

ADOPTED: 14 May 2020

doi: 10.2903/j.efsa.2020.6154

Scientific Opinion on Flavouring Group Evaluation 91, Revision 3 (FGE.91Rev3): consideration of aliphatic, aromatic and α,β -unsaturated sulfides and thiols evaluated by JECFA (53rd, 61st, 68th and 76th meetings), structurally related to substances in FGE.08Rev5

EFSA Panel on Food Additives and Flavourings (FAF),
Maged Younes, Gabriele Aquilina, Laurence Castle, Karl-Heinz Engel, Paul Fowler,
Maria Jose Frutos Fernandez, Peter Fürst, Ursula Gundert-Remy, Rainer Gürtler, Trine Husøy,
Melania Manco, Peter Moldeus, Agneta Oskarsson*, Sabina Passamonti, Romina Shah,
Ine Waalkens-Berendsen, Detlef Wölfle, Matthew Wright, Romualdo Benigni, Claudia Bolognesi,
Kevin Chipman, Eugenia Cordelli, Gisela Degen, Daniel Marzin, Camilla Svendsen, Giorgia Vianello
and Wim Mennes

Abstract

The EFSA Panel on Food Additives and Flavourings was requested to evaluate 49 flavouring substances assigned to the Flavouring Group Evaluation 91 (FGE.91), using the Procedure as outlined in the Commission Regulation (EC) No 1565/2000. Forty-four substances have been considered in FGE.91 and its revisions (FGE.91Rev1 and FEG.91Rev2). With regard to the remaining five flavouring substances considered in this revision 3 of FGE.91: two ([FL-no: 12.065 and 12.079]) have been cleared with respect to genotoxicity in FGE.201Rev2; two ([FL-no: 12.169 and 12.241]) were originally allocated to FGE.74Rev4 and one ([FL-no: 12.304]) to FGE.08Rev5. The Panel considered the flavouring substance [FL-no: 12.169] representative for the tertiary monothiols [FL-no: 12.038, 12.085, 12.137, 12.138, 12.145, 12.252, 12.259, 12.241 and 12.304]. The substances were evaluated through a stepwise approach that integrates information on the structure–activity relationships, intake from current uses, toxicological threshold of concern (TTC), and available data on metabolism and toxicity. The Panel concluded that none of these 49 substances gives rise to safety concerns at their levels of dietary intake, estimated on the basis of the 'Maximised Survey-derived Daily Intake' (MSDI) approach. The specifications for the materials of commerce have also been considered and found adequate for all 49 flavouring substances. For five substances [FL-no: 12.077, 12.162, 12.265, 12.267 and 17.036], evaluated through the Procedure in FGE.91Rev2, no normal and maximum use levels are available. For 10 substances [FL-no: 12.065, 12.038, 12.079, 12.108, 12.139, 12.264, 12.274, 12.252, 12.284 and 12.304], the modified Theoretical Added Maximum Daily Intake (mTAMDI) intake estimates are above the TTC for their structural class. Therefore, for these 15 substances, more detailed data on uses and use levels should be provided in order to refine their exposure assessments and to finalise their safety evaluations.

© 2020 European Food Safety Authority. *EFSA Journal* published by John Wiley and Sons Ltd on behalf of European Food Safety Authority.

Keywords: Flavourings, α,β -unsaturated carbonyls and precursors, FGE.91Rev2, JECFA

Requestor: European Commission

* Member of the EFSA Panel on Food Additives and Flavourings (FAF) and of its Working Group on Flavourings until 31 December 2019.

Question numbers: EFSA-Q-2020-00266, EFSA-Q-2020-00023, EFSA-Q-2020-00022, EFSA-Q-2020-00021, EFSA-Q-2020-00020, EFSA-Q-2020-00019, EFSA-Q-2020-00018, EFSA-Q-2020-00017, EFSA-Q-2020-00016, EFSA-Q-2020-00015, EFSA-Q-2018-00840, EFSA-Q-2018-00839

Correspondence: fip@efsa.europa.eu

Panel members: Gabriele Aquilina, Laurence Castle, Karl-Heinz Engel, Paul Fowler, Maria Jose Frutos, Fernandez, Peter Fürst, Rainer G Gürtler, Ursula Gundert-Remy, Trine Husøy, Melania Manco, Wim Mennes, Peter Moldeus, Agneta Oskarsson*, Sabina Passamonti, Romina Shah, Ine Waalkens-Berendsen, Detlef Wölfle, Matthew Wright and Maged Younes.

Acknowledgments: The Panel wishes to thank the hearing experts Vibe Beltoft and Karin Nørby for the support provided to this scientific output.

Suggested citation: EFSA FAF Panel (EFSA Panel on Food Additives and Flavourings), Younes M, Aquilina G, Castle L, Engel K-H, Fowler P, Frutos Fernandez P, Fürst P, Gundert-Remy U, Gürtler R, Husøy T, Manco M, Moldeus P, Oskarsson A, Passamonti S, Shah R, Waalkens-Berendsen I, Wölfle D, Wright M, Benigni R, Bolognesi C, Chipman K, Cordelli E, Degen G, Marzin D, Svendsen C, Vianello G and Mennes W, 2020. Scientific Opinion on Flavouring Group Evaluation 91, Revision 3 (FGE.91Rev3): consideration of aliphatic, aromatic and α,β -unsaturated sulfides and thiols evaluated by JECFA (53rd, 61st, 68th and 76th meetings), structurally related to substances in FGE.08Rev5. *EFSA Journal* 2020;18(6):6154, 49 pp. <https://doi.org/10.2903/j.efsa.2020.6154>

ISSN: 1831-4732

© 2020 European Food Safety Authority. *EFSA Journal* published by John Wiley and Sons Ltd on behalf of European Food Safety Authority.

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



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



Table of contents

Abstract.....	1
1. Introduction.....	4
1.1. Background and Terms of Reference as provided by the requestor.....	4
1.1.1. Background to Mandate of FGE.201Rev2 (M-2017-0048).....	4
1.1.2. Terms of Reference of Mandate from FGE.201Rev2 (M-2017-0048).....	4
1.1.3. Background to Mandate of FGE.91Rev3 joint with FGE.74Rev4 (M-2020-0004)	5
1.1.4. Terms of Reference of FGE.91Rev3 joint with FGE.74Rev4 (M-2020-0004).....	6
1.2. Interpretation of the Terms of Reference.....	6
1.2.1. History of the evaluation of the substances in Flavouring Group Evaluation 91.....	6
2. Data and methodologies	8
2.1. Data.....	8
2.2. Methodologies.....	8
2.2.1. Procedure for the safety evaluation of flavouring substances	8
2.2.2. Approach used for the calculation of exposure.....	9
3. Assessment.....	9
3.1. Specifications	9
3.2. Estimation of intake.....	10
3.3. Biological and toxicological data	11
3.3.1. ADME data	11
3.3.2. Genotoxicity data	12
3.3.2.1. <i>In vitro</i> micronucleus assay on human peripheral blood lymphocytes on 2-methyl-4-oxopentane-2-thiol (4-mercapto-4-methyl-2-pentanone) (Documentation provided to EFSA nr: 6).....	12
3.3.3. Toxicological data.....	12
3.3.3.1. Subchronic toxicity study on dimethyl sulfide [FL-no: 12.006] (FGE.08Rev5, EFSA CEF Panel, 2012a) ..	
3.3.3.2. Toxicological studies on 2-methyl-4-oxopentane-2-thiol (4-mercapto-4-methyl-2-pentanone) (Documentation provided to EFSA nr: 5).....	13
3.4. Application of the Procedure	14
4. Discussion	16
5. Conclusions.....	16
6. Recommendations.....	16
7. Documentation provided to EFSA	17
References.....	17
Abbreviations.....	18
Appendix A – Procedure of the safety evaluation	20
Appendix B – Specifications.....	23
Appendix C – Exposure estimates	33
Appendix D – Summary of safety evaluations	41
Appendix E – Repeated dose toxicity studies	49

1. Introduction

The objective of this revision of FGE.91 (FGE.91Rev3) is to assess:

- two α,β -unsaturated substituted sulfides (i.e. [FL-no: 12.065 and 12.079]), cleared with respect to genotoxicity in FGE.201Rev2.¹
- The additional genotoxicity, toxicity and exposure data submitted to complete the safety evaluation of 10 tertiary monothiols, represented by flavouring substance [FL-no: 12.169], which were originally allocated to FGE.74Rev4 ([FL-no: 12.169 and 12.241]), FGE.91Rev2 ([FL-no: 12.038, 12.085, 12.137, 12.138, 12.145, 12.252 and 12.259]) and FGE.08Rev5 ([FL-no: 12.304]).

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

1.1.1. Background to Mandate of FGE.201Rev2 (M-2017-0048)

The use of flavouring is regulated under Regulation (EC) No 1334/2008² of the European Parliament and Council of 16 December 2008 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 872/2012³. The list includes a number of flavouring substances for which the safety evaluation should be completed in accordance with Commission Regulation (EC) No 1565/2000⁴.

The substances in this group were included in the Union list with a footnote 1 (under evaluation by EFSA).

In its opinion about this subgroup of 2012, the EFSA Panel considered that the mutagenicity hazard could not be cleared by the endpoints evaluated in the *in vivo* micronucleus assay submitted. The Panel therefore conclude that further data were required in order to clarify the genotoxic potential of this subgroup. The Panel considered the Comet assay with [FL-no: 05.095] as test material and performed on liver, blood and first site of contact, as a preferred option to further investigate the genotoxicity *in vivo*.

