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Flavouring Group Evaluation 21 Revision 6 (FGE.21Rev6): thiazoles, thiophenes, thiazoline and thienyl derivatives from chemical groups 29 and 30

EFSA Panel on Food Additives and Flavourings (FAF),
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Abstract

The Panel on Food additives and Flavourings (FAF) was requested to evaluate the flavouring substances 2,4-dimethyl-3-thiazoline [FL-no: 15.060] and 2-isobutyl-3-thiazoline [FL-no: 15.119] in Flavouring Group Evaluation 21 revision 6 (FGE.21Rev6). FGE.21Rev6 deals with 41 flavouring substances of which 39 have been already evaluated to be of no safety concern when based on the MSDI approach. For [FL-no: 15.060 and 15.119], a concern for genotoxicity was raised in FGE.21. Genotoxicity data have been submitted for the supporting substance 4,5-dimethyl-2-isobutyl-3-thiazoline [FL-no: 15.032] evaluated in FGE.76Rev2. The concerns for gene mutations and clastogenicity are ruled out for [FL-no: 15.032] and for the structurally related substances [FL-no: 15.060 and 15.119], but not for aneugenicity. Therefore, the aneugenic potential of [FL-no: 15.060 and 15.119] should be investigated in studies with the individual substances. For [FL-no: 15.054, 15.055, 15.057, 15.079 and 15.135], (more reliable) information on uses and use levels is needed to (re)calculate the mTAMDIs in order to finalise their evaluation. Provided that information is submitted for [FL-no: 15.060 and 15.119] with respect to potential aneugenicity, that would allow evaluation of these substances through the Procedure, also for these two substances, more reliable data on uses and use levels would be required. Upon submission of such data, additional data on toxicity may become necessary for all seven substances. For [FL-no: 15.054, 15.057, 15.079 and 15.135], information on the actual percentages of stereoisomers in the material of commerce based on analytical data should be provided.

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1. Introduction

1.1. Background and terms of reference as provided by the requestor

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[1] 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[2]. The list includes flavouring substances for which the scientific evaluation should be completed in accordance with Commission Regulation (EC) No 1565/2000[3].

On 24 October 2013, the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and processing Aids (CEF) adopted the following three opinions:

- an opinion on Flavouring Group Evaluation 76Rev1 (FGE.76 Rev1)
- an opinion on Flavouring Group Evaluation 21Rev4 (FGE.21 Rev4)
- an opinion on Flavouring Group Evaluation 93Rev1 (FGE.93 Rev1)

In the opinion on FGE.76Rev1, the Panel requested *in vivo* genotoxicity data concerning three thiazolines [FL-no: 15.029, 15.030 and 15.032] as the representative flavouring substance 15.032 is considered to have genotoxic potential *in vitro*. Additionally, the Panel noted the presence of a terminal conjugated bond in the substances [FL-no: 15.018] and [FL-no: 15.005] which raises genotoxicity concern and therefore requested additional data on them.

In the opinion on FGE.21Rev4, the Panel concluded that for two substances [FL-no: 15.060] and [FL-no: 15.119] additional genotoxicity data were required.

In the opinion on FGE.93Rev1, the Panel concluded that for two substances [FL-no: 15.130 and 15.131] additional genotoxicity data were required.

The applicant has submitted additional data in response to these three EFSA evaluations.

Terms of Reference

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.

1.2. Interpretation of the terms of reference

In FGE.21, the AFC Panel considered that the substances 2,4-dimethyl-3-thiazoline [FL-no: 15.060] and 2-isobutyl-3-thiazoline [FL-no: 15.119] cannot be evaluated through the Procedure due to a concern for genotoxicity from *in vitro* studies on the structurally related substances 2-methylthiazolidine [FL-no: 15.090] and 2-propylthiazolidine [FL-no: 15.099].

In FGE.21Rev5, the CEF Panel confirmed the need to investigate the potential genotoxicity of 2,4-dimethyl-3-thiazoline [FL-no: 15.060] and 2-isobutyl-3-thiazoline [FL-no: 15.119]. Industry communicated that they have no further interest in the evaluation of [FL-no: 15.090, 15.099]; therefore, no further data were submitted. However, genotoxicity data have been submitted for the supporting substance 4,5-dimethyl-2-isobutyl-3-thiazoline [FL-no: 15.032] evaluated in FGE.76Rev2.

Based on structural similarity, the Panel considered that the substances 5-ethyl-4-methyl-2-(2-methylpropyl)-thiazoline [FL-no: 15.130] and 5-ethyl-4-methyl-2-(2-butyl)-thiazoline [FL-no: 15.131] originally allocated to FGE.93 can be evaluated in FGE.76 (FGE.76Rev2). Therefore, the genotoxicity data for [FL-no: 15.032] can support the evaluation of [FL-no: 15.029, 15.030, 15.130 and 15.131] in FGE.76Rev2 and of [FL-no: 15.060 and 15.119] in FGE.21Rev6.

[1] 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, pp. 34–50.

[2] 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, pp. 1–161.

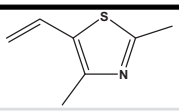
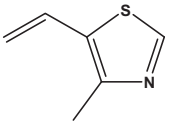
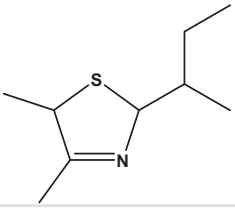
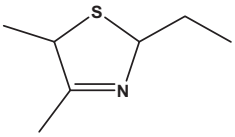
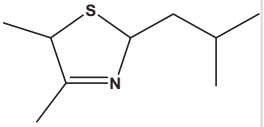
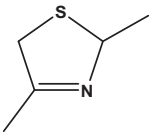
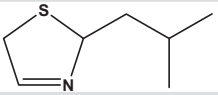
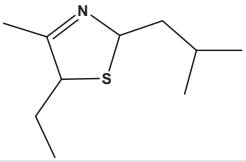
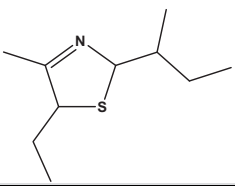
[3] Commission Regulation (EC) No 1565/2000 of 18 July 2000 laying down the measures necessary for the adoption of an evaluation programme in application of Regulation (EC) No 2232/96. OJ L 180, 19.7.2000, pp. 8–16.

Industry submitted genotoxicity data also for 4-methyl-5-vinylthiazole [FL-no: 15.018], a representative substance for 2,4-dimethyl-5-vinylthiazole [FL-no: 15.005]. These substances are evaluated in FGE.76Rev2 (EFSA FAF Panel, 2023). However, since vinyl thiazoles are not present in FGE.21, [FL-no: 15.018] is not representative for any of the flavouring substances in FGE.21 and the submitted data for this substance will not be further discussed in FGE.21Rev6.

Following the submission of these new data, the European Commission requests EFSA to carry out a safety assessment in accordance with Commission Regulation (EC) No 1565/2000[3].

Table 1 summarises the flavouring substances whose genotoxicity evaluation is covered by the two representative substances [FL-no: 15.018 and 15.032] in FGE.76Rev2, FGE.21Rev6 and FGE.93Rev1.

Table 1: Substances in FGE.76Rev2, FGE.21Rev6 and FGE.93Rev1 for which the genotoxicity evaluation is based on the representative substances [FL-no: 15.018] or [FL-no: 15.032]

FGE	FL-no JECFA no	Chemical name	Structural formula	Representative substance	Group
FGE.76	15.005 1039	2,4-Dimethyl-5-vinylthiazole			Thiazoles
FGE.76	15.018 1038	4-Methyl-5-vinylthiazole		REPRESENTATIVE	Thiazoles
FGE.76	15.029 1059	2-(sec-Butyl)-4,5-dimethyl-3-thiazoline			3-Thiazolines
FGE.76	15.030 1058	4,5-Dimethyl-2-ethyl-3-thiazoline			3-Thiazolines
FGE.76	15.032 1045	4,5-Dimethyl-2-isobutyl-3-thiazoline		REPRESENTATIVE	3-Thiazolines
FGE.21	15.060	2,4-Dimethyl-3-thiazoline			3-Thiazolines
FGE.21	15.119	2-Isobutyl-3-thiazoline			3-Thiazolines
FGE.93	15.130 1761	5-Ethyl-4-methyl-2-(2-methylpropyl)-thiazoline			3-Thiazolines
FGE.93	15.131 1762	5-Ethyl-4-methyl-2-(2-butyl)-thiazoline			3-Thiazolines

1.3. History of the evaluation of the substances in FGE.21Rev6

FGE.21

In FGE.21 (EFSA, 2007), 54 flavouring substances from chemical groups 29 and 30 (Annex I of Commission Regulation (EC) No 1565/2000) were considered. These substances fall into the chemical groups of thiazoles, thiophene, thiazoline and thienyl derivatives.

The data available for 2-methylthiazolidine [FL-no: 15.090] and 2-propylthiazolidine [FL-no: 15.099][4] (subgroup B-III: thiazolidines) indicated a potential for genotoxicity *in vitro* (see Appendix D, Table D.1). Therefore, the Panel on Food Additives, Flavourings, Processing Aids and Materials in contact with Food (AFC Panel) decided that the Procedure should not be applied to these two flavouring substances nor to the three structurally related substances 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] (subgroup B-II: thiazolines) until adequate genotoxicity data become available.

Forty-nine substances were evaluated through the procedure.

Valid toxicological data providing a NOAEL which, when compared to the estimated intake from use as flavouring substances, indicate adequate margins of safety only for 26 flavouring substances in subgroup A-Ic (thiophenes with thiol-containing ring substituents) and subgroup A-II (thiazoles).

For the remaining 23 flavouring substances belonging to the subgroups A-Ia (thiophene), A-Ib (thiophenes with non-thiol-containing ring substituents), A-III (benzothiazoles), B-I (dihydrothiophenes), B-IV (dithiazines) and B-V (dihydrothiazines), the AFC Panel considered that there were insufficient data available to provide a margin of safety from their use as flavouring substances and that additional toxicity data were needed.

The estimated intake based on the mTAMDI approach was above the threshold of toxicological concern (TTC, 90 μg/person per day) for four structural class III substances [FL-no: 15.042, 15.055, 15.088, 15.114].

Adequate specifications including complete purity criteria and identity tests for the materials of commerce were provided for all 49 flavouring substances, except that information on chirality was missing for eight substances [FL-no: 15.042, 15.054, 15.055, 15.060, 15.077, 15.090, 15.099 and 15.119].

The AFC Panel concluded that 26 substances would present no safety concern at their estimated levels of intake estimated on the basis of the MSDI approach: [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, 15.082, 15.084, 15.085, 15.087, 15.089, 15.098, 15.108, 15.115, 15.116 and 15.118].

FGE.21Rev1

Revision 1 of FGE.21 (FGE.21Rev1) included the assessment of two additional candidate substances [FL-no: 15.129 and 15.133] for which no toxicity and/or metabolism data were provided. In this revision, the Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF Panel) evaluated 56 flavouring substances (EFSA, 2009).

For the two candidate substances, 6-acetyl-2,3-dihydro-1,4-thiazine [FL-no: 15.114] (Register[5] name: 5-acetyl-2,3-dihydro-1,4-thiazine) and 5-acetyl-2,3-dihydro-1,4-thiazine [FL-no: 15.133] from subgroup B-V, which are α,β-unsaturated ketones, i.e. they have a structural alert for genotoxicity, there were no genotoxicity data available and accordingly a concern for genotoxicity could not be ruled out. For the substances [FL-no: 15.090, 15.099] (subgroup B-III) and [FL-no: 15.060, 15.086, 15.119] (subgroup B-II), no additional genotoxicity data were available. The CEF Panel concluded that in the absence of further genotoxicity data, the Procedure could not be applied to these seven substances. In addition, information on stereoisomerism and/or identity test was not provided for five of these seven substances [FL-no: 15.060, 15.090, 15.099, 15.119 and 15.133].

[4] The use of 2-methylthiazolidine [FL-no: 15.090] and 2-propylthiazolidine [FL-no: 15.099] as flavouring substances was no longer of interest for industry and no further data were provided. Therefore, these substances were not included in the Union List, i.e. 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.

[5] Commission Decision of 23 February 1999 adopting a register of flavouring substances used in or on foodstuffs drawn up in application of Regulation (EC) No 2232/96 of the European Parliament and of the Council of 28 October 1996. OJ L 84, 27.3.1999, pp. 1–137.

The Procedure was applied to 49 substances.

The CEF Panel confirmed that 26 of the 49 candidate substances evaluated through the Procedure [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, 15.082, 15.084, 15.085, 15.087, 15.089, 15.098, 15.108, 15.115, 15.116 and 15.118] are not of safety concern at their estimated levels of intake based on the MSDI approach. Whereas for 23 candidate substances, additional toxicological data are required [FL-no: 15.037, 15.040, 15.042, 15.043, 15.045, 15.054, 15.055, 15.064, 15.070, 15.072, 15.074, 15.076, 15.077, 15.088, 15.091, 15.092, 15.093, 15.094, 15.096, 15.097, 15.106, 15.107 and 15.129].

For [FL-no: 15.129] and for [FL-no: 15.042, 15.055, 15.088], the mTAMDI values are above the TTC for structural class II (540 μg/person per day) and for structural class III (90 μg/person per day), respectively.

The final evaluation of the materials of commerce cannot be performed for the five substances [FL-no: 15.042, 15.054, 15.055, 15.077 and 15.129], pending further information on stereoisomerism and/or identity test.

FGE.21Rev2

In revision 2 of FGE.21 (FGE.21Rev2), the same group of 56 substances was considered (EFSA CEF Panel, 2011).

The CEF Panel confirmed that for seven substances [FL-no: 15.060, 15.086, 15.090, 15.099, 15.114, 15.119 and 15.133], genotoxicity data were needed. Therefore, as in FGE.21Rev1, the Procedure was applied to 49 substances, confirming that for 23 substances, additional toxicity data were needed and that 26 substances would present no safety concern at their estimated levels of intake estimated on the basis of the MSDI approach.

Additional toxicity data were provided for thiophene [FL-no: 15.106] (subgroup AIa) and 2-pentylthiophene [FL-no: 15.096] (subgroup A-Ib), but the CEF Panel concluded that these data were not valid for the purposes of establishing a NOAEL.

Further, information on stereoisomeric composition was provided for nine substances [FL-no: 15.042, 15.054, 15.055, 15.060, 15.077, 15.090, 15.099, 15.119 and 15.129] (EFFA, 2010, 2011b). For [FL-no: 15.042], the ratio of diastereoisomers was not provided and for two substances [FL-no: 15.129 and 15.133], identity tests were missing.

The CEF Panel confirmed that for four substances [FL-no: 15.042, 15.055, 15.088 and 15.129], mTAMDI values are above the TTC for their respective structural class and that additional information is required.

FGE.21Rev3

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]. Therefore, in FGE.21Rev3, the CEF Panel evaluated 59 substances (EFSA CEF Panel, 2012).

The CEF Panel confirmed that for seven substances [FL-no: 15.060, 15.086, 15.090, 15.099, 15.114, 15.119 and 15.133], genotoxicity data were needed. Therefore, the Procedure was applied to 52 substances, confirming that 26 substances would present no safety concern at their estimated levels of intake based of the MSDI approach. For 26 substances (including [FL-no: 15.057, 15.079 and 15.135]), additional toxicity data were needed.

For four substances [FL-no: 15.042, 15.057, 15.079 and 15.135], the stereoisomeric composition was not specified sufficiently.

For two substances [FL-no: 15.057 and 15.079], no use levels were provided. More reliable exposure data were required for [FL-no: 15.042, 15.055, 15.057, 15.079, 15.088, 15.129 and 15.135].

FGE.21Rev4

Since the publication of FGE.21Rev3, industry communicated that they have no further interest in the evaluation of 18 substances [FL-no: 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]. Therefore, no further data were submitted (DG SANCO, 2012, 2013) and these substances will not be considered any further (the 18 substances are listed in Table 2). Therefore, in FGE.21Rev4, the CEF Panel evaluated 41 substances (EFSA CEF Panel, 2013a).

Additional toxicity data were provided for the supporting substance 5,6-dihydro-2,4,6-tris(2-methylpropyl)-4H-1,3,5-dithiazine [FL-no: 15.113] (from FGE.76Rev1), which is considered to be structurally related to the candidate substances [FL-no: 15.054, 15.055, 15.057, 15.079, 15.135].

Furthermore, new *in vitro* genotoxicity studies have become available on the supporting substance 2-acetyl-2-thiazoline [FL-no: 15.010], which is considered to be structurally related to the candidate substance [FL-no: 15.086].

