remarks
15.072	2-Ethylthiophene		0.0012	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		No longer supported by Industry (DG SANCO, 2013).
15.074	5-Ethylthiophene-2-carbaldehyde		0.037	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	d		
15.076 1764	2-Hexylthiophene		0.46	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	d		
15.078	2-Isobutyl-4,5-dimethylthiazole		0.12	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	d	f	
15.080	2-Isopropyl-4,5-dimethylthiazole		0.012	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	d	f	
15.084	5-Methyl-2-pentylthiazole		0.0037	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	d	f	
15.085	4-Methyl-2-propionylthiazole		0.0037	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	d	f	

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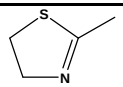
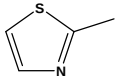
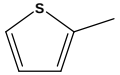
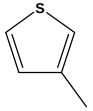
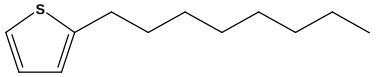
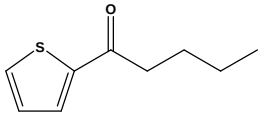
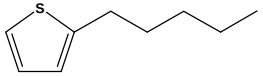
FL-no	EU Register name	Structural formula	MSDI [(a)] (µg/*capita* per day)	Class [(b)] Evaluation procedure path [(c)]	Outcome on the named compound [(d) or (e)]	Outcome on the material of commerce [(f), (g) or (h)]	Evaluation remarks
15.086	2-Methyl-2-thiazoline		0.012	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	d	f	
15.089	2-Methylthiazole		0.018	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	d	f	
15.091	2-Methylthiophene		0.019	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		No longer supported by Industry (DG SANCO, 2013).
15.092	3-Methylthiophene		0.12	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		No longer supported by Industry (DG SANCO, 2013).
15.093	2-Octylthiophene		0.012	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	d		
15.094	2-Pentanoylthiophene		0.0012	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		No longer supported by Industry (DG SANCO, 2012).
15.096 2106	2-Pentylthiophene		1.6	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	d		

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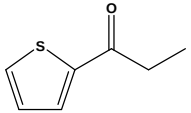
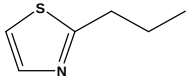
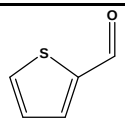
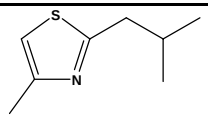
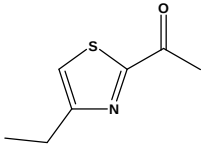
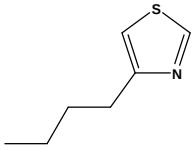
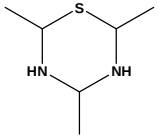
FL-no	EU Register name	Structural formula	MSDI [(a)] (µg/*capita* per day)	Class [(b)] Evaluation procedure path [(c)]	Outcome on the named compound [[(d)] or [(e)]]	Outcome on the material of commerce [[(f)], [(g)] or [(h)]]	Evaluation remarks
15.097	2-Propionylthiophene		0.012	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	d		
15.098	2-Propylthiazole		0.085	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	d	f	
15.107	Thiophene-2-carbaldehyde		0.21	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		No longer supported by Industry (DG SANCO, 2012).
15.115	2-Isobutyl-4-methyl thiazole		0.011	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	d	f	
15.116	2-Acetyl-4-ethylthiazole		0.024	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	d	f	
15.118	4-Butylthiazole		1.3	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	d	f	
15.129	Tetrahydro-2,4,6-trimethyl-1,3,5(2H)-thiadiazine		0.61	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		No longer supported by Industry (DG SANCO, 2012).

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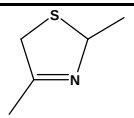
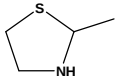
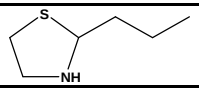
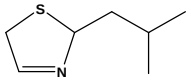
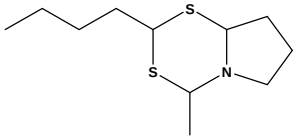
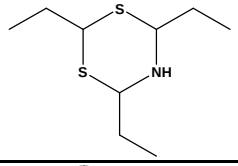
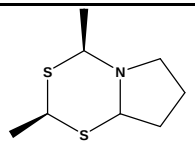
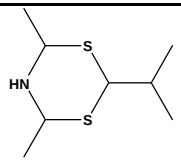
FL-no	EU Register name	Structural formula	MSDI [(a)] (µg/*capita* per day)	Class [(b)] Evaluation procedure path [(c)]	Outcome on the named compound [(d) or (e)]	Outcome on the material of commerce [(f), (g) or (h)]	Evaluation remarks
15.060	2,4-Dimethyl-3-thiazoline		0.012	Class II No evaluation			Concern for genotoxicity.
15.090	2-Methylthiazolidine		0.024	Class II No evaluation			No longer supported by Industry (DG SANCO, 2012).
15.099	2-Propylthiazolidine		0.012	Class II No evaluation			No longer supported by Industry (DG SANCO, 2012).
15.119	2-Isobutyl-3-thiazoline		0.011	Class II No evaluation			Concern for genotoxicity.
15.042	2-Butyl-4-methyl(4H)pyrrolidino[1,2d]-1,3,5-dithiazine		0.0012	Class III B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		No longer supported by Industry (DG SANCO, 2012).
15.054	Dihydro-2,4,6-triethyl-1,3,5(4H)-dithiazine		0.85	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	d	f	
15.055 1763	[2S-(2a,4a,8ab)] 2,4-Dimethyl(4H)pyrrolidino[1,2e]-1,3,5-dithiazine		0.012	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	d	f	
15.057	4,6-Dimethyl-2-(1-methylethyl)dihydro-1,3,5-dithiazine		1.6	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	d	f	

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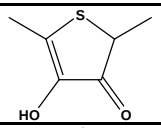
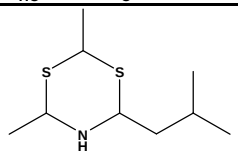
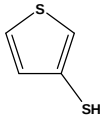
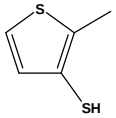
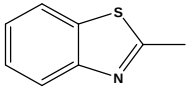
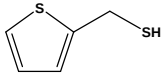
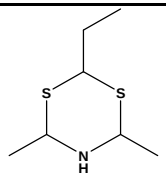
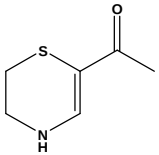
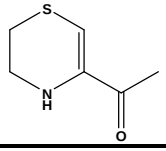
FL-no	EU Register name	Structural formula	MSDI [(a)] (µg/*capita* per day)	Class [(b)] Evaluation procedure path [(c)]	Outcome on the named compound [[(d)] or [(e)]]	Outcome on the material of commerce [[(f)], [(g)] or [(h)]]	Evaluation remarks
15.077	4-Hydroxy-2,5-dimethylthiophen-3(2H)-one		0.12	Class III B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		No longer supported by Industry (DG SANCO, 2012).
15.079	2-Isobutyldihydro-4,6-dimethyl-1,3,5-dithiazine		5.7	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	d	f	
15.082	3-Mercaptothiophene		0.011	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	d	f	
15.087	2-Methyl-3-mercaptothiophene		0.12	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	d	f	
15.088	2-Methyl-4,5-benzothiazole		0.0085	Class III B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		No longer supported by Industry (DG SANCO, 2012).
15.106	Thiophene		0.12	Class III B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		No longer supported by Industry (DG SANCO, 2012).
15.108	2-Thiophenemethanethiol		0.0073	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	d	f	

Table 10: Summary of Safety Evaluation Applying the Procedure

FL-no	EU Register name	Structural formula	MSDI [(a)] (µg/*capita* per day)	Class [(b)] Evaluation procedure path [(c)]	Outcome on the named compound [[(d)] or [(e)]]	Outcome on the material of commerce [[(f)], [(g)] or [(h)]]	Evaluation remarks
15.135	Ethyl thialdine		0.012	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	d	f	
15.114	6-Acetyl-2,3-dihydro-1,4-thiazine		0.012	Class III No evaluation			No longer supported by Industry (DG SANCO, 2012). Concern for genotoxicity.
15.133 1766	5-Acetyl-2,3-dihydro-1,4-thiazine		0.61	Class III No evaluation			No longer supported by Industry (DG SANCO, 2012). Concern for genotoxicity.

(a): EU MSDI: Quantity added to food as flavour in (kilograms per year) x 10E9 / (0.1 x population in Europe (= 375 × 10E6) × 0.6 × 365) = µg/capita per day.
(b): Thresholds of concern: Class I = 1800 µg/person per day, Class II = 540 µg/person per day, Class III = 90 µg/person per day.
(c): Procedure path A substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot.
(d): No safety concern based on intake calculated by the MSDI approach of the named compound.
(e): Data must be available on the substance or closely related substances to perform a safety evaluation.
(f): No safety concern at the estimated level of intake of the material of commerce meeting the specification requirement (based on intake calculated by the MSDI approach).
(g): Tentatively regarded as presenting no safety concern (based on intake calculated by the MSDI approach) pending further information on the purity of the material of commerce and/or information on stereoisomerism.
(h): No conclusion can be drawn because of a lack of information on the purity of the material of commerce.

SUPPORTING SUBSTANCES SUMMARY

Table 11: Supporting Substances Summary

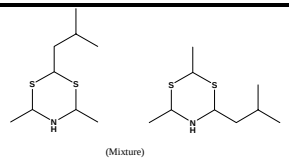
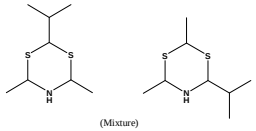
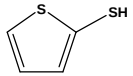
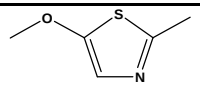
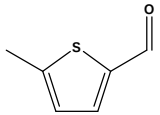
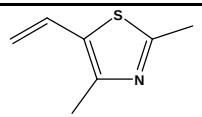
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) [a] (μg/*capita* per day)	SCF status [b] JECFA status [c] CoE status [d]	Comments
	2-Isobutyl-4,6-dimethyldihydro-1,3,5-dithiazine and 4-isobutyl-2,6-dimethyldihydro-1,3,5-dithiazine (mixture)	 (Mixture)	3781 101517-87-7 and 101517-86-6	1046 JECFA specification (JECFA, 2002b)	0.1	No safety concern (JECFA, 2002a)	Not in EU-Register
	2-Isopropyl-4,6-dimethyl 2,6-dimethyldihydro-1,3,5-dithiazine and 4-isopropyl-2,6-dimethyldihydro-1,3,5-dithiazine (mixture)	 (Mixture)	3782 104691-41-0 and 104691-40-9	1047 JECFA specification (JECFA, 2002b)	ND	No safety concern (JECFA, 2002a)	Not in EU-Register
15.001	2-Mercaptothiophene		3062 478 7774-74-5	1052 JECFA specification (JECFA, 2002b).	0.012	No safety concern (JECFA, 2002a) Deleted (CoE, 1992)	
15.002	2-Methyl-5-methoxythiazole		3192 736 38205-64-0	1057 JECFA specification (JECFA, 2002b).	0.012	No safety concern (JECFA, 2002a) Category B (CoE, 1992)	
15.004	5-Methyl-2-thiophenecarbaldehyde		3209 2203 13679-70-4	1050 JECFA specification (JECFA, 2002b).	0.73	No safety concern (JECFA, 2002a) Deleted (CoE, 1992)	
15.005	2,4-Dimethyl-5-vinylthiazole		3145 2237 65505-18-2	1039 JECFA specification (JECFA, 2002b).	0.012	No safety concern (JECFA, 2002a) Category B (CoE, 1992)	

Table 11: Supporting Substances Summary

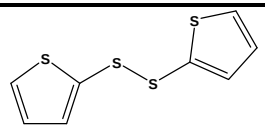
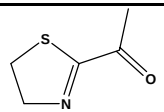
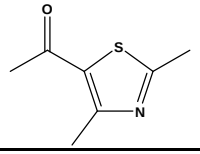
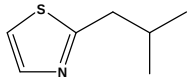
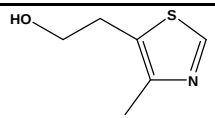
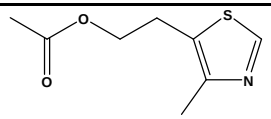
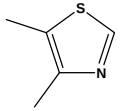
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) [a] (μg/*capita* per day)	SCF status [b] JECFA status [c] CoE status [d]	Comments
15.008	2-Thienyl disulfide		3323 2333 6911-51-9	1053 JECFA specification (JECFA, 2002b).	0.061	No safety concern (JECFA, 2002a) Category B (CoE, 1992)	
15.010	2-Acetyl-2-thiazoline		3817 2335 29926-41-8	1759	0.51	Category B (CoE, 1992)	
15.011	5-Acetyl-2,4-dimethylthiazole		3267 2336 38205-60-6	1055 JECFA specification (JECFA, 2002b).	0.012	No safety concern (JECFA, 2002a) Category B (CoE, 1992)	
15.013	2-Isobutylthiazole		3134 11618 18640-74-9	1034 JECFA specification (JECFA, 2002b).	2.3	No safety concern (JECFA, 2002a)	
15.014	5-(2-Hydroxyethyl)-4-methylthiazole		3204 11621 137-00-8	1031 JECFA specification (JECFA, 2002b).	150	No safety concern (JECFA, 2002a)	
15.015	4-Methyl-5-(2-acetoxyethyl)thiazole		3205 11620 656-53-1	1054 JECFA specification (JECFA, 2002b).	8.6	No safety concern (JECFA, 2002a)	
15.017	4,5-Dimethylthiazole		3274 11606 3581-91-7	1035 JECFA specification (JECFA, 2002b).	0.18	No safety concern (JECFA, 2002a)	

Table 11: Supporting Substances Summary

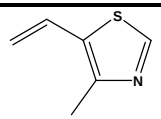
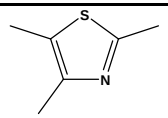
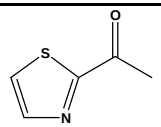
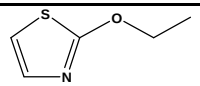
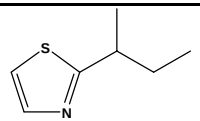
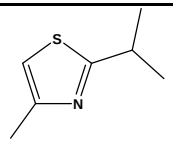
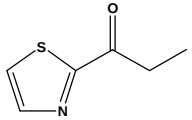
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) [a] (μg/*capita* per day)	SCF status [b] JECFA status [c] CoE status [d]	Comments
15.018	4-Methyl-5-vinylthiazole		3313 11633 1759-28-0	1038 JECFA specification (JECFA, 2002b).	2.1	No safety concern (JECFA, 2002a)	
15.019	2,4,5-Trimethylthiazole		3325 11650 13623-11-5	1036 JECFA specification (JECFA, 2002b).	0.61	No safety concern (JECFA, 2002a)	
15.020	2-Acetylthiazole		3328 11726 24295-03-2	1041 JECFA specification (JECFA, 2002b).	9.7	No safety concern (JECFA, 2002a)	
15.021	2-Ethoxythiazole		3340 11611 15679-19-3	1056 JECFA specification (JECFA, 2002b).	0.012	No safety concern (JECFA, 2002a)	
15.022	2-(*sec*-Butyl)thiazole		3372 11598 18277-27-5	1033 JECFA specification (JECFA, 2002b).	0.024	No safety concern (JECFA, 2002a)	JECFA evaluated 2-(1-methylpropyl)thiazole (CASrn as in Register). (R)- or (S)- enantiomer not specified by CASrn in Register.
15.026	2-Isopropyl-4-methylthiazole		3555 15679-13-7	1037 JECFA specification (JECFA, 2002b).	19	No safety concern (JECFA, 2002a)	
15.027	2-Propionylthiazole		3611 43039-98-1	1042 JECFA specification (JECFA, 2002b).	0.056	No safety concern (JECFA, 2002a)	