The additional data submitted by the applicant consist essentially of:

- a transgenic mutation assay in combination with an *in vivo* micronucleus assay for the substance 2-methylcrotonaldehyde [FL-no: 05.095]
- a combination of a Comet/micronucleus assay for the substance 2-methylpent-2-enal [FL-no: 05.090]

The Panel also considered in this opinion on FGE.201 rev.1 that the additional data on 2-methylcrotonaldehyde [FL-no: 05.095] could also be considered representative for the following substances: 2,8-dithianon-4-en-4-carboxaldehyde [FL-no: 12.065] and 2-(methylthiomethyl)but-2-enal [FL-no: 12.079].

1.1.2. Terms of Reference of Mandate from FGE.201Rev2 (M-2017-0048)

The European Commission requests the European Food Safety Authority (EFSA) to evaluate the new information submitted on 2-methylpent-2-enal [FL-no: 05.090] and 2-methylcrotonaldehyde [FL-no: 05.095] including also 2,8-dithianon-4-en-4-carboxaldehyde [FL-no: 12.065] and 2-(methylthiomethyl)but-2-enal [FL-no: 12.079] and, depending on the outcome, proceed to the full evaluation of the substances of this group listed in the table below, in accordance with Commission Regulation (EC) No

¹ According to the Mandate and Term of Reference of this FGE, when for a flavouring substance the concern for genotoxicity is ruled out, EFSA proceeds to the full evaluation of these flavouring substances, taking into account the requirements of the Commission Regulation (EC) No 1565/2000 and of Regulation (EU) No 1334/2008.

² Regulation (EC) No 1334/2008 of the European Parliament and of the Council of 16 December 2008 on flavourings and certain food ingredients with flavouring properties for use in and on foods and amending Council Regulation (EEC) No 1601/91, Regulations (EC) No 2232/96 and (EC) No 110/2008 and Directive 2000/13/EC. OJ L 354, 31.12.2008, p. 34–50.

³ Commission implementing Regulation (EU) No 872/2012 of 1 October 2012 adopting the list of flavouring substances provided for by Regulation (EC) No 2232/96 of the European Parliament and of the Council, introducing it in Annex I to Regulation (EC) No 1334/2008 of the European Parliament and of the Council and repealing Commission Regulation (EC) No 1565/2000 and Commission Decision 1999/217/EC. OJ L 267, 2.10.2012, p. 1–161.

⁴ 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, p. 8–16.

1565/2000, within nine months. In case the genotoxic potential cannot be ruled out or the procedure cannot be applied EFSA is asked to characterise the hazards and also quantify the exposure.

As regards the substance 2,6-Dimethyl-2,5,7-octatriene-1-ol acetate ([FL-no: 09.931] CAS no 999999-91-4) the applicant indicate that it is included in this subgroup 1.1.2 of FGE19 (FGE.201). However, this substance has been already evaluated by EFSA in FGE 207 and FGE 72 Rev.1 of 2013.

As regards substance 4-methyl-3-hepten-5-one ([FL-no: 07.261] CAS no 22319-31-9) EFSA indicated in its opinion FGE.204 that 'the 2-methyl substituted alpha, beta-unsaturated aldehydes in FGE.201Rev1 can be considered as structurally related to it [FL-no: 07.261]. Thus the final conclusion on [FL-no: 07.261] will be drawn based on the outcome of the evaluation of FGE.201Rev1'.

1.1.3. Background to Mandate of FGE.91Rev3 joint with FGE.74Rev4 and FGE.08Rev5 (M-2020-0004)

The use of flavourings is regulated under Regulation (EC) No 1334/2008 of the European Parliament and Council of 16 December 2008 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. The list contains flavouring substances for which the scientific evaluation should be completed in accordance with Commission Regulation (EC) No 1565/2000 and taking into account also the provisions of Regulation (EC) No 1334/2008.

Additional information was submitted regarding the group of substances belonging to the Flavouring Group Evaluation 74. In January the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids adopted the opinion on the Flavouring Group Evaluation 74 Revision 4, (FGE.74Rev4)⁵ and concluded that for the two tertiary thiols [FL-no: 12.169 and 12.241] further data are required. The Panel did not agree with JECFA that appropriate studies were available for deriving NOAELs for these two tertiary thiols. Therefore, the evaluation could not be considered completed for these substances.

On December 2017 the applicant submitted additional information consisting of a 90-day study (on substance [FL-no: 12.169]) and also poundage and usage information concerning substances from FGE.74 and FGE.91. As EFSA is currently evaluating two newly included substances in the group FGE.91 revision 3, not considered in earlier revisions of this FGE, it is now appropriate to also consider this additional information submitted on the substances represented by 2-methyl-4-oxopentane-2-thiol [FL-no: 12.169], in order to finalize their safety assessment through the Procedure.

The current request also concerns the flavouring substance [FL-no: 12.304]. This substance [FL-no: 12.304] is structurally related to the other 9 tertiary monothiols in FGE.91Rev3 and FGE.74Rev4 and therefore can be represented by 2-methyl-4-oxopentane-2-thiol [FL-no: 12.169]. The substance [FL-no: 12.304] is currently included in the FGE.08Rev5.⁶ FGE.08Rev5 is a supporting FGE both for FGE.91 and FGE.74. FGE.08 includes non-JECFA-evaluated substances structurally related to the ones in FGE.91 and 74. The safety evaluation through the Procedure of four substances included in FGE.08Rev5 with [FL-no: 12.304, 12.172, 12.174 and 16.057], was not concluded as the assessment of their genotoxicity potential could not be carried out at the time of the finalization of revision 5 of FGE.08. However, [FL-no: 12.172, 12.174 and 16.057] were no longer supported by industry and they were not included in the Union List of flavourings. Therefore, the only safety evaluation through the Procedure which is still pending for the substances in FGE.08Rev5 is the one for the remaining substance [FL-no: 12.304]. The concern for genotoxicity for this substance could be addressed by considering the genotoxicity data made available on the representative substance for the tertiary monothiols, i.e. [FL-no: 12.169], mentioned in the initial request. In addition, since [FL-no: 12.304] was evaluated by JECFA in 2012, its safety assessment could be adequately finalized in FGE.91Rev3 since now [FL-no: 12.304] is a JECFA-evaluated substance (FGE.50 up to FGE.99 contain only JECFA-evaluated substances).

⁵ Scientific Opinion on Flavouring Group Evaluation 74, Revision 4 (FGE.74Rev4): Consideration of Simple Aliphatic Sulphides and Thiols evaluated by JECFA (53rd and 61st meeting) Structurally related to Aliphatic and Alicyclic Mono-, Di-, Tri-, and Polysulphides with or without Additional Oxygenated Functional Groups from Chemical Group 20 evaluated by EFSA in FGE.08Rev5 (2012). EFSA Journal 2018;16(3):5167, 58 pp. <https://doi.org/10.2903/j.efsa.2018.5167>

⁶ Scientific Opinion on Flavouring Group Evaluation 08, Revision 5 (FGE.08Rev5): Aliphatic and alicyclic mono-, di-, tri-, and polysulphides with or without additional oxygenated functional groups from chemical groups 20 and 30. <https://doi.org/10.2903/j.efsa.2012.2837>. Available online: www.efsa.europa.eu/efsajournal

1.1.4. Terms of Reference of FGE.91Rev3 joint with FGE.74Rev4 and FGE.08Rev5 (M-2020-0004)

The European Commission requests the European Food Safety Authority (EFSA) to evaluate this new information and to proceed with the full evaluation of these flavouring substances in accordance with Commission Regulation (EC) No 1565/2000 and taking into account also Regulation (EC) No 1334/2008. The assessment should be carried out within 6 months from the receipt of this letter, e.g. up to 30th June 2020.

1.2. Interpretation of the Terms of Reference

The Panel considered that for the two sulfides [FL-no 12.065 and 12.079] the evaluation of their possible genotoxic properties in FGE.201Rev2 was justified considering the structural similarity, with respect to the α,β -unsaturated carbonyl moiety, to the other substances within FGE.201. Since the concern for genotoxicity has been alleviated in FGE.201Rev2, the assessment can proceed in the current revision of FGE.91 as the substances in this group are structurally similar with respect to the sulfur function.

In a previous submission, industry also provided an additional *in vitro* genotoxicity assay on flavouring substance [FL-no: 12.169] (Documentation provided to EFSA nr: 6). This assay was not considered in FGE.74Rev4 where the genotoxicity potential of this substance, and its other structurally related substances, was initially investigated and ruled out. Since this flavouring substance [FL-no: 12.169] will be included in the current revision of FGE.91, this study will now be included and assessed in this revision.

1.2.1. History of the evaluation of the substances in Flavouring Group Evaluation 91

The EFSA consideration in FGE.91 (EFSA CEF Panel, 2010) dealt with 45 substances, 40 simple aliphatic and aromatic sulfides and thiols evaluated by JECFA at the 68th meeting (JECFA, 2007) and five tertiary thiols evaluated by JECFA at the 53rd meeting (JECFA, 2000). For seven tertiary mono thiols [FL-no: 12.038, 12.085, 12.137, 12.138, 12.145, 12.252 and 12.259], it was concluded that adequate genotoxicity data were needed before the substances could be evaluated through the Procedure.

The first revision of FGE.91, FGE.91Rev1 (EFSA CEF Panel, 2011a), included the assessment of two additional substances, benzyl methyl sulfide [FL-no: 12.077] and methyl phenyl sulfide [FL-no: 12.162] (in total 47 substances). These substances were evaluated by JECFA at its 53rd meeting (JECFA, 2000).