New information on European production figures was provided for five substances [FL-no: 15.054, 15.055, 15.057, 15.079 and 15.135] (EFFA, 2012a), and information on missing stereoisomeric composition for [FL-no: 15.057, 15.079 and 15.135] was also included (EFFA, 2013b).

The stereoisomeric composition was specified for all candidate substances.

The CEF Panel concluded that 32 candidate substances, evaluated through the Procedure [FL-no: 15.038, 15.039, 15.044, 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, 15.078, 15.079, 15.080, 15.082, 15.084, 15.085, 15.086, 15.087, 15.089, 15.098, 15.108, 15.115, 15.116, 15.118 and 15.135], are not of safety concern at their estimated levels of intake based on the MSDI approach, whereas for seven candidate substances [FL-no: 15.040, 15.045, 15.074, 15.076, 15.093, 15.096 and 15.097], additional toxicological data are required.

For two substances [FL-no: 15.060 and 15.119], the CEF Panel reiterated that additional genotoxicity data were required.

The CEF Panel confirmed that more reliable exposure data were required for [FL-no: 15.055, 15.057, 15.079 and 15.135].

Table 2: Substances for which industry indicated no further interest in their evaluation as flavouring substances; therefore, no further data were submitted

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

FGE.21Rev5

The fifth revision of FGE.21 (FGE.21Rev5) included the assessment of additional toxicity data for:

- 2-pentylthiophene [FL-no: 15.096] supporting substance for [FL-no: 15.045, 15.076 and 15.093]
- 5-ethylthiophene-2-carbaldehyde [FL-no: 15.074] supporting substance for [FL-no: 15.040 and 15.097].

Updated information on European production figures were provided by industry for these seven substances [FL-no: 15.040, 15.045, 15.074, 15.076, 15.093, 15.096 and 15.097] (IOFI, 2013a,b).

For two substances [FL-no: 15.060 and 15.119], additional genotoxicity data were required.

The CEF Panel concluded that 39 flavouring substances evaluated using the Procedure [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] would present no safety concerns at their estimated levels of intake based on the MSDI approach (EFSA CEF Panel, 2015).

The CEF Panel reiterated that more reliable exposure data were required for [FL-no: 15.055, 15.057, 15.079 and 15.135].[6]

The present revision, FGE.21Rev6, is due to the submission of genotoxicity data on 4,5-dimethyl-2 isobutyl-3-thiazoline [FL-no: 15.032]. This substance is representative for the group of 3-thiazolines and has been evaluated in FGE.76Rev2 (EFSA FAF Panel, 2023). It is a supporting substance for 2,4-dimethyl-3-thiazoline [FL-no: 15.060] and 2-isobutyl-3-thiazoline [FL-no: 15.119] in FGE.21. FGE.21Rev6 deals in total with 41 flavouring substances of which 39 have been already evaluated to be of no safety concern based on the MSDI approach in the previous revisions of FGE.21. A summary of the history of the evaluation of the substances in FGE.21 is presented in Figure 1.

For the sake of completeness, the information on identity of all substances is maintained in various tables of this FGE. Information on specifications is only maintained for the substances which are currently in the Union List (see Appendix B). For substances that are no longer in the Union List, FGE.21Rev5 should be consulted.

Table 3 gives information on adoption dates and links to the published scientific opinions.

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

FGE	Adopted	Link	Substances
FGE.21	8 February 2007	https://www.efsa.europa.eu/en/efsajournal/pub/455	54
FGE.21Rev1	26 March 2009	https://www.efsa.europa.eu/en/efsajournal/pub/1023	56
FGE.21Rev2	4 February 2011	https://www.efsa.europa.eu/en/efsajournal/pub/1989	56
FGE.21Rev3	23 November 2011	https://www.efsa.europa.eu/en/efsajournal/pub/2457	59
FGE.21Rev4	24 October 2013	https://www.efsa.europa.eu/en/efsajournal/pub/3451	41
FGE.21Rev5	19 March 2015	https://www.efsa.europa.eu/en/efsajournal/pub/4066	41
FGE.21Rev6	13 December 2022	https://www.efsa.europa.eu/en/efsajournal/pub/7777	41

[6] In previous version of this FGE, substance [FL-no: 15.054] was allocated to structural class II and its mTAMDI was below the TTC for this structural class. Then, in FGE.21Rev5, the structural class of [FL-no: 15.054] was changed from II to class III. With this change, the mTAMDI (160 μg/person per day) was above the TTC for class III. This was not explained in FGE.21Rev5 and more detailed information on uses and use levels should have been requested already in this revision 5.

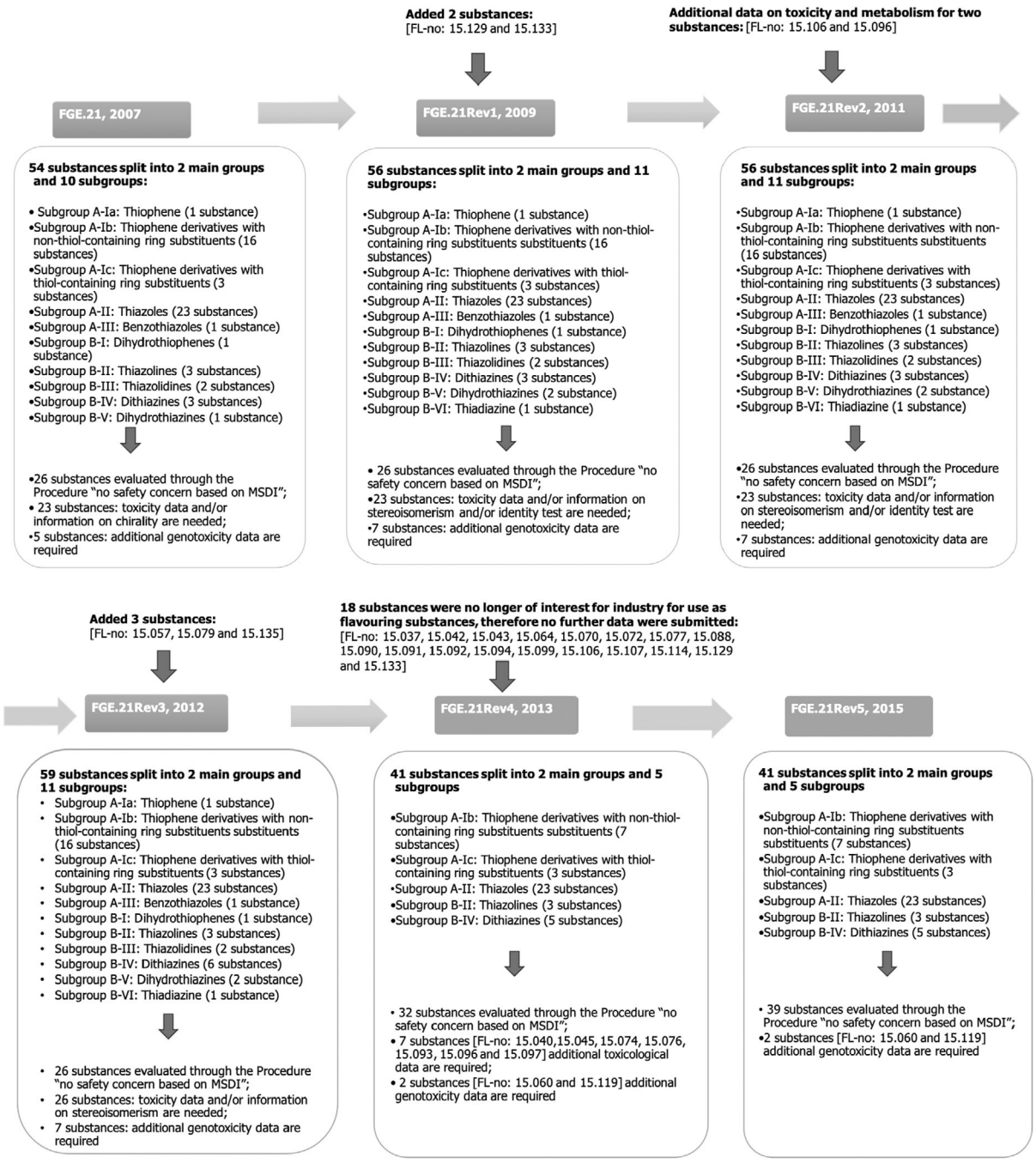


Figure 1: Summary of the history of evaluation of the substances in FGE.21

1.4. Presentation of the substances in FGE.21Revision 6

FGE.21Rev6 deals with 41 flavouring substances (candidate substances) from chemical groups 29 ('Thiazoles, thiophene, thiazoline and thienyl derivatives') and 30 ('Miscellaneous substances'), Annex I of Commission Regulation (EC) No 1565/2000.

All 41 candidate substances in FGE.21 are structurally related to 28 flavouring substances (supporting substances) evaluated at the 59th and 68th JECFA meetings (JECFA, 2002, 2003, 2007, 2008) in the group of 'sulfur-containing heterocyclic compounds'. Two of these supporting substances (Table 4) are mixtures (one of two isomeric isobutyl-substituted and one of two isomeric isopropyl-substituted dimethyldihydrodithiazine derivatives) that were not included in the register[5] and are not in the Union List of flavourings.[1]

The assignment of the individual 41 substances to the different subgroups in FGE.21Rev6 and the supporting substances for each subgroup are reported in Table 4. In the last column of Table 4, the status of the evaluation by EFSA, when based on the MSDI approach, of the supporting substances and of individual members of FGE.21 is presented, based on the evaluation in FGE.21Rev5, i.e. before consideration of the information received by EFSA that leads to the present revision 6 of this FGE. The substances previously allocated to subgroup A-Ia, A-III, BI, B-III, B-V and B-VI are no longer of interest for Industry for use as flavouring substances in Europe; therefore, no further data were submitted.

Only subgroup B-II, which includes the two substances that will be evaluated in FGE.21Rev6, is described. No descriptions are given for subgroups A-Ib, A-Ic, AII and BIV, which can be found in FGE.21Rev5 (EFSA CEF Panel, 2015).

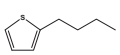
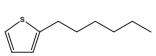
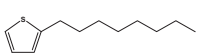
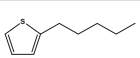
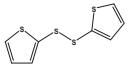
Group B includes non-aromatic substances. Thiazolines are allocated in subgroup B-II: three candidate substances and seven supporting substances. All substances in this subgroup are five-membered heterocycles containing a sulfur and a nitrogen atom in the 1- and 3-ring positions, respectively. In the ring, one double bond is present (in contrast to the thiazoles, which have two double bonds (aromatic)).

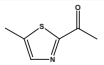
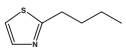
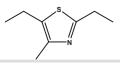
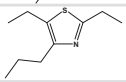
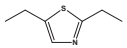
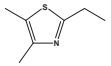
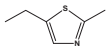
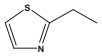
The two candidate substances in subgroup B-II [FL-no: 15.060 and 15.119] are 3-thiazolines. These two candidate substances are structurally related to five supporting substances [FL-no: 15.029, 15.030, 15.032, 15.130 and 15.131] evaluated at the 59th, and 68th JECFA meeting (JECFA, 2002, 2007) in a group of 'sulfur-containing heterocyclic compounds'. These five supporting substances have been considered by EFSA in FGE. 76Rev2 (EFSA FAF Panel, 2023). The names and structures of these supporting substances (from FGE.76Rev2) are presented in Appendix G (Table G.1), together with their evaluation status (EFSA FAF Panel, 2023).

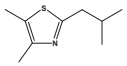
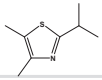
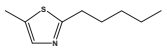
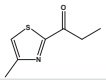
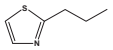
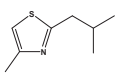
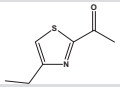
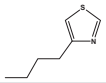
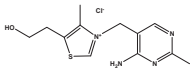
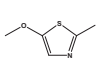
The substance 2-methyl-2-thiazoline [FL-no: 15.086] in subgroup B-II was evaluated in FGE.21Rev4 (EFSA CEF Panel, 2013a), based on additional data on the supporting substance 2-acetyl-2-thiazoline [FL-no: 15.010] from FGE.93Rev1 (EFSA CEF Panel, 2013c).

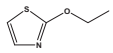
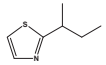
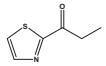
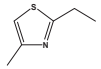
A summary of the safety evaluation of the flavouring substances in FGE.21 and further revisions is presented in Appendix F (Table F.1).

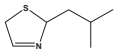
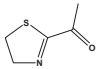
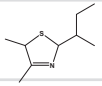
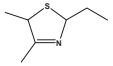
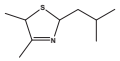
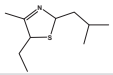
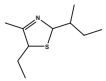
Table 4: Candidate and supporting substances divided into subgroups of related chemical structures. Substances listed in bold are the candidate substances. The supporting substances from the 59th and 68th JECFA meetings (JECFA, 2002, 2003, 2007, 2008) and from FGE.76 (EFSA, 2008) and FGE.76Rev1 (EFSA CEF Panel, 2013b) and FGE.93Rev1 (EFSA CEF Panel, 2013c) are in normal type face

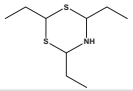
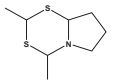
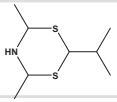
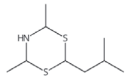
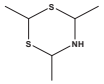
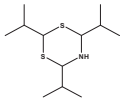
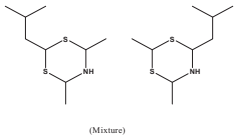
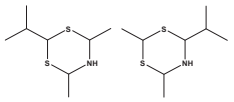
FL-no JECFA no	Chemical name	Structural formula	Structural Class	EFSA status according to FGE.21Rev5
A-Ia Thiophene – The substance previously allocated to the group is no longer of interest for Industry for use as flavouring substances in Europe; therefore, no further data were submitted.				
A-Ib Thiophenes (with non-thiol-containing ring substituents)				
15.040	**2-Acetylthiophene**		Class II	FGE.21Rev5 – no safety concern
15.045	**2-Butylthiophene**		Class II	FGE.21Rev5 – no safety concern
15.074	**5-Ethylthiophene-2-carbaldehyde**		Class II	FGE.21Rev5 – no safety concern
15.076	**2-Hexylthiophene**		Class II	FGE.21Rev5 – no safety concern
15.093	**2-Octylthiophene**		Class II	FGE.21Rev5 – no safety concern
15.096	**2-Pentylthiophene**		Class II	FGE.21Rev5 – no safety concern
15.097	**2-Propionylthiophene**		Class II	FGE.21Rev5 – no safety concern
15.004 1050	5-Methyl-2-thiophenecarbaldehyde[(b)]		Class II	FGE.76Rev1 – no safety concern
A-Ic Thiophenes (with thiol-containing ring substituents)				
15.082	**3-Mercaptothiophene**		Class III	FGE.21 – no safety concern
15.087	**2-Methyl-3-mercaptothiophene**		Class III	FGE.21 – no safety concern
15.108	**2-Thiophenemethanethiol**		Class III	FGE.21 – no safety concern
15.001 1052	2-Mercaptothiophene[(b)]		Class III	FGE.76 – no safety concern
15.008 1053	2-Thienyl disulfide[(b)]		Class III	FGE.76Rev1 – no safety concern

FL-no JECFA no	Chemical name	Structural formula	Structural Class	EFSA status according to FGE.21Rev5
A-II Thiazoles				
15.038	**2-Acetyl-4-methylthiazole**		Class II	FGE.21 – no safety concern
15.039	**2-Acetyl-5-methylthiazole**		Class II	FGE.21 – no safety concern
15.044	**2-Butylthiazole**		Class II	FGE.21 – no safety concern
15.050	**2,5-Diethyl-4-methylthiazole**		Class II	FGE.21 – no safety concern
15.051	**2,5-Diethyl-4-propylthiazole**		Class II	FGE.21 – no safety concern
15.052	**2,5-Diethylthiazole**		Class II	FGE.21 – no safety concern
15.058	**4,5-Dimethyl-2-ethylthiazole**		Class II	FGE.21 – no safety concern
15.061	**2,5-Dimethyl-4-ethylthiazole**		Class II	FGE.21 – no safety concern
15.062	**2,4-Dimethylthiazole**		Class II	FGE.21 – no safety concern
15.063	**2,5-Dimethylthiazole**		Class II	FGE.21 – no safety concern
15.067	**4-Ethyl-2-methylthiazole**		Class II	FGE.21 – no safety concern
15.068	**5-Ethyl-2-methylthiazole**		Class II	FGE.21 – no safety concern
15.069	**4-Ethyl-5-methylthiazole**		Class II	FGE.21 – no safety concern
15.071	**2-Ethylthiazole**		Class II	FGE.21 – no safety concern