Table 11: Supporting Substances Summary

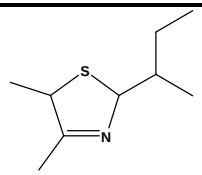
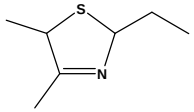
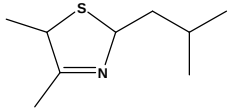
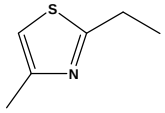
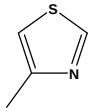
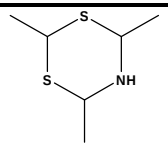
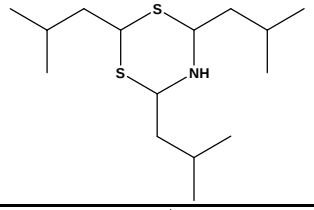
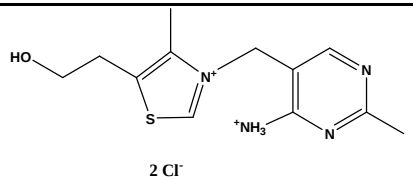
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) [a] (μg/*capita* per day)	SCF status [b] JECFA status [c] CoE status [d]	Comments
15.029	2-(*sec*-Butyl)-4,5-dimethyl-3-thiazoline		3619 65894-82-8	1059 JECFA specification (JECFA, 2002b).	0.012	No safety concern (JECFA, 2002a)	JECFA evaluated 2-(2-Butyl)-4,5-dimethyl-3-thiazoline (CASrn as in Register). (R)- or (S)-enantiomer not specified by CASrn in Register.
15.030	4,5-Dimethyl-2-ethyl-3-thiazoline		3620 76788-46-0	1058 JECFA specification (JECFA, 2002b).	0.012	No safety concern (JECFA, 2002a)	JECFA evaluated 4,5-dimethyl-2-ethyl-3-thiazoline (CASrn as in Register). (R)- or (S)-enantiomer not specified by CASrn in Register.
15.032	4,5-Dimethyl-2-isobutyl-3-thiazoline		3621 65894-83-9	1045 JECFA specification (JECFA, 2002b).	0.012	No safety concern (JECFA, 2002a)	JECFA evaluated 4,5-dimethyl-2-isobutyl-3-thiazoline (CASrn as in Register). (R)- or (S)-enantiomer not specified by CASrn in Register.
15.033	2-Ethyl 4-methylthiazole		3680 11612 15679-12-6	1044 JECFA specification (JECFA, 2002b).	3.2	No safety concern (JECFA, 2002a)	
15.035	4-Methylthiazole		3716 11627 693-95-8	1043 JECFA specification (JECFA, 2002b).	0.097	No safety concern (JECFA, 2002a)	

Table 11: Supporting Substances Summary

FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) [a] (µg/*capita* per day)	SCF status [b] JECFA status [c] CoE status [d]	Comments
15.109	2,4,6-Trimethyldihydro-1,3,5(4H)-dithiazine		4018 11649 638-17-5	1049 JECFA specification (JECFA, 2002b).	1.1	No safety concern (JECFA, 2002a)	JECFA evaluated 2,4,6-trimethyldihydro-4H-1,3,5-dithiazine (CASrn as in Register). (R)- or (S)- enantiomer not specified by CASrn in Register.
15.113	5,6-Dihydro-2,4,6,tris(2-methylpropyl)-4H-1,3,5-dithiazine		4017 74595-94-1	1048 JECFA specification (JECFA, 2002b).	2.4	No safety concern (JECFA, 2002a)	JECFA evaluated 2,4,6-triisobutyl-5,6-dihydro-4H-1,3,5-dithiazine (CASrn as in Register). (R)- or (S)- enantiomer not specified by CASrn in Register.
16.027	Thiamine hydrochloride		3322 10493 67-03-8	1030 JECFA specification (JECFA, 2002b).	300	No safety concern (JECFA, 2002a)	

(a): EU MSDI: Quantity added to food as flavouring substance in (kilograms per year) × 10E9 / (0.1 × population in Europe (= 375 × 10E6) × 0.6 × 365) = µg/capita/day.
(b): Category 1: Considered safe in use, Category 2: Temporarily considered safe in use, Category 3: Insufficient data to provide assurance of safety in use, Category 4: Not acceptable because of evidence of toxicity.
(c): No safety concern at estimated levels of intake.
(d): Category A: Flavouring substance, which may be used in foodstuffs, Category B: Flavouring substance which can be used provisionally in foodstuffs.
ND: No intake data reported

TOXICITY DATA

Table 12: Acute Toxicity

Chemical Name [FL-no][(a)]	Species	Sex	Route	LD_{50} (mg/kg bw)	Reference
(2-Thienyl disulfide [15.008])	Mouse	M, F	Gavage	400	(Moran et al., 1980)
(2-Methyl-5-methoxythiazole [15.002])	Rat	NR	Oral	1250	(Posternak et al., 1975)
(2,4-Dimethyl-5-vinylthiazole [15.005])	Mouse	M, F	Oral	400 – 800	(Moran et al., 1980)
(5-Acetyl-2,4-dimethylthiazole [15.011])	Mouse	M, F	Gavage	975	(Moran et al., 1980)
	Mouse	M, F	Gavage	M: 610, F: 620	(Shellenberger, 1971)
(5-(2-Hydroxyethyl)-4-methylthiazole [15.014])	Mouse	M, F	Oral	4800	(Calvary and Nelson, 1944)
(4-Methyl-5-vinylthiazole [15.018])	Mouse	M, F	Gavage	400 – 800	(Oser, 1970)
(2-Propionylthiazole [15.027])	Mouse	M, F	Gavage	2113	(Moran et al., 1980)
(4-Methylthiazole [15.035])	Mouse	F	Gavage	0.36 ml/kg (360 mg/kg, based on an assumed specific gravity of 1.0 g/ml)	(O'Neal et al., 1978)
	Rat	M, F	Gavage	930	(Mondino, 1981)
(4,5-Dimethylthiazole [15.017])	Rat	M, F	Gavage	964.9	(Piccirillo et al., 1982a)
(2-Ethoxythiazole [15.021])	Rat	M, F	Gavage	910.9	(Piccirillo et al., 1982b)
(2-Isopropyl-4-methylthiazole [15.026])	Rat	M, F	Gavage	0.83 ml/kg (832 mg/kg based on a specific gravity of 1.003 g/ml)	(Griffiths and Babish, 1977)
(2-Ethyl 4-methylthiazole [15.033])	Rat	M, F	Oral	540	(Moreno et al., 1981)
(Thiamine hydrochloride [16.027])	Rat	M	Gavage	3710	(Sprince et al., 1974)
(2-(2-Butyl)-4,5-dimethyl-3-thiazoline [15.029])	Mouse	M, F	Gavage	2827	(Moran et al., 1980)
(4,5-Dimethyl-2-ethyl-3-thiazoline [15.030])	Mouse	M, F	Gavage	1265	(Moran et al., 1980)
(4,5-Dimethyl-2-isobutyl-3-thiazoline [15.032])	Mouse	M, F	Gavage	3067	(Moran et al., 1980)

(a): Supporting substances are listed in brackets.

Table 13: Subacute / Subchronic / Chronic / Carcinogenicity Studies

Chemical Name [FL-no][(a)]	Species; Sex No./Group	Route	Dose levels	Duration (days)	NOAEL (mg/kg bw per day)	Reference	Comments
(Thiophene (former [15.106]))	Rat; M 5	Gavage	0, 50, 100, 500, 1000 mg/kg bw per day	Up to 19 days	100	(O'Donoghue, 1979)	Inadequately reported study of short duration.
	Rat; M/F 13	Gavage	0, 25, 100, 400 mg/kg bw per day	42	25	(Nagao, 2006)	Only a translated summary report available.
2-Pentylthiophene [15.096]	Rat; M/F 5	Gavage	0, 15, 150, 500 mg/kg bw per day	28		(Dhinsa et al., 2006)	Signs of haemolytic anaemia were seen in all dosage groups, in the lowest dosage group statistically significant raised bilirubin.
	Rat; M/F 5	Gavage	0, 3 mg/kg bw per day	28	3	(Marr and Watson, 2007)	This study is a follow up of (Dhinsa et al., 2006), since in this study effects were seen at the lowest dose.
	Rat; M, F 3/20	Diet	0, 1.0, 5.5 and 24.4 mg/kg bw (M) 0, 1.1, 6.2 and 28.4 mg/kg bw (F)	90 days	5.5	(Bauter, 2013b)	OECD Guideline study (408). Dose levels recalculated to compensate for low stability of the test substance in the dietary matrix, see text.
(Thiophene-2-carbaldehyde (former [15.107]))	Rat; sex not reported 5	Diet	0, 0.1, and 1.0 % in diet equal to 0, 95 and 840 mg/kg bw per day	11 days	95	(Sharp, 1979)	Reduced food consumption and reduced absolute kidney weight. Inadequately reported study of short duration.
(5-Methyl-2-thiophenecarbaldehyde [15.004])	Rat; M, F 5/sex/group	Diet	10 mg/kg bw per day	14 days	10	(Gill and Van Miller, 1987)	One dose level, GLP study, comprehensively reported, but no haematology or clinical chemistry carried out, only a limited number of organs examined at autopsy and only liver and kidney examined histopathologically. Short duration, limited study design.
(2-Thienyl disulfide [15.008])	Rat; M, F 15/sex	Diet	0.29 mg/kg bw per day	90 days	0.29	(Morgareidge and Oser, 1970)	Study carried out at a one low dose level. Standard toxicological parameters were monitored (body weight, food consumption, haematology, urinalysis, histopathology). Parameters assessed were reasonably comprehensive. Study considered valid.
(5-Acetyl-2,4-dimethylthiazole [15.011])	Rat; M, F 23/sex	Diet	approx. 25 mg/kg bw per day.	90 days	M: 25.2 F:24.4	(Shellenberger , 1971)	Old study carried out at a one dose level. Standard toxicological parameters were monitored (body weight, food consumption, haematology, urinalysis, histopathology). Parameters assessed were reasonably comprehensive. Study considered valid.

Table 13: Subacute / Subchronic / Chronic / Carcinogenicity Studies

Chemical Name [FL-no][(a)]	Species; Sex No./Group	Route	Dose levels	Duration (days)	NOAEL (mg/kg bw per day)	Reference	Comments
(2-Acetylthiazole [15.020])	Rat; M 5	Diet	5000 and 10 000 mg/kg in diet equivalent to 250 and 500 mg/kg bw per day	28 days	< 250	(Wheldon et al., 1970)	Preliminary dose-range finding study for the 90-day study described below, very limited experimental details provided.
	Rat; M 10	Diet	0, 100, 1000, 10 000 mg/kg in diet equivalent to 0.5, 50, 500 mg/kg bw per day	90 days	50	(Wheldon et al., 1970)	Study considered to be of limited validity, since no clinical chemistry was undertaken, limited number of organs weighed, number of organs taken for histopathology was limited, and histopathological review restricted to 5 animals/sex/group.
(2-Methyl-5-methoxythiazole [15.002])	Rat; M, F 10-16/sex	Diet	approx. 9 mg/kg bw per day	90 days	M: 8.83 F: 8.63	(Posternak et al., 1975)	Toxicity data reported as part of a summary publication on a large number of flavouring substances. Body weight, food utilisation, haematological and histopathology examination undertaken, clinical chemistry restricted to blood urea nitrogen, organ weight changes restricted to liver and kidney. Due to reporting limitations data quality could not be assessed.
(2,4-Dimethyl-5-vinylthiazole [15.005])	Rat; M, F 10-16/sex	Diet	approx. 1 mg/kg bw per day	90 days	M: 0.92 F: 1.0	(Posternak et al., 1969)	Toxicity data reported as part of a summary publication on a large number of flavouring substances,. Body weight, food utilisation, haematological and histopathology examination undertaken, clinical chemistry restricted to blood urea nitrogen, organ weight changes restricted to liver and kidney. Due to reporting limitations data quality could not be assessed.
(2-Acetyl-2-thiazoline [15.010])	Rat; M, F 8/sex	Diet	0, 197, 492, 983, 1966, 2949, 3932 mg/kg in diet equivalent to 0, 0.09, 0.23, 0.45, 0.90, 1.4, 1.8 mg/kg bw per day	90 days	1.8	(Munday and Kirkby, 1971)	Flavour coctail consisted of 4-hydroxy-5-methyl-2,3-dihydrofuran-3one [FL-no:13.085] (74 %), 2,3-dimethyl-4-hydroxy-2,5-dihydrofuran-5-one [FL-no: 10.030] (23 %), 2-acetyl-2-thiazole [FL-no: 15.020] (1.8 %) and 2-acetyl-2-thazoline [FL-no: 15.010] (0.9 %).
2-(*sec*-butyl)-4,5-dimethyl-3-thiazoline [15.029])	Rat; M, F 15/sex	Oral	1.21(M) /1.26 (F) mg/kg bw per day	90 days	M: 1.21 F: 1.26	(Babish and Re, 1978)	Old study carried out at a one dose level. Standard toxicological parameters were monitored (body weight, food consumption, haematology, urinalysis, histopathology). Study considered valid.

Table 13: Subacute / Subchronic / Chronic / Carcinogenicity Studies

Chemical Name [FL-no][(a)]	Species; Sex No./Group	Route	Dose levels	Duration (days)	NOAEL (mg/kg bw per day)	Reference	Comments
(5,6-Dihydro-2,4,6,tris(2-methylpropyl)-*4H*-1,3,5-dithiazine [15.113])	Rat M, F 3/6	Diet	0, 11, 111, 217 mg/kg bw (M) 0, 11, 107, 206 mg/kg bw (F)	14 days	>217	(Bauter, 2012a)	OECD Guideline study (407).
	Rat M, F 3/20	Diet	0, 9, 68, 132 mg/kg bw (M) 0, 11, 77, 154 mg/kg bw (F)	90 days	9.3 (M) 11.0 (F)	(Bauter, 2013a)	OECD Guideline study (408).
5-Ethylthiophene-2-carbaldehyde [15.074]	Rat M, F 3/6	Diet	0, 22, 194, 676 mg/kg bw per day (M) 0, 22, 195, 686 mg/kg bw per day (F)	14 days	M: 21.9 F: 686.2	(Bauter, 2012b)	OECD Guideline study (407).
	Rat M, F 3/20	Gavage	0, 2, 8, 75 mg/kg bw per day	90 days	75	(Bauter, 2013c)	OECD Guideline study (408).
(2-Isobutyl-4,6-dimethyldihydro-1,3,5-dithiazine and 4-isobutyl-2,6-dimethyldihydro-1,3,5-dithiazine (mixture))	Rat, M, F 5/sex	Oral	10 mg/kg bw per day	14 days	M: 11.5 F: 11.1	(Rush, 1989a)	One dose level study of short duration, no haematological or clinical chemistry parametes were examined and histopathological examinations were restricted to liver and kidney.
(2-Isopropyl-4, 6-dimethyldihydro-1,3,5-dithiazine and 4-isopropyl-2,6-dimethyldihydro-1,3,5-dithiazine (mixture))	Rat, M, F 5/sex	Oral	10 mg/kg bw per day	14 days	M: 11.8 F: 11.1	(Rush, 1989b)	One dose level study of short duration, no haematological or clinical chemistry parametes were examined and histopathological examinations were restricted to liver and kidney.