Since publication of FGE.91Rev1, additional genotoxicity data have become available for 4-mercapto-4-methyl-2-pentanone [FL-no: 12.169] from FGE.74Rev3 (EFSA CEF Panel, 2014b). This substance was considered representative for the seven tertiary monothiols [FL-no: 12.038, 12.085, 12.137, 12.138, 12.145, 12.252 and 12.259] for which a concern with respect to genotoxicity was identified in FGE.91.

After the publication of FGE.91Rev1, three (i.e. [FL-no: 12.114, 12.256 and 12.272]) candidate substances were no longer supported by the industry for use as flavouring substances in Europe⁷ and therefore they were not considered any further. The second revision of FGE.91 (EFSA CEF Panel, 2014a), FGE.91Rev2 (in total including 44 substances), concerned the assessment of the newly submitted genotoxicity data on [FL-no: 12.169] used to cover the seven tertiary monothiols. The available data were limited but the CEF Panel considered that these genotoxicity data did not preclude the evaluation of the substances in FGE.91Rev2 through the Procedure. In FGE.91Rev2, the Panel concluded that for the tertiary mono thiols [FL-no: 12.038, 12.085, 12.137, 12.138, 12.145, 12.252 and 12.259], contrary to the JECFA, there are no adequate no observed adverse effect levels (NOAELs) and that additional toxicity data are required to finalise the evaluation of these seven substances. In addition, the modified Theoretical Added Maximum Daily Intake (mTAMDI) figures for five substances [FL-no: 12.264, 12.284, 12.274, 12.108 and 12.139] were above the threshold of concern for their structural classes. For these substances more detailed data on uses and use levels are needed. For 10 substances [FL-no: 12.038, 12.077, 12.085, 12.137, 12.138, 12.145, 12.162, 12.265, 12.267 and 17.036], no use levels have been provided.

⁷ DG SANCO (Directorate General for Health and Consumer Affairs), 2013a. Information from DG SANCO 14/05 2013, concerning a list of 18 non-supported substances. FLAVIS.2.26.

The present revision of FGE.91, FGE.91Rev3, includes the safety evaluation of two additional sulfides: 2-(methylthiomethyl)but-2-enal [FL-no: 12.079] and 2,8-dithianon-4-en-4-carboxaldehyde [FL-no: 12.065]. These substances were evaluated by JECFA in its 53rd meeting (JECFA, 2000) and cleared with respect to genotoxicity in FGE.201Rev2 (EFSA CEF Panel, 2018b). By expert judgement, these substances have been included in the current revision 3 of FGE.91 on the basis of their structural similarity with the substances considered in this FGE. The present revision includes also the completion of the safety evaluation of seven tertiary monothiols [FL-no: 12.038, 12.085, 12.137, 12.138, 12.145, 12.252 and 12.259], which were pending from the previous revision (FGE.91Rev2) owing to lack of toxicological data (i.e. a 90-day study to identify a NOAEL). A 90-day toxicity study performed on 4-mercapto-4-methyl-2-pentanone [FL-no: 12.169] (from FGE.74Rev4, EFSA CEF Panel, 2018a), chosen as representative substance to cover the evaluation of tertiary monothiols in FGE.74 ([FL-no: 12.169 and 12.241]) and FGE.91 ([FL-no: 12.038, 12.085, 12.137, 12.138, 12.145, 12.252 and 12.259]), has been provided and a NOAEL can be identified. The Panel considers that the chemical structures of the two substances from FGE.74Rev4 are sufficiently structurally related to those of the tertiary monothiols in FGE.91 and therefore agrees to include these two substances in FGE.91Rev3. New poundage and use levels data have been submitted for these nine substances [FL-no: 12.169, 12.241, 12.038, 12.085, 12.137, 12.138, 12.145, 12.252 and 12.259] and these new exposure data will be included in the present opinion (Documentation provided to EFSA nr: 4). In addition, this revision 3 of FGE.91 includes the finalisation of the safety evaluation of substance ethyl-2-mercapto-2-methyl propanoate [FL-no: 12.304] which was originally allocated in FGE.08Rev5 (EFSA CEF Panel, 2012a). The safety evaluation through the Procedure of this flavouring substance was not concluded in FGE.08Rev5 as the genotoxicity concern could not be ruled out due to lack of data. The concern for genotoxicity for this substance could now be ruled out by the genotoxicity data available on the representative substance for the tertiary monothiols, i.e. [FL-no: 12.169]. Moreover, since [FL-no: 12.304] had been evaluated by JECFA at its 76th meeting (JECFA-no: 2085, JECFA, 2012), the Panel agrees to finalise its safety assessment directly in FGE.91Rev3 as now it is a JECFA-evaluated substance and it is structurally related to the other nine tertiary monothiols in FGE.91Rev3.

Therefore, together with the 44 substances that were already considered in FGE.91Rev2 and the five newly included substances, i.e. [FL-no: 12.169 and 12.241] from FGE.74Rev4, [FL-no: 12.079 and 12.065] from FGE.201Rev2 and [FL-no: 12.304] from FGE.08Rev5, the current revision comprises 49 substances. The Panel agrees that all 49 JECFA-substances in FGE.91Rev3 are structurally related to the aliphatic and alicyclic mono-, di-, tri- and polysulfides with or without additional oxygenated functional groups evaluated by EFSA in the FGE.08Rev5 (EFSA CEF Panel, 2012a).

The 44 flavouring substances for which the evaluation was finalised in FGE.91Rev2 will not further be discussed. Nevertheless, for the sake of completeness their information is maintained in the various tables in this FGE.

FGE	Adopted by EFSA	Link	No. of substances
FGE.91	24 September 2009	https://www.efsa.europa.eu/en/efsajournal/pub/688	45
FGE.91Rev1	23 November 2011	https://www.efsa.europa.eu/en/efsajournal/pub/1026	47
FGE.91Rev2	21 May 2014	https://www.efsa.europa.eu/en/efsajournal/pub/3707	44
FGE.91Rev3	14 May 2020	https://www.efsa.europa.eu/en/efsajournal/pub/6154	49

2. Data and methodologies

2.1. Data

The present revision of the opinion on FGE.91 is based on the following data as provided by the applicant:

FL-no	Chemical name	Data provided for the current revision 3 of FGE.91	Appendix (Table nr)/ relevant section of the opinion	Documentation provided to EFSA/Reference
12.065	2,8-Dithianon-4-en-4-carboxaldehyde	Specifications, EU poundage data (MSDI), use levels (mTAMDI)	Appendix B (Table B.1), Appendix C (Tables C.1 and C.4)	Documentation provided to EFSA nr: 1, 2, 3
12.079	2-(Methylthiomethyl)-but-2-enal			
12.038	8-Mercapto- <i>p</i> -menthan-3-one	EU poundage data (MSDI), use levels (mTAMDI)	Appendix C (Tables C.1 and C.4)	Documentation provided to EFSA nr: 4
12.085	<i>p</i> -Menth-1-ene-8-thiol			
12.137	3-Mercapto-3-methylbutan-1-ol			
12.138	3-Mercapto-3-methylbutyl formate			
12.145	4-Methoxy-2-methylbutane-2-thiol			
12.252	4-Mercapto-4-methyl-2-pentanol			
12.259	1-Mercapto- <i>p</i> -menthan-3-one			
12.241	2-Mercapto-2-methylpentan-1-ol			
12.169	2-Methyl-4-oxopentane-2-thiol	EU poundage data (MSDI), use levels (mTAMDI), Genotoxicity and toxicity data	Appendix C (Tables C.1 and C.4), Section 3.3.2.1. Appendix E (Table E.1)	Documentation provided to EFSA nr: 4, 5, 6

FL-no: FLAVIS number; MSDI: Maximised Survey-derived Daily Intake; mTAMDI: modified Theoretical Added Maximum.

In addition, the following references were used:

- JECFA specifications for the two newly allocated flavouring substances [FL-no: 12.065 and 12.079] (JECFA, 2003, 2005).
- 53rd, 61st and 76th JECFA reports (JECFA, 2000, 2004, 2012).
- Genotoxicity data evaluated in FGE.201Rev2 (EFSA CEF Panel, 2018b).
- EFSA scientific opinions on FGE.91 and its revisions (EFSA CEF Panel, 2010, 2011a, 2014).
- EFSA scientific opinion on FGE.74Rev4 (EFSA CEF Panel, 2018a).
- EFSA scientific opinion on FGE.08Rev5 (EFSA CEF Panel, 2012a).

2.2. Methodologies

This opinion follows the principles described in the EFSA Guidance on transparency with regard to scientific aspects of risk assessment (EFSA Scientific Committee, 2009) and the relevant existing guidance documents from the EFSA Scientific Committee. The assessment strategy applied for the evaluation programme of flavouring substances, as laid down in Commission Regulation (EC) No 1565/2000, 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 safety evaluation of chemically defined flavouring substances as referred to in Commission Regulation (EC) No 1565/2000, 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 (Section C.2 'mTAMDI calculation').

3. Assessment

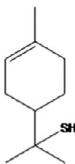
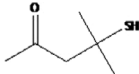
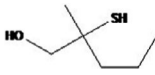
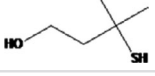
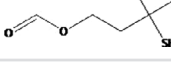


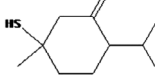
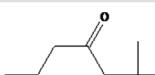

3.1. Specifications

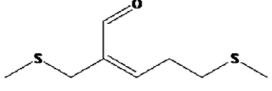
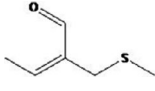
JECFA specifications are available for all 49 flavouring substances in FGE.91Rev3 (JECFA, 2003, 2005, 2012). All flavouring substances are shown in Table B.1 – Appendix B.