FL-no JECFA no	Chemical name	Structural formula	Structural Class	EFSA status according to FGE.21Rev5
15.078	**2-Isobutyl-4,5-dimethylthiazole**		Class II	FGE.21 – no safety concern
15.080	**2-Isopropyl-4,5-dimethylthiazole**		Class II	FGE.21 – no safety concern
15.084	**5-Methyl-2-pentylthiazole**		Class II	FGE.21 – no safety concern
15.085	**4-Methyl-2-propionylthiazole**		Class II	FGE.21 – no safety concern
15.089	**2-Methylthiazole**		Class II	FGE.21 – no safety concern
15.098 	**2-Propylthiazole**		Class II	FGE.21 – no safety concern
15.115	**2-Isobutyl-4-methylthiazole**		Class II	FGE.21 – no safety concern
15.116	**2-Acetyl-4-ethylthiazole**		Class II	FGE.21 – no safety concern
15.118	**4-Butylthiazole**		Class II	FGE.21 – no safety concern
16.027 1030	Thiamine hydrochloride[(b)]		Class II	FGE.76 – no safety concern
15.002 1057	2-Methyl-5-methoxythiazole[(b)]		Class III	FGE.76Rev1 – no safety concern
15.005 1039	2,4-Dimethyl-5-vinylthiazole[(b)]		Class III	To be evaluated in FGE.76Rev2
15.011 1055	5-Acetyl-2,4-dimethylthiazole[(b)]		Class III	FGE.76 – no safety concern

FL-no JECFA no	Chemical name	Structural formula	Structural Class	EFSA status according to FGE.21Rev5
15.013 1034	2-Isobutylthiazole[(b)]		Class II	FGE.76 – no safety concern
15.014 1031	5-(2-Hydroxyethyl)-4-methylthiazole[(b)]		Class II	FGE.76 – no safety concern
15.015 1054	4-Methyl-5-(2-acetoxyethyl)thiazole[(b)]		Class II	FGE.76 – no safety concern
15.017 1035	4,5-Dimethylthiazole[(b)]		Class II	FGE.76 – no safety concern
15.018 1038	4-Methyl-5-vinylthiazole[(b)]		Class III	To be evaluated in FGE.76Rev2
15.019 1036	2,4,5-Trimethylthiazole[(b)]		Class II	FGE.76 – no safety concern
15.020 1041	2-Acetylthiazole[(b)]		Class II	FGE.76 – no safety concern
15.021 1056	2-Ethoxythiazole[(b)]		Class III	FGE.76 – no safety concern
15.022 1033	2-(*sec*-Butyl)thiazole[(b)]		Class II	FGE.76Rev1 – no safety concern
15.026 1037	2-Isopropyl-4-methylthiazole[(b)]		Class II	FGE.76 – no safety concern
15.027 1042	2-Propionylthiazole[(b)]		Class II	FGE.76Rev1 – no safety concern
15.033 1044	2-Ethyl 4-methylthiazole[(b)]		Class II	FGE.76 – no safety concern
15.035 1043	4-Methylthiazole[(b)]		Class II	FGE.76 – no safety concern

FL-no JECFA no	Chemical name	Structural formula	Structural Class	EFSA status according to FGE.21Rev5
A-III Benzothiazoles – The substance previously allocated to the group is no longer of interest for Industry for use as flavouring substances in Europe; therefore, no further data were submitted.				
B-I Dihydrothiophenes – The substance previously allocated to the group is no longer of interest for Industry for use as flavouring substances in Europe; therefore, no further data were submitted.				
B-II Thiazolines				
15.060	**2,4-Dimethyl-3-thiazoline**		Class III	To be evaluated in FGE.21Rev6
15.086	**2-Methyl-2-thiazoline**		Class II	FGE.21Rev4 – no safety concern
15.119	**2-Isobutyl-3-thiazoline**		Class III	To be evaluated in FGE.21Rev6
15.010 1759	2-Acetyl-2-thiazoline[(b)]		Class II	FGE.93Rev1 – no safety concern
15.029 1059	2-(*sec*-Butyl)-4,5-dimethyl-3-thiazoline[(b)]		Class III	To be evaluated in FGE.76Rev2
15.030 1058	4,5-Dimethyl-2-ethyl-3-thiazoline[(b)]		Class III	To be evaluated in FGE.76Rev2
15.032 1045	4,5-Dimethyl-2-isobutyl-3-thiazoline[(b)]		Class III	To be evaluated in FGE.76Rev2
15.128 1760	2-Propionyl-2-thiazoline[(b)]		Class II	FGE.93Rev1 – no safety concern
15.130 1761	5-Ethyl-4-methyl-2-(2-methylpropyl)-thiazoline[(b)]		Class III	Substance from FGE.93Rev1 to be evaluated in FGE.76Rev2
15.131 1762	5-Ethyl-4-methyl-2-(2-butyl)-thiazoline[(b)]		Class III	Substance from FGE.93Rev1 to be evaluated in FGE.76Rev2
B-III Thiazolidines – The substances previously allocated to the group are no longer of interest for Industry for use as flavouring substances in Europe, therefore, no further data were submitted.				

FL-no JECFA no	Chemical name	Structural formula	Structural Class	EFSA status according to FGE.21Rev5
B-IV Dithiazines				
15.054	**Dihydro-2,4,6-triethyl-1,3,5(4H)-dithiazine**		Class III	FGE.21Rev4 – no safety concern
15.055	**[2 S-(2a,4a,8ab)]2,4-Dimethyl(4H)pyrrolidino[1,2 e]-1,3,5-dithiazine**		Class III	FGE.21Rev4 – no safety concern
15.057	**4,6-Dimethyl-2-(1-methylethyl)dihydro-1,3,5-dithiazine**		Class III	FGE.21Rev4 – no safety concern
15.079	**2-Isobutyldihydro-4,6-dimethyl-1,3,5-dithiazine**		Class III	FGE.21Rev4 – no safety concern
15.135	**Ethyl thialdine**		Class III	FGE.21Rev4 – no safety concern
15.109 1049	2,4,6-Trimethyldihydro-1,3,5(4H)-dithiazine[(b)]		Class II	FGE.76Rev1 – no safety concern
15.113 1048	(5,6-Dihydro-2,4,6,tris(2-methylpropyl)-*4H*-1,3,5-dithiazine[(b)]		Class II	FGE.76Rev1 – no safety concern
(Not in EU Register[(a)] or in the Union List)	(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[(a)] or in the Union List)	(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 of interest for Industry for use as flavouring substances in Europe; therefore, no further data were submitted.				

FL-no JECFA no	Chemical name	Structural formula	Structural Class	EFSA status according to FGE.21Rev5
B-VI Thiadiazines – The substance previously allocated to the group is no longer of interest for Industry for use as flavouring substances in Europe; therefore, no further data were submitted.				

(a): Commission Decision of 23 February 1999 adopting a register of flavouring substances used in or on foodstuffs drawn up in application of Regulation (EC) No 2232/96 of the European Parliament and of the Council of 28 October 1996. OJ L 84, 27.3.1999, pp. 1–137.
(b): Supporting substances for each subgroup.

2. Data and methodologies

2.1. Data

Following the concern for genotoxicity for the substances [FL-no: 15.060, 15.119] expressed by the CEF Panel in FGE.21Rev5 and former revisions, new genotoxicity data have been provided for the representative and supporting substance 4,5-dimethyl-2-isobutyl-3-thiazoline [FL-no: 15.032] evaluated in FGE.76Rev2.

For [FL-no: 15.032], the following studies were submitted: an *in vitro* reverse gene mutation assay in bacteria (Covance, 2012), an *in vitro* micronucleus assay (Covance, 2013) and an *in vivo* combined bone marrow micronucleus test and Comet assay in liver (Covance, 2014).

A 90-day toxicity study was submitted for 2-(sec-butyl)-4,5-dimethyl-3-thiazoline [FL-no: 15.029] (Food and Drug Research Laboratories, 1978).[7]

Moreover, industry provided updated poundage data (EFFA, 2018).

Additional information was provided by the applicant during the assessment process in response to requests from EFSA sent on 19 February 2015, 11 June 2018 and 11 August 2021 (Covance, 2015; BioReliance, 2018; EFFA, 2018, 2022; Charles River Laboratories, 2020). Following the first request for additional data, two technical hearings were held with the applicant on 19 January 2016 (EFSA, 2016) and on 24 January 2017 (EFSA, 2017). A summary of the information requested for the supporting substance [FL-no: 15.032] is reported in FGE.76Rev2 (EFSA FAF Panel, 2023).

The newly submitted data considered in the present revision of FGE.21 are summarised in Table 5.

Table 5: Newly submitted data evaluated in FGE.21Rev6 and FGE.76Rev2

FL-no JECFA no	Chemical name	Data provided for the current revision 6 of FGE.21	Appendix (Table nr) and relevant section of the opinion	Documentation provided to EFSA/ reference
15.029 1059	2-(sec-butyl)-4,5-dimethyl-3-thiazoline[(a)]	90-day toxicity study Use levels and poundage data	Section 3.3	Food and Drug research laboratories (1978); EFFA (2018, 2022)
15.032 1045	4,5-Dimethyl-2-isobutyl-3-thiazoline[(a)]	Genotoxicity data Use levels and poundage data	Appendix E (Tables E.1 and E.2); Section 3.3	Covance (2012); Covance (2013); Covance (2014, 2015); BioReliance (2018); Charles River Laboratories (2020); EFFA (2018, 2022)
15.060	2,4-Dimethyl-3-thiazoline	Use levels and poundage data	Appendix C (Tables C.3 and C.6); Section 3.2	EFFA (2018, 2022)
15.119	2-Isobutyl-3-thiazoline	Use levels and poundage data	Appendix C (Tables C.3 and C.6); Section 3.2	EFFA (2018, 2022)

(a): Data on the supporting substances 2-(sec-butyl)-4,5-dimethyl-3-thiazoline [FL-no: 15.029] and 4,5-dimethyl-2-isobutyl-3-thiazoline [FL-no: 15.032] are evaluated in FGE.76Rev2.

In addition, the following references were used:

- EFSA scientific opinion on FGE.76Rev1 (EFSA CEF Panel, 2013b)
- EFSA scientific opinion on FGE.76Rev2 (EFSA FAF Panel, 2023)
- EFSA scientific opinion on FGE.21Rev5 (EFSA CEF Panel, 2015)

2.2. Methodologies

This opinion was prepared following the principles described in the EFSA Guidance on transparency with regard to scientific aspects of risk assessment (EFSA Scientific Committee, 2009) and following the relevant existing Guidelines from the EFSA Scientific Committee. The assessment strategy applied for the evaluation programme of flavouring substances, as laid down in Commission Regulation (EC)

[7] Study previously reported as Babish and Re (1978) in FGE.21Rev5 and former revisions

No 1565/2000[3], is based on the Opinion on a Programme for the Evaluation of Flavouring substances of the Scientific Committee on Food (SCF, 1999).

2.2.1. Procedure for the safety evaluation of flavouring substances

The approach for the safety evaluation of chemically defined flavouring substances as referred to in Commission Regulation (EC) No 1565/2000[3], named the 'Procedure', is described in Appendix A.

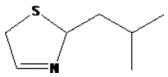
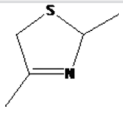
2.2.2. Approach used for the calculation of exposure

The approach used for calculation of the intake of the flavouring substances is described in Appendix A (point 'a) (*Intake*') and in Appendix C (Appendix C.2 'mTAMDI calculation').

3. Assessment

The 39 flavouring substances already evaluated in the previous revisions of FGE.21 will not be further discussed. Thus, in FGE.21Rev6, only two substances [FL-no: 15.060 and 15.119] will be evaluated. Nevertheless, for the sake of completeness, the information for all 41 substances is maintained in the various tables of this FGE. The substances under consideration in FGE.21Rev6 are reported in Table 6.

Table 6: Flavouring substances under evaluation in FGE.21Rev6

FL-no	Chemical structure	Chemical name	Structural class*
15.119		2-Isobutyl-3-thiazoline	III
15.060		2,4-Dimethyl-3-thiazoline	III

*: Determined with OECD Toolbox (version 4.4.1 available at https://www.oecd.org/chemicalsafety/risk-assessment/oecd-qsar-toolbox.htm).

3.1. Specifications

Purity criteria for the candidate substances were provided by the flavouring industry (EFFA, 2004d, 2011a; Flavour Industry, 2010). No new information for the two substances [FL-no: 15.119 and 15.060] has become available since FGE.21Rev5.

Judged against the requirements in Annex II of Commission Regulation (EC) No 1565/2000, the available information on specification was considered adequate for all 41 substances (see Appendix B).

Regarding [FL-no: 15.057 and 15.079], the Panel noted their low purities of 44% and 64%, respectively. However, for [FL-no: 15.057], this was considered acceptable, taking into account that all other components are 1,3,5-dithiazine positional isomers with only isopropyl- or methyl- substituents at the 2-, 4- or 6- positions of the ring. The same reasoning applies to [FL-no: 15.079] for which the only structural difference is the presence of isobutyl- rather than isopropyl- substituents.

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], four possess two chiral centres [FL-no: 15.054, 15.057, 15.079 and 15.135] and one possess three chiral centres [FL-no: 15.055].

The information on the stereochemical composition was considered sufficient for [FL-no: 15.055, 15.060 and 15.119]. However, the information on the stereochemical composition was considered inadequate for [FL-no: 15.054, 15.057, 15.079 and 15.135]. Information on the percentages of the stereoisomers in the material of commerce based on analytical data should be provided (see Table B.1 – Appendix B).

3.2. Intake data

3.2.1. Natural occurrence in food

A search in the VCF online database (VCF, 2022) of volatile compounds in food did not reveal any new data about natural occurrence of the two substances [FL-no: 15.060 and 15.119]. [FL-no: 15.060] has been reported in chicken, pork and beef without any quantitative data listed and [FL-no: 15.119] has not been reported in any food item according to the database.

Information on natural occurrence of the other 39 substances in this FGE can be found in FGE.21Rev5.

3.2.2. Estimated daily per capita intake (MSDI approach)

The intake estimation is based on the 'Maximised Survey-derived Daily Intake' (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[8] (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, 2010; 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] account 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 (Appendix C).

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] and of 2-pentylthiophene [FL-no: 15.096] is 1.6 μg and of 4-butylthiazole [FL-no: 15.118] is 1.3 μg. For the remaining 37 substances, the estimated daily *per capita* intakes are in the range of 0.0012–0.85 μg (Appendix C).

New information on production figures for the two candidate substances under consideration has been provided. Poundage data for both [FL-no: 15.060 and 15.119] are 0.1 kg per year (EFFA, 2018). Based on these data, an estimated daily *per capita* intake (MSDI) of 0.012 μg has been calculated for both [FL-no: 15.060 and 15.119] from use as flavouring substances.

3.2.3. 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.

[8] 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.

For the evaluation of the candidate substances, information on food categories and normal and maximum use levels were submitted by the Flavour Industry (EFFA, 2004c,d, 2007, 2022; Flavour Industry, 2004–2005, 2010) for 39 substances. No information on use levels has 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. 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.

According to the Flavour Industry, the normal use levels range from 0.04 to 2 mg/kg food, and the maximum use levels range from 0.2 to 12 mg/kg (EFFA, 2004c,d, 2007, 2022; Flavour Industry, 2004–2005, 2010) for the 39 substances for which use levels have been provided (Appendix C, Table C.3).

The mTAMDI values for the 31 candidate substances from structural class (SC) II (see Appendix C, Table C.6) range from 78 to 220 μg/person per day. For eight candidate substances from SC III, the mTAMDI range from 78 to 605 μg/person per day, and for five of these [FL-no: 15.054, 15.055, 15.060, 15.119, and 15.135], the mTAMDI values are above the TTC for their SC III (90 μg/person per day).

Since the previous version of this FGE, no information has been submitted on uses and use levels for [FL-no: 15.057 and 15.079] and consequently, for these two substances, no mTAMDI can be calculated.

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

3.2.4. 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. Furthermore, 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 combined exposure is calculated for each subgroup considering also the supporting substances.

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 and are therefore not considered in the calculation of the combined intake which thus includes only the 39 candidate substances evaluated through the Procedure in FGE.21Rev5.