(a): Supporting substances are listed in brackets.

Table 14: Developmental and reproductive toxicity studies

Chemical Name [FL-no]	Species/ Sex No./ group	Route	Dose Levels	Duration	NOAEL (mg/kg bw per day), Including information of possible maternel toxicity	Reference	Comments
Thiophene (former [FL-no: 15.106])	Rats/M, F 13	Gavage	0, 25, 100, 400 mg/kg bw per day	42 days	M: 400 mg/kg bw F: 25 mg/kg bw	(Nagao, 2006)	Only a translated summary report available.

Table 15: Genotoxicity (*In Vitro*)

Chemical Name [FL-no]*	Test System	Test Object	Concentration	Result	Reference	Comments
Subgroup A-Ia						
Thiophene (former [FL-no: 15.106])	Ames assay (plate incorporation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537	3 µmol/plate (all strains) (252 µg/plate)	Negative (±S9)	(Florin et al., 1980)	Published non-GLP study. Qualitative screening in a spot-test with three strains, quantitative study (4 doses, 0.03, 0.3, 3, 30 µmol/plate) with TA100 only. Limited report of experimental details and results. Insufficient quality, study not considered adequate for the evaluation of mutagenic activity.
	Ames assay (preincubation method)	*S. typhimurium* TA97;TA98; TA100; TA1535; TA1537	Up to 10,000 µg/plate	Negative (±S9) [(a)]	(Zeiger et al., 1987)	Non-GLP study roughly in accordance with OECD guideline 471. The study is considered valid.
	Ames assay (preincubation method)	*S. typhimurium* TA98; TA100; TA102	0.01 - 1.2 mmol/plate (100,968 µg/plate)	Negative (±S9)	(Aeschbacher et al., 1989)	Greatest effects are quantified by "mutation factor," no numbers are given for negative results. Limited quality (only 3 strains used), but otherwise acceptable study.
	Ames assay (plate incorporation method)	*S. typhimurium* TA98; TA100	Up to 100 µmol/plate (8414 µg/plate)	Negative (±S9)	(Lee et al., 1994)	Only two strains used but otherwise acceptable study.
	Ames assay	*S. typhimurium* TA98, TA100, TA1535, TA1537 *E. coli* WP2 uvrA	0, 78, 156, 313, 625, 1250 µg/plate 0, 78, 156, 313, 625, 1250, 2500, 5000 µg/plate	Negative (±S9) Negative (±S9)	(Shibuya, 2006)	Valid study according to OECD Test Guidelines and Guidelines for screening mutagenicity testing of chemicals (Japan), provided as a translation of the original report in Japanese.
	Chromosomal Abberation	Chinese hamster lung cells	0, 210, 420, 840 µg/ml	Negative (±S9)	(Tanaka, 2006)	Valid study according to Guidelines for screening mutagenicity testing of chemicals (Japan), provided as a translation of the original report in Japanese.
Subgroup A-Ib						
2-Methylthiophene (former [FL-no: 15.091])	Ames assay (preincubation method)	*S. typhimurium* TA98; TA100; TA102	0.00001 - 1.0 mmol/plate (98,170 µg/plate)	Negative (±S9)	(Aeschbacher et al., 1989)	Greatest effects are quantified by "mutation factor," no numbers are given for negative results. Limited quality (only 3 strains used), but otherwise acceptable study.
	Ames assay (plate incorporation	*S. typhimurium* TA98; TA100	Up to 100 µmol/plate (9817 µg/plate)	Negative (±S9)	(Lee et al., 1994)	Only two strains used but otherwise acceptable study.

Table 15: Genotoxicity (*In Vitro*)

Chemical Name [FL-no]*	Test System	Test Object	Concentration	Result	Reference	Comments
	method)					
3-Methylthiophene (former [FL-no: 15.092])	Ames assay (preincubation method)	*S. typhimurium* TA98; TA100; TA102	0.01 - 1.0 mmol/plate (98,170 µg/plate)	Negative (±S9)	(Aeschbacher et al., 1989)	Greatest effects are quantified by "mutation factor," no numbers are given for negative results. Limited quality (only 3 strains used), but otherwise acceptable study.
	Ames assay (plate incorporation method)	*S. typhimurium* TA98; TA100	Up to 100 µmol/plate (9817 µg/plate)	Negative (±S9)	(Lee et al., 1994)	Only two strains used but otherwise acceptable study.
2,5-Dimethylthiophene (former [FL-no: 15.064])	Ames assay (plate incorporation method)	*S. typhimurium* TA98; TA100	Up to 100 µmol/plate (11,219 µg/plate)	Negative (±S9)	(Lee et al., 1994)	Only two strains used but otherwise acceptable study.
2-Acetylthiophene [15.040]	Ames assay (plate incorporation method)	*S. typhimurium* TA98; TA100	Up to 100 µmol/plate (12,618 µg/plate)	Negative (±S9)	(Lee et al., 1994)	Only two strains used but otherwise acceptable study.
	SOS Chromotest	*E. coli*	NR	Negative with rat S9, positive without rat S9	(Mosier et al., 2003)	Study endpoint inappropriate for the estimation of genotoxic potential.
2-Acetyl-3-Methylthiophene (former [FL-no: 15.037])	SOS Chromotest	*E. coli*	NR	Negative with rat S9, positive without rat S9	(Mosier et al., 2003)	Study endpoint inappropriate for the estimation of genotoxic potential.
Thiophene-2-carbaldehyde (former [FL-no: 15.107])	SOS Chromotest	*E. coli*	NR	Negative with rat S9, positive without rat S9	(Mosier et al., 2003)	Study endpoint inappropriate for the estimation of genotoxic potential.
5-Ethylthiophene-2-carbaldehyde [15.074]	SOS Chromotest	*E. coli*	NR	Negative with rat S9, positive without rat S9	(Mosier et al., 2003)	Study endpoint inappropriate for the estimation of genotoxic potential.
(5-Methyl-2-thiophenecarbaldehyde [15.004])	Ames assay (plate incorporation method)	*S. typhimurium* TA98; TA100	Up to 100 µmol/plate (12,618 µg/plate)	Negative (±S9)	(Lee et al., 1994)	Only two strains used but otherwise acceptable study.

Table 15: Genotoxicity (*In Vitro*)

Chemical Name [FL-no]*	Test System	Test Object	Concentration	Result	Reference	Comments
Subgroup A-II						
2,4-Dimethylthiazole [15.062]	Ames assay (plate incorporation method)	*S. typhimurium* TA100	9.3 and 94 mmol/l top agar (10,639 µg/ml)	Negative (-S9)	(Voogd et al., 1983)	Insufficient quality (one test strain as well as without metabolic activation only).
(4,5-Dimethylthiazole [15.017])	Ames assay (plate incorporation method)	*S. typhimurium* TA98; TA100	Up to 100 µmol/plate (11,318 µg/plate)	Negative (±S9)	(Lee et al., 1994)	Only two strains used but otherwise acceptable study.
(4-Methylthiazole [15.035])	Ames assay (plate incorporation method)	*S. typhimurium* TA98; TA100	Up to 100 µmol/plate (9916 µg/plate)	Negative (±S9)	(Lee et al., 1994)	Only two strains used but otherwise acceptable study.
Subgroup B-II						
2-Acetyl-2-thiazoline [15.010]	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537 and TA102	5, 16, 50, 158, 500, 1581 and 5000 µg/plate[(b)]	Negative	(Mc Garry, 2012)	OECD Guideline study (471).
			156, 312, 625, 1250, 2500 and 5000 µg /plate[(c)]	Negative		
	Micronucleus induction	Human peripheralblood lymphocytes	600, 1000 and 1292 µg /ml[(d)]	Negative	(Watters, 2012)	OECD Guideline study (487).
			600, 1000 and 1292 µg /ml[(e)]			
			100, 200, 400 and 600 µg /ml[(f)]	Negative		

* Supporting substances are listed in brackets. NR: Not Reported.
(a): With and without rat and hamster S9 metabolic activation.

No *in vivo* mutagenicity/genotoxicity data are available for either the candidate substance of the present flavouring group evaluation nor for the supporting substances evaluated by the JECFA at the 59th and 68th meetings.

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3. Bauter MR, 2013a. 5,6-Dihydro-2,4,6-tri(2-methylpropyl)-4H-1,3,5-dithiazine: A 90-day dietary study in rats. Product Safety Labs, study no 34089. January 11 2013. Unpublished report submitted by EFFA to FLAVIS Secretariat.

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APPENDICES

Appendix A. Procedure for the safety evaluation

The approach for a safety evaluation of chemically defined flavouring substances, as referred to in Commission Regulation (EC) No 1565/2000 (EC, 2000), named the "Procedure", is shown in schematic form in Figure A.1. The Procedure is based on the opinion of the SCF expressed on 2 December 1999 (SCF, 1999), which is derived from the evaluation Procedure developed by JECFA at its 44th, 46th and 49th meetings (JECFA, 1995, 1996, 1997, 1999).

The Procedure is a stepwise approach that integrates information on intake from current uses, structure–activity relationships, metabolism and, when needed, toxicity. One of the key elements in the Procedure is the subdivision of flavourings into three SCs (I, II, III) for which thresholds of concern (human exposure thresholds) have been specified. Exposures below these thresholds are not considered to present a safety concern.

Class I contains flavourings that have simple chemical structures and efficient modes of metabolism, which would suggest a low order of oral toxicity. Class II contains flavourings that have structural features that are less innocuous, but are not suggestive of toxicity. Class III comprises flavourings that have structural features that permit no strong initial presumption of safety, or may even suggest significant toxicity (Cramer et al., 1978). The thresholds of concern for these structural classes of 1 800, 540 or 90 µg/person per day, respectively, are derived from a large database containing data on subchronic and chronic animal studies (JECFA, 1996).

In Step 1 of the Procedure, the flavourings are assigned to one of the SCs. The further steps address the following questions:

- Can the flavourings be predicted to be metabolised to innocuous products[13] (Step 2)?
- Do their exposures exceed the threshold of concern for the SC (Step A3 and B3)?
- Are the flavourings or their metabolites endogenous[14] (Step A4)?
- Does a NOAEL exist on the flavourings or on structurally related substances (Step A5 and B4)?

In addition to data provided for the flavouring substances to be evaluated (candidate substances), toxicological background information available for compounds structurally related to the candidate substances is considered (supporting substances), in order to assure that these data are consistent with the results obtained after application of the Procedure.

The Procedure is not to be applied to flavourings with existing unresolved problems of toxicity. Therefore, the right is reserved to use alternative approaches if data on specific flavourings warranted such actions.

[13] "Innocuous metabolic products": Products that are known or readily predicted to be harmless to humans at the estimated intakes of the flavouring agent (JECFA, 1997).

[14] "Endogenous substances": Intermediary metabolites normally present in human tissues and fluids, whether free or conjugated; hormones and other substances with biochemical or physiological regulatory functions are not included (JECFA, 1997).

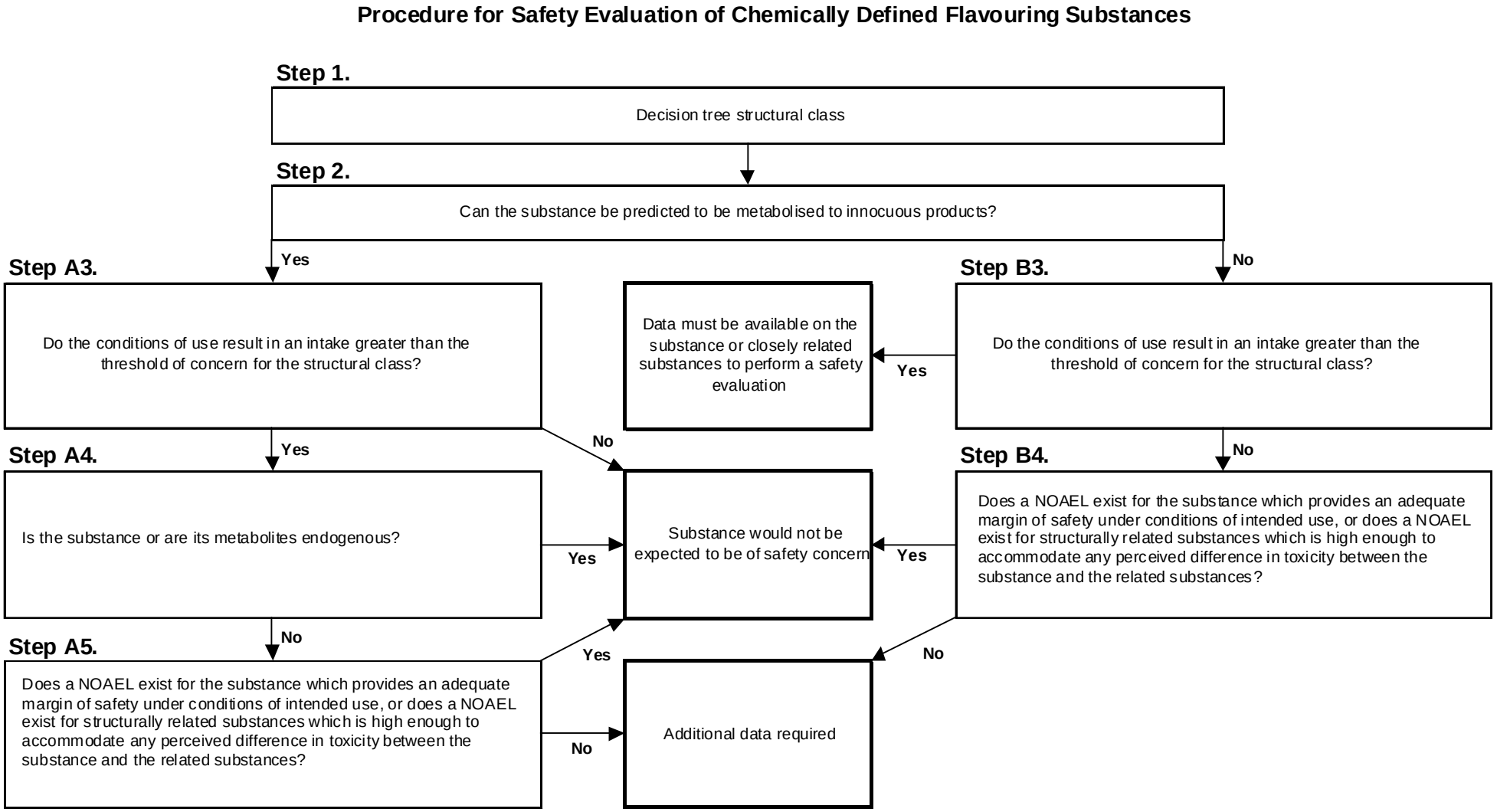


Figure A.1: Procedure for safety evaluation of chemically defined flavouring substances

Appendix B. Use levels/mTAMDI

B.1 Normal and maximum use levels

For each of the 18 food categories (Table B.1.1) in which the candidate substances are used, Flavour Industry reports a "normal use level" and a "maximum use level" (EC, 2000). According to the Industry, "normal use" is defined as the average of reported usages and "maximum use" is defined as the 95th percentile of reported usages (EFFA, 2002). The normal and maximum use levels in different food categories have been extrapolated from figures derived from 12 model flavouring substances (EFFA, 2004a).