EFSA considerations

Table 1 shows the chemical structures of the substances considered in this revision of FGE.91 (FGE.91Rev3).

Table 1: Flavouring substances under evaluation in FGE.91Rev3.

FL-no	Chemical structure	Chemical name	Structural class*
12.085		<i>p</i> -Menth-1-ene-8-thiol	I
12.169		2-Methyl-4-oxopentane-2-thiol (4-mercapto-4-methyl-2-pentanone)	III
12.241		2-Mercapto-2-methylpentan-1-ol	III
12.137		3-Mercapto-3-methylbutan-1-ol	III
12.138		3-Mercapto-3-methylbutyl formate	III
12.145		4-Methoxy-2-methylbutane-2-thiol	III
12.252		4-Mercapto-4-methyl-2-pentanol	III
12.259		1-Mercapto- <i>p</i> -menthan-3-one	III
12.038		8-Mercapto- <i>p</i> -menthan-3-one	III
12.304		Ethyl-2-mercapto-2-methyl propanoate	III

FL-no	Chemical structure	Chemical name	Structural class*
12.065		2,8-Dithianon-4-en-4- carboxaldehyde	III
12.079		2-(Methylthiomethyl)-but-2-enal	III

FL-no: FLAVIS number.

*: Determined with OECD Toolbox (version 4.3.1 available at <https://www.oecd.org/chemicalsafety/risk-assessment/oecd-qsar-toolbox.htm>).

The two newly allocated substances [FL-no: 12.065 and 12.079] are α,β -unsaturated substituted aldehydes and they can exist as geometrical stereoisomers. The applicant provided adequate information with respect to the composition of the stereoisomeric mixtures (see Table B.1 – Appendix B) (Documentation provided to EFSA nr: 1). For the remaining candidate substances in FGE.91Rev3, the specifications were considered in FGE.74Rev4 ([FL-no: 12.169 and 12.241]), FGE.91Rev2 ([FL-no: 12.038, 12.085, 12.137, 12.138, 12.145, 12.252 and 12.259]) and FGE.08Rev5 ([FL-no: 12.304]) and found adequate (EFSA CEF Panel, 2012a, 2014a, 2018a).

According to the information provided by industry, flavouring substance [FL-no: 12.169] is supplied as 1% solution in propylene glycol of a mixture of 2-methyl-4-oxopentane-2-thiol (48%) and 4-methyl-3-penten-2-one (48–50%) (EFSA CEF Panel, 2018a). The Panel noted that the latter is an authorised flavouring substance ([FL-no: 07.101]). Moreover, as already indicated in FGE.74Rev4, the chemical name 2-methyl-4-oxopentane-2-thiol should be changed to *4-mercapto-4-methyl-2-pentanone*.

The most recent specifications data for all substances in FGE.91Rev3 are summarised in Table B.1 – Appendix B.

3.2. Estimation of intake

JECFA status

For all 49 flavouring substances, evaluated through the JECFA Procedure, intake data are available for the EU (JECFA, 2000, 2004, 2012).

EFSA considerations

EU production figures for all flavouring substances under evaluation in FGE.91Rev3 ([FL-no: 12.065, 12.079, 12.038, 12.085, 12.137, 12.138, 12.145, 12.252, 12.259, 12.169, 12.241 and 12.304]) have been provided by industry (EFSA CEF Panel 2012a, 2014a, 2018a; Documentation provided to EFSA nr: 2, 3, 4) and MSDI values have been calculated ranging from 0.012 to 37 $\mu\text{g}/\text{capita}$ per day.

Normal and maximum use levels have been provided for flavouring substances [FL-no: 12.065, 12.079, 12.038, 12.085, 12.137, 12.138, 12.145, 12.252, 12.259, 12.169, 12.241 and 12.304], (Documentation provided to EFSA n. 3 and 4) and mTAMDI intake values have been calculated. The mTAMDI intake estimate calculated from these data for flavouring substance [FL-no: 12.085] is below the toxicological threshold of concern (TTC) for its structural class I. The mTAMDI intake estimates for flavouring substances [FL-no: 12.137, 12.138, 12.145, 12.169 and 12.241] are below the TTC for their structural class III. The mTAMDI intake estimates for flavouring substances [FL-no: 12.065, 12.079, 12.304, 12.038 and 12.252] are above the TTC for their structural class III. From the previous revision (FGE.91Rev2) the mTAMDI intake values for flavouring substances [FL-no: 12.264, 12.284, 12.274, 12.108 and 12.139] are above the TTC for their structural classes. Therefore, for these 10 substances ([FL-no: 12.065, 12.079, 12.304, 12.038, 12.252, 12.264, 12.284, 12.274, 12.108 and 12.139]), more detailed data on uses and uses levels should be provided in order to refine the exposure assessment and to finalise their safety evaluations.

No normal and maximum use levels have been provided for flavouring substances [FL-no: 12.077, 12.162, 12.265, 12.267 and 17.036], previously evaluated in FGE.91Rev2.

The MSDI values and the mTAMDI intake estimates for substances in FGE.91Rev3 are shown in Table C.4 – Appendix C.

3.3. Biological and toxicological data

3.3.1. ADME data

The two candidate substances [FL-no: 12.065 and 12.078] are α,β -unsaturated sulfides which were evaluated by JECFA at its 53rd meeting, within a group of 137 sulfur-containing substances, particularly in the '*subgroup ii acyclic sulfides with oxidised side-chains*' (JECFA, 2000). In the 53rd JECFA report (JECFA, 2000), these substances are described as sufficiently lipophilic to be absorbed from the intestine and they would be metabolised via many different pathways. The presence of other functional groups, such the α,β -unsaturated aldehydes in the two candidate substances, provides greater polarity and an additional site for the biotransformation of these sulfides. According to JECFA, the presence of these polar groups would also enhance the renal excretion (JECFA, 2000). Concurrent metabolic pathways of various sulfides substituted with oxygenated functions were reported, but sulfoxide formation, via enzymatic catalysis (P450 and flavin-containing monooxygenases), is the predominant metabolic pathway of detoxification. Sulfoxides may be further oxidised to sulfones and these two are the main urinary metabolites of sulfides.

The other 10 candidate substances are tertiary monothiols. Seven of them were evaluated in the 53rd JECFA meeting within the '*subgroup v thiols with oxidised side-chains*' (JECFA, 2000) ([FL-no: 12.038, 12.085, 12.137, 12.138, 12.145, 12.252 and 12.259]), two substances at the 61st JECFA meeting (JECFA, 2004) ([FL-no: 12.169 and 12.241]) and one substance ([FL-no: 12.304]) at the 76th JECFA meeting (JECFA, 2012). Substances both from 61st and 76th JECFA meetings were evaluated within the same *subgroup v* of the original group of 137 sulfur-containing substances, previously evaluated at the 53rd JECFA meeting. According to JECFA, the metabolism of thiols with oxidised side-chains is predicted to involve a combination of pathways for simple thiols together with further oxidation or conjugation of the oxidised side-chain. Metabolic options for thiols in mammals are thiol methyltransferase catalysed S-methylation to yield the corresponding thioethers and sulfides, which are then oxidised to methyl sulfoxides and methyl sulfones. S-adenosyl-L-methionine is required as a methyl group donor. Simple thiols may undergo oxidation to form unstable sulfenic acids (RSOH) which are oxidised to sulfinic acids (RSO₂H) and then to sulfonic acids (RSO₃H) or combined with nucleophilic sites. Thiols may also react with glutathione (GSH) and other endogenous thiols to generate disulfides via bio-catalysis with microsomal and cytosolic thioltransferases. The disulfides can be reduced back to thiols, oxidatively desulfurated or oxidised to sulfonic acid.

JECFA could not conclude that the expected resulting metabolites are innocuous and evaluated the 12 candidate substances [FL-no: 12.065, 12.078, 12.169, 12.241, 12.038, 12.085, 12.137, 12.138, 12.145, 12.252, 12.259 and 12.304] along the B-side of the Procedure scheme.

EFSA considerations

Regarding the two oxygenated sulfides [FL-no: 12.065 and 12.079], the Panel observed that one of the candidate flavouring substance, i.e. [FL-no: 12.065], was used as a supporting substance in the evaluation of the *acyclic sulfides* subgroup in FGE.08Rev5 (EFSA CEF Panel, 2012a). In FGE.08Rev5 (EFSA CEF Panel, 2012a), there is an extensive description of the possible metabolic routes for the acyclic sulfides, which are in line with those outlined by JECFA. These substances are sufficiently lipophilic to be absorbed and they rapidly undergo oxidation catalysed by monooxygenase systems (P450 and FMO) to yield sulfoxides (S-oxidation). Sulfoxides may be further transformed to sulfones via an irreversible oxidation catalysed by P450. However, the sulfoxide is generally the predominant urinary metabolite of simple sulfides, such as methyl sulfide. When a sulfide contains also an oxygenated functional group, like the two candidate substances [FL-no: 12.065 and 12.078], for the oxygenated functional group the common biotransformations of alcohol, acid and carbonyl functional groups (C-oxidation and/or conjugation) would be expected. For oxygenated sulfides, C-oxidation and/or conjugation may compete with S-oxidation, nevertheless sulfoxide formation is usually the major metabolic pathway.