On the basis of the reported annual production volumes in Europe (EFFA, 2004b, 2011a, 2012a; Flavour Industry, 2010; IOFI, 2013a,b), the combined estimated daily *per capita* intakes as flavourings of the candidate substances, calculated for each subgroup (with more than one substance) and 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 (five 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, 2002, 2003, 2007, 2008). The total combined daily *per capita* intakes (in Europe) of candidate and supporting substances in each of the four 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) 0.2 μg; subgroup A-II (35 substances from SC II) 500 μg; subgroup B-II (three substances from SC II) 0.7 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 SC II and III, respectively.

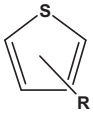
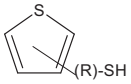
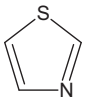
3.3. Biological and toxicological data

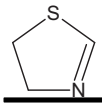
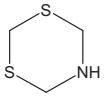
3.3.1. Absorption, distribution, metabolism and elimination

The 41 candidate substances are structurally related to 28 supporting substances evaluated by the JECFA in 'Sulfur-containing heterocyclic compounds' (JECFA, 2002, 2003, 2007, 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 (sulfur or sulfur 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 7 (the substances previously allocated to subgroup A-Ia, A-III, BI, B-III, B-V and B-VI are no longer of interest for Industry for use as flavouring substances in Europe; therefore, no further data were submitted).

In FGE.21Rev6, only information relevant for subgroup B-II is reported. Information on all other subgroups can be retrieved in FGE.21Rev5 (EFSA CEF Panel, 2015).

Table 7: Candidate substances divided into subgroups of related chemical structures

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

Subgroup and common ring structure	Chemical name	FL-no
	2-Acetyl-5-methylthiazole	15.039
	2-Acetyl-4-ethylthiazole	15.116
	4-Methyl-2-propionylthiazole	15.085
A-III: Benzothiazoles The substance previously allocated to the group is no longer of interest for Industry for use as flavouring substances in Europe; therefore, no further data were submitted.		
B	Non-aromatic subgroups	
B-I: Dihydrothiophenes	The substance previously allocated to the group is no longer of interest for Industry for use as flavouring substances in Europe; therefore, no further data were submitted.	
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 of interest for Industry for use as flavouring substances in Europe; therefore, no further data were submitted.		
B-IV: Dithiazines 	Dihydro-2,4,6-triethyl-1,3,5(4*H*)-dithiazine	15.054
	[2 S-(2a,4a,8ab)] 2,4-Dimethyl(4H)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 of interest for Industry for use as flavouring substances in Europe; therefore, no further data were submitted.		
B-VI: Thiadiazine The substance previously allocated to the group is no longer of interest for Industry for use as flavouring substance in Europe; therefore, no further data were submitted.		

Metabolism of the substances in subgroups B-II

No specific information was available on the metabolism of the thiazoline derivatives or related substances for any of these non-aromatic substances in the B-II subgroup. From general knowledge on biotransformation, the Panel anticipated that the substances in this group may be metabolised by ring *S*- or *N*-oxidation. In addition, metabolism of the ring substituents is likely to occur.

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

3.3.2. Genotoxicity data

In FGE.21 (EFSA, 2007), the AFC Panel considered that the two candidate substances 2-methylthiazolidine [FL-no: 15.090] and 2-propylthiazolidine [FL-no: 15.099] in subgroup B-III have been reported to be positive in the Ames tests (S. Typhimurium TA98 and TA100) (Mihara and Shibamoto, 1980), see Appendix D.

Owing to limited reporting, the data could not be properly evaluated. Nevertheless, these reports do raise the possibility of a genotoxic potential of these thiazolidines. Accordingly, the AFC Panel did not to evaluate the candidate substances 2-methylthiazolidine [FL-no: 15.090] and 2-propylthiazolidine [FL-no: 15.099] through the Procedure.

Considering the structural similarities between these two thiazolidines in subgroup B-III and 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 AFC Panel concluded that in the absence of further genotoxicity data, the Procedure could not be applied to these five substances from subgroup B-II and B-III (EFSA, 2007).

In FGE.21Rev4 (EFSA CEF Panel, 2013a), based on experimental data on the supporting substance 2-acetyl-2-thiazoline [FL-no: 15.010], the CEF Panel concluded that 2-methyl-2-thiazoline [FL-no: 15.086] does not give rise to concern with respect to genotoxicity and it can accordingly be evaluated using the Procedure. In the same opinion, it was indicated that no data were provided for the

substances in subgroup B-III, because their evaluation was not supported by Industry (EFSA CEF Panel, 2013a). Therefore, no genotoxicity data were available for the evaluation of 2,4-dimethyl-3-thiazoline [FL-no: 15.060] and 2-isobutyl-3-thiazoline [FL-no: 15.119].

Industry has submitted *in vitro* and *in vivo* genotoxicity data for 4,5-dimethyl-2-isobutyl-3-thiazoline [FL-no: 15.032], representative substance of the group of 3-thiazolines in FGE.76Rev2 and supporting substance for [FL-no: 15.060 and 15.119], the 3-thiazolines in subgroup B-II of FGE.21Rev6. The new studies on [FL-no: 15.032] have been evaluated in FGE.76Rev2. The summary of that evaluation is reported below, for detailed description of the evaluation of the genotoxicity studies on [FL-no: 15.032], see FGE.76Rev2 (EFSA FAF Panel, 2023).

3.3.2.1. Genotoxicity evaluation of the supporting substance 4,5-dimethyl-2-isobutyl-3-thiazoline [FL-no: 15.032] from FGE.76Rev2 (EFSA FAF Panel, 2023)

4,5-Dimethyl-2-isobutyl-3-thiazoline [FL-no: 15.032] did not induce gene mutations in *S. Typhimurium* (TA98, TA100, TA1535, TA1537 and TA102) in the absence or in the presence of metabolic activation (Covance, 2012).

In an *in vitro* MN assay in human peripheral blood lymphocytes, 4,5-dimethyl-2-isobutyl-3-thiazoline induced a statistically significant increase in micronucleated cell frequency at 3 + 21 h in the presence of metabolic activation and a weak, but statistically significant, increase in micronucleated cell frequency when tested for 3 + 21 h in the absence of S9-mix (Covance, 2013).

In TK6 cells, 4,5-dimethyl-2-isobutyl-3-thiazoline [FL-no: 15.032] did not induce an increase in micronucleated cell frequency at any conditions used in the test (BioReliance, 2018). Since this result is in contrast to the positive results observed in primary cultures of human lymphocytes, the Panel requested to repeat the assay in conditions similar to the *in vitro* MN assay performed in human lymphocytes and applying fluorescence *in situ* hybridisation (FISH) analysis instead of kinetochores staining (CREST).

An *in vitro* micronucleus assay combined with FISH analysis was provided by the applicant to investigate the mechanism inducing MN *in vitro* (clastogenicity or aneugenicity). The test was applied in human peripheral blood lymphocytes using the cytokinesis-block method (Charles River Laboratories, 2020). 4,5-Dimethyl-2-isobutyl-3-thiazoline was tested up to 10 mM, the highest concentration recommended by OECD TG 487 (OECD, 2016) with a short-term treatment with and without S9-mix and with 24 h continuous treatment. A statistically significant and concentration-related increase in percent of cells with MN was detected at all the conditions of treatment. The FISH analysis applied on the samples from the 24 h treatment showed 81% of MN positive for the DNA probe demonstrating the aneugenic mechanism of the compound.

Based on these observations, the FAF Panel concluded in FGE.76Rev2 (EFSA FAF Panel, 2023) that the concerns for gene mutations and clastogenicity are ruled out for the representative substance [FL-no: 15.032]. Consequently, the Panel reached the same conclusion for the structurally related substances [FL-no: 15.060 and 15.119] in the current revision 6 of FGE.21.

An *in vivo* combined bone marrow micronucleus test and comet assay in liver and duodenum of rats was performed to follow-up the observed *in vitro* chromosomal damage (Covance, 2014). 4,5-Dimethyl-2-isobutyl-3-thiazoline [FL-no: 15.032] did not induce DNA damage in duodenum and liver of treated rats, confirming that this substance is not clastogenic. In the *in vivo* micronucleus assay, there was no significant increase in micronucleated cells in bone marrow, but this study was inconclusive due to insufficient evidence of bone marrow exposure. Thus, the concern for aneugenicity could not be ruled out based on this study (EFSA FAF Panel, 2023).

Following the recommendations for risk assessment in the EFSA Scientific Committee guidance on aneugenicity (EFSA Scientific Committee, 2021), the Panel compared for [FL-no: 15.032] the concentration resulting in aneugenicity *in vitro* (97.2 μg/mL) with the estimated concentration of the substance in the GIT following ingestion of food or beverage (highest normal use level is 0.49 mg/kg (i.e. 0.49 μg/g) and highest maximum use level is 2 mg/kg (i.e. 2 μg/g)). Based on that comparison, the Panel concluded for [FL-no: 15.032] in FGE.76Rev2 that the use of this substance at the reported use levels would not raise a concern for aneugenicity. Accordingly, this substance can be evaluated following the Procedure for evaluation of flavouring substances (EFSA FAF Panel, 2023).

Based on structural similarity, for the two 3-thiazolines in FGE.21Rev6 [FL-no: 15.060 and 15.119], an aneugenic potential may also be anticipated. However, the lowest concentration resulting in aneugenicity *in vitro* for [FL-no: 15.032] cannot be used for the safety assessment for these two substances, because quantitative extrapolation of this concentration to estimate the aneugenic potency of these two substances would be connected to a high level of uncertainty. Consequently, similar to

the conclusion that was reached in FGE.76Rev2 for the substances structurally related to [FL-no: 15.032], the FAF Panel concluded that [FL-no: 15.060 and 15.119] in FGE.21Rev6 could currently not be evaluated following the Procedure for evaluation of flavouring substances.

For these two substances, individual data are needed to establish whether they have aneugenic potential. They should be screened in an *in vitro* MN test preferably with human lymphocytes and application of a FISH technique for centromere analysis. In case of an increase in micronucleated cells frequency, an appropriate follow-up test should be done, according to the EFSA guidance on genotoxicity testing strategy (EFSA Scientific Committee, 2011) and the EFSA guidance on aneugenicity (EFSA Scientific Committee, 2021).

3.3.3. Toxicity studies for the 3-thiazolines in subgroup B-II

There were no toxicological data available for [FL-no: 15.060 and 15.119].

For the group of 3-thiazolines, industry submitted a 90-day toxicity study for 2-(sec-butyl)-4,5-dimethyl-3-thiazoline [FL-no: 15.029] (Food and Drug Research Laboratories, 1978).[7]

2-(Sec-butyl)-4,5-dimethyl-3-thiazoline was administered via diet to male and female Sprague Dawley rats (15/sex) for 90 days at one-dose level of 1.2 mg/kg body weight (bw) per day. Body weight changes, food consumption, limited haematological and clinical chemistry parameters were assessed, and urinalysis was undertaken.

A significant increase in the percentage of eosinophils was observed in treated males; however according to the study report, the value was within the normal percentage range for 'the rat', with a general reference to scientific data from the public domain (from the report of the study with [FL-no: 15.029], it is not clear whether this reference is strain specific). No other differences were observed. The authors stated that 'based on the data evaluated the material produced no overt toxicity'.

The Panel noted that the study had several shortcomings, e.g. only one dose level, purity of test material was not specified. Pathology and histopathology were studied, but detailed results were not included in the study report.

The Panel considered that despite the limitations of the toxicity data available, the NOAEL of 1.2 mg/kg bw per day, from the study on [FL-no: 15.029], can be used for the calculation of a margin of exposure (MOE) for the structurally related substances [FL-no: 15.060 and 15.119].

3.4. Application of the procedure

In FGE.21Rev5, the CEF Panel concluded that none of the 39 candidate substances evaluated through the Procedure is of safety concern at their estimated levels of intake when based on the MSDI approach (see Appendix F).

In the present revision (FGE.21Rev6), the substances [FL-no: 15.060 and 15.119] are not evaluated through the Procedure because genotoxicity data are needed to investigate their potential aneugenicity.

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

The estimated intakes, based on the mTAMDI, for the 31 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 six candidate substances assigned to SC III and evaluated using the Procedure, range from 78 to 250 μg/person per day. For three of these candidate substances [FL-no: 15.054, 15.055 and 15.135], the mTAMDI values are above the threshold of concern for SC III substances of 90 μg/person per day. The same applies for the two SC III substances [FL-no: 15.060 and 15.119], which were not evaluated through the Procedure. For three SC III substances [FL-no: 15.082, 15.087 and 15.108], the mTAMDI estimates were below the TTC for their structural class. For the two remaining SC III candidate substances [FL-no: 15.057 and 15.079], no information on use levels has been provided.

Therefore, further information is required for five substances [FL-no: 15.054, 15.055, 15.057, 15.079 and 15.135]. This would include more reliable data on uses and use levels. On the basis of such additional data, these flavouring substances should be reconsidered along the steps of the Procedure. Provided that information is submitted for [FL-no: 15.060 and 15.119] with respect to potential aneugenicity, that would allow evaluation of these substances through the Procedure, also for

these two substances, more reliable data on uses and use levels would be required. Following this procedure, additional toxicological data might become necessary.

For comparison of the MSDI and mTAMDI values, see Appendix C.

4. Discussion

In total FGE.21Rev6 comprises 41 flavouring substances. In FGE.21Rev5, the CEF 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] evaluated using the Procedure, would present no safety concern at their estimated levels of intake when based on the MSDI approach.

Seven substances possess one or more chiral centres [FL-no: 15.054, 15.055, 15.057, 15.060, 15.079 15.119 and 15.135]. The information on the stereochemical composition is sufficient for [FL-no: 15.055, 15.060 and 15.119]. However, the information on the stereochemical composition is inadequate for [FL-no: 15.054, 15.057, 15.079 and 15.135]. Information on the percentages of the stereoisomers in the material of commerce based on analytical data should be provided.

For the remaining two substances [FL-no: 15.060 and 15.119], new genotoxicity data have been provided for the supporting substance [FL-no: 15.032], from FGE.76Rev2, and considered in the present revision (FGE.21Rev6).

Based on the new available data, the concern for gene mutation and clastogenicity is ruled out for the representative substance [FL-no: 15.032] and thus also for the structurally related substances [FL-no: 15.060 and 15.119].

4,5-Dimethyl-2-isobutyl-3-thiazoline [FL-no: 15.032] induced micronuclei *in vitro* through an aneugenic mode of action. The available *in vivo* micronucleus study in bone marrow was not adequate to rule out the potential aneugenicity of [FL-no: 15.032] *in vivo*, because there was insufficient evidence of adequate target tissue exposure. Based on structural similarity, for the 3-thiazoline substances in this FGE [FL-no: 15.060 and 15.119], an aneugenic potential may also be anticipated. Applying the recommendations for risk assessment of aneugenic substances (EFSA Scientific Committee, 2021), the Panel noted that the lowest concentration resulting in aneugenicity *in vitro* for [FL-no: 15.032] cannot be used for the safety assessment for these two substances, because quantitative extrapolation of this concentration to estimate the aneugenic potency of these two substances would be connected to a high level of uncertainty. Therefore, for [FL-no: 15.060 and 15.119], individual data are needed to establish whether they have aneugenic potential; consequently, these substances have not been evaluated through the Procedure in FGE.21Rev6.

For the candidate substances [FL-no: 15.054, 15.055, 15.060, 15.119 and 15.135], the estimated intakes, based on the mTAMDI approach, are above the threshold of concern for SC III substances of 90 μg/person per day.

The Panel concluded that more reliable data on uses and use levels are required for [FL-no: 15.054, 15.055 and 15.135]. On the basis of such additional data, these flavouring substances should be re-evaluated using the Procedure. Provided that information is submitted for [FL-no: 15.060 and 15.119] with respect to potential aneugenicity, that would allow evaluation of these substances through the Procedure, also for these two substances more reliable data on uses and use levels would be required. Following this procedure, additional toxicological data might become necessary.

For two candidate substances [FL-no: 15.057 and 15.079], no use levels were provided. For these two substances, data on uses and use levels are needed to calculate the mTAMDIs. Upon submission of such data, additional data on toxicity may become necessary.

5. Conclusions

The Panel concluded that 39 flavouring substances evaluated using the Procedure in this FGE, would present no safety concern at their estimated levels of intake when based on the MSDI approach.