Table B.1.1: Food categories according to Commission Regulation (EC) No 1565/2000

Food category	Description
01.0	Dairy products, excluding products of category 02.0
02.0	Fats and oils, and fat emulsions (type water-in-oil)
03.0	Edible ices, including sherbet and sorbet
04.1	Processed fruit
04.2	Processed vegetables (including mushrooms and fungi, roots and tubers, pulses and legumes), and nuts and seeds
05.0	Confectionery
06.0	Cereals and cereal products, including flours and starches from roots and tubers, pulses and legumes, excluding bakery
07.0	Bakery wares
08.0	Meat and meat products, including poultry and game
09.0	Fish and fish products, including molluscs, crustaceans and echinoderms
10.0	Eggs and egg products
11.0	Sweeteners, including honey
12.0	Salts, spices, soups, sauces, salads, protein products, etc.
13.0	Foodstuffs intended for particular nutritional uses
14.1	Non-alcoholic ("soft") beverages, excluding dairy products
14.2	Alcoholic beverages, including alcohol-free and low-alcoholic counterparts
15.0	Ready-to-eat savouries
16.0	Composite foods (e.g. casseroles, meat pies, mincemeat)—foods that could not be placed in categories 01.0–15.0

The "normal and maximum use levels" are provided by Industry for 39 of 41 candidate substances, for which use levels have been provided, in the present flavouring group (Table B.1.2).

Table B.1.2 Normal and Maximum use levels (mg/kg) for the candidate substances FGE.21Rev5 (EFFA, 2004c; EFFA, 2004d; EFFA, 2007; Flavour Industry, 2004-5; Flavour Industry, 2010b).

FL-no	Food Categories																	
	Normal use levels (mg/kg) Maximum use levels (mg/kg)																	
	01.0	02.0	03.0	04.1	04.2	05.0	06.0	07.0	08.0	09.0	10.0	11.0	12.0	13.0	14.1	14.2	15.0	16.0
15.038	0,4 2	0,2 1	0,4 2	0,3 1,5	- -	0,4 2	0,2 1	0,4 2	0,1 0,4	0,1 0,4	- -	- -	0,2 1	0,4 2	0,2 1	0,4 2	1 5	0,2 1
15.039	0,4 2	0,2 1	0,4 2	0,3 1,5	- -	0,4 2	0,2 1	0,4 2	0,1 0,4	- -	- -	- -	0,2 1	0,4 2	0,2 1	0,4 2	1 5	0,2 1
15.040	0,2 1	0,1 0,5	0,2 1	0,2 1	- -	0,2 1	0,1 0,5	0,2 1	0,1 0,2	0,1 0,2	- -	- -	0,1 0,5	0,2 1	0,1 0,5	0,2 1	0,4 2	0,1 0,5
15.044	0,4 2	0,2 1	0,4 2	0,3 1,5	- -	0,4 2	0,2 1	0,4 2	0,1 0,4	0,1 0,4	- -	- -	0,2 1	0,4 2	0,2 1	0,4 2	1 5	0,2 1
15.045	0,2 1	0,1 0,5	0,2 1	0,2 1	- -	0,2 1	0,1 0,5	0,2 1	0,1 0,2	0,1 0,2	- -	- -	0,1 0,5	0,2 1	0,1 0,5	0,2 1	0,4 2	0,1 0,5
15.050	0,4 2	0,2 1	0,4 2	0,3 1,5	- -	0,4 2	0,2 1	0,4 2	0,1 0,4	0,1 0,4	- -	- -	0,2 1	0,4 2	0,2 1	0,4 2	1 5	0,2 1
15.051	0,4 2	0,2 1	0,4 2	0,3 1,5	- -	0,4 2	0,2 1	0,4 2	0,1 0,4	0,1 0,4	- -	- -	0,2 1	0,4 2	0,2 1	0,4 2	1 5	0,2 1
15.052	0,4 2	0,2 1	0,4 2	0,3 1,5	- -	0,4 2	0,2 1	0,4 2	0,1 0,4	0,1 0,4	- -	- -	0,2 1	0,4 2	0,2 1	0,4 2	1 5	0,2 1
15.054	0,4 2	0,2 1	0,4 2	0,3 1,5	- -	0,4 2	0,2 1	0,4 2	0,1 0,4	0,1 0,4	- -	- -	0,2 1	0,4 2	0,2 1	0,4 2	1 5	0,2 1
15.055	0,4 2	0,2 1	0,4 2	0,3 1,5	- -	0,4 2	0,2 0,4	0,4 2	0,1 0,4	0,1 0,4	- -	- -	0,2 1	0,4 2	0,2 1	0,4 2	1 5	0,2 1
15.058	0,4 2	0,2 1	0,4 2	0,3 1,5	- -	0,4 2	0,2 1	0,4 2	0,1 0,4	0,1 0,4	- -	- -	0,2 1	0,4 2	0,1 0,5	0,4 2	1 5	0,2 1
15.060	0,4 2	0,2 1	0,2 1	0,3 1,5	- -	0,4 2	0,2 1	0,4 2	0,1 0,4	0,1 0,4	- -	- -	0,2 1	0,4 2	0,2 1	0,4 2	1 5	0,2 1
15.061	0,4 2	0,2 1	0,4 2	0,3 1,5	- -	0,4 2	0,2 1	0,4 2	0,1 0,4	0,1 0,4	- -	- -	0,2 1	0,4 2	0,2 1	0,4 2	1 5	0,2 1
15.062	0,4 2	0,2 1	0,4 2	0,3 1,5	0,4 2	- -	0,2 1	0,4 2	0,1 0,4	0,1 0,4	- -	- -	0,2 1	0,4 2	0,2 1	- -	1 5	0,2 1
15.063	0,4 2	0,2 1	0,4 2	0,3 1,5	- -	0,4 2	0,2 1	0,4 2	0,1 0,4	0,1 0,4	- -	- -	0,2 1	0,4 2	0,2 1	0,4 2	1 5	0,2 1
15.067	0,4 2	0,2 1	0,4 2	0,3 1,5	- -	0,4 2	0,2 1	0,4 2	0,1 0,4	0,1 0,4	- -	- -	0,2 1	0,4 2	0,2 1	0,4 2	1 5	0,2 1
15.068	0,4 2	0,2 1	0,4 2	0,3 1,5	- -	0,4 2	0,2 1	0,4 2	0,1 0,4	0,1 0,4	- -	- -	0,2 1	1 5	0,2 1	0,4 2	- -	0,2 1

Table B.1.2 Normal and Maximum use levels (mg/kg) for the candidate substances FGE.21Rev5 (EFFA, 2004c; EFFA, 2004d; EFFA, 2007; Flavour Industry, 2004-5; Flavour Industry, 2010b).

FL-no	Food Categories																	
	Normal use levels (mg/kg) Maximum use levels (mg/kg)																	
	01.0	02.0	03.0	04.1	04.2	05.0	06.0	07.0	08.0	09.0	10.0	11.0	12.0	13.0	14.1	14.2	15.0	16.0
15.069	0,4 2	0,2 1	0,4 2	0,3 1,5	- -	0,4 2	0,2 1	0,4 2	0,1 0,4	0,1 0,4	- -	- -	0,2 1	0,4 2	0,2 1	0,4 2	1 5	0,2 1
15.071	0,4 2	0,2 1	0,4 2	0,3 1,5	- -	0,4 2	0,2 1	0,4 2	0,1 0,4	0,1 0,4	- -	- -	0,2 1	0,4 2	0,2 1	0,4 2	1 5	0,2 1
15.074	0,2 1	0,1 0,5	0,2 1	0,2 1	- -	0,2 1	0,1 0,5	0,2 1	0,1 0,2	0,1 0,2	- -	- -	0,1 0,5	0,2 1	0,1 0,5	0,2 1	0,4 2	0,1 0,5
15.076	0,2 1	0,1 0,5	0,2 1	0,2 1	- -	0,2 1	0,1 0,5	0,2 1	0,1 0,2	0,1 0,2	- -	- -	0,1 0,5	0,2 1	0,1 0,5	0,2 1	0,4 2	0,1 0,5
15.078	0,4 2	0,2 1	0,4 2	0,3 1,5	- -	0,4 2	0,2 1	0,4 2	0,1 0,4	0,1 0,4	- -	- -	0,2 1	0,4 2	0,2 1	0,4 2	1 5	0,2 1
15.080	0,4 2	0,2 1	0,4 2	0,3 1,5	- -	0,4 2	0,2 1	0,4 2	0,1 0,4	0,1 0,4	- -	- -	0,2 1	0,4 2	0,2 1	0,4 2	1 5	0,2 1
15.082	0,2 1	0,1 0,5	0,2 1	0,2 1	- -	0,2 1	0,1 0,5	0,2 1	0,1 0,2	0,1 0,2	- -	- -	0,1 0,5	0,2 1	0,1 0,5	0,2 1	0,4 2	0,1 0,5
15.084	0,4 2	0,2 1	0,4 2	0,3 1,5	- -	0,4 2	0,2 1	0,4 2	0,1 0,4	0,1 0,4	- -	- -	0,2 1	0,4 2	0,2 1	0,4 2	1 5	0,2 1
15.085	0,4 2	0,2 1	0,4 2	0,3 1,5	- -	0,4 2	0,2 1	0,4 2	0,1 0,4	0,1 0,4	- -	- -	0,2 1	0,4 2	0,2 1	0,4 2	1 5	0,2 1
15.086	0,4 2	0,2 1	0,4 2	0,3 1,5	- -	0,4 2	0,2 1	0,4 2	0,1 0,4	0,1 0,4	- -	- -	0,2 1	0,4 2	0,2 1	0,4 2	1 5	0,2 1
15.087	0,2 1	0,1 0,5	0,2 1	0,2 1	- -	0,2 1	0,1 0,5	0,2 1	0,1 0,2	0,1 0,2	- -	- -	0,1 0,5	0,2 1	0,1 0,5	0,2 1	0,4 2	0,1 0,5
15.089	0,4 2	0,2 1	0,4 2	0,3 1,5	- -	0,4 2	0,2 1	0,4 2	0,1 0,4	0,1 0,4	- -	- -	- -	0,4 2	0,2 1	0,2 1	1 5	0,2 1
15.093	0,4 2	0,2 1	0,4 2	0,3 1,5	- -	0,4 2	0,2 1	0,4 2	0,1 0,4	0,1 0,4	- -	- -	0,2 1	0,4 2	0,2 1	0,4 2	1 5	0,2 1
15.096	0,4 2	0,2 1	0,4 2	0,3 1,5	- -	0,4 2	0,2 1	0,4 2	0,1 0,4	0,1 0,4	- -	- -	0,2 1	0,4 2	0,2 1	0,4 2	1 5	0,2 1
15.097	0,4 2	0,2 1	0,4 2	0,3 1,5	- -	0,4 2	0,2 1	0,4 2	0,1 0,4	0,1 0,4	- -	- -	0,2 1	0,4 2	0,2 1	0,4 2	1 5	0,2 1
15.098	0,4 2	0,2 1	0,4 2	0,3 1,5	- -	0,4 2	0,2 1	0,4 2	0,1 0,4	0,1 0,4	- -	- -	0,2 1	0,4 2	0,2 1	0,4 2	1 5	0,2 1
15.108	0,2 1	0,1 0,5	0,2 1	0,2 1	- -	0,2 1	0,1 0,5	0,2 1	0,1 0,2	0,1 0,2	- -	- -	0,1 0,5	0,2 1	0,1 0,5	0,2 1	0,4 2	0,2 1

Table B.1.2 Normal and Maximum use levels (mg/kg) for the candidate substances FGE.21Rev5 (EFFA, 2004c; EFFA, 2004d; EFFA, 2007; Flavour Industry, 2004-5; Flavour Industry, 2010b).

FL-no	Food Categories																	
	Normal use levels (mg/kg) Maximum use levels (mg/kg)																	
	01.0	**02.0**	**03.0**	**04.1**	**04.2**	**05.0**	**06.0**	**07.0**	**08.0**	**09.0**	**10.0**	**11.0**	**12.0**	**13.0**	**14.1**	**14.2**	**15.0**	**16.0**
15.115	0,4 2	0,2 1	0,4 2	0,3 1,5	- -	0,4 2	0,2 1	0,4 2	0,1 0,4	0,1 0,4	- -	- -	0,2 1	0,4 2	0,2 1	0,4 2	1 5	0,2 1
15.116	0,4 2	0,2 1	0,4 2	0,3 1,5	- -	0,4 2	0,2 1	0,4 2	0,1 0,4	0,1 0,4	- -	- -	0,2 1	0,4 2	0,2 1	0,4 2	1 5	0,2 1
15.118	0,4 2	0,2 1	0,4 2	0,3 1,5	- -	0,4 2	0,2 1	0,4 2	0,1 0,4	0,1 0,4	- -	- -	0,2 1	0,4 2	0,2 1	0,4 2	1 5	0,2 1
15.119	0,4 2	0,2 1	0,4 2	0,3 1,5	- -	0,4 2	0,2 1	0,4 2	0,1 0,4	0,1 0,4	- -	- -	0,2 1	0,4 2	0,2 1	0,4 2	1 5	0,2 1
15.135	1,5 3	1 2	- -	0,5 1	0,5 1	- -	0,5 1	1,5 3	1,5 3	- -	- -	- -	2 12	- -	- -	- -	0,5 1	- -

B.2 mTAMDI calculations

The method for calculation of mTAMDI values is based on the approach used by SCF up to 1995 (SCF, 1995). The assumption is that a person may consume the quantities of flavourable foods and beverages listed in Table B.2.1. These consumption estimates are then multiplied by the reported use levels in the different food categories and summed up.

Table B.2.1: Estimated quantities of flavourable foods, beverages and exceptions assumed to be consumed per person per day (SCF, 1995)

Class of product category	Intake estimate (grams per day)
Beverages (non-alcoholic)	324.0
Foods	133.4
Exception a: Candy, confectionery	27.0
Exception b: Condiments, seasonings	20.0
Exception c: Alcoholic beverages	20.0
Exception d: Soups, savouries	20.0
Exception e: Others, e.g. chewing gum	e.g. 2.0 (chewing gum)

The mTAMDI calculations are based on the normal use levels reported by Industry. The seven food categories used in the SCF TAMDI approach (SCF, 1995) correspond to the 18 food categories as outlined in Commission Regulation (EC) No 1565/2000 and reported by the Flavour Industry in the following way (see Table B.2.2):

- Beverages correspond to food category 14.1.
- Foods correspond to the food categories 1, 2, 3, 4.1, 4.2, 6, 7, 8, 9, 10, 13 and/or 16.
- Exception a corresponds to food category 5 and 11.
- Exception b corresponds to food category 15.
- Exception c corresponds to food category 14.2.
- Exception d corresponds to food category 12.
- Exception e corresponds to others, e.g. chewing gum.