With regard to the 10 tertiary monothiols, under evaluation in FGE.91Rev3 ([FL-no: 12.169, 12.241, 12.038, 12.085, 12.137, 12.138, 12.145, 12.252, 12.259 and 12.304]), some of them were used as supporting substances in the evaluation of the thiols subgroup in FGE.08Rev5 (EFSA CEF Panel, 2012a) and [FL-no: 12.304] was originally allocated to FGE.08Rev5. According to FGE.08Rev5, and in line with JECFA evaluations, these substances would be expected to be detoxified via a combination of pathways including S-oxidation, methylation, oxidative desulfuration, alkylation, and conjugation with

GSH and/or glucuronic acid. The electrophilic metabolites, resulting from the S-oxidation and/or other reactions (i.e. oxidative desulfuration, alkylation, hydrolysis), can react with endogenous thiols present in cellular macromolecules (such as cysteine or reduced GSH) or with other nucleophilic cellular sites leading to the formation of perthiols. These substances are strong reductants and can interact with free radicals to produce perthiyl radicals which undergo prooxidative reactions.

Therefore, based on the reactivity of the expected metabolites, the Panel agrees to evaluate all the 12 candidate substances [FL-no: 12.065, 12.079, 12.169, 12.241, 12.038, 12.085, 12.137, 12.138, 12.145, 12.252, 12.259 and 12.304] via the B-side of the Procedure scheme (see Appendix A).

3.3.2. Genotoxicity data

This revision involves the inclusion of two flavouring substances [FL-no: 12.065 and 12.079], for which in FGE.19 a concern for genotoxicity had been identified based on the presence of a structural alert (i.e. α,β -unsaturated carbonyl substance or precursor for that), precluding their evaluation through the Procedure (EFSA CEF Panel, 2011b). Therefore, these substances needed further attention in FGE.201 and its revision 2 (FGE.201Rev2), where their genotoxic potential has been assessed and the concern for genotoxicity was ruled out (EFSA CEF Panel, 2018b). Therefore, the safety evaluation through the Procedure can be performed for these flavouring substances [FL-no: 12.065 and 12.079].

FGE.91Rev3 also deals with the finalisation of the safety evaluation through the Procedure for nine tertiary monothiols [FL-no: 12.169, 12.241, 12.038, 12.085, 12.137, 12.138, 12.145, 12.252 and 12.259]. The genotoxicity of these substances was investigated and the concern for the genotoxicity was ruled out in FGE.91Rev2 and FGE.74Rev4 (EFSA CEF Panel, 2014a, 2018a) based on genotoxicity data on the representative substance 2-methyl-4-oxopentane-2-thiol (4-mercapto-4-methyl-2-pentanone) [FL-no: 12.169] and other genotoxicity data (from JECFA and FGE.08Rev5) on structurally related substances. With regard to the flavouring substance [FL-no: 12.304], originally allocated in FGE.08Rev5 (EFSA CEF Panel, 2012a), its safety evaluation could not be finalised in FGE.08Rev5 as the genotoxicity concern was not ruled out. The concern for genotoxicity of [FL-no: 12.304] can now be ruled out based on the available genotoxicity data on the representative substance 2-methyl-4-oxopentane-2-thiol (4-mercapto-4-methyl-2-pentanone) and thus [FL-no: 12.304] can be evaluated through the Procedure in this FGE.

3.3.2.1. *In vitro* micronucleus assay on human peripheral blood lymphocytes on 2-methyl-4-oxopentane-2-thiol (4-mercapto-4-methyl-2-pentanone) (Documentation provided to EFSA nr: 6)

The flavouring substance 2-methyl-4-oxopentane-2-thiol (4-mercapto-4-methyl-2-pentanone) [FL-no: 12.169] (purity 99.9%) in dimethyl sulfoxide (DMSO) solution was tested in an *in vitro* micronucleus (MN) assay using duplicate human lymphocyte cultures in a single experiment consisting of five separate trials, as in accordance to OECD guideline 487. The vehicle control was DMSO solution. Mitomycin C (MMC) and Vinblastine (VIN) were used as clastogenic and aneugenic positive control, respectively.

Based on the results of a range-finding test, cultures were treated up to 1,323 $\mu\text{g/mL}$, equivalent to 10 mM, for 3 + 21 h in the absence and presence of S-9 and up to a cytotoxic concentration (90.00 $\mu\text{g/mL}$) for 24 + 0 h in the absence of S-9. The test article concentrations for MN analysis were selected by evaluating the effect of 2-methyl-4-oxopentane-2-thiol on the replication index. Cultures from all three treatment conditions were analysed for micronuclei at four concentrations in 2,000 binucleated per concentration cells.

From the study results, the Panel concludes that 2-methyl-4-oxopentane-2-thiol (4-mercapto-4-methyl-2-pentanone) [FL-no: 12.169] did not induce biologically relevant increases in the frequency of micronuclei in cultured human peripheral blood lymphocytes under the tested conditions. This further supports the conclusions on genotoxicity previously reached in FGE.74Rev4 (EFSA CEF Panel, 2018a) and confirms that flavouring substance [FL-no: 12.169], and its structurally related substances [FL-no: 12.038, 12.085, 12.137, 12.138, 12.145, 12.241, 12.252, 12.259 and 12.304], do not pose a concern with respect to genotoxicity.

3.3.3. Toxicological data

One subacute toxicity study is available for candidate substance [FL-no: 12.065] (Central Institute for Nutrition and Food Research; 1974, see Appendix E). This study is not suitable for the assessment of the two sulfides candidate substances ([FL-no: 12.065 and 12.079]) because of the too short

duration. In FGE.08Rev5, a subchronic toxicity study is available for dimethyl sulfide [FL-no: 12.006]. This study was used for the evaluation of substances in FGE.08Rev5 (acyclic sulfides) that are structurally related to the two sulfides candidate substances in FGE.91Rev3.

For flavouring substance 2-Methyl-4-oxopentane-2-thiol (4-mercapto-4-methyl-2-pentanone) [FL-no: 12.169], selected as representative substance for the tertiary monothiols thiols in FGE.91Rev3 ([FL-no: 12.241, 12.038, 12.085, 12.137, 12.138, 12.145, 12.252 and 12.259 and 12.304]), industry submitted toxicological studies which are described below (Documentation provided to EFSA nr: 5).

All the available toxicity studies are summarised in Table E.1 of Appendix E.

3.3.3.1. Subchronic toxicity study on dimethyl sulfide [FL-no: 12.006] (FGE.08Rev5, EFSA CEF Panel, 2012a)

Four groups of 15 Wistar rats per sex were given dimethyl sulfide by daily gavage in corn oil at dose levels of 2.5, 25 or 250 mg/kg body weight (bw) for 14 weeks. The control group received the same volume of corn oil only. Additional two groups (five/sex per dose) were given daily doses of 0.25 or 250 mg/kg bw for 2 or 6 weeks, respectively. The animals were weighed on day 0 and then weekly throughout the study. Food and water consumption were measured over a 24-h period preceding the day of weighing. Urine samples were collected during weeks 2, 6 and 14, and examined for volume, appearance, specific gravity, microscopic constituents, and content of glucose, ketones, bile salts and blood. At sacrifice, blood was taken for haematological examinations. Gross abnormalities and organ weights were recorded. Histological examinations were also performed. There was no adverse effect at any level in treated rats, and therefore, 250 mg/kg bw per day, the highest dose tested, was considered as the NOAEL.

3.3.3.2. Toxicological studies on 2-methyl-4-oxopentane-2-thiol (4-mercapto-4-methyl-2-pentanone) (Documentation provided to EFSA nr: 5)

Repeated dose range-finding study

Forty adult CrI: Sprague–Dawley CD[®] IGS rats (20 males and 20 females) were equally distributed into four Groups (5/sex per group). Dose levels of 15, 65 and 130 µg/kg bw per day of 2-methyl-4-oxopentane-2-thiol as well as a vehicle control (propylene glycol) were administered via gavage for 14 days.

Cage side observations were recorded daily for all animals and a battery of detailed clinical observations was performed once weekly. Body weights and food consumption were collected once weekly and body weight gain and food efficiency were calculated.

There were no mortalities or clinical effects. Investigations of body weight, body weight gain, food consumption, or food efficiency did not reveal any changes attributable to 2-methyl-4-oxopentane-2-thiol administration.

There were no macroscopic changes attributable to 2-methyl-4-oxopentane-2-thiol administration. Under the conditions of this study and based on the toxicological endpoints evaluated, male and female rats are expected to tolerate dose levels of 130 µg/kg bw per day in a study of longer duration.

Subchronic toxicity study

Sprague–Dawley CD[®] IGS rats (10/sex per group) received by gavage a 1% solution of 2-methyl-4-oxopentane-2-thiol in propylene glycol, resulting in nominal dose levels of 0 (control, 10% propylene glycol, 10 mL/kg bw per day), 130, 200 and 260 µg/kg bw per day for 90 consecutive days.

The stability of the test material, 2-methyl-4-oxopentane-2-thiol, was investigated via head space gas chromatography–mass spectrometry. The nominal dose levels mentioned above reflect the actual exposure in the different dose groups.

The study was conducted in compliance with OECD guideline 408 (from 1998) which was applicable when the study was conducted. The Panel noted that the highest dose levels did not induce toxicity as required by this OECD guideline. However, the strong odour of the tested substance precluded inclusion of higher doses in the study design, which would have resulted in a wider dose range.

All animals survived the treatment period. No treatment-related clinical signs were observed. There were no treatment-related changes in haematology, coagulation or urinalysis parameters. At the highest dose tested (260 µg/kg bw per day) on day 44, a slight increase in cholesterol was observed in females. However, this clinical chemistry change was not considered adverse and it was not correlated to any histopathology findings. Pathology and histopathology revealed no increase in the

absolute and relative organ weights. There were no treatment-related macroscopic or microscopic findings in any of the treated rats examined.