For two remaining substances [FL-no: 15.060 and 15.119], individual data are needed to determine if they have aneugenic potential, according to the EFSA guidance on genotoxicity testing strategy (EFSA Scientific Committee, 2011) and the EFSA guidance on aneugenicity (EFSA Scientific Committee, 2021).

For two substances [FL-no: 15.057 and 15.079], use levels are needed to calculate the mTAMDIs. For three substances [FL-no: 15.054, 15.055 and 15.135], more reliable information on uses and normal and maximum use levels are needed. With this information, the Panel could (re)calculate the mTAMDI estimates for these five substances in order to finalise their evaluation. Provided that information is submitted for [FL-no: 15.060 and 15.119] with respect to potential aneugenicity, that would allow evaluation of these substances through the Procedure, also for these two substances more reliable data on uses and use levels would be required. Upon submission of such data, additional data on toxicity may become necessary for all seven substances.

Adequate specifications including complete purity criteria and identity for the materials of commerce have been provided for 37 flavouring substances. For [FL-no: 15.054, 15.057, 15.079 and 15.135], information on the actual percentages of stereoisomers in the material of commerce based on analytical data should be provided.

6. Recommendation

The Panel recommends the European Commission to consider the following:

- for the substances [FL-no: 15.057 and 15.079], to change the information currently reported in column 6 of the Union list[1] according to the comments provided in Appendix B – Table B.1.
- the name of the substance [FL-no: 15.055] in the Union List should be changed to [2S-(2α,4α,8αβ)]-2,4-dimethyl(4H)pyrrolidino[1,2e]-1,3,5-dithiazine.

7. Documentation as provided to EFSA

1) BioReliance, 2018. *In vitro* Mammalian Cell Micronucleus Assay in TK6 Cells. 4,5-dimethyl-2-isobutyl-3-thiazoline. BioReliance Corporation, BioReliance Study Number AE84HN.361.BTL. April 2018. Unpublished final report submitted by EFFA to EFSA.
2) Charles River Laboratories, 2020. 4,5-Dimethyl-2-isobutyl-3-thiazoline, *in vitro* Micronucleus Assay in Cultured Human Peripheral Blood Lymphocytes. Testing Facility Study No. 00968013. February 2020. Unpublished final report submitted by EFFA to EFSA.
3) Covance, 2012. Reverse mutation in five histidine-requiring strains of Salmonella typhimurium, 4,5-dimethyl-2-isobutyl-3-thiazoline. Covance Laboratories Ltd. Study No. 8252805. August 2012. Unpublished final report submitted by EFFA to EFSA.
4) Covance, 2013. Induction of micronuclei in cultured human peripheral blood lymphocytes: 4,5-dimethyl-2-isobutyl-3-thiazoline. Covance Laboratories Ltd. Study No. 8266504. May 2013. Unpublished final report submitted by EFFA to EFSA.
5) Covance, 2014. 4,5-Dimethyl-2-isobutyl-3-thiazoline: Combined bone marrow micronucleus test and Comet assay in the liver of treated rats. Covance Laboratories Ltd. Study No. 8297734. July 2014. Unpublished final report submitted by EFFA to EFSA.
6) Covance, 2015. 4,5-Dimethyl-2-isobutyl-3-thiazoline: analysis of duodenum comet slides from Covance Study 8297734. Covance Laboratories Ltd. Study No. 8321902. July 2015. Unpublished final report submitted by EFFA to EFSA.
7) DG SANCO (Directorate General for Health and Consumer Affairs), 2012. Information from DG SANCO 07/022012, concerning two lists of 85 and 15 non-supported substances and one list of 30 substances for which no data have been submitted or which are duplicates. FLAVIS.2.23rev1.
8) DG SANCO (Directorate General for Health and Consumer Affairs), 2013. Information from DG SANCO 14/052013, concerning a list of 18 non-supported substances. FLAVIS.2.26.
9) EFFA (European Flavour and Fragrance Association), 2002. Letter from EFFA to the Danish Veterinary and Food Administration. 31 October 2002. Re.: Second group of questions. FLAVIS/8.26.
10) EFFA (European Flavour and Fragrance Association), 2004a. Intake—Collection and collation of usage data for flavouring substances. Letter from EFFA to EFSA. 31 May 2004.
11) EFFA (European Flavour and Fragrance Association), 2004b. Submission 2003–10. Flavouring group evaluation of 54 flavouring substances (candidate chemicals) of the chemical group 29 (Annex I of 1565/2000/EC) structurally related to sulfur-containing heterocyclic compounds [JECFA/WHO FAS 50/59] used as flavouring substances. 4 February 2004. FLAVIS/8.33. European inquiry on volume of use. IOFI, International Organisation of the Flavour Industry, 1995. Private communication to FEMA. Unpublished report submitted by EFFA to FLAVIS Secretariat.

12) EFFA (European Flavour and Fragrance Association), 2004c. Submission 2003–9. Flavouring group evaluation of 23 flavouring substances (candidate chemicals) of the chemical group 25 (Annex I of 1565/2000/EC) structurally related to phenol and phenol derivatives [JECFA/WHOFAS 46/55] used as flavouring substances. 9 April 2004. Unpublished report submitted by EFFA to FLAVIS Secretariat. FLAVIS/8.34.
13) EFFA (European Flavour and Fragrance Association), 2004d. Submission 2003–10. Flavouring group evaluation of 54 flavouring substances (candidate chemicals) of the chemical group 29(Annex I of 1565/2000/EC) structurally related to sulfur-containing heterocyclic compounds [JECFA/WHO FAS 50/59] used as flavouring substances. 4 February 2004. Unpublished report submitted by EFFA to FLAVIS Secretariat. FLAVIS/8.33.
14) EFFA (European Flavour and Fragrance Association), 2007. E-mail from EFFA to FLAVIS Secretariat, National Food Institute, Technical University of Denmark. 8 February 2007. Re: FLAVIS submissions—use levels for Category 14.2—Alcoholic beverages. FLAVIS/8.70.
15) EFFA (European Flavour Association), 2010. EFFA Letters to EFSA for clarification of specifications and isomerism for which data were requested in published FGEs.
16) EFFA (European Flavour Association), 2011a. Specifications and poundage data for 42 register substances submitted by EFFA/Industry to FLAVIS Secretariat. August 2011. FLAVIS/8.124.
17) EFFA (European Flavour Association), 2011b. Email from EFFA to FLAVIS Secretariat, Danish Food Institute, Technical University of Denmark. 26 August 2011. Re.: FGE.21Rev1: [FL-no: 15.042, 15.054, 15.119 and 15.129] specifications on isomers. FLAVIS/8.121.
18) EFFA (European Flavour Association), 2012a. Addendum of additional data of 7 flavouring substances relevant to the flavouring group evaluation of the chemical groups 29 and 30 (Annex I of 1565/2000/EC) thiazoles, thiophene, thiazoline and thienyl derivatives as evaluated by EFSA in FGE.21Rev3 and FGE.76. Addendum to FGE.21Rev3 and FGE.76 December 2012. FLAVIS/8.178.
19) EFFA (European Flavour Association), 2012b. Addendum of additional data relevant to the flavouring group evaluation of the chemical group 29 (Annex I of 1565/2000/EC) consideration of sulfur containing heterocyclic compounds evaluated by JECFA (68th meeting) structurally related to thiazoles, thiophene, thiazoline and thienyl derivatives evaluated by EFSA inFGE.21Rev1(2009). Addendum to FGE.93 May 2012. FLAVIS/8.175.
20) EFFA (European Flavour Association), 2013a. Email from EFFA to FLAVIS Secretariat, Danish Food Institute, Technical University of Denmark, dated 25 February 2013. Information on three substances evaluated in FGE.76Rev1 [FL-no: 15.005, 15.029 and 15.030]. FLAVIS/8.180.
21) EFFA (European Flavour Association), 2013b. Email from EFFA to FLAVIS Secretariat, Danish Food Institute, Technical University of Denmark, dated 6 March and 25 October 2013. Information on substances evaluated in FGE.21Rev4, FGE.25Rev3, FGE.76Rev1, FGE.90Rev1, FGE.93Rev1. FLAVIS/8.185.
22) EFFA (European Flavour Association), 2018. Updated Addendum of Additional Data Relevant to the Flavouring Group Evaluation of the Chemical Group 29 Subgroups AII and BII (Annex I of 1565/2000/EC) Consideration of sulfur containing heterocyclic compounds evaluated by JECFA (59th and 68th meetings) structurally related to thiazoles, thiophene, thiazoline and thienyl derivatives from group 29, miscellaneous substances from chemical group 30 evaluated by EFSA in FGE.21 (2008) and FGE.21Rev1 (2009). Updated Addendum to FGE.76, FGE.21 and FGE.93 prepared by International Organisation of the Flavour Industry, 3 March 2018. Unpublished report submitted by EFFA to EFSA.
23) EFFA (European Flavour Association), 2022. Revised Submission of EFFA data: use level information for nine substances of FGE.21/76/93: Thiazoles/Thiazolines. 25 February 2022. Submitted by EFFA to EFSA.
24) Flavour Industry, 2004–2005. Information submitted by Flavour Industry to DG SANCO and forwarded to FLAVIS. A-21Rev1.
25) Flavour Industry, 2010. Unpublished information submitted by Flavour Industry to EFSA and forwarded to FLAVIS Secretariat. A-21Rev3 [FL-no: 15.135].
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Abbreviations

AFC	Food Additives, Flavourings, Processing Aids and Materials in Contact with Food
CAS	Chemical Abstract Service
CEF	Food Contact Materials, Enzymes, Flavourings and Processing Aids
CoE	Council of Europe
CREST	Calcinosis, Raynaud's phenomenon, oesophageal dysmotility, sclerodactyly and telangiectasia
EFFA	European Flavour Association
FAF	Food Additives and Flavourings
FAO	Food and Agriculture Organisation of the United Nations
FEMA	Flavour and Extract Manufacturers Association
FGE	Flavouring Group Evaluation
FISH	Fluorescence *in situ* hybridisation
FLAVIS (FL)	Flavour Information System (database)
GLP	Good Laboratory Practice
ID	Identity
IOFI	International Organisation of the Flavour Industry
IR	Infrared spectroscopy
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
MN	Micronucleus
MOE	Margin of Exposure
MSDI	Maximised Survey-Derived Daily Intake
mTAMDI	Modified Theoretical Added Maximum Daily Intake
NOAEL	No Observed Adverse Effect Level
OECD	Organisation for Economic Co-operation and Development
SCF	Scientific Committee on Food
TAMDI	Theoretical Added Maximum Daily Intake
TTC	Threshold of Toxicological Concern
WHO	World Health Organisation

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[3], named the 'Procedure', is shown in schematic form in Figure A.1. The Procedure is based on the Opinion of the Scientific Committee on Food expressed on 2 December 1999 (SCF, 1999), which is derived from the evaluation Procedure developed by the Joint FAO/WHO Expert Committee on Food Additives at its 44th, 46th and 49th meetings (JECFA, 1995, 1996, 1997, 1999), hereafter named the 'JECFA Procedure'.[9]

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 structural classes (I, II and III) for which toxicological thresholds of concern (TTCs) (human exposure thresholds) have been specified. Exposures below these TTCs 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 TTCs 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 structural classes. The further steps address the following questions:

- Can the flavourings be predicted to be metabolised to innocuous[10] products (step 2)?
- Do their exposures exceed the TTC for the structural class (steps A3 and B3)?
- Are the flavourings or their metabolites endogenous[10] (step A4)?
- Does a NOAEL exist on the flavourings or on structurally related substances (steps A5 and B4)?

In addition to the 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.

[9] The FAF Panel is aware that a Revised Procedure for the Safety Evaluation of Flavouring agents has been agreed by JECFA (JECFA, 2016). Also, the EFSA Scientific Committee has recently developed a modified procedure for evaluation of substances based on the TTC approach (EFSA Scientific Committee, 2019). However, these developments have no impact on the present evaluation, which should follow the requirements as set out in Commission Regulation (EC) No 1565/2000.

[10] *Innocuous products*: products that are known or readily predicted to be harmless to humans at the estimated intake of the flavouring agent (JECFA, 1997). *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).

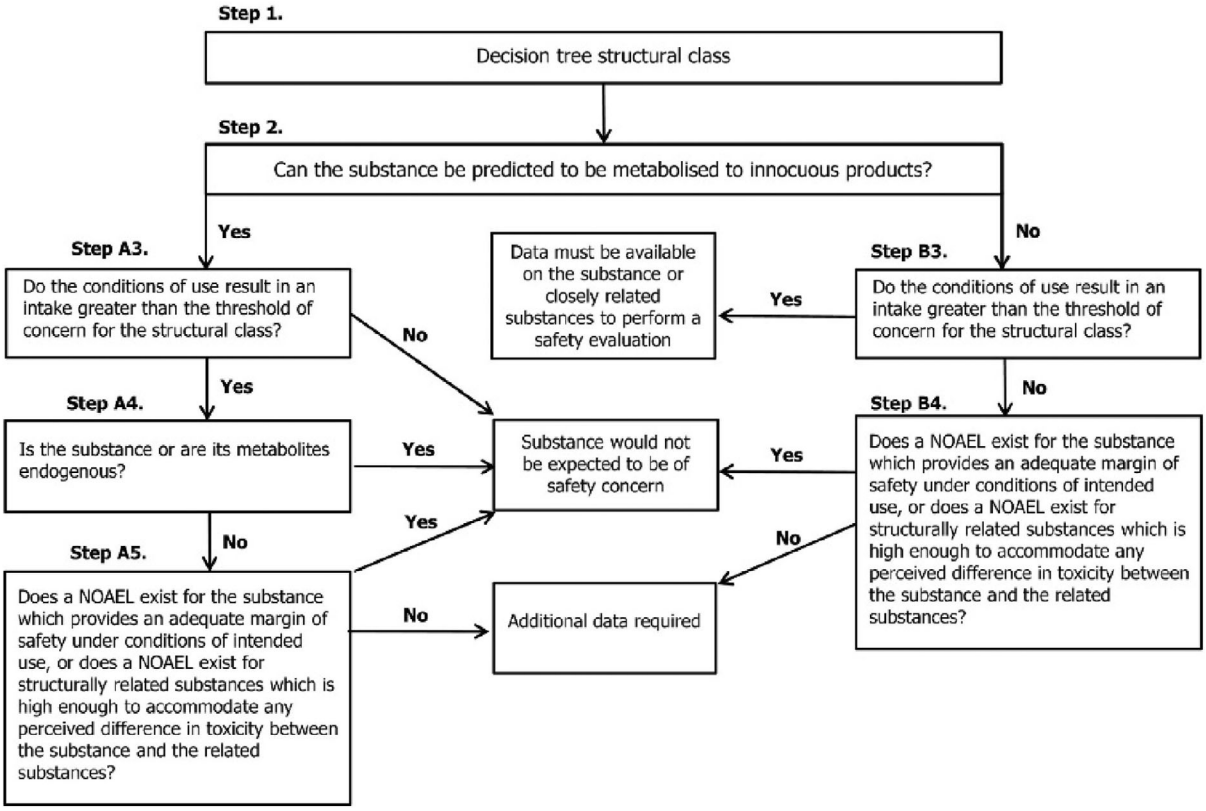


Figure A.1: Procedure for the safety evaluation of chemically defined flavouring substances

The following issues are of special importance:

a) Intake

Annual production volumes of the flavouring substances as surveyed by the Industry can be used to calculate the 'Maximised Survey-derived Daily Intake' (MSDI)[11] 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

[11] EU MSDI: Amount added to food as flavour in (kg/year) $\times$ 10^9/(0.1 $\times$ population in Europe (= 375 $\times$ 10^6) $\times$ 0.6 $\times$ 365) = μg/capita per day.

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).

The method for the modified TAMDI (mTAMDI) calculations is described in Appendix C.

To gather information on the occurrence and levels of a flavouring substance in natural sources, the volatile compounds in food (VCF) database is used (available at the following link https://www.vcf-online.nl/VcfHome.cfm).

b) Genotoxicity

As reflected in the opinion of SCF (SCF, 1999), the Panel has in its evaluation focussed on a possible genotoxic potential of the flavouring substances or of structurally related substances. Generally, substances for which the Panel has concluded that there is an indication of genotoxic potential *in vitro*, will not be evaluated using the EFSA Procedure until further genotoxicity data are provided. Substances for which a genotoxic potential *in vivo* has been concluded, will not be evaluated through the Procedure.