Table B.2.2: Distribution of the 18 food categories listed in Commission Regulation (EC) No 1565/2000 (EC, 2000) into the seven SCF food categories used for TAMDI calculation (SCF, 1995)

Key	Food categories according to Commission Regulation 1565/2000 Food category	Distribution of the seven SCF food categories Food	Beverages	Exceptions
01.0	Dairy products, excluding products of category 02.0	Food		
02.0	Fats and oils, and fat emulsions (type water-in-oil)	Food		
03.0	Edible ices, including sherbet and sorbet	Food		
04.1	Processed fruit	Food		
04.2	Processed vegetables (including mushrooms and fungi, roots and tubers, pulses and legumes), and nuts and seeds	Food		
05.0	Confectionery			Exception a
06.0	Cereals and cereal products, including flours and starches from roots and tubers, pulses and legumes, excluding bakery	Food		
07.0	Bakery wares	Food		

Key	Food categories according to Commission Regulation 1565/2000 Food category	Distribution of the seven SCF food categories Food	Beverages	Exceptions
08.0	Meat and meat products, including poultry and game	Food		
09.0	Fish and fish products, including molluscs, crustaceans and echinoderms	Food		
10.0	Eggs and egg products	Food		
11.0	Sweeteners, including honey			Exception a
12.0	Salts, spices, soups, sauces, salads, protein products, etc.			Exception d
13.0	Foodstuffs intended for particular nutritional uses	Food		
14.1	Non-alcoholic ("soft") beverages, excluding dairy products		Beverages	
14.2	Alcoholic beverages, including alcohol-free and low-alcoholic counterparts			Exception c
15.0	Ready-to-eat savouries			Exception b
16.0	Composite foods (e.g. casseroles, meat pies, mincemeat)—foods that could not be placed in categories 01.0–15.0	Food		

The mTAMDI values (see Table B.2.3) are presented for 41 candidate substances in the present flavouring group, for which Industry has provided use and use levels (EFFA, 2004c, d, 2007; Flavour Industry, 2004–5, 2010b). The mTAMDI values are given for only the highest reported normal use levels (see Table B.2.3). Table B.2.3 also contains the mTAMDI values for the 18 substances no longer supported by Industry for use as flavouring substances in Europe: [FL-nos: 15.037, 15.042, 15.043, 15.064, 15.070, 15.072, 15.077, 15.088, 15.090, 15.091, 15.092, 15.094, 15.099, 15.106, 15.107, 15.114, 15.129 and 15.133] (DG SANCO, 2012, 2013).

Table B.2.3: Estimated intakes based on the mTAMDI approach

FL-no	EU register name	mTAMDI (µg/person per day)	Structural class	Threshold of concern (µg/person per day)
15.037	2-Acetyl-3-methylthiophene	78	Class II	540
15.038	2-Acetyl-4-methylthiazole	160	Class II	540
15.039	2-Acetyl-5-methylthiazole	160	Class II	540
15.040	2-Acetylthiophene	78	Class II	540
15.043	2-Butyl-5-ethylthiophene	78	Class II	540
15.044	2-Butylthiazole	160	Class II	540
15.045	2-Butylthiophene	78	Class II	540
15.050	2,5-Diethyl-4-methylthiazole	160	Class II	540
15.051	2,5-Diethyl-4-propylthiazole	160	Class II	540
15.052	2,5-Diethylthiazole	160	Class II	540
15.058	4,5-Dimethyl-2-ethylthiazole	130	Class II	540
15.061	2,5-Dimethyl-4-ethylthiazole	160	Class II	540
15.062	2,4-Dimethylthiazole	140	Class II	540
15.063	2,5-Dimethylthiazole	160	Class II	540
15.064	2,5-Dimethylthiophene	78	Class II	540
15.067	4-Ethyl-2-methylthiazole	160	Class II	540
15.068	5-Ethyl-2-methylthiazole	220	Class II	540
15.069	4-Ethyl-5-methylthiazole	160	Class II	540
15.070	2-Ethyl-5-methylthiophene	78	Class II	540
15.071	2-Ethylthiazole	160	Class II	540
15.072	2-Ethylthiophene	78	Class II	540
15.074	5-Ethylthiophene-2-carbaldehyde	78	Class II	540
15.076	2-Hexylthiophene	78	Class II	540
15.078	2-Isobutyl-4,5-dimethylthiazole	160	Class II	540
15.080	2-Isopropyl-4,5-dimethylthiazole	160	Class II	540

FL-no	EU register name	mTAMDI (µg/person per day)	Structural class	Threshold of concern (µg/person per day)
15.084	5-Methyl-2-pentylthiazole	160	Class II	540
15.085	4-Methyl-2-propionylthiazole	160	Class II	540
15.086	2-Methyl-2-thiazoline	160	Class II	540
15.089	2-Methylthiazole	150	Class II	540
15.091	2-Methylthiophene	78	Class II	540
15.092	3-Methylthiophene	78	Class II	540
15.093	2-Octylthiophene	160	Class II	540
15.094	2-Pentanoylthiophene	160	Class II	540
15.096	2-Pentylthiophene	160	Class II	540
15.097	2-Propionylthiophene	160	Class II	540
15.098	2-Propylthiazole	160	Class II	540
15.107	Thiophene-2-carbaldehyde	78	Class II	540
15.115	2-Isobutyl-4-methyl thiazole	160	Class II	540
15.116	2-Acetyl-4-ethylthiazole	160	Class II	540
15.118	4-Butylthiazole	160	Class II	540
15.129	Tetrahydro-2,4,6-trimethyl-1,3,5(2*H*)-thiadiazine	4 000	Class II	540
15.060	2,4-Dimethyl-3-thiazoline	160	Class II	540
15.090	2-Methylthiazolidine	160	Class II	540
15.099	2-Propylthiazolidine	160	Class II	540
15.119	2-Isobutyl-3-thiazoline	160	Class II	540
15.042	2-Butyl-4-methyl(4*H*)pyrrolidino[1,2*d*]–1,3,5-dithiazine	160	Class III	90
15.054	Dihydro-2,4,6-triethyl-1,3,5(4*H*)-dithiazine	160	Class III	90
15.055	[2*S*-(2*a*,4*a*,8*ab*)] 2,4-Dimethyl(4*H*)pyrrolidino[1,2*e*]-1,3,5-dithiazine	160	Class III	90
15.057	4,6-Dimethyl-2-(1-methylethyl)dihydro-1,3,5-dithiazine		Class III	90
15.077	4-Hydroxy-2,5-dimethylthiophen-3(2*H*)-one	78	Class III	90
15.079	2-Isobutyldihydro-4,6-dimethyl-1,3,5-dithiazine		Class III	90
15.082	3-Mercaptothiophene	78	Class III	90
15.087	2-Methyl-3-mercaptothiophene	78	Class III	90
15.088	2-Methyl-4,5-benzothiazole	160	Class III	90
15.106	Thiophene	78	Class III	90
15.108	2-Thiophenemethanethiol	78	Class III	90
15.135	Ethyl thialdine	250	Class III	90
15.114	6-Acetyl-2,3-dihydro-1,4-thiazine	160	Class III	90
15.133	5-Acetyl-2,3-dihydro-1,4-thiazine	4 000	Class III	90

Appendix C. Metabolism

C.1 Introduction

The candidate substances in this FGE are structurally related to the 28 flavouring substances evaluated in "Safety Evaluations of Groups of Related Flavouring Agents: Sulfur-Containing Heterocyclic Compounds" (JECFA, 2002a, 2003, 2007, 2008).

The candidate substances from EU chemical groups 29 and 30 in this FGE include five- and six-membered sulphur-containing aromatic and non-aromatic heterocycles, which in the previous versions of FGE.21 have been arranged into nine subgroups in order to facilitate comparisons of the datasets between the groups. This division was done on the basis of degree of aromaticity and according to the presence of other heteroatoms (i.e. nitrogen). Subgroup A-I has been further divided into three subgroups: A-Ia (thiophene), A-Ib (thiophenes with alkyl, acyl or carbaldehyde ring substituents) and A-Ic (thiophenes with thiol-containing ring substituents). Therefore, a total of 11 subgroups. In this revision 4, FGE.21Rev5, 18 substances are no longer supported by Industry for the use as flavouring substances and have accordingly been deleted from the text unless the information is considered relevant for the remaining substances. This means that the following subgroups are no longer considered: A-Ia, A-III, B-I, B-III, B-V and B-VI.

The substances and subgroups are presented in Table C.1.

Table C.1 Division of candidate and supporting substances into structurally related subgroups – supporting substances are listed in brackets

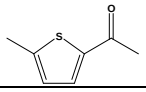
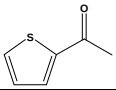
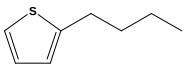
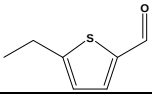
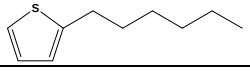
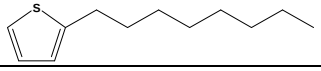
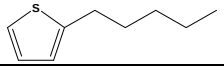
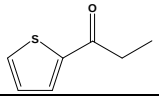
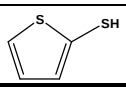
FL-no	EU Register name	Structural formula
A-Ia	Thiophene - The substance previously allocated to the group is no longer supported for use as flavouring substance in Europe by Industry	
A-Ib	Thiophenes (with non-thiol-containing ring substituents)	
(15.004)	(5-Methyl-2-thiophenecarbaldehyde)	
15.040	2-Acetylthiophene	
15.045	2-Butylthiophene	
15.074	5-Ethylthiophene-2-carbaldehyde	
15.076	2-Hexylthiophene	
15.093	2-Octylthiophene	
15.096	2-Pentylthiophene	
15.097	2-Propionylthiophene	
A-Ic	Thiophenes (with thiol-containing ring substituents)	
(15.001)	(2-Mercaptothiophene)	

Table C.1 Division of candidate and supporting substances into structurally related subgroups – supporting substances are listed in brackets

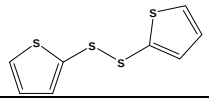
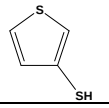
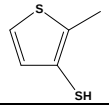
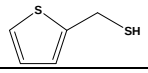
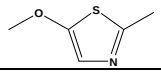
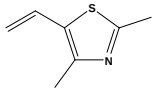
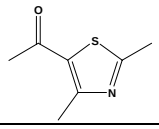
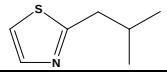
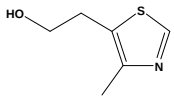
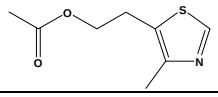
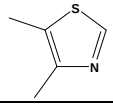
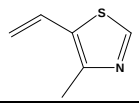
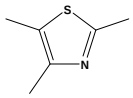
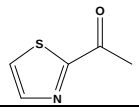
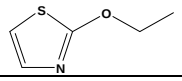
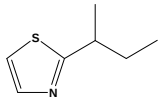
FL-no	EU Register name	Structural formula
(15.008)	(2-Thienyl disulfide)	
15.082	3-Mercaptothiophene	
15.087	2-Methyl-3-mercaptothiophene	
15.108	2-Thiophenemethanethiol	
A-II Thiazoles		
(15.002)	(2-Methyl-5-methoxythiazole)	
(15.005)	(2,4-Dimethyl-5-vinylthiazole)	
(15.011)	(5-Acetyl-2,4-dimethylthiazole)	
(15.013)	(2-Isobutylthiazole)	
(15.014)	(5-(2-Hydroxyethyl)-4-methylthiazole)	
(15.015)	(4-Methyl-5-(2-acetoxyethyl)thiazole)	
(15.017)	(4,5-Dimethylthiazole)	
(15.018)	(4-Methyl-5-vinylthiazole)	
(15.019)	(2,4,5-Trimethylthiazole)	
(15.020)	(2-Acetylthiazole)	
(15.021)	(2-Ethoxythiazole)	
(15.022)	(2-(*sec*-Butyl)thiazole)	

Table C.1 Division of candidate and supporting substances into structurally related subgroups – supporting substances are listed in brackets

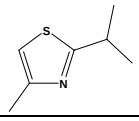
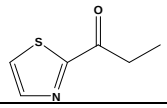
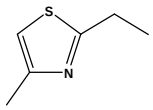
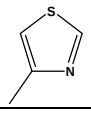
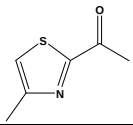
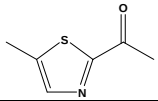
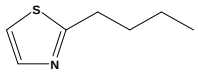
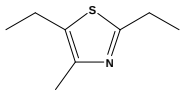
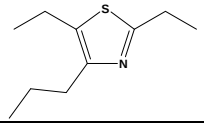
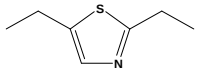
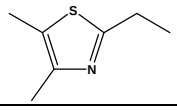
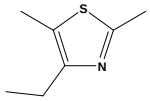
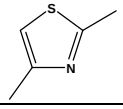
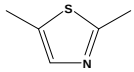
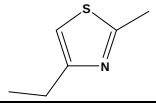
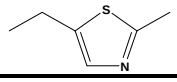
FL-no	EU Register name	Structural formula
(15.026)	(2-Isopropyl-4-methylthiazole)	
(15.027)	(2-Propionylthiazole)	
(15.033)	(2-Ethyl 4-methylthiazole)	
(15.035)	(4-Methylthiazole)	
15.038	2-Acetyl-4-methylthiazole	
15.039	2-Acetyl-5-methylthiazole	
15.044	2-Butylthiazole	
15.050	2,5-Diethyl-4-methylthiazole	
15.051	2,5-Diethyl-4-propylthiazole	
15.052	2,5-Diethylthiazole	
15.058	4,5-Dimethyl-2-ethylthiazole	
15.061	2,5-Dimethyl-4-ethylthiazole	
15.062	2,4-Dimethylthiazole	
15.063	2,5-Dimethylthiazole	
15.067	4-Ethyl-2-methylthiazole	
15.068	5-Ethyl-2-methylthiazole	

Table C.1 Division of candidate and supporting substances into structurally related subgroups – supporting substances are listed in brackets

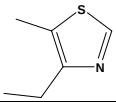
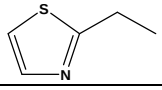
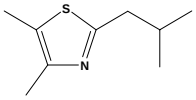
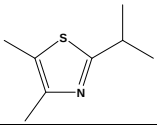
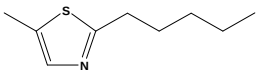
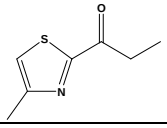
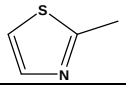
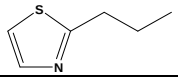
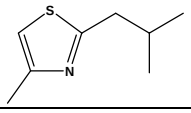
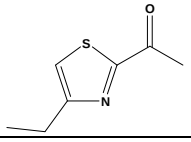
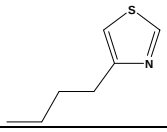
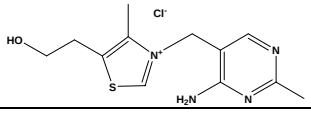
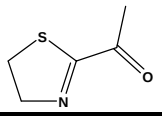
FL-no	EU Register name	Structural formula
15.069	4-Ethyl-5-methylthiazole	
15.071	2-Ethylthiazole	
15.078	2-Isobutyl-4,5-dimethylthiazole	
15.080	2-Isopropyl-4,5-dimethylthiazole	
15.084	5-Methyl-2-pentylthiazole	
15.085	4-Methyl-2-propionylthiazole	
15.089	2-Methylthiazole	
15.098	2-Propylthiazole	
15.115	2-Isobutyl-4-methylthiazole	
15.116	2-Acetyl-4-ethylthiazole	
15.118	4-Butylthiazole	
(16.027)	(Thiamine hydrochloride)	
A-III Benzothiazoles - The substance previously allocated to the group is no longer supported for use as flavouring substance in Europe by Industry		
B-I Dihydrothiophenes - The substance previously allocated to the group is no longer supported for use as flavouring substance in Europe by Industry		
B-II Thiazolines		
(15.010)	2-Acetyl-2-thiazoline	

Table C.1 Division of candidate and supporting substances into structurally related subgroups – supporting substances are listed in brackets