In an additional report for the same study, the influence of the test substance on reproduction function parameters (including oestrus cyclicity and sperm motility, epididymal sperm count, homogenisation-resistant spermatid count and morphology) in male and females was described. None of these study parameters was affected by the exposure to the test substance.

The NOAEL for the 90-day study was the highest dose administered, i.e. 260 µg/kg bw per day of 2-methyl-4-oxopentane-2-thiol. The Panel agrees with the conclusions of the study report.

The Panel noted that, according to the information provided in the study report, the test item in the repeated dose toxicity study was a 1% solution of 2-methyl-4-oxopentane-2-thiol in propylene glycol that did not contain 4-methyl-3-penten-2-one [FL-no: 07.101], which is a secondary component in the material of commerce of [FL-no: 12.169].

EFSA Considerations

Regarding the available subchronic toxicity study for dimethyl sulfide [FL-no: 12.006] available in FGE.08Rev5, the Panel noted that this compound has been used as a representative substance to cover the evaluation of the subgroup of acyclic sulfides, with and without oxygenated functional groups, in FGE.08Rev5 (EFSA CEF Panel, 2012a). The Panel considered that the two sulfides [FL-no: 12.065 and 12.079], according to their chemical structures, can be considered structurally related to the substances in this subgroup. In addition, based on the available information on ADME (see Section 3.3.1), the expected metabolic pathways involved would be the same for all the acyclic sulfides (different chain length and with or without oxygenated functional groups). Therefore, the Panel agreed that the NOAEL (250 mg/kg bw per day) for dimethyl sulfide [FL-no: 12.006] can be used to derive a margin of safety for the two sulfides [FL-no: 12.065 and 12.079].

3.4. Application of the Procedure

Application of the Procedure to two substances from subgroup ii – acyclic sulfides with oxidised side-chains and 10 substances from subgroup v – thiols with oxidised side-chains (JECFA, 2000, 2004, 2012).

Subgroup ii – acyclic sulfides with oxidised side-chains

In the 53rd JECFA meeting report, the two sulfides [FL-no: 12.065 and 12.079], under evaluation in FGE.91Rev3, were allocated to structural class I, according to Cramer et al. (1978).

JECFA considered that these two flavouring substances cannot be anticipated to be metabolised to innocuous products and accordingly they should be evaluated along the B-side of the Procedure scheme. JECFA considered the available NO(A)ELs for structurally substances 2-(methylthiomethyl)-3-phenylpropenal (JECFA-no: 505) and dimethyl sulfide (JECFA-no: 452, [FL-no: 12.006]) inappropriate for evaluating the toxicity of the two candidate substances [FL-no: 12.065 and 12.079], since JECFA expected these substances ([FL-no: 12.065 and 12.079]) to be potentially more reactive and toxic. Therefore, the JECFA evaluation proceeded to step B5 of their Procedure where JECFA concluded that the substances [FL no: 12.065 and 12.079] do not raise a safety concern because their exposure estimates (based on MSDIs) were below the threshold of 1.5 µg/person per day (see Appendix A).

Subgroup v – thiols with oxidised side-chains

At their 53rd, JECFA (2000) allocated the tertiary monothiols to structural class I [FL-no: 12.137, 12.138, 12.145 and 12.252] and structural class II [FL-no: 12.038, 12.085 and 12.259], according to the decision tree approach presented Cramer et al. (1987). JECFA decided that these substances cannot be anticipated to be converted to innocuous metabolites. Therefore, their evaluation proceeded along the B-side of the JECFA procedure. JECFA evaluated these flavouring substances by comparison of their MSDI exposure estimates with the NOAELs of the secondary thiol 2-mercapto-3-butanol ([FL-no: 12.024], JECFA-no: 546) for [FL-no: 12.038, 12.085, 12.137, 12.138, 12.145, 12.252 and 12.259] and of the secondary thiol cyclopentanethiol ([FL-no: 12.029], JECFA-no: 546) for [FL-no: 12.085]. Adequate margins of safety could be derived and therefore JECFA concluded, at step B4 of the Procedure, that the candidate flavouring substances [FL-no: 12.137, 12.138, 12.145, 12.252, 12.038, 12.085 and 12.259] would not pose a safety concern at their estimated levels of exposure based on the MSDI approach.

At their 61st meeting, JECFA (2004) allocated the candidate substances [FL-no: 12.169 and 12.241] to structural class I, according to Cramer et al. (1987) and decided that these two thiols should be evaluated along the B-side of the procedure as no anticipated conversion into innocuous metabolites is expected. JECFA calculated adequate margins of safety by comparing exposure estimate (based on MSDI approach) of [FL-no: 12.169] with a NOAEL for the secondary thiol 3-mercapto-2-pentanone ([FL-no: 12.031], JECFA-no: 560) and exposure estimate of [FL-no: 12.241] with a NOAEL for the secondary thiol 2-mercapto-3-butanol ([FL-no: 12.024], JECFA-no: 546).

At their 76th meeting, JECFA (2012) allocated the candidate substance [FL-no: 12.304] to structural class I, according to Cramer et al. (1987) and decided to evaluate this along the B-side of the procedure as no anticipated conversion into innocuous metabolites is expected. JECFA calculated adequate margins of safety by comparing exposure estimate of [FL-no: 12.304] with NOAELs for the secondary thiol 2-mercapto-3-butanol ([FL-no: 12.024], JECFA-no: 546), α -methyl- β -hydroxypropyl α -methyl- β -mercaptopropyl sulfide (JECFA-no: 547) and 3-mercapto-2-pentanone ([FL-no: 12.031], JECFA-no: 560).

Therefore, JECFA concluded that flavouring substances [FL-no: 12.169, 12.241 and 12.304] would not pose a safety concern.

The JECFA safety evaluations of flavouring substances in FGE.91Rev3 are summarised in Table D.1 – Appendix D.

EFSA considerations

The FAF Panel disagrees with JECFA with respect to the allocation of the two sulfides [FL-no: 12.065 and 12.079] to structural class I. According to the structural analysis using the OECD (Q)SAR Toolbox (version 4.3.1), the Panel assigns the substances to structural class III.

In line with JECFA, the Panel considers that the two flavouring substances are not expected to be metabolised to innocuous products (step 2) and accordingly evaluates these substances along the B-side of the Procedure.

The estimated daily intake, based on MSDI approach, of the two substances is below the TTC for their structural class III (step B3). The Panel considers that the available NOAEL on the structurally related substance dimethyl sulfide ([FL-no: 12.006]) is suitable for a further evaluation of these substances (see EFSA considerations in section 3.3.3.2). Comparison of the MSDIs of [FL-no: 12.065 and 12.079] with the NOAEL of the 90-day study with dimethyl sulfide (250 mg/kg bw per day) provides adequate margins of safety (12.5×10^8 and 6.25×10^8 , respectively) for both substances.

Therefore, the Panel concluded, at step B4 of the Procedure scheme, that these two sulfides [FL-no: 12.065 and 12.079] do not pose a safety concern when used as flavouring substances at the estimated levels of intake, based on MSDI approach.

The FAF Panel allocated the tertiary monothiol [FL-no: 12.085] to structural class I, whereas the remaining monothioles [FL-no: 12.038, 12.085, 12.137, 12.138, 12.145, 12.169, 12.241, 12.252, 12.259 and 12.304] to structural class III, in accordance to the structural analysis using the OECD (Q)SAR Toolbox (version 4.3.1).

The Panel agrees with JECFA that these substances cannot be anticipated to be converted into innocuous metabolites, and therefore these substances will be evaluated along the B-side of the Procedure. The MSDIs of all tertiary monothioles are below the TTC for their structural classes (step B3). The Panel does not make use of the NOAELs, derived from secondary thiols, selected by JECFA for the finalisation of the safety evaluations of the 10 tertiary monothioles [FL-no: 12.038, 12.085, 12.137, 12.138, 12.169, 12.145, 12.241, 12.252, 12.259 and 12.304]. With respect to these 10 substances, the Panel considered that the newly available 90-day toxicity study on 2-methyl-4-oxopentane-2-thiol (4-mercapto-4-methyl-2-pentanone) is suitable to identify a NOAEL of 260 μ g/kg bw per day (the highest dose tested). The Panel noted that this 90-day toxicity study has been performed with a test material that did not contain the secondary component 4-methyl-3-penten-2-one, differently from the material of commerce of [FL-no: 12.169] which contains 48–50% of this compound. However, 4-methyl-3-penten-2-one has been evaluated as of no safety concern by EFSA in FGE.63Rev2⁸ (EFSA CEF Panel, 2013) and it is an authorised flavouring substance in the EU Union List under [FL-no: 07.101]. Therefore, the Panel considers the NOAEL of this study with 2-methyl-4-oxopentane-2-thiol (4-mercapto-4-methyl-2-pentanone) suitable for the evaluation of the 10 tertiary

⁸ The genotoxicity concern for [FL-no: 07.101] was ruled out in FGE.204 (EFSA CEF Panel, 2012b) and then [FL-no: 07.101] was concluded at step A3 of the Procedure as of no safety concern at the estimated levels of intake as flavouring substance, based on the MSDI approach, in FGE.63Rev2 (EFSA CEF Panel, 2013).

monothiols in FGE.91Rev3. With this NOAEL, adequate margins of safety (> 400) for these 10 tertiary monothiols [FL-no: 12.038, 12.085, 12.137, 12.138, 12.169, 12.145, 12.241, 12.252, 12.259 and 12.304] have been calculated. Therefore, the FAF Panel concludes at step B4 of the Procedure scheme that none of these 10 substances would raise a safety concern when used as flavouring substances at their estimated levels of exposure based on the MSDI approach.