Appendix B – Specifications

Table B.1: Summary of specifications for flavouring substances in FGE.21Rev6 (for chemical structures, see Appendix F) that are included in the Union List. Substances which are not included in the EU Union list are referenced in FGE.21Rev5 (EFSA CEF Panel, 2015)

Information included in the EU Union list Regulation (EC) No. 1334/2008 as amended			Most recent available specifications data[a]				EFSA comments
FL-no FEMA no CoE no CAS no	**Chemical name**	**Purity of the named compound**	**Phys. form Mol. formula Mol. weight**	**Solubilityc[c] Solubility in ethanol[d]**	**Boiling point, °C[e] Melting point, °C ID test Assay minimum (isomers distribution/ secondary components)**	**Refrac. Index[f] Spec. gravity[g]**	
15.038 – 11589 7533-07-5	2-Acetyl-4-methylthiazole	[b]	Solid C_6H_7NOS 141.19	Practically insoluble or insoluble Freely soluble	95 (16 hPa) 34 MS 95%	n.a. n.a.	
15.039 – – 59303-17-2	2-Acetyl-5-methylthiazole	[b]	Solid C_6H_7NOS 141.19	Practically insoluble or insoluble Freely soluble	93 (16 hPa) 30 MS 95%	n.a. n.a.	
15.040 – 11728 88-15-3	2-Acetylthiophene	[b]	Solid C_6H_6OS 126.17	Practically insoluble or insoluble Freely soluble	213 34 MS 98%	1.563–1.569 1.164–1.171	
15.044 – 11597 37645-61-7	2-Butylthiazole	[b]	Solid $C_7H_{11}NS$ 141.23	Practically insoluble or insoluble Freely soluble	212 79 NMR 95%	n.a. n.a.	
15.045 – – 1455-20-5	2-Butylthiophene	[b]	Liquid $C_8H_{12}S$ 140.24	Practically insoluble or insoluble Freely soluble	182 MS 95%	1.504–1.510 0.951–0.957	

Information included in the EU Union list Regulation (EC) No. 1334/2008 as amended			Most recent available specifications data[a]				EFSA comments
FL-no **FEMA no** **CoE no** **CAS no**	**Chemical name**	**Purity of the named compound**	**Phys. form** **Mol. formula** **Mol. weight**	**Solubilityc[c]** **Solubility in ethanol[d]**	**Boiling point, °C[e]** **Melting point, °C** **ID test** **Assay minimum (isomers distribution/ secondary components)**	**Refrac. Index[f]** **Spec. gravity[g]**	
15.050 – – 41981-71-9	2,5-Diethyl-4-methylthiazole	(b)	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 – – 4276-68-0	2,5-Diethyl-4-propylthiazole	(b)	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 – – 15729-76-7	2,5-Diethylthiazole	(b)	Solid $C_7H_{11}NS$ 141.23	Practically insoluble or insoluble Freely soluble	187 92 MS 95%	n.a. n.a.	
15.054 – – 54717-17-8	Dihydro-2,4,6-triethyl-1,3,5(4H)-dithiazine	(b) Mixture of diastereoisomers ((R/R), (R/S), (S/R) & (S/S))	Solid $C_9H_{19}NS_2$ 205.38	Practically insoluble or insoluble Freely soluble	287 188 MS 95% According to EFFA (2011b) 'there are eventually 4 enantiomers: 2 cis forms and 2 trans forms in the ratio 25/25/25/25.'	n.a. n.a.	Information on the actual percentages of the stereoisomers in the material of commerce is inadequate
15.055 4321 – 116505-60-3	[2S-(2a,4a,8ab)] 2,4-Dimethyl(4H)pyrrolidino [1,2e]-1,3,5-dithiazine	(b)	Solid $C_8H_{15}NS_2$ 189.34	Practically insoluble or insoluble Freely soluble	235 130 MS 95%	n.a. n.a.	JECFA: 1763 (JECFA, 2007) The name should be changed to [2S-(2α,4α,8αβ)]-2,4-Dimethyl(4H)pyrrolidino [1,2e]-1,3,5-dithiazine

Information included in the EU Union list Regulation (EC) No. 1334/2008 as amended			Most recent available specifications data[a]				EFSA comments
FL-no **FEMA no** **CoE no** **CAS no**	**Chemical name**	**Purity of the named compound**	**Phys. form** **Mol. formula** **Mol. weight**	**Solubilityc[c]** **Solubility in ethanol[d]**	**Boiling point, °C[e]** **Melting point, °C** **ID test** **Assay minimum (isomers distribution/ secondary components)**	**Refrac. Index[f]** **Spec. gravity[g]**	
15.057 3782 – 104691-40-9	4,6-Dimethyl-2-(1-methylethyl)dihydro-1,3,5-dithiazine	At least 44% isopropyl-4,6-dimethyl and 27% 4-isopropyl-2,6-dimethyl; secondary components at least 24% 2,4,6-trimethyldihydro-1,3,5-dithiazine; 6-methyl-2,4-diisopropyl-1,3,5-dithiazine; 4-methyl-2,6-diisopropyl-1,3,5-dithiazine; 2,4,6-triisopropyl-dihydro-1,3,5-dithiazine	Liquid $C_8H_{17}NS_2$ 191.36	Slightly soluble Soluble	109 (0.23 hPa) MS At least 44% According to EFFA (2013b): '8 isomers each of them with 12.5% chance'	1.496–1.500 0.951–0.959	Information on the actual percentages of the stereoisomers in the material of commerce is inadequate. Proposal to correct the text in the Union List as follows: At least 44% 2-isopropyl-4,6-dimethyl; secondary components: at least 27% 4-isopropyl-2,6-dimethyl, 24% 2,4,6-trimethyldihydro-1,3,5-dithiazine, 6-methyl-2,4-diisopropyl-1,3,5-dithiazine, 4-methyl-2,6-diisopropyl-1,3,5-dithiazine, 2,4,6-triisopropyl-dihydro-1,3,5-dithiazine
15.058 – – 873–64-3	4,5-Dimethyl-2-ethylthiazole	(b)	Solid $C_7H_{11}NS$ 141.23	Practically insoluble or insoluble Freely soluble	185 104 MS 96%	n.a. n.a.	
15.060 – – 60755-05-7	2,4-Dimethyl-3-thiazoline	(b)	Solid C_5H_9NS 115.19	Practically insoluble or insoluble Freely soluble	52 (15 hPa) 102 MS 95% (racemate, EFFA, 2010)	n.a. n.a.	

Information included in the EU Union list Regulation (EC) No. 1334/2008 as amended			Most recent available specifications data[a]				EFSA comments
FL-no FEMA no CoE no CAS no	**Chemical name**	**Purity of the named compound**	**Phys. form Mol. formula Mol. weight**	**Solubilityc[c] Solubility in ethanol[d]**	**Boiling point, °C[e] Melting point, °C ID test Assay minimum (isomers distribution/ secondary components)**	**Refrac. Index[f] Spec. gravity[g]**	
15.061 – – 32272-57-4	2,5-Dimethyl-4-ethylthiazole	(b)	Solid $C_7H_{11}NS$ 141.23	Practically insoluble or insoluble Freely soluble	185 104 MS 95%	n.a. n.a.	
15.062 – 11605 541-58-2	2,4-Dimethylthiazole	(b)	Solid C_5H_7NS 113.18	Practically insoluble or insoluble Freely soluble	145 69 MS 95%	n.a. n.a.	
15.063 – – 4175-66-0	2,5-Dimethylthiazole	(b)	Solid C_5H_7NS 113.18	Practically insoluble or insoluble Freely soluble	152 69 MS 95%	n.a. n.a.	JECFA: 1758 (JECFA, 2007)
15.067 – – 32272-48-3	4-Ethyl-2-methylthiazole	(b)	Solid C_6H_9NS 127.20	Practically insoluble or insoluble Freely soluble	91 (89 hPa) 80 MS 95%	n.a. n.a.	
15.068 – – 19961-52-5	5-Ethyl-2-methylthiazole	(b)	Solid C_6H_9NS 127.20	Practically insoluble or insoluble Freely soluble	170 80 MS 95%	n.a. n.a.	
15.069 – – 52414-91-2	4-Ethyl-5-methylthiazole	(b)	Solid C_6H_9NS 127.20	Practically insoluble or insoluble Freely soluble	174 80 MS 95%	n.a. n.a.	
15.071 – – 15679-09-1	2-Ethylthiazole	(b)	Liquid C_5H_7NS 113.18	Practically insoluble or insoluble Freely soluble	148 MS 95%	1.511–1.517 1.058–1.064	

Information included in the EU Union list Regulation (EC) No. 1334/2008 as amended			Most recent available specifications data[a]				EFSA comments
FL-no **FEMA no** **CoE no** **CAS no**	**Chemical name**	**Purity of the named compound**	**Phys. form** **Mol. formula** **Mol. weight**	**Solubilityc[c]** **Solubility in ethanol[d]**	**Boiling point, °C[e]** **Melting point, °C** **ID test** **Assay minimum (isomers distribution/ secondary components)**	**Refrac. Index[f]** **Spec. gravity[g]**	
15.074 – – 36880–33-8	5-Ethylthiophene-2-carbaldehyde	(b)	Solid C_7H_8OS 140.20	Practically insoluble or insoluble Freely soluble	104 (12 hPa) 62 MS 95%	n.a. n.a.	
15.076 4137 11616 18794-77-9	2-Hexylthiophene	(b)	Solid $C_{10}H_{16}S$ 168.30	Practically insoluble or insoluble Freely soluble	92 (13 hPa) 41 MS 95%	n.a. n.a.	JECFA: 1764 (JECFA, 2007)
15.078 – 11617 53498-32-1	2-Isobutyl-4,5-dimethylthiazole	(b)	Solid $C_9H_{15}NS$ 169.29	Practically insoluble or insoluble Freely soluble	267 112 MS 97%	n.a. n.a.	
15.079 3781 – 101517-87-7	2-Isobutyldihydro-4,6-dimethyl-1,3,5-dithiazine	At least 64% 2-isobutyl-4,6-dimethyl and 18% 4-isobutyl-2,6-dimethyl; secondary components at least 13% 2,4,6-trimethyl-1,3,5-dithiazine; 2,4-diisobutyl-6-methyl-1,3,5-dithiazine; 2,6-dimethyl-4-butyldihydro-1,3,5-dithiazine; substituted 1,3,5-thiadiazine	Liquid $C_9H_{19}NS_2$ 205.39	Slightly soluble Soluble	115 (0.33 hPa) MS At least 64% According to EFFA (2013b): '8 isomers each of them with 12.5% chance'	1.488–1.492 0.961–0.967	Information on the actual percentages of the stereoisomers in the material of commerce is inadequate. Proposal to correct the text in the Union List as follows: At least 64% 2-isobutyl-4,6-dimethyl; secondary components: 18% 4-isobutyl-2,6-dimethyl; at least 13% 2,4,6-trimethyl-1,3,5-dithiazine, 2,4-diisobutyl-6-methyl-1,3,5-dithiazine, 2,6-dimethyl-4-butyldihydro-1,3,5-dithiazine, substituted 1,3,5-thiadiazine

Information included in the EU Union list Regulation (EC) No. 1334/2008 as amended			Most recent available specifications data[a]				EFSA comments
FL-no **FEMA no** **CoE no** **CAS no**	**Chemical name**	**Purity of the named compound**	**Phys. form** **Mol. formula** **Mol. weight**	**Solubilityc[c]** **Solubility in ethanol[d]**	**Boiling point, °C[e]** **Melting point, °C** **ID test** **Assay minimum (isomers distribution/ secondary components)**	**Refrac. Index[f]** **Spec. gravity[g]**	
15.080 – – 53498-30-9	2-Isopropyl-4,5-dimethylthiazole	(b)	Solid $C_8H_{13}NS$ 155.26	Practically insoluble or insoluble Freely soluble	244 101 MS 95%	n.a. n.a.	
15.082 – – 7774-73-4	3-Mercaptothiophene	(b)	Liquid $C_4H_4S_2$ 116.20	Slightly soluble Freely soluble	171 MS 95%	1.617–1.623 1.248–1.254	
15.084 – – 86290-21-3	5-Methyl-2-pentylthiazole	(b)	Solid $C_9H_{15}NS$ 169.26	Practically insoluble or insoluble Freely soluble	262 114 NMR 95%	n.a. n.a.	
15.085 – 11622 13679-83-9	4-Methyl-2-propionylthiazole	(b)	Solid C_7H_9NOS 155.21	Practically insoluble or insoluble Freely soluble	86 (12 hPa) 142 NMR 95%	n.a. n.a.	
15.086 – – 2346-00-1	2-Methyl-2-thiazoline	(b)	Solid C_4H_7NS 101.17	Slightly soluble Freely soluble	144 62 MS 95%	n.a. n.a.	
15.087 – – 2527-76-6	2-Methyl-3-mercaptothiophene	(b)	Solid $C_5H_6S_2$ 130.22	Slightly soluble Freely soluble	73 (15 hPa) 27 MS 95%	n.a. n.a.	
15.089 – 11626 3581-87-1	2-Methylthiazole	(b)	Liquid C_4H_5NS 99.15	Slightly soluble Freely soluble	128 MS 95%	1.511–1.517 1.109–1.116	

Information included in the EU Union list Regulation (EC) No. 1334/2008 as amended			Most recent available specifications data[a]				EFSA comments
FL-no **FEMA no** **CoE no** **CAS no**	**Chemical name**	**Purity of the named compound**	**Phys. form** **Mol. formula** **Mol. weight**	**Solubilityc[c]** **Solubility in ethanol[d]**	**Boiling point, °C[e]** **Melting point, °C** **ID test** **Assay minimum (isomers distribution/ secondary components)**	**Refrac. Index[f]** **Spec. gravity[g]**	
15.093 – – 880-36-4	2-Octylthiophene	[b]	Solid $C_{12}H_{20}S$ 196.35	Practically insoluble or insoluble Freely soluble	259 64 MS 95%	n.a. n.a.	
15.096 4387 11634 4861-58-9	2-Pentylthiophene	[b]	Liquid $C_9H_{14}S$ 154.27	Practically insoluble or insoluble Freely soluble	201 MS 95%	1.495–1.501 0.940–0.946	JECFA: 2106 (JECFA, 2012)
15.097 – 11635 13679-75-9	2-Propionylthiophene	[b]	Solid C_7H_8OS 140.20	Practically insoluble or insoluble Freely soluble	225 57 MS 95%	n.a. n.a.	
15.098 – – 17626-75-4	2-Propylthiazole	[b]	Solid C_6H_9NS 127.20	Practically insoluble or insoluble Freely soluble	172 78 MS 95%	n.a. n.a.	
15.108 – – 6258-63-5	2-Thiophenemethanethiol	[b]	Liquid $C_5H_6S_2$ 130.22	Slightly soluble Freely soluble	82 (16 hPa) MS 95%	1.571–1.578 1.165–1.171	
15.115 – – 61323–24-8	2-Isobutyl-4-methyl thiazole	[b]	Solid $C_8H_{13}NS$ 155.26	Slightly soluble Freely soluble	189 88 MS 95%	n.a. n.a.	

Information included in the EU Union list Regulation (EC) No. 1334/2008 as amended			Most recent available specifications data[a]				EFSA comments
FL-no **FEMA no** **CoE no** **CAS no**	**Chemical name**	**Purity of the named compound**	**Phys. form** **Mol. formula** **Mol. weight**	**Solubilityc[c]** **Solubility in ethanol[d]**	**Boiling point, °C[e]** **Melting point, °C** **ID test** **Assay minimum (isomers distribution/ secondary components)**	**Refrac. Index[f]** **Spec. gravity[g]**	
15.116 – – 233665-91-3	2-Acetyl-4-ethylthiazole	(b)	Solid C_7H_9NOS 155.22	Slightly soluble Freely soluble	270 142 NMR 95%	n.a. n.a.	
15.118 – – 53833-33-3	4-Butylthiazole	(b)	Solid $C_7H_{11}NS$ 141.23	Practically insoluble or insoluble Freely soluble	212 79 MS 95%	n.a. n.a.	
15.119 – – 39800-92-5	2-Isobutyl-3-thiazoline	(b)	Solid $C_7H_{13}NS$ 143.25	Very slightly soluble Freely soluble	197 80 MS 95% (racemate EFFA, 2011b)	n.a. n.a.	
15.135 4667 – 54717-14-5	Ethyl thialdine	At least 90%, secondary components less than 5% 3,5- diethyl-1,2,4-trithiolane, less than 2% thialdine, less than 3% other impurities	Liquid $C_7H_{15}NS_2$ 177.33	Poorly soluble Soluble	75 IR NMR MS 90% According to EFFA 'only 4 isomers' (EFFA, 2013b)	1.5344–1.5544 1.0745–1.0765	Information on the actual percentages of the stereoisomers in the material of commerce is inadequate.