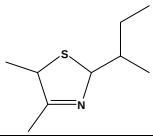
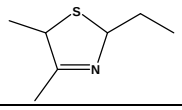
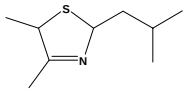
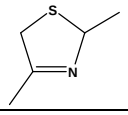
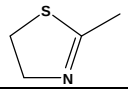
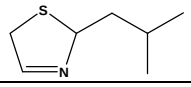
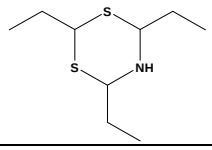
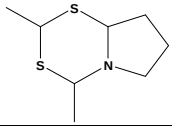
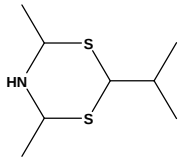
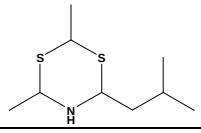
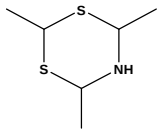
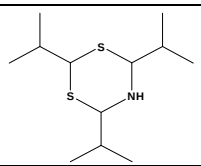
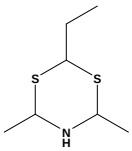
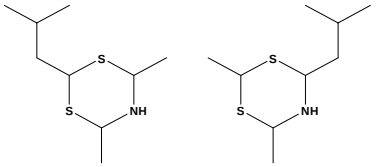
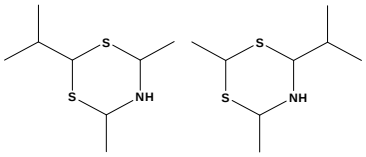
FL-no	EU Register name	Structural formula
(15.029)	(2-(*sec*-Butyl)-4,5-dimethyl-3-thiazoline)	
(15.030)	(4,5-Dimethyl-2-ethyl-3-thiazoline)	
(15.032)	(4,5-Dimethyl-2-isobutyl-3-thiazoline)	
15.060	2,4-Dimethyl-3-thiazoline	
15.086	2-Methyl-2-thiazoline	
15.119	2-Isobutyl-3-thiazoline	
B-III Thiazolidines - The substances previously allocated to the group are no longer supported for use as flavouring substances in Europe by Industry		
B-IV Dithiazines		
15.054	Dihydro-2,4,6-triethyl-1,3,5(4H)-dithiazine	
15.055	[2S-(2a,4a,8ab)] 2,4-Dimethyl(4H)pyrrolidino[1,2e]-1,3,5-dithiazine	
15.057	4,6-Dimethyl-2-(1-methylethyl)dihydro-1,3,5-dithiazine	
15.079	2-Isobutyldihydro-4,6-dimethyl-1,3,5-dithiazine	
(15.109)	(2,4,6-Trimethyldihydro-1,3,5(4H)-dithiazine)	
(15.113)	(5,6-Dihydro-2,4,6,tris(2-methylpropyl)-*4H*-1,3,5-dithiazine)	

Table C.1 Division of candidate and supporting substances into structurally related subgroups – supporting substances are listed in brackets

FL-no	EU Register name	Structural formula
15.135	Ethyl thialdine	
(Not in EU Register)	(2-Isobutyl-4,6-dimethyldihydro-1,3,5-dithiazine and 4-isobutyl-2,6-dimethyldihydro-1,3,5-dithiazine (mixture))	 (Mixture)
(Not in EU Register)	(2-Isopropyl-4,6-dimethyl 2,6-dimethyldihydro-1,3,5-dithiazine and 4-isopropyl-2,6-dimethyldihydro-1,3,5-dithiazine (mixture))	 (Mixture)
B-V Dihydrothiazines - The substances previously allocated to the group are no longer supported for use as flavouring substances in Europe by Industry		
B-VI Thiadiazines - The substance previously allocated to the group is no longer supported for use as flavouring substance in Europe by Industry		

The following is a description of the characteristic features of the subgroups:

A Aromatic group

Subgroup A-I: Thiophenes (10 candidate substances and three supporting substances). Aromatic heterocyclic five-membered ring substances with a sulphur atom at ring position 1. This group is further divided into subgroups: A-Ib with seven ring-substituted thiophenes, which have one or more alkyl, acyl or carbaldehyde substituents to the ring, and subgroup A-Ic with three ring-substituted thiophenes, which have a free thiol group in their ring-substituent group (subgroup A-Ia no longer supported).

Subgroup A-II: Thiazoles (23 candidate substances and 17 supporting substances). Five-membered *aromatic* heterocycles containing one sulphur and one nitrogen atom in the 1- and 3-ring positions, respectively. To this heterocyclic ring one or more alkyl- or acyl- substituents may be attached.

B Non-aromatic group

Subgroup B-II: Thiazolines (three candidate substances and four supporting substances). Five-membered heterocycles containing a sulphur and a nitrogen atom in the 1- and 3-ring positions, respectively. In the ring, one double bond is present (in contrast to the thiazoles, which have two double bonds (aromatic)).

Subgroup B-IV: Dithiazines (five candidate substances and four supporting substances). Six-membered non-aromatic heterocycles containing two sulphur atoms and one nitrogen atom with various ring substituents, including a fused second ring in one of these.

C.2 Absorption, distribution and elimination

Only limited data were submitted for the evaluation of the absorption, distribution and elimination of the candidate flavouring substances in this group. The absorption and elimination were studied for thiophene[15] [former FL-no: 15.106]. Data were also submitted for a few, more or less, related substances. Because the information is so fragmentary, it is not useful to present it (sub)group-wise. In addition, some of the studies are related to oral exposure and some others to parenteral dosing, and the relevance of the latter may be limited.

Single doses of 33 mg/kg bw [2,5-^{14}C]-thiophene were administered to mice via oral and rectal intubation. At 48 hours after the oral gavage dose, 78 % of the radioactivity was found in the urine, while 5 % was found in both faeces and expired air. At 24 hours after the rectal dose, 43 % was found in the urine, but 40 % was found in the expired air. The difference in the elimination pattern after oral vs. rectal administration was attributed to an extensive first-pass effect after the oral dose. In addition, the oral dose resulted in liver radioactivity levels 10 times higher than the rectal dose. With thin-layer chromatography two urinary metabolites were observed. However, neither these metabolites nor the radioactivity in tissues or excreta were further identified (Chanal et al., 1974).

Female rats (200–250 g) and rabbits (2–3 kg) were administered thiophene as single oral gavage doses of 60 mg and 450 mg, respectively. Urine was collected over a 24-hour period following dosing. Table C.2 provides a summary of the quantitative determinations of metabolites in this study.

Table C.2: Summary of excretion of metabolites of thiophene in rabbits and rats

Metabolites	Rabbit (N = 6)	Rat (N = 3)
(Pre)-mercapturic acids in urine	38 (32–49)	40 (36–45)
Free thiophene expired	Not determined	32 (29–35)
Free thiophene in faeces	Not determined	0.48 (0.25–0.68)

Tabulated data are percentage of administered dose (%)

Other urinary metabolites, in particular conjugates of 2-hydroxy- or 3-hydroxythiophene, but no acid-hydrolysable products of thiophene or free thiophene were found (Bray et al., 1971).

When male Sprague–Dawley rats were injected intraperitoneally with a 200 mg/kg bw dose of tritiated (at C2 and C5 positions) thiophene in maize oil, approximately 31 % of the radioactivity was excreted between 0 and 15 hours, while 4 % of the radioactivity was excreted between 15 and 50 hours. More than 94 % of the urinary radioactivity was accounted for by a single metabolite, the 2-mercapturic acid derivative of 1,4-dihydrothiophene-*S*-oxide (Dansette et al., 1992).

In rats, a single subcutaneous dose of ^{35}*S*-thiophene was rapidly absorbed into the bloodstream. Thiophene produced a peak blood concentration within 30 minutes and peak levels in the brain within three hours. Radioactivity was excreted in the urine at 61.1 % of the dose within 34 hours and 74.4 % of the dose within three days (Bikbulatov and Nigmatullina, 1976; O'Donoghue, 2000).

[15] Structural formula: 

Non-candidate substances

In two male Sprague–Dawley rats, approximately 20 % of the radioactivity of an intraperitoneal injection of 30 mg/kg bw of the 3-aroylthiophene (tienilic acid isomer 1)[16] (^{14}C at the keto position) is recovered in the urine within 24 hours. Approximately 15 % of urinary radioactivity could be accounted for as a mixture of two diastereoisomers of the mercapturic acid conjugate of the 3-aroyl-4,5-dihydrothiophene. The study was designed to identify these two metabolites. The rest of the urinary radioactivity was identified as parent substance (ca. 17 % of the dose) and an unidentified metabolite (ca. 1 % of the dose), presumably a derivative of one or both of the already identified mercapturates (Valadon et al., 1996).

When the benzothiazole derivative fostedil[17] was administered to dogs via intravenous infusion or orally as a solution, a suspension or as an encapsulated substance, mean relative bioavailabilities for the parent substance after oral dosing could be calculated as 71, 64 or 42 %, respectively. The substance was cleared from the plasma with a terminal half-life of 6–9 hours and, based on high biliary excretion, enterohepatic circulation has been suggested. When the substance was administered with a radiolabel (no further details) 80 % of the radioactivity was found in the faeces (Thomas, 1984; Thomas and Bopp, 1984).

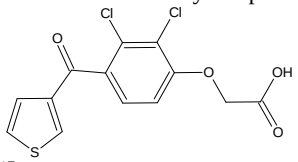
In a previous FGE.24Rev1 (EFSA, 2008), data on the absorption and elimination of indole[18] have been discussed. As this substance bears some resemblance to the benzothiazoles in subgroup A-III, these data are also briefly mentioned here. After oral dosing of 2-^{14}C-indole to rats, 62–82 % of the administered radioactivity was excreted via urine or expired air within 48–72 hours post dosing.

The substances in subgroup B-IV (the dithiazines with FL-no: 15.054, 15.055, 15.057, 15.079 and 15.135) may be regarded as cyclic thioacetals, which could be subject to acid hydrolysis in the stomach, similar to oxygen-containing acetals. However, thioacetals are more resistant to hydrolysis than oxygen acetals. In addition, chances of hydrolysis would be reduced even more by their cyclic character (Smith and March, 2001; EFSA CEF Panel, 2011c). It is thus anticipated that these substances may be expected to reach the intestinal lumen intact and may also be absorbed as such.

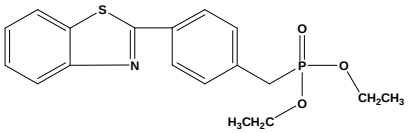
C.3 Metabolism

In the sections below, the available metabolism data have been presented according to the division into subgroups of the candidate flavouring substances in this FGE.

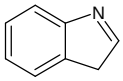
[16] Structure of 3-aroylthiophene (isomer of tienilic acid (2-aroylthiophene)):



[17] Structure of fostedil:



[18] Structure of indole:



Aromatic candidate substances

Subgroup A-Ib: Thiophenes with non-thiol-containing ring substituents

(Candidate substances [FL-no: 15.040, 15.045, 15.074, 15.076, 15.093, 15.096 and 15.097])

Data on the structurally related substance thiophene

Thiophene former [FL-no: 15.106] was administered to female rats (200–250 g) and rabbits (2–3 kg) as single oral gavage doses of 60 mg and 450 mg, respectively. Urine was collected over a 24-hour period following dosing. The predominant metabolites excreted in the urine were a mercapturic acid and a premercapturic acid, which, according to the authors, could result from the conjugation of glutathione with an intermediate 2,3-thiophene-epoxide. 2-Thienylmercapturic acid was excreted in relatively small quantities and 3-hydroxy-2,3-dihydro-2-thienyl-mercapturic acid was the major metabolite excreted in urine. The metabolites were identified by IR spectroscopy, gas–liquid chromatography (GLC) and MS techniques, and further by characteristic chemical reactions of thiophene derivatives (desulphuration following treatment of the premercapturic acid with Raney nickel resulting in the formation of 2-butanol) (Bray et al., 1971).

More recently, male Wistar rats (190–400 g) were injected intraperitoneally with 0.2 mmol (≈ 17 mg) thiophene in peanut oil, and mercapturic acids were not found to be important thioethers (i.e. comprising not more than about 1 % of the dose) excreted in the urine (Hickman et al., 1992), contrary to the results of Bray et al. (1971). It is noted that 2-thienyl mercapturic acid was also identified as a less important metabolite by Bray et al. (1971) and that the two studies differ in route of administration. The oral route of administration (Bray et al., 1971) results in higher metabolic conversion than the intraperitoneal one (Hickman et al., 1992). In addition, identification of thioethers by Hickman et al. (1992) was carried out only by reaction with Ellman's reagent following extraction and hydrolysis of the urine samples. No attempts were made to identify any other thiophene metabolite in urine samples.

When male Sprague–Dawley rats were injected (intraperitoneal) with 200 mg/kg bw [2,5-^{3}H]-labelled thiophene, more than 94 % of the urinary radioactivity was accounted for by the 2-mercapturic acid derivative of 2,5-dihydrothiophene-*S*-oxide, as identified by NMR, IR and mass spectroscopical techniques. A small second peak in the high-performance liquid chromatography (HPLC) diagram of the urine radioactivity was also observed, but this peak was not further studied. The authors concluded that its formation occurred by *S*-oxidation yielding the very reactive thiophene sulphoxide, which undergoes Michael-type addition of glutathione at the 2-position. In subsequent reactions, the glutathione moiety of this metabolite is converted into an *N*-acetyl cysteine group. The final metabolite is subsequently excreted via urine, and may be converted spontaneously into 2-thienyl-mercapturic acid (Dansette et al., 1992). The authors considered the formation of the epoxide intermediate, as suggested by Bray et al. (1971), highly unlikely.

Based on subsequent studies with chemically defined reaction mixtures and with microsomal incubations, the formation of the 2,5-dihydrothiophene-*S*-oxide was further substantiated by the formation of two diastereoisomeric thiophene *S*-oxide dimers (3*a*,4,7,7*a*-tetrahydro-4,7epithio-benzo[*b*]thiophene 1,8 dioxide) one of which (the 1-*syn*,-*8*-*syn*,*endo*-isomer) was suggested having been observed in the *in vivo* studies by Dansette et al. (ca. 10 % of urinary radioactivity, comprising 2 % of the oral dose in the above study; the unidentified second peak (see above)). The formation of the dimers may result from a Diels−Alder reaction. Thus, the metabolic fate for the thiophene *S*-oxide *in vivo* involves, predominantly, reaction with nucleophiles such as glutathione (catalysed by glutathione *S*-transferase) leading eventually to mercapturate derivatives that are excreted in the urine, or to a lesser extent it involves dimerisation and the resulting dimer is also excreted via the urine (Treiber et al., 1997).

Non-candidate substances

In two male Sprague–Dawley rats injected intraperitoneally with the 3-aroylthiophene tienilic acid isomer 1 (see above), 15 % of urinary radioactivity (≈ 2 % of the dose) could be accounted for as a mixture of two diastereoisomers of the mercapturic acid conjugate of 3-aroyl-4,5-dihydrothiophene. In the ultimate urinary metabolite, the *N*-acetylcysteine residue (mercapturate) is found in the 4-position of the thiophene ring. This residue may be introduced at that position after initial Michael-type addition of glutathione to the primary thiophene sulphoxide metabolite (see Figure C.1) at ring position 2 (i.e. the C next to the S atom) and several rearrangements, including ring opening/closing and reaction with second glutathione molecules. Results of *in vitro* experiments with rat liver microsomes indicate that the thiophene sulphoxide is a reactive intermediate in the conversion of the 3-aroylthiophene to the dihydromercapturic acid metabolite (Valadon et al., 1996).