The stepwise evaluations of all flavouring substances in FGE.91Rev3 are summarised in Table D.1 – Appendix D.

4. Discussion

This revision 3 of FGE.91 comprises in total 49 substances, 44 of which had already been considered before in FGE.91Rev2. Two additional flavouring substances [FL-no: 12.065 and 12.079] have been included in this revision, following an extensive evaluation in FGE.201Rev2 of their possible genotoxic potential due to a structural alert for genotoxicity (i.e. α,β -unsaturated carbonyl compounds or precursors for that). Three additional substances [FL-no: 12.169, 12.241] from FGE.74Rev4 and [FL-no: 12.304] from FGE.08Rev5 have been included in the present revision 3 of FGE.91 for finalisation of their safety evaluation. The Panel considered these substances sufficiently structurally related to the other tertiary monothiols in this FGE and thus agreed to conclude their evaluation directly in the same FGE.

Because the concern for genotoxicity was ruled out and based on consideration of structural class, metabolism and toxicological data and the MSDI exposure estimates, the FAF Panel concludes that the flavouring substances considered in this revision of FGE.91 (FGE.91Rev3) do not raise a safety concern at step B4 of the Procedure scheme as a NOAEL which provides an adequate margin of safety for all candidate substances exists.

For 44 substances, including the newly added flavouring substances ([FL-no: 12.065, 12.079, 12.169, 12.241 and 12.304]) and the seven tertiary monothiols from FGE.91Rev2 ([FL-no: 12.038, 12.085, 12.137, 12.138, 12.145, 12.252 and 12.259]), normal and maximum use levels have been provided from which mTAMDI exposure estimates have been calculated. The mTAMDI intake estimates for flavouring substances [FL-no: 12.085, 12.137, 12.138, 12.145, 12.169 and 12.241] are below the TTC for their structural classes (I and III). The mTAMDI figures for five substances [FL-no: 12.264, 12.284, 12.274, 12.108, 12.139] from the previous revision (FGE.91Rev2) and five substances in the current revision [FL-no: 12.065, 12.079, 12.038, 12.252 and 12.304] are above the TTC for their structural classes. No normal and maximum use levels have been provided for flavouring substances [FL-no: 12.077, 12.162, 12.265, 12.267 and 17.036], previously evaluated in FGE.91Rev2. Therefore, for these 15 substances (more detailed), data on uses and use levels are needed to refine their exposure assessment. On the basis of such data, these flavouring substances should be reconsidered using the Procedure. In order to determine whether the conclusion for the 49 JECFA-evaluated substances can be applied to the materials of commerce, it is necessary to consider the available specifications. Adequate specifications, including complete purity criteria and identity data, are available for all 49 flavouring substances.

5. Conclusions

The Panel concludes that for 49 flavouring substances in FGE.91Rev3 there is no safety concern at the estimated levels of intake as flavouring substances, based on the MSDI approach. For 10 flavouring substances [FL-no: 12.065, 12.038, 12.079, 12.108, 12.139, 12.264, 12.274, 12.252, 12.284 and 12.304], the mTAMDI value estimates are above the TTC for their structural classes. Therefore, for these 10 substances more detailed data on uses and use levels are needed in order to refine the exposure assessment and to finalise their safety evaluations. No normal and maximum use levels have been provided for flavouring substances [FL-no: 12.077, 12.162, 12.265, 12.267 and 17.036] which preclude the calculation of their mTAMDI values for comparison with the TTC.

6. Recommendations

The Panel recommends the European Commission to consider:

- to change the chemical name of [FL-no: 12.169] from 2-methyl-4-oxopentane-2-thiol to 4-mercapto-4-methyl-2-pentanone as indicated in Table B.1 – Appendix B;
- to request normal and maximum use levels for flavouring substances [FL-no: 12.077, 12.162, 12.265, 12.267 and 17.036].

- to request more detailed data on uses and use levels for flavouring substances [FL-no: 12.065, 12.038, 12.079, 12.108, 12.139, 12.264, 12.274, 12.252, 12.284 and 12.304] in order to refine the exposure assessment and to finalise their safety evaluations.

7. Documentation provided to EFSA

- 1) EFFA (European Flavour Association), Submission of additional information on isomeric composition of substances of FGE.91Rev3 ([FL-no: 12.065 and 12.079]).
- 2) IOFI (International Organization of the Flavor Industry), 1995b. European inquiry on volume of use. IOFI, International Organization of the Flavor Industry, 1995.
- 3) EFFA (European Flavour Association), 2018d. EFFA 2018 use levels for 63 flavouring substances from FGE.67, 76, 91, 201, 204, 212, 213, 215, 216 and 222. Unpublished data submitted from EFFA to DG SANTE. Dated December 2018.
- 4) EFFA (European Flavour Association), 2020a. Flavouring Substances-thiols-FGE91_poundages & Refined-UL_upd 030320. Updated information on nine substances to be evaluated in FGE.91Rev3. Unpublished data submitted by EFFA to EFSA, dated 03/03/2020.
- 5) EFFA (European Flavour Association), 2017. Submission of Footnote-10 Dossier ("Thiols"): toxicological studies on Repr. Material: 2-Methyl-4-oxopentane2-thiol [FL-no: 12.169] (FGE.74Rev3 & FGE.91Rev2) and tonnage data – Footnote-10 substances. Unpublished data submitted by EFFA to EFSA. Dated 12/12/17.
- 6) M Lloyd BSs, 2014. Induction of micronuclei in cultured human peripheral blood lymphocytes. 2-Mercapto-4-methylpentan-2-one. Covance Laboratories Ltd. Study no. 8261929. May 2014. Unpublished report submitted by EFFA to EFSA.

References

- Cramer GM, Ford RA and Hall RL, 1978. Estimation of toxic hazard – a decision tree approach. *Food and Cosmetics Toxicology*, 16, 255–276.
- Butterworth KR, Carpanini FMB, Gaunt IF, Hardy J, Kiss IS and Gangolli SD, 1975. Short-term toxicity of dimethyl sulphide in the rat. *Food and Cosmetics Toxicology*, 13, 15–22.
- Cramer GM, Ford RA and Hall RL, 1978. Estimation of toxic hazard – a decision tree approach. *Food and Cosmetics Toxicology*, 16, 255–276.
- EFSA CEF Panel (EFSA panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2010. Scientific opinion on Flavouring Group Evaluation 91 (FGE.91): consideration of simple aliphatic and aromatic sulphides and thiols evaluated by JECFA (53rd and 68th meetings) structurally related to aliphatic and alicyclic mono-, di-, tri-, and polysulphides with or without additional oxygenated functional groups evaluated by EFSA in FGE.08Rev1. *EFSA Journal* 2010;8(10):1337. <https://doi.org/10.2903/j.efsa.2010.1337>
- EFSA CEF Panel (EFSA panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2011a. Scientific opinion on Flavouring Group Evaluation 91 Revision 1(FGE.91Rev1): consideration of simple aliphatic and aromatic sulphides and thiols evaluated by JECFA (53rd and 68th meetings) structurally related to aliphatic and alicyclic mono-, di-, tri-, and polysulphides with or without additional oxygenated functional groups evaluated by EFSA in FGE.08Rev3 (2011). *EFSA Journal* 2011;9(12):2459, 72 pp. <https://doi.org/10.2903/j.efsa.2011.2459>
- EFSA CEF Panel (EFSA panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2011b. Statement on data provided for subgroup 1.1.1 of FGE.19. *EFSA Journal* 2011;9(2):2086, 5 pp. <https://doi.org/10.2903/j.efsa.2011.2086>
- EFSA CEF Panel (EFSA panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2012a. Scientific Opinion on Flavouring Group Evaluation 08, Revision 5 (FGE.08Rev5): aliphatic and alicyclic mono-, di-, tri-, and polysulphides with or without additional oxygenated functional groups from chemical groups 20 and 30. *EFSA Journal* 2012;10(7):2837, 154 pp. <https://doi.org/10.2903/j.efsa.2012.2837>
- EFSA CEF Panel (EFSA panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2012b. Scientific Opinion on Flavouring Group Evaluation 204 (FGE.204): consideration of genotoxicity data on representatives for 18 mono-unsaturated, aliphatic, α,β -unsaturated ketones and precursors from chemical subgroup 1.2.1 of FGE.19 by EFSA. *EFSA Journal* 2012;10(12):2992, 25 pp. <https://doi.org/10.2903/j.efsa.2012.2992>
- EFSA CEF Panel (EFSA panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2013. Scientific opinion on Flavouring Group Evaluation 63 Revision 2 (FGE.63Rev2): consideration of aliphatic secondary alcohols, ketones and related esters evaluated by JECFA (59th and 69th meetings) structurally related to saturated and unsaturated aliphatic secondary alcohols, ketones and esters of secondary alcohols and saturated linear or branched-chain carboxylic acids evaluated by EFSA in FGE.07Rev4. *EFSA Journal* 2013;11(4):3188, 45 pp. <https://doi.org/10.2903/j.efsa.2013.3188>