CAS: Chemical Abstract Service; CoE: Council of Europe; FEMA: Flavor and Extract Manufacturers Association; FL-No: FLAVIS number; ID: identity; IR: infrared spectroscopy; n.a.: not applicable; NMR: nuclear magnetic resonance; MS: mass spectrometry.
(a): Documentation provided to EFSA: EFFA (2010, 2011b, 2013b).
(b): At least 95% unless otherwise specified.
(c): Solubility in water, if not otherwise stated.
(d): Solubility in 95% ethanol, if not otherwise stated.
(e): At 1013.25 hPa, if not otherwise stated.
(f): At 20°C, if not otherwise stated.
(g): At 25°C, if not otherwise stated.

Appendix C – Exposure estimate

C.1. Normal and maximum use levels

For each of the 18 food categories (Table C.1) in which the candidate substances are used, Flavour Industry reports a 'normal use level' and a 'maximum use level'. 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). Based on the reported uses and use levels provided by industry, the distribution of 39 flavouring substances in the 18 food categories is summarised in Table C.2.

Table C.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 (incl. mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds
05.0	Confectionery
06.0	Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & 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, excl. dairy products
14.2	Alcoholic beverages, incl. 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

Table C.2: Food categories in which 39 substances in FGE.21Rev6 are used

Food category	Description	Flavourings used[(a)]
01.0	Dairy products, excluding products of category 2	39
02.0	Fats and oils, and fat emulsions (type water-in-oil)	39 except [FL-no: 15.060, 15.119]
03.0	Edible ices, including sherbet and sorbet	39 except [FL-no: 15.135, 15.060, 15.119]
04.1	Processed fruits	39 except [FL-no: 15.060, 15.119]
04.2	Processed vegetables (incl. mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds	Only [FL-no: 15.062 and 15.135]
05.0	Confectionery	39 except [FL-no: 15.062 and 15.135]
06.0	Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery	39
07.0	Bakery wares	39
08.0	Meat and meat products, including poultry and game	39

Food category	Description	Flavourings used[(a)]
09.0	Fish and fish products, including molluscs, crustaceans and echinoderms	39 except [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.	39 except [FL-no: 15.089]
13.0	Foodstuffs intended for particular nutritional uses	39 except [FL-no: 15.060, 15.199, 15.135]
14.1	Non-alcoholic ('soft') beverages, excl. dairy products	39 except [FL-no: 15.135]
14.2	Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts	39 except [FL-no: 15.060, 15.062, 15.119 and 15.135]
15.0	Ready-to-eat savouries	39 except [FL-no: 15.068]
16.0	Composite foods (e.g. casseroles, meat pies, mincemeat) – foods that could not be placed in categories 1–15	39 except [FL-no: 15.060, 15.119, 15.135]

(a): No use levels are available for [FL-no: 15.057 and 15.079].

The 'normal and maximum use levels' for the candidate substances in the present flavouring group, for which use levels have been provided by Industry, are shown in Table C.3.

Table C.3: Normal and maximum use levels (mg/kg) for the candidate substances in FGE.21Rev6

FL-no		Food categories																
		Normal use levels (mg/kg)[a],[b]																
		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	1.5 2	– –	– –	– –	– –	1 2.8	1 1	1.75 3.77	0.45 1.92	0.1 0.4	– –	– –	1 1.5	– –	1 1.3	– –	0.04 0.23	– –
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

FL-no		Food categories																
		Normal use levels (mg/kg)[a],[b]																
		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
15.067	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
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

FL-no		Food categories																
		Normal use levels (mg/kg)[(a),(b)]																
		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
15.096	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
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	1.5 2	– –	– –	– –	– –	1 2.8	1 1	1.75 3.77	0.45 1.92	0.1 0.4	– –	– –	1 1.5	– –	1 1.3	– –	0.04 0.23	– –
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	– –

FL-No: FLAVIS number; '– ' no value for normal or maximum use level was provided.
(a): 'Normal use' is defined as the average of reported usages and 'maximum use' is defined as the 95th percentile of reported usages (EFFA, 2002).
(b): 'normal and maximum use levels' provided by industry for 39 of the 41 candidate substances in the present flavouring group (EFFA, 2004c,d, 2007, 2022; Flavour Industry, 2004–2005, 2010).

C.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 C.4. These consumption estimates are then multiplied by the reported use levels in the different food categories and summed up.

Table C.4: 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 C.5):

- 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 C.5: Distribution of the 18 food categories listed in Commission Regulation (EC) No 1565/2000 into the seven SCF food categories used for TAMDI calculation (SCF, 1995)

Key	Food categories according to Commission Regulation 1565/2000	Distribution of the seven SCF food categories		
	Food category	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		
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		

Key	Food categories according to Commission Regulation 1565/2000	Distribution of the seven SCF food categories		
	Food category	Food	Beverages	Exceptions
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 C.6) are reported for 39 candidate substances in the present flavouring group, for which industry has provided use and use levels (EFFA, 2004c,d, 2007, 2022; Flavour Industry, 2004–2005, 2010). No use levels are available for [FL-no: 15.057 and 15.079].

The MSDI values for the 41 candidate substances are reported in Table C.6.

Table C.6: Estimated intakes based on the MSDI approach and the mTAMDI approach

FL-no	EU Union List name	MSDI – EU (μg/*capita* per day)	mTAMDI (μg/person per day)	Structural class	TTC (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

FL-no	EU Union List name	MSDI – EU (μg/*capita* per day)	mTAMDI (μg/person per day)	Structural class	TTC (μg/person per day)
15.118	4-Butylthiazole	1.3	160	Class II	540
15.060	2,4-Dimethyl-3-thiazoline	0.012	605	Class III	90
15.119	2-Isobutyl-3-thiazoline	0.012	605	Class III	90
15.054	Dihydro-2,4,6-triethyl-1,3,5(4H)-dithiazine	0.85	160	Class III	90
15.055	[2S-(2a,4a,8ab)] 2,4-Dimethyl(4H)pyrrolidino[1,2e]-1,3, 5-dithiazine	0.012	160	Class III	90
15.057	4,6-Dimethyl-2-(1-methylethyl)dihydro-1,3,5-dithiazine	1.6	n.a.	Class III	90
15.079	2-Isobutyldihydro-4,6-dimethyl-1,3,5-dithiazine	5.7	n.a.	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

FL-No: FLAVIS number; MSDI: maximised survey-derived daily intake; mTAMDI: modified theoretical added maximum daily intake; n.a.: not available.

Appendix D – Genotoxicity data considered in FGE.21Rev3

In FGE.21Rev6, only genotoxicity data relevant for subgroup B-II are reported. Data relevant for other subgroups can be retrieved in FGE.21Rev5 (EFSA CEF Panel, 2015).

Table D.1: *In vitro* genotoxicity data considered in FGE.21Rev3 for subgroup B-III (EFSA CEF Panel, 2012)

Chemical name[(a)]	Test system	Test object	Concentration	Result	Reference	Comments
Subgroup B-III						
2-Propylthiazolidine [15.099][(b)]	Ames assay	S. Typhimurium TA98, TA100	1, 10, 100 μg/ml	1 and 10 μg/ml: positive in TA100 (±S9); 100 μg/ml: positive in TA98 and TA100 (±S9)	Mihara and Shibamoto (1980)	The results were stated to be positive; however, the magnitude and a positive dose effect relationship could not be assessed (no numbers are given).
2-Methylthiazolidine [15.090][(b)]	Ames assay	S. Typhimurium TA98, TA100	1, 10, 100 μg/ml	1 and 10 μg/ml: positive in TA100 (±S9); 100 μg/ml: positive in TA98 and TA100 (±S9)	Mihara and Shibamoto (1980)	The results were stated to be positive; however, the magnitude and a positive dose effect relationship could not be assessed (no numbers are given).
(2-Ethylthiazolidine)	Ames assay	*S. typhimurium* TA98, TA100	1, 10, 100 μg/ml	1 μg/ml: positive in TA100 (±S9) and TA98 (-S9); 10 μg/ml: positive in TA100 (±S9); 100 μg/ml: positive in TA98 and TA100 (±S9)	Mihara and Shibamoto (1980)	The results were stated to be positive; however, the magnitude and a positive dose effect relationship could not be assessed (no numbers are given).
(2-Isopropylthiazolidine)	Ames assay	*S. typhimurium* TA98, TA100	1, 10, 100 μg/ml	1 and 10 μg/ml: positive in TA100 (±S9); 100 μg/ml: positive in TA100 (±S9) and TA98 (−S9)	Mihara and Shibamoto (1980)	The results were stated to be positive; however, the magnitude and a positive dose effect relationship could not be assessed (no numbers are given).
(2-Butylthiazolidine)	Ames assay	S. Typhimurium TA98, TA100	1, 10, 100 μg/ml	1 μg/ml: positive in TA100 (+S9); 10 μg/ml: positive in TA100 (±S9); 100 μg/ml: positive in TA100 (±S9) and TA98 (−S9)	Mihara and Shibamoto (1980)	The results were stated to be positive; however, the magnitude and a positive dose effect relationship could not be assessed (no numbers are given).
(2-Isobutylthiazolidine)	Ames assay	*S. typhimurium* TA98, TA100	1, 10, 100 μg/ml	1 μg/ml: positive in TA98 and TA100 (+S9); 10 μg/ml: positive in TA98 and TA100 (±S9); 100 μg/ml: positive in TA98 and TA100 (±S9)	Mihara and Shibamoto (1980)	The results were stated to be positive; however, the magnitude and a positive dose effect relationship could not be assessed (no numbers are given).

(a): Substances in brackets are structurally related substances for subgroup B-III in FGE.21. They have no FL-no and are not authorised for use as flavouring substances in EU. These substances have been studied for genotoxicity in the same publication as [FL-no: 15.090 and 15.099]. Since also for these structurally related substances genotoxicity was observed, they supported the concern for genotoxicity raised for [FL-no: 15.090 and 15.099] already in FGE.21 (EFSA, 2007).

(b): The use of 2-methylthiazolidine [FL-no: 15.090] and 2-propylthiazolidine [FL-no: 15.099] as flavouring substances in Europe was no longer of interest for Industry and no further data were provided. Therefore, these substances were not included in the Union List, i.e. 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.

Appendix E – Genotoxicity studies on supporting substance from FGE.76Rev2

Table E.1: Summary of *in vitro* genotoxicity data for [FL-no: 15.032] evaluated in FGE.76Rev2

Chemical Name [FL-no]	Test system	Test object	Concentrations of substance and test conditions	Result	Reference	Comments
4,5-Dimethyl-2-isobutyl-3-thiazoline [15.032]	Reverse bacterial mutation assay	Experiment 1: S. Typhimurium (TA98, TA100, TA1535, TA1537 and TA102)	5–5,000 μg/plate[a, b, c]	Negative	Covance (2012)	Reliable without restrictions. Study performed in compliance with GLP and OECD TG 471.
		Experiment 2: TA100	78.13–5,000 μg/plate[a, c] 156.3–5,000 μg/plate[b, d]			
		TA100				
		TA98, TA1535, TA1537 and TA102	156.3–5,000 μg/plate [a, c), (b, d]			
		Experiment 3: TA98, TA100, TA1535, TA1537 and TA102	39–1250 μg/plate[b, d]			
	Micronucleus assay	Human blood lymphocytes	4, 8, 9 μg/mL[e] 10, 20, 60, 80 μg/mL[f] 40, 160, 210 μg/mL[g]	Negative Positive[f] Positive[g]	Covance (2013)	Reliable without restrictions. Study performed in compliance with GLP and OECD TG 487. The given concentrations are those for the cultures that were scored for micronuclei.
	Micronucleus assay	Mammalian TK6 cells	5, 15, 25 μg/mL[h] 5, 50, 100 μg/mL[i] 25, 125, 150 μg/mL[j]	Negative	BioReliance (2018)	Reliable with restrictions. Study performed in compliance with GLP and OECD TG 487. The given concentrations are those for the cultures that were scored for micronuclei.
	Micronucleus assay with FISH analysis	Human blood lymphocytes	129, 196, 228 μg/mL[k] 33.9, 70.9, 97.2 μg/mL[e] 222, 263, 272 μg/mL[l]	Positive	Charles River Laboratories (2020)	Reliable without restrictions. Study performed in compliance with GLP and OECD TG 487. FISH analysis indicates that 4,5-dimethyl-2-isobutyl-3-thiazoline induced MN via an aneugenic mechanism. The given concentrations are those for the cultures that were scored for micronuclei.

FL-No: FLAVIS number.
(a): Plate incorporation method.
(b): With S9-mix.
(c): Without S9-mix.

(d): Pre-incubation method.
(e): 24-h treatment without S9-mix.
(f): 3 + 21-h treatment without S9-mix.
(g): 3 + 21-h treatment with S9-mix.
(h): 27-h treatment without S9-mix.
(i): 4 + 23-h treatment without S9-mix.
(j): 4 + 23-h treatment with S9-mix.
(k): 4 + 20-h treatment without S9-mix.
(l): 4 + 20-h treatment with S9-mix.

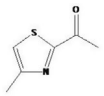
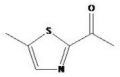
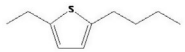
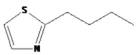
Table E.2: Summary of *in vivo* genotoxicity data for [FL-no: 15.032] evaluated in FGE.76Rev2

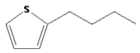
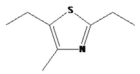
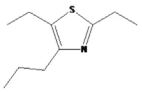
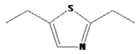
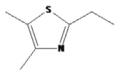
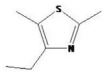
Chemical name [FL-no]	Test system	Test object	Route	Dose, mg/kg bw per day	Result	Reference	Comments
4,5-Dimethyl-2-isobutyl-3-thiazoline [15.032]	Micronucleus assay (bone marrow)	Han Wistar Rat; M	Gavage	87.5, 175 and 350 mg/kg bw per day[(a)]	Inconclusive (negative, but insufficient evidence of bone marrow exposure)	Covance (2014, 2015)	Reliable with restrictions. Study performed in compliance with GLP and OECD TG 474.
	Comet assay (liver and duodenum)	Han Wistar Rat; M	Gavage		Negative		Reliable without restrictions. The study was performed in compliance with recommendations of the Comet and IWGT workshop, Japanese Center for the Validation of Alternative Methods (JaCVAM) and current literature.

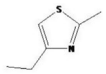
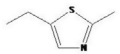
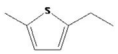
FL-No: FLAVIS number; M: Male.
(a): Administered via gavage, 3 doses at times 0, 24 and 45 h with sacrifice and harvest at 48 h.