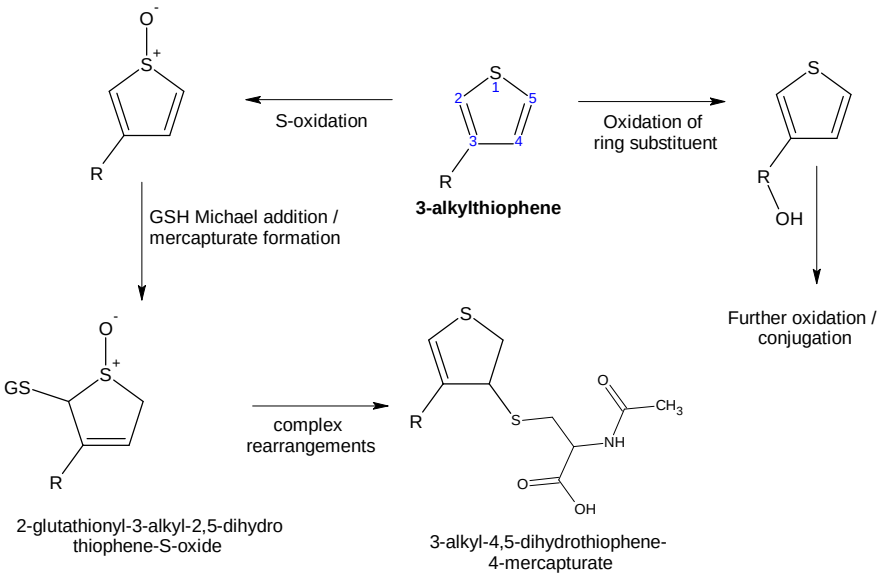


Figure C.1: Proposed metabolism of 3-alkylthiophenes

Oxidation of 2-phenylthiophene by rat liver microsomes, in the presence of nicotinamide adenine dinucleotide phosphate, reduced form, (NADPH) and glutathione involves two initial reactions, resulting in three subsequent types of metabolites. The first reaction involves *S*-oxidation to yield 2-phenylthiopene *S*-oxide that then dimerises by Diels–Alder-type reaction of the *S*-oxide. Alternatively, the *S*-oxide may form a glutathione adduct via a 1,4-Michaël-type addition of glutathione to the C5 position of 2-phenylthiopene *S*-oxide. The second metabolic reaction involves formation of a 2-phenylthiophene-4,5-epoxide, which may be subject to subsequent conjugation with glutathione at C4 of the thiophene ring. Subsequent dehydration of the resulting hydroxyl–glutathione conjugate yields the corresponding glutathione conjugate of phenylthiophene, which is excreted mainly as the 2-mercapturic derivative. Oxidation of 2-phenylthiophene by recombinant, human cytochrome P450 1A1, in the presence of NADPH and glutathione, also led to these metabolites. These results provide the evidence that cytochrome P450 (1A1) may catalyse the oxidation of thiophene compounds with the simultaneous formation of two reactive intermediates, a thiophene-*S*-oxide and a thiophene epoxide (Dansette et al., 2005).

The addition of alkyl substituents can result in a significant change in metabolism. Biotransformation of a thiophene derivative was studied in six healthy male volunteers after administration of a single

oral dose of 12.5 mg olanzapine[19]. Mean radiocarbon recovery was 87 %, with 30 % appearing in the faeces and 57 % excreted in the urine. In addition to other metabolites, the methyl substituent on the thiophene ring underwent oxidation to the corresponding 2-hydroxymethyl and 2-carboxylic acid derivatives. There was no evidence for glutathione conjugation of the thiophene *S*-oxide or epoxidation of the thiophene ring double bond for alkyl-substituted thiophenes (Kassahun et al., 1997). However, the total recovery and identification of metabolites in urine or faeces was far from complete and some metabolites may have been missed in the analysis. In addition, alternative reactions to *S*-oxidation (e.g. *N*-oxidation or *N*-glucuronidation) may greatly reduce the relevance of the *S*-oxidation in this substance as than more simple alkyl-substituted thiophenes.

Subgroup A-Ic: Thiophenes with a thiol group in the ring substituent

Candidate substances [FL-no: 15.082, 15.087 and 15.108]

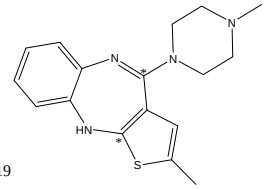
There is no specific information on any of the candidate substances in this subgroup, or on structurally related thiophene derivatives. It may be anticipated that some of the metabolic conversions described above for subgroups A-Ia and A-Ib may also apply to this subgroup.

In addition, the mercapto-group (thiol) in the ring-substituent chain may undergo *S*-methylation to produce the corresponding methyl thioether or sulphide, with further oxidation to the corresponding sulphoxide and sulphone. They may also react with glutathione or other endogenous thiol substances to form mixed disulphides, which may undergo reduction to thiols or oxidative desulphuration. Alternatively, they may undergo enzymatic oxygenation resulting in the formation of the corresponding sulphinic or sulphonic acid. All these metabolites are expected to be excreted in the urine. A more detailed discussion on the metabolism of sulphur compounds may be found in another EFSA CEF opinion on other sulphur-containing flavouring substances (EFSA CEF Panel, 2011b).

Additional considerations for the candidate substances in subgroup A-I

No information was submitted that would indicate whether side chain oxidation of the alkyl-substituted thiophenes or keto-reduction of the acyl-substituted thiophenes could occur. Such reactions may be anticipated (e.g. omega or omega-1 oxidations). Similar reactions have been discussed for alkylated pyrazines and furan in previous opinions (EFSA CEF Panel, 2011a, b). In addition, the aldehyde candidate substance, 5-ethylthiophene-2-carbaldehyde [FL-no: 15.074] may be expected to be oxidised to the corresponding carboxylic acid. Where applicable, conjugation with amino acids (e.g. glycine) or glucuronic acid may also be expected to occur (EFSA, 2009; EFSA CEF Panel, 2011a, b).

Rance (1989) has indicated that unsubstituted thiophene may be subject to *S*-oxidation and ring substitution. It was stated that *S*-oxides of thiophene are highly reactive. In this review several studies have been quoted with C2 substituted thiophene derivatives (in particular the pharmaceuticals tienilic acid, morantel and pyrantel) for which it was demonstrated that ring C5 hydroxylation of these substituted thiophenes may occur to a considerable extent. In contrast C2, C5 bisubstituted thiophenes are less susceptible to ring hydroxylation (Rance, 1989).



19 Structure of olanzapine; the radioactive carbons are indicated with *.

Subgroup A-II: Thiazoles

Candidate substances [FL-no: 15.038, 15.039, 15.044, 15.050, 15.051, 15.052, 15.058, 15.061, 15.062, 15.063, 15.067, 15.068, 15.069, 15.071, 15.078, 15.080, 15.084, 15.085, 15.089, 15.098, 15.115, 15.116 and 15.118]

No specific information was available for any of the candidate substances in this subgroup.

Non-candidate substances

Thiamine hydrochloride [FL-no: 16.027] and 5-(2-hydroxyethyl)-4-methylthiazole [FL-no: 15.014]

Thiamine (see Figure C.2) is excreted in the urine as an unchanged substance but also in the form of 25–30 metabolites, some of which still contain the pyrimidine moiety, while other metabolites are generated as a result of molecular cleavage.

In fish and microorganisms, breakdown of thiamine is catalysed by two enzymes, namely thiaminase I and thiaminase II (Tanphaichitr, 1976; Tietz, 1986). Based on comparative studies in which rodents were given thiamine intravenously or orally, it has been demonstrated that, in the gastro-intestinal tract, thiamine may already be split into a pyrimidine and a thiazole derivative, namely 5-(2-hydroxyethyl)-4-methylthiazole, which together with its oxidation product (4-methylthiazole-5-acetic acid) and parent thiamine have been identified in urine. The fate of the pyrimidine derivative will not be further discussed. The relevance of the intraintestinal cleavage may be different among various animal species (Informatics Inc., 1974).

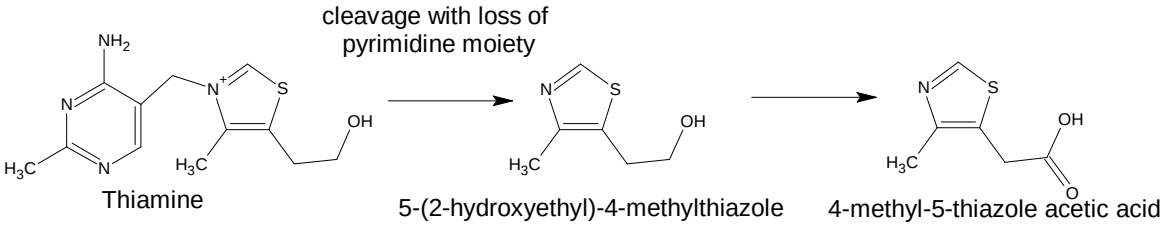


Figure C.2: Metabolism of thiamine and 5-(2-hydroxyethyl)-4-methylthiazole

Chlormethiazole

The therapeutic agent chlormethiazole (5-(2-chloroethyl)-4-methylthiazole; see Figure C.3), is an alkyl- and chloroalkyl-substituted thiazole derivative that is metabolised by *C*-oxidation of the alkyl- and chloroalkyl-substituents and by *S*- and *N*-oxidation. In human males, a 1 000 mg oral dose of chlormethiazole is metabolised via side chain *C*-oxidation of the ethyl C1-position to yield 5-(1-hydroxyethyl)-4-methylthiazole, 5-acetyl-4-methylthiazole, 5-(1-hydroxy-2-chloroethyl)-4-methyl-thiazole, 4,5-dimethylthiazole and 4-methylthiazole-5-acetic acid, which is the major C-oxidation urinary metabolite (see Figure C.3). Presumably, the 5-methyl derivative is formed via decarboxylation of the 5-acetic acid metabolite. In addition, oxidation of the methyl group at C4 in 5-(2-hydroxyethyl)-4-methylthiazole yields 5-(2-hydroxyethyl)-4-thiazolecarboxylic acid lactone.

Separate ring sulphoxide and ring *N*-oxide products were not identified, but a combined ring *S*- and *N*-oxide of a side chain-oxidised metabolite was also eliminated in the 12-hour urine of volunteers. This was the first reported example of a sulphoxidation of a thiazole sulphur and simultaneous oxidation of two different heteroatoms in the same heteroaromatic ring *in vivo* (Offen et al., 1985).

Moore et al. (1975) reported the excretion of urinary metabolites of chlormethiazole after oral administration of this anticonvulsant drug (in the form of its unstable chlormethiazole ethanedisulphonate derivative) to humans. The substance was given in capsules to three volunteers (sex not reported) at the total dose of 768 mg. Urinary samples were collected for up to 36 hours post-

dosing and processed for the identification and quantification of metabolites by gas chromatography–mass spectrometry (GC–MS) techniques. Results of urinalysis were compared with results obtained using reference substances. After oral administration, about 16 % of the dose could be identified in the 36-hour urine as four substances: parent chlormethiaziole (< 0.01 % of the dose), 5-acetyl-4-methylthiazole, 5-(1-hydroxyethyl)-4-methylthiazole (free and as unspecified conjugate) and 4-methylthiazole-5-acetic acid (> 75 % of all metabolites identified). 5-(2-Hydroxyethyl)-4-methylthiazole was found in only trace amounts. In addition, a metabolite was tentatively identified as 4-methyl-5-thiazole-acetaldehyde. The authors noted that at that time 80 % of the dose was still unaccounted for, which might indicate extensive metabolism of the substance in humans (Moore et al., 1975).

It has also been reported that chlormethiazole undergoes nucleophilic attack at C2, which would ultimately result in ring opening. The exact mechanism for the formation of ring-opening products has not been well-described/identified, but the products are nonetheless found in human urine. It was suggested that *N*-oxidation followed by glutathione conjugation and subsequent glutathione breakdown would yield the 2-thiomethyl derivative of chlormethiazole, whereas hydroxide ion attack would yield 2,4-pentanedione-3-thiol and thiodiacetic acid. The authors suggest that the *N*-oxidation would make the C2 prone to nucleophilic attack (Grupe and Spiteller, 1982; Pal and Spiteller, 1982). According to data from Grupe and Spiteller (1982), urinary excretion of thiodiacetic acid may comprise up to about 5 % of the dose of chlormethiazole in humans. For the other metabolites (see Figure C.3) no quantitative data were provided.

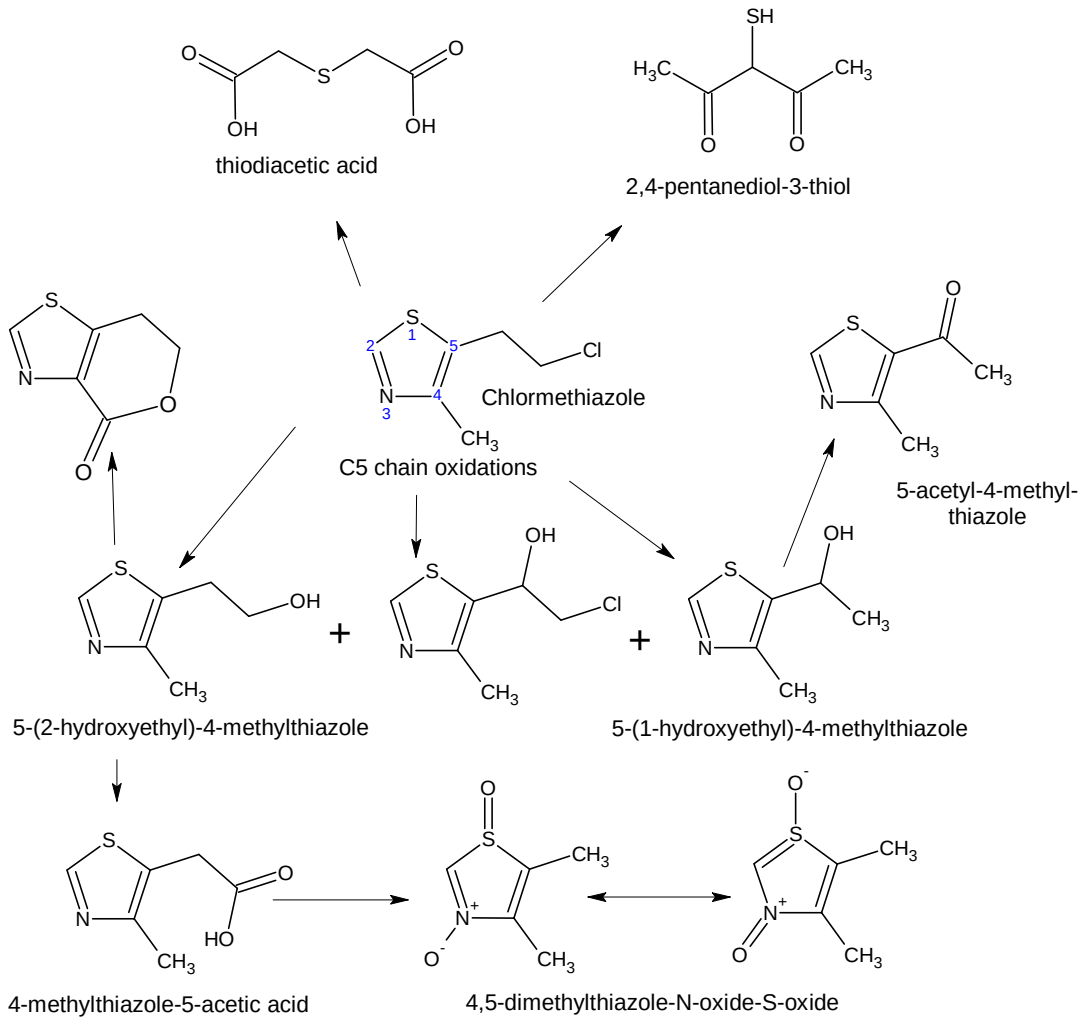


Figure C.3: Metabolism of chlormethiazole

Herbertz et al. (1973) studied the metabolism of (2-^{14}C)-chlormethiazole-ethane disulphonate in isolated recycling-perfused rat liver. After two hours, 35 % of the system's radioactivity was found in the bile, which was associated with a polar metabolite not observed in previous *in vivo* studies in urine. This metabolite was identified as a mixture of *N*,4-dimethyl-thiazole-5-acetic acid, 2-hydroxy-*N*,4-dimethyl-thiazole-5-acetic acid and the glycine conjugate of the latter. Other substances in the perfusate were chlormethiazole, 2-hydroxy-4-methyl-5-(2-chloroethyl)thiazole, 4-methyl-5-(2-hydroxyethyl)thiazole, 4-methylthiazole-5-acetic acid, 2-hydroxy-4-methyl-thiazole-5-acetic acid and its glycine conjugate (Herbertz et al., 1973).

In vivo, the urine was identified as the primary route of elimination (70 % of the dose within three hours in a study by Allgen et al. (1963) (cited by Herbertz et al., 1973)), with no formation of *N*-methylated products. Hence, it was speculated that *in vivo* enterohepatic recycling of chlormethiazole metabolites would occur, in particular of the *N*-methylated substances, which would be de-methylated in the gastro-intestinal tract and subsequently be excreted via the kidneys. It was noted that the difference between the observations *in vivo* and the perfused system may have occurred because of the build-up of (secondary) metabolite levels in the (closed) perfused system (Herbertz et al., 1973).

Mizutani et al. (1993) investigated the relationship between the toxicological profile of several thiazole derivatives and the toxicity of the ring cleavage products, in particular the thioamide metabolites (see Figure C.4). The various thiazoles were nephrotoxic *in vivo* in glutathione-depleted mice, which toxicity decreased upon substitution at the ring 4 and 5 carbons. The larger the substituents, the less nephrotoxic the substances appeared to be. In addition to being nephrotoxic, some of the thiazoles studied were also hepatotoxic. In the ring C4- and C5-substituted derivatives, ring substitution at the 2 position carbon (the one between the sulphur and the nitrogen atoms) markedly reduced nephrotoxicity, but hepatotoxicity was maintained, except when the substituted was a hydroxyl group (e.g. 2-hydroxy-4-methylthiazole). It was also demonstrated that thioformamide was both nephrotoxic and hepatotoxic, but only in glutathione-depleted animals. Thioamides from ring C2-substituted thiazoles appeared to be only hepatotoxic, both after glutathione-depletion and in normal animals, although in the normal animals the hepatotoxicity was less pronounced (Mizutani et al., 1993). Thus, the toxicity appears to be associated with glutathione-depletion, which is a high-dose phenomenon.

In additional studies, again with mice, administration of another non-flavouring thiazole derivative with known hepatotoxic and nephrotoxic properties, 4-*t*-butyl-2-methylthiazole, at a dose of 312 mg/kg bw by gavage, resulted in limited ring *C*-oxidation. Only 0.25 % of the administered dose was recovered as a ring fragmentation product (3,3-dimethyl-2-oxobutanal; an alpha-dicarbonyl metabolite) in the urine within 24 hours. In addition, a thioamide metabolite (thioacetamide) was found; but this was not quantified. Other metabolites were not studied. Based on the structure of the dicarbonyl fragment, it was postulated that the 4,5-double bond of the thiazole ring undergoes epoxidation followed by hydrolysis to yield 4,5-diol. The diol then undergoes hydrolytic cleavage to yield the corresponding carbonyl derivatives and thioacetamide. Similar, thiazole ring opening has also been observed with thiabendazole (= 2-(thiazol-4-yl)benzimidazole) and with 2-(*p*-methoxyphenyl)-4-methylthiazole (Mizutani et al., 1994).

Additional information for candidate substances in subgroup A-II

In an extensive review on the metabolism of heterocyclic substances, Rance (1989) indicated that several alternative metabolic pathways are available to thiazoles. One involves *S*-oxidation to form the corresponding sulphoxide and sulphone. Electrophilic attack at the ring N (resulting in quaternisation, e.g. *N*-methylation) is stated to be even more preferential than *S*-oxidation. The C2 carbon atom is electron deficient and substituents at this position are reactive and subject to nucleophilic displacement, in particular if the ring nitrogen is quaternised. Another pathway involves electrophilic attack at ring carbons, preferably C5. The C5 hydroxythiazoles exist as two tautomeric forms. C5-

hydroxylation, however, is quantitatively unimportant because thiazoles do not readily undergo electrophilic substitution reactions (Rance, 1989).

Alkyl- and acyl-substituted thiazole derivatives can be metabolised via side chain oxidation and ring *S*- and *N*-oxidation (Dalvie et al., 2002). The major metabolites are readily excreted in the urine either free or as glutathione conjugates. In addition, it may be expected that the presence of alkyl- and acyl-substituents at the thiazole ring increases the number of metabolic options. It has also been demonstrated that, to a very small extent, ring *C*-oxidation may be followed by ring cleavage to yield alpha-diketone and thioamide fragments (see Figure C.4). The acyl-substituted thiazoles [FL-nos: 15.038, 15.039, 15.085 and 15.116] may also be expected to undergo keto-reduction, possibly followed by conjugation, similar to acetyl derivatives of furane (EFSA CEF Panel, 2011b) and pyrazine (EFSA CEF Panel, 2011a). However, there is very limited information to evaluate the extent of metabolism of ring substituent groups, and it is also very difficult, based on the information available, to assess how the presence of ring substituents and their positions at the ring might influence the metabolism of the ring itself.

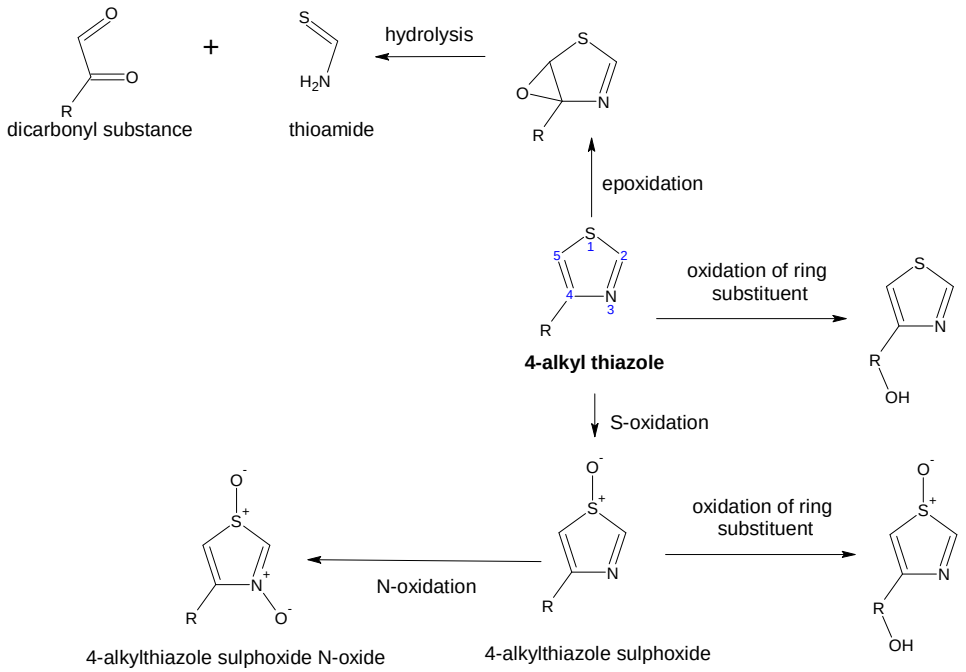


Figure C.4: Metabolism of 4-alkylthiazols

Non-aromatic candidate substances

Subgroups B-II and B-IV: Thiazoline and dithiazine derivatives

Candidate substances [FL-no: 15.060, 15.086 and 15.119] (thiazolines from subgroup B-II) and candidate substances [FL-no: 15.054, 15.055, 15.057, 15.079 and 15.135] (dithiazines from subgroup B-IV)

No specific information was available to evaluate the metabolism of any of the candidate substances mentioned in these two subgroups (B-II and B-IV).

It may be speculated that the substances in these chemical groups, which contain a partially or completely reduced thiazole or dithiazine, are metabolised primarily by ring *S*-oxidation or via *N*-oxidation, similar to the aromatic thiazole compounds. In addition, metabolism of the ring substituents is likely to occur.

C.4 Summary on metabolism

The candidate substances in this FGE are structurally related to 28 flavouring substances evaluated by the JECFA in "Safety Evaluations of Groups of Related Flavouring Agents: Sulfur-Containing Heterocyclic Compounds". The candidate and 28 supporting substances in this group were originally subdivided into 11 subgroups based on the nature of the ring (aromatic (clustered in subgroups A-I to A-III) vs. non-aromatic (clustered in subgroups B-I to B-VI)), type and number of ring heteroatoms (sulphur or sulphur with nitrogen) and the degree of saturation in the non-aromatic rings. In this revision, FGE.21Rev5, the use of 18 candidate substances as flavourings are no longer supported by Industry, reducing the number of subgroups to five.

For the evaluation of the metabolism of the candidate substances in this group, only very limited data were available. These were confined to a few references on thiophene, and some thiophene and thiazole derivatives (i.e. only directly relevant for the evaluation of A subgroups the aromatic candidate substances). Other information was found in several review papers. Virtually no data were found on the possible metabolism of the non-aromatic ring structures in the B subgroups.

A subgroups (substances with aromatic ring structures)

Very few data are available on absorption, distribution and excretion. Some of the studies are related to oral exposure and some others to parenteral dosing, and the relevance of the latter may be limited. In mice, a thiazolobenzimidazole derivative was rapidly absorbed after oral dosing, *S*-oxidised and eliminated from plasma. The structurally related substance thiophene has been shown to be absorbed rapidly from the gastro-intestinal tract and eliminated via urine as metabolites, or via the lungs. In addition, the thiazole derivative fostedil is absorbed from the gastro-intestinal tract (42–71 % of the dose), metabolised and eliminated with a plasma half-life of about six to nine hours in dogs. Hence, from data on absorption and elimination that are available for the aromatic candidate substances in this FGE, it may be speculated that most of them will be fairly well absorbed and eliminated after biotransformation. Some volatile substances may also be eliminated via the lungs. For the non-aromatic candidate substances no information is available.

From available metabolism data, it may be anticipated that the sulphur- and nitrogen-containing heterocyclic and heteroaromatic derivatives participate in metabolic pathways, principally involving side chain *C*-oxidation, ring *S*- and *N*-oxidation to yield sulphoxide or sulphones and *N*-oxides, respectively. Sulphoxide metabolites may conjugate with glutathione or may undergo dimerisation via a Diels–Adler reaction. For substituted thiophenes, epoxidation of the double bonds has also been reported, which may also be followed by glutathione conjugation and mercapturic acid formation. As far as studied, ring *C*-oxidation may, to a very minor extent, be accompanied by heterocyclic ring cleavage, which for thiazoles could result in the formation of reactive thioamide intermediates.

Additional alkyl and acyl substituents in the thiazole ring may increase the alternative metabolic pathways available to thiazole derivatives, as was demonstrated for chlormethiazole. Alkyl- and acyl-substituted thiazole derivatives are primarily metabolised via side chain oxidation and ring *S*- and *N*-oxidation. The major metabolites are readily excreted in the urine either free or as glutathione conjugates. Metabolites of the side chain oxidation pathways may be expected to be conjugated, e.g. to glucuronic acid. To a very small extent, ring *C*-oxidation may be followed by ring cleavage to yield alpha-diketone and thioamide fragments. The acyl-substituted thiazoles [FL-no: 15.038, 15.039, 15.085 and 15.116] may also be expected to undergo keto-reduction, possibly followed by conjugation, similar to acyl derivatives of furanes (EFSA CEF Panel, 2011b) and pyrazines (EFSA CEF Panel, 2011a).

The structurally related thiophene and the ring-substituted thiophenes (subgroups A-Ib and A-Ic) undergo *S*-oxidation and glutathione conjugation. In addition, from some studies with thiazole derivatives, it can be seen that their metabolites may react spontaneously with glutathione, and it is likely that they also have reactivity towards protein thiols, which may result in toxicity. For example, for the thiazole ring cleavage products (thioamide intermediates) a relationship with nephrotoxicity

and hepatotoxicity has been established, especially after glutathione depletion, even though this is only a very minor metabolite.

Limited information was submitted that indicates that side chain oxidation of the alkyl- or acyl-substituted thiophenes (subgroups A-Ib and A-Ic) or thiazoles (subgroup A-II) may also occur. Such reactions may be anticipated (e.g. omega or omega-1 oxidations). Similar reactions have been discussed for alkylated pyrazines (EFSA CEF Panel, 2011a). In addition, the candidate substance with an aldehyde group [FL-no: 15.074] may be expected to be oxidised to the corresponding carboxylic acid. Where applicable, conjugation with amino acids (e.g. glycine) or glucuronic acid may also be expected to occur (Scheline, 1968; EFSA, 2005; EFSA CEF Panel, 2011a).

Based on the MSDI exposure estimates (see Table 9, Section 7, of the main text) it seems reasonable to assume that saturation of the metabolic pathways for the candidate substances in this FGE is unlikely, given these low levels of exposure to the candidate substances from their use as flavouring substances.

Overall, there is insufficient quantitative information to evaluate the extent of metabolism of ring substituent groups, and it is also very difficult, based on available data, to assess how the presence of ring substituents and their positions at the ring influences metabolism of the rings itself, for any of the subgroups within this FGE. With respect to sulphydryl reactivity in thiophenes (subgroup A-Ic), this property was a reason to consider such substances, e.g. FGE.13, as not being metabolised through pathways leading to innocuous metabolites. In concordance with this and based on the information presented above, it is concluded that for all candidate flavouring substances in A subgroups of the present FGE it cannot be anticipated that they are metabolised to innocuous metabolites.

B subgroups (substances with non-aromatic ring structures)

No specific information was available on the metabolism of the thiazoline or dithiazine derivatives or of related substances for any of the (non-aromatic) substances in the B subgroups. It may be speculated that the substances in these chemical groups are metabolised primarily by ring *S*-oxidation or, if applicable, via *N*-oxidation, similar to the aromatic thiazole and thiophene compounds. In addition, metabolism of the ring substituents is likely to occur. Owing to the lack of metabolism data on the substances in the B subgroups, it cannot be concluded that the candidate flavouring substances in the respective B subgroups will be metabolised to innocuous products.

ABBREVIATIONS

bw	body weight
CAS	Chemical Abstract Service
CEF	Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CoE	Council of Europe
CRL	Charles River Laboratories
DNA	Deoxyribonucleic acid
EC	European Commission
EFFA	European Flavour and Fragrance Association
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FEMA	Flavor and Extract Manufacturers Association
FGE	Flavouring Group Evaluation
GC–MS	gas chromatography–mass spectrometry
GLC	gas–liquid chromatography
GLP	Good Laboratory Practice
FLAVIS (FL)	Flavour Information System (database)
HPLC	high-performance liquid chromatography
ID	identity
IOFI	International Organization of the Flavour Industry
IR	infrared spectroscopy
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LD_{50}	Lethal Dose, 50 %; median lethal dose
MNBN	micronucleated binucleate cells
MS	mass spectrometry
MSDI	Maximised Survey-derived Daily Intake
MTS	Minimum Toxicity Screen
mTAMDI	modified Theoretical Added Maximum Daily Intake
NADPH	Nicotinamide Adenine Dinucleotide Phosphate, reduced form
NMR	nuclear magnetic resonance
No	number
NOAEL	no-observed-adverse-effect level
OECD	Organisation for Economic Co-operation and Development
RI	Replication Index
SC	structural class
SCF	Scientific Committee on Food
TAMDI	Theoretical Added Maximum Daily Intake
TNO	Nederlandse Organisatie voor Toegepast Natuurwetenschappelijk Onderzoek
WHO	World Health Organization