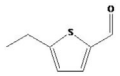
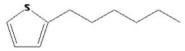
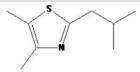
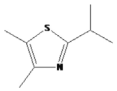
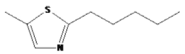
Appendix F – Summary of safety evaluations for flavouring substances in FGE.21Rev6

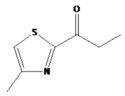
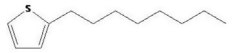
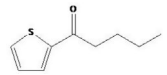
Table F.1: Summary of Safety Evaluation Applying the Procedure for substances in FGE.21Rev6 (based on intakes calculated by the MSDI approach)

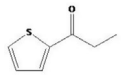
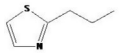
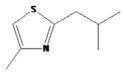
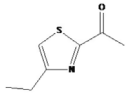
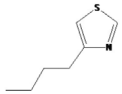
FL-no	EU Union List chemical name	Structural formula	MSDI[a] (μg/*capita* per day)	Class[b] Evaluation procedure path[c] Outcome on the named compound and on the material of commerce	EFSA comments
15.037	2-Acetyl-3-methylthiophene		0.18	Class II B3: Intake below threshold, B4: No adequate NOAEL	No longer of interest for Industry for use as flavouring substances in Europe (DG SANCO, 2012). No further data were submitted.
15.038	2-Acetyl-4-methylthiazole		0.0049	Class II B3: Intake below threshold, B4: Adequate NOAEL exists No safety concern based on intakes calculated by the MSDI approach	Concluded in FGE.21
15.039	2-Acetyl-5-methylthiazole		0.0024	Class II B3: Intake below threshold, B4: Adequate NOAEL exists No safety concern based on intakes calculated by the MSDI approach	Concluded in FGE.21
15.040	2-Acetylthiophene		0.049	Class II B3: Intake below threshold, B4: Adequate NOAEL exists No safety concern based on intakes calculated by the MSDI approach	Concluded in FGE.21Rev5
15.043	2-Butyl-5-ethylthiophene		0.0012	Class II B3: Intake below threshold, B4: No adequate NOAEL	No longer of interest for Industry for use as flavouring substances in Europe (DG SANCO, 2012). No further data were submitted.
15.044	2-Butylthiazole		0.011	Class II B3: Intake below threshold, B4: Adequate NOAEL exists No safety concern based on intakes calculated by the MSDI approach	Concluded in FGE.21

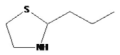
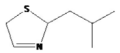
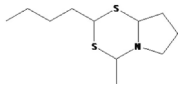
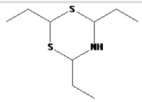
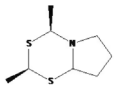
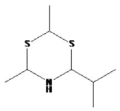
FL-no	EU Union List chemical name	Structural formula	MSDI[(a)] (μg/*capita* per day)	Class[(b)] Evaluation procedure path[(c)] Outcome on the named compound and on the material of commerce	EFSA comments
15.045	2-Butylthiophene		0.012	Class II B3: Intake below threshold, B4: Adequate NOAEL exists No safety concern based on intakes calculated by the MSDI approach	Concluded in FGE.21Rev5
15.050	2,5-Diethyl-4-methylthiazole		0.012	Class II B3: Intake below threshold, B4: Adequate NOAEL exists No safety concern based on intakes calculated by the MSDI approach	Concluded in FGE.21
15.051	2,5-Diethyl-4-propylthiazole		0.0012	Class II B3: Intake below threshold, B4: Adequate NOAEL exists No safety concern based on intakes calculated by the MSDI approach	Concluded in FGE.21
15.052	2,5-Diethylthiazole		0.015	Class II B3: Intake below threshold, B4: Adequate NOAEL exists No safety concern based on intakes calculated by the MSDI approach	Concluded in FGE.21
15.058	4,5-Dimethyl-2-ethylthiazole		0.015	Class II B3: Intake below threshold, B4: Adequate NOAEL exists No safety concern based on intakes calculated by the MSDI approach	Concluded in FGE.21
15.061	2,5-Dimethyl-4-ethylthiazole		0.011	Class II B3: Intake below threshold, B4: Adequate NOAEL exists No safety concern based on intakes calculated by the MSDI approach	Concluded in FGE.21

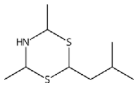
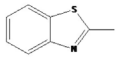
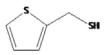
FL-no	EU Union List chemical name	Structural formula	MSDI[a] (μg/*capita* per day)	Class[b] Evaluation procedure path[c] Outcome on the named compound and on the material of commerce	EFSA comments
15.062	2,4-Dimethylthiazole		0.61	Class II B3: Intake below threshold, B4: Adequate NOAEL exists No safety concern based on intakes calculated by the MSDI approach	Concluded in FGE.21
15.063	2,5-Dimethylthiazole		0.0061	Class II B3: Intake below threshold, B4: Adequate NOAEL exists No safety concern based on intakes calculated by the MSDI approach	Concluded in FGE.21 JECFA: 1758 (JECFA, 2007)
15.064	2,5-Dimethylthiophene		0.23	Class II B3: Intake below threshold, B4: No adequate NOAEL	No longer of interest for Industry for use as flavouring substances in Europe (DG SANCO, 2013). No further data were submitted.
15.067	4-Ethyl-2-methylthiazole		0.0037	Class II B3: Intake below threshold, B4: Adequate NOAEL exists No safety concern based on intakes calculated by the MSDI approach	Concluded in FGE.21
15.068	5-Ethyl-2-methylthiazole		0.0061	Class II B3: Intake below threshold, B4: Adequate NOAEL exists No safety concern based on intakes calculated by the MSDI approach	Concluded in FGE.21
15.069	4-Ethyl-5-methylthiazole		0.012	Class II B3: Intake below threshold, B4: Adequate NOAEL exists No safety concern based on intakes calculated by the MSDI approach	Concluded in FGE.21
15.070	2-Ethyl-5-methylthiophene		0.061	Class II B3: Intake below threshold, B4: No adequate NOAEL	No longer of interest for Industry for use as flavouring substances in Europe (DG SANCO, 2012). No further data were submitted.

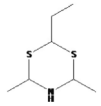
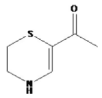
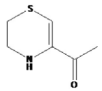
FL-no	EU Union List chemical name	Structural formula	MSDI[(a)] (μg/*capita* per day)	Class[(b)] Evaluation procedure path[(c)] Outcome on the named compound and on the material of commerce	EFSA comments
15.071	2-Ethylthiazole		0.028	Class II B3: Intake below threshold, B4: Adequate NOAEL exists No safety concern based on intakes calculated by the MSDI approach	Concluded in FGE.21
15.072	2-Ethylthiophene		0.0012	Class II B3: Intake below threshold, B4: No adequate NOAEL	No longer of interest for Industry for use as flavouring substances in Europe (DG SANCO, 2013). No further data were submitted.
15.074	5-Ethylthiophene-2-carbaldehyde		0.037	Class II B3: Intake below threshold, B4: Adequate NOAEL exists No safety concern based on intakes calculated by the MSDI approach	Concluded in FGE.21Rev5
15.076	2-Hexylthiophene		0.46	Class II B3: Intake below threshold, B4: Adequate NOAEL exists No safety concern based on intakes calculated by the MSDI approach	Concluded in FGE.21Rev5 JECFA: 1764 (JECFA, 2007)
15.078	2-Isobutyl-4,5-dimethylthiazole		0.12	Class II B3: Intake below threshold, B4: Adequate NOAEL exists No safety concern based on intakes calculated by the MSDI approach	Concluded in FGE.21
15.080	2-Isopropyl-4,5-dimethylthiazole		0.012	Class II B3: Intake below threshold, B4: Adequate NOAEL exists No safety concern based on intakes calculated by the MSDI approach	Concluded in FGE.21
15.084	5-Methyl-2-pentylthiazole		0.0037	Class II B3: Intake below threshold, B4: Adequate NOAEL exists No safety concern based on intakes calculated by the MSDI approach	Concluded in FGE.21

FL-no	EU Union List chemical name	Structural formula	MSDI[(a)] (μg/*capita* per day)	Class[(b)] Evaluation procedure path[(c)] Outcome on the named compound and on the material of commerce	EFSA comments
15.085	4-Methyl-2-propionylthiazole		0.0037	Class II B3: Intake below threshold, B4: Adequate NOAEL exists No safety concern based on intakes calculated by the MSDI approach	Concluded in FGE.21
15.086	2-Methyl-2-thiazoline		0.012	Class II B3: Intake below threshold, B4: Adequate NOAEL exists No safety concern based on intakes calculated by the MSDI approach	Concluded in FGE.21Rev4
15.089	2-Methylthiazole		0.018	Class II B3: Intake below threshold, B4: Adequate NOAEL exists No safety concern based on intakes calculated by the MSDI approach	Concluded in FGE.21
15.091	2-Methylthiophene		0.019	Class II B3: Intake below threshold, B4: No adequate NOAEL	No longer of interest for Industry for use as flavouring substances in Europe (DG SANCO, 2013). No further data were submitted.
15.092	3-Methylthiophene		0.12	Class II B3: Intake below threshold, B4: No adequate NOAEL	No longer of interest for Industry for use as flavouring substances in Europe (DG SANCO, 2013). No further data were submitted.
15.093	2-Octylthiophene		0.012	Class II B3: Intake below threshold, B4: Adequate NOAEL exists No safety concern based on intakes calculated by the MSDI approach	Concluded in FGE.21Rev5
15.094	2-Pentanoylthiophene		0.0012	Class II B3: Intake below threshold, B4: No adequate NOAEL	No longer of interest for Industry for use as flavouring substances in Europe (DG SANCO, 2012). No further data were submitted.
15.096	2-Pentylthiophene		1.6	Class II B3: Intake below threshold, B4: Adequate NOAEL exists No safety concern based on intakes calculated by the MSDI approach	Concluded in FGE.21Rev5 JECFA: 2106 (JECFA, 2012)

FL-no	EU Union List chemical name	Structural formula	MSDI[(a)] (μg/*capita* per day)	Class[(b)] Evaluation procedure path[(c)] Outcome on the named compound and on the material of commerce	EFSA comments
15.097	2-Propionylthiophene		0.012	Class II B3: Intake below threshold, B4: Adequate NOAEL exists No safety concern based on intakes calculated by the MSDI approach	Concluded in FGE.21Rev5
15.098	2-Propylthiazole		0.085	Class II B3: Intake below threshold, B4: Adequate NOAEL exists No safety concern based on intakes calculated by the MSDI approach	Concluded in FGE.21
15.107	Thiophene-2-carbaldehyde		0.21	Class II B3: Intake below threshold, B4: No adequate NOAEL	No longer of interest for Industry for use as flavouring substances in Europe (DG SANCO, 2012). No further data were submitted.
15.115	2-Isobutyl-4-methyl thiazole		0.011	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	Concluded in FGE.21
15.116	2-Acetyl-4-ethylthiazole		0.024	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	Concluded in FGE.21
15.118	4-Butylthiazole		1.3	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	Concluded in FGE.21
15.129	Tetrahydro-2,4,6-trimethyl-1,3,5(2H)-thiadiazine		0.61	Class II B3: Intake below threshold, B4: No adequate NOAEL	No longer of interest for Industry for use as flavouring substances in Europe (DG SANCO, 2012). No further data were submitted.
15.060	2,4-Dimethyl-3-thiazoline		0.012	Class III No evaluation	Genotoxicity data required
15.090	2-Methylthiazolidine		0.024	Class II No evaluation	No longer of interest for Industry for use as flavouring substances in Europe (DG SANCO, 2012). No further data were submitted.

FL-no	EU Union List chemical name	Structural formula	MSDI[a] (μg/*capita* per day)	Class[b] Evaluation procedure path[c] Outcome on the named compound and on the material of commerce	EFSA comments
15.099	2-Propylthiazolidine		0.012	Class II No evaluation	No longer of interest for Industry for use as flavouring substances in Europe (DG SANCO, 2012). No further data were submitted.
15.119	2-Isobutyl-3-thiazoline		0.012	Class III No evaluation	Genotoxicity data required
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	No longer of interest for Industry for use as flavouring substances in Europe (DG SANCO, 2012). No further data were submitted.
15.054	Dihydro-2,4,6-triethyl-1,3,5 (4H)-dithiazine		0.85	Class III B3: Intake below threshold, B4: Adequate NOAEL exists No safety concern based on intakes calculated by the MSDI approach. Information on the stereochemical composition of the material of commerce is inadequate.	Concluded in FGE.21Rev4
15.055	[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 No safety concern based on intakes calculated by the MSDI approach	Concluded in FGE.21Rev4 JECFA: 1763 (JECFA, 2007)
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 No safety concern based on intakes calculated by the MSDI approach. Information on the stereochemical composition of the material of commerce is inadequate.	Concluded in FGE.21Rev4

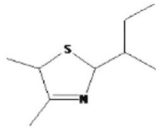
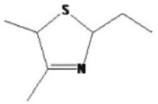
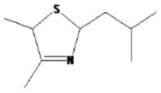
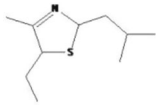
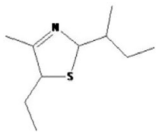
FL-no	EU Union List chemical name	Structural formula	MSDI[a] (μg/*capita* per day)	Class[b] Evaluation procedure path[c] Outcome on the named compound and on the material of commerce	EFSA comments
15.077	4-Hydroxy-2,5-dimethylthiophen-3(2H)-one		0.12	Class III B3: Intake below threshold, B4: No adequate NOAEL	No longer of interest for Industry for use as flavouring substances in Europe (DG SANCO, 2012). No further data were submitted.
15.079	2-Isobutyldihydro-4,6-dimethyl-1,3,5-dithiazine		5.7	Class III B3: Intake below threshold, B4: Adequate NOAEL exists No safety concern based on intakes calculated by the MSDI approach. Information on the stereochemical composition of the material of commerce is inadequate.	Concluded in FGE.21Rev4
15.082	3-Mercaptothiophene		0.011	Class III B3: Intake below threshold, B4: Adequate NOAEL exists No safety concern based on intakes calculated by the MSDI approach	Concluded in FGE.21
15.087	2-Methyl-3-mercaptothiophene		0.12	Class III B3: Intake below threshold, B4: Adequate NOAEL exists No safety concern based on intakes calculated by the MSDI approach	Concluded in FGE.21
15.088	2-Methyl-4,5-benzothiazole		0.0085	Class III B3: Intake below threshold, B4: No adequate NOAEL	No longer of interest for Industry for use as flavouring substances in Europe (DG SANCO, 2012). No further data were submitted.
15.106	Thiophene		0.12	Class III B3: Intake below threshold, B4: No adequate NOAEL	No longer of interest for Industry for use as flavouring substances in Europe (DG SANCO, 2012). No further data were submitted.
15.108	2-Thiophenemethanethiol		0.0073	Class III B3: Intake below threshold, B4: Adequate NOAEL exists No safety concern based on intakes calculated by the MSDI approach	Concluded in FGE.21

FL-no	EU Union List chemical name	Structural formula	MSDI[(a)] (μg/*capita* per day)	Class[(b)] Evaluation procedure path[(c)] Outcome on the named compound and on the material of commerce	EFSA comments
15.135	Ethyl thialdine		0.012	Class III B3: Intake below threshold, B4: Adequate NOAEL exists No safety concern based on intakes calculated by the MSDI approach. Information on the stereochemical composition of the material of commerce is inadequate	Concluded in FGE.21Rev4
15.114	6-Acetyl-2,3-dihydro-1,4-thiazine		0.012	Class III No evaluation	No longer of interest for Industry for use as flavouring substances in Europe (DG SANCO, 2012). No further data were submitted.
15.133	5-Acetyl-2,3-dihydro-1,4-thiazine		0.61	Class III No evaluation	No longer of interest for Industry for use as flavouring substances in Europe (DG SANCO, 2012). No further data were submitted. JECFA: 1766 (JECFA, 2007)

(a): EU MSDI: Amount added to food as flavour in (kg/year) × 10^9/(0.1 × population in Europe (= 375 × 10^6) × 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.

Appendix G – Summary of safety evaluations for supporting substances from FGE.76Rev2

Table G.1: Summary of safety evaluations performed by JECFA and EFSA conclusions on supporting flavouring substances from FGE.76Rev2 for FGE.21Rev6

FL-no JECFA-no	EU Union List chemical name	Structural formula	JECFA conclusions	EFSA conclusions
			Class[a] Evaluation procedure path[b],[c] Outcome on the named compound based on the MSDI approach	Procedural path if different from JECFA, Conclusion based on the MSDI[d] approach on the named compound and on the material of commerce
15.029 1059	2-(sec-Butyl)-4,5-dimethyl-3-thiazoline		Class III B3: Intake below threshold B4: Adequate NOAEL (1.2 mg/kg bw per day) exists	Genotoxicity data required
15.030 1058	4,5-Dimethyl-2-ethyl-3-thiazoline		Class III B3: Intake below threshold B4: Adequate NOAEL (1.2 mg/kg bw per day) exists	Genotoxicity data required
15.032 1045	4,5-Dimethyl-2-isobutyl-3-thiazoline		Class III B3: Intake below threshold B4: Adequate NOAEL (1.2 mg/kg bw per day) exists	
15.130 1761	5-Ethyl-4-methyl-2-(2-methylpropyl)-thiazoline		Class III B3: Intake below threshold B4: Adequate NOAEL (1.2 mg/kg bw per day) exists	Genotoxicity data required
15.131 1762	5-Ethyl-4-methyl-2-(2-butyl)-thiazoline		Class III B3: Intake below threshold B4: Adequate NOAEL (1.2 mg/kg bw per day) exists	Genotoxicity data required

FL-No: FLAVIS number; FGE: Flavouring Group Evaluation; JECFA: The Joint FAO/WHO Expert Committee on Food Additives; NOAEL: No observed adverse effect level; bw: body weight.
(a): Thresholds of concern: Class I = 1800 μg/person/day, Class II = 540 μg/person/day, Class III = 90 μg/person/day.
(b): JECFA (2002, 2003).
(c): JECFA (2007, 2008).
(d): EU MSDI: Amount added to food as flavouring in (kg/year) × 10^9/(0.1 × population in Europe (= 375 × 10^6) × 0.6 × 365) = μg/capita per day.