- EFSA CEF Panel (EFSA panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2014a. Scientific opinion on Flavouring Group Evaluation 91 Revision 2 (FGE.91Rev2): consideration of simple aliphatic and aromatic sulphides and thiols evaluated by the JECFA (53rd and 68th meetings) structurally related to aliphatic and alicyclic mono-, di-, tri-, and polysulphides with or without additional oxygenated functional groups evaluated by EFSA in FGE.08Rev5 (2012). EFSA Journal 2014;12(6):3707, 77 pp. <https://doi.org/10.2903/j.efsa.2014.3707>
- EFSA CEF Panel (EFSA panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2014b. Scientific opinion on Flavouring Group Evaluation 74 Revision 3 (FGE.74Rev3): consideration of simple aliphatic sulphides and thiols evaluated by the JECFA (53rd and 61st meeting) structurally related to aliphatic and alicyclic mono-, di-, tri-, and polysulphides with or without additional oxygenated functional groups from chemical group 20 evaluated by EFSA in FGE.08Rev5 (2012). EFSA Journal 2014;12(6):3710, 62 pp. <https://doi.org/10.2903/j.efsa.2014.3710>
- EFSA CEF Panel (EFSA panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2018a. Scientific Opinion on Flavouring Group Evaluation 74, Revision 4 (FGE.74Rev4): consideration of aliphatic sulphides and thiols evaluated by JECFA (53rd and 61st meeting) structurally related to aliphatic and alicyclic mono-, di-, tri- and polysulphides with or without additional oxygenated functional groups from chemical group 20 evaluated by EFSA in FGE.08Rev5. EFSA Journal 2018;16(3):5167, 58 pp. <https://doi.org/10.2903/j.efsa.2018.5167>
- EFSA CEF Panel (EFSA panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2018b. Scientific Opinion on Flavouring Group Evaluation 201 Revision 2 (FGE.201Rev2): 2-alkylated, aliphatic, acyclic alpha, beta-unsaturated aldehydes and precursors, with or without additional double-bonds, from chemical subgroup 1.1.2 of FGE.19. EFSA Journal 2018;16(10):5423, 33 pp. <https://doi.org/10.2903/j.efsa.2018.5423>
- EFSA Scientific Committee, 2009. Guidance of the Scientific Committee on transparency in the scientific aspects of risk assessments carried out by EFSA. Part 2: general principles. EFSA Journal 2009;7(7):1051, 22 pp. <https://doi.org/10.2903/j.efsa.2009.1051>
- EFSA Scientific Committee, 2019. Guidance on the use of the Threshold of Toxicological Concern approach in food safety assessment. EFSA Journal 2019;17(6):5708, 17 pp. <https://doi.org/10.2903/j.efsa.2019.5708>
- JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1995. Evaluation of certain food additives and contaminants. Forty-fourth Meeting of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, no. 859. Geneva.
- JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1996. Toxicological evaluation of certain food additives. The forty-fourth meeting of the Joint FAO/WHO Expert Committee on Food Additives and contaminants.
- JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1997. Evaluation of certain food additives and contaminants. Forty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, 6-15 February 1996. WHO Technical Report Series, no. 868. Geneva.
- JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1999. Evaluation of certain food additives and contaminants. Forty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives. Rome, 17-26 June 1997. WHO Technical Report Series, no. 884. Geneva.
- JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2000. Evaluation of certain food additives and contaminants. Fifty-third meeting of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series no. 896. Geneva, 1-10 June 1999.
- JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2003. Compendium of food additive specifications. Addendum 11. Joint FAO/WHO Expert Committee of Food Additives 61st session. Rome, 10 -19 June 2003. Food and Nutrition paper 52 Add.11.
- JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2004. Evaluation of certain food additives. Sixty-first report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, no. 922. Rome, 10-19 June 2003.
- JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2005. Compendium of food additive specifications. Addendum 12. Joint FAO/WHO Expert Committee of Food Additives 63rd session. Rome, 8-17 June 2004. FAO Food and Nutrition paper 52 Add. 12.
- JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2006. Evaluation of certain food additives. Sixty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, no. 934. Geneva, 7-16 June 2005.
- JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2007. Evaluation of certain food additives and contaminants, Sixty-eighth report of the Joint FAO/WHO Expert committee on Food Additives, WHO Technical Report Series, no. 947, 2007, Geneva, Switzerland.
- JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2012. Evaluation of certain food additives. Seventy-sixth Meeting of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, 5-14 June 2012.
- JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2016. Evaluation of certain food additives, eightysecond report of the Joint FAO/WHO Expert Committee on Food Additives, WHO technical report series; no. 1000, 2016, Geneva, Switzerland.

SCF (Scientific Committee on Food), 1995. First annual report on chemically defined flavouring substances. May 1995, 2nd draft prepared by the SCF Working Group on Flavouring Substances (Submitted by the SCF Secretariat, 17 May 1995). CS/FLAV/FL/140-Rev2. Annex 6 to Document III/5611/95, European Commission, Directorate-General III, Industry.

SCF (Scientific Committee on Food), 1999. Opinion on a programme for the evaluation of flavouring substances (expressed on 2 December 1999). SCF/CS/FLAV/TASK/11 Final 6/12/1999. Annex I the minutes of the 119th Plenary meeting. European Commission, Health & Consumer Protection Directorate-General.

Abbreviations

ADME	absorption, distribution, metabolism and excretion
bw	body weight
CEF	EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
DMSO	dimethyl sulfoxide
EFFA	European Flavour Association
FAF	EFSA Panel on Food Additives and Flavourings
FAO	Food and Agriculture Organization of the United Nations
FEMA	Flavor and Extract Manufacturers Association
FGE	Flavouring Group Evaluation
FL-no	FLAVIS number
FLAVIS	Flavour Information System (database)
GSH	glutathione
IOFI	International Organization of the Flavor Industry
JECFA	Joint FAO/WHO Expert Committee on Food Additives
MMC	mitomycin C
MN	micronucleus
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
TTC	threshold of toxicological concern
VIN	vinblastine
WHO	World Health Organization

Appendix A – Procedure of the safety evaluation

The approach for a safety evaluation of chemically defined flavouring substances as referred to in Commission Regulation (EC) No 1565/2000, 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 products¹⁰ (step 2)?
- Do their exposures exceed the TTC for the structural class (steps A3 and B3)?
- Are the flavourings or their metabolites endogenous¹¹ (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). The EFSA Scientific Committee has 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).

¹¹ *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).

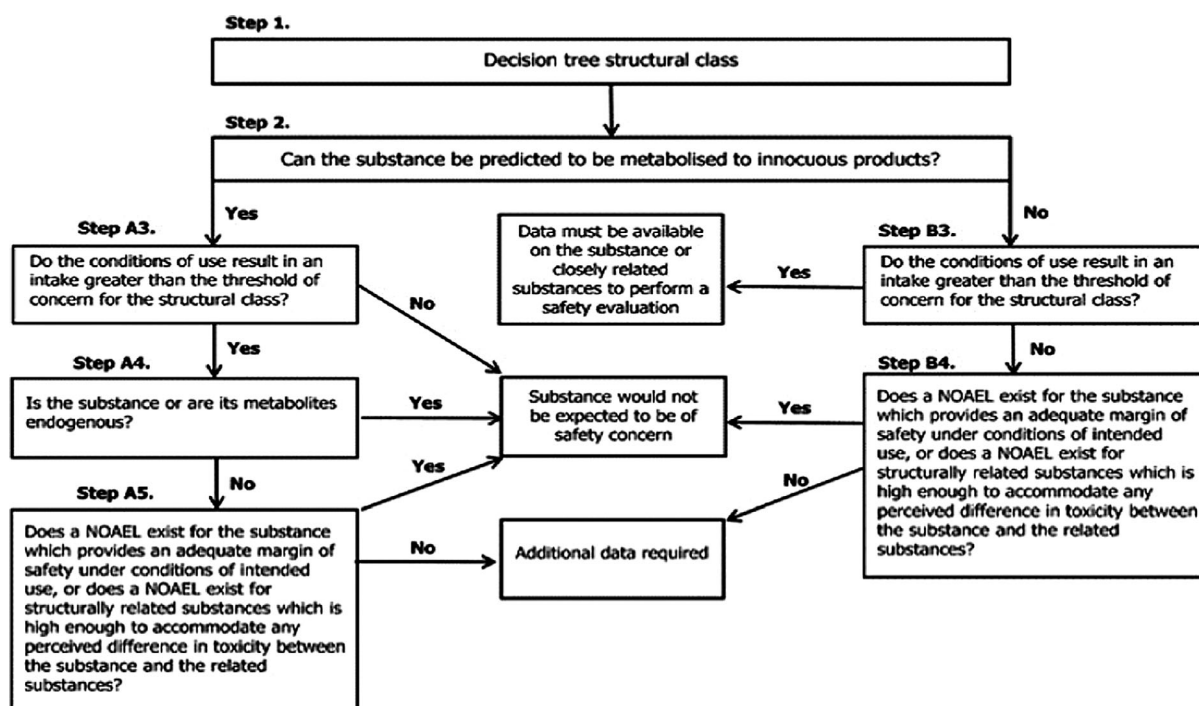


Figure A.1: Procedure for the safety evaluation of chemically defined flavouring substances

For the flavouring substances considered in this Flavouring Group Evaluation (FGE), the EFSA Panel on Food Additives and Flavourings (FAF) compares the JECFA evaluation of structurally related substances with the result of a corresponding EFSA evaluation, focussing on specifications, intake estimations and toxicity data, especially genotoxicity data. The considerations by EFSA will conclude whether the flavouring substances are of no safety concern at their estimated levels of intake, whether additional data are required or whether certain substances should not be evaluated through the EFSA Procedure.

The following issues are of special importance:

a) Intake

In its evaluation, the Panel as a default uses the 'maximised survey-derived daily intake' (MSDI)¹² approach to estimate the per capita intakes of the flavouring substances in Europe.

In its evaluation, JECFA includes intake estimates based on the MSDI approach derived from both European and USA production figures. The highest of the two MSDI figures is used in the evaluation by JECFA. It is noted that in several cases, only the MSDI figures from the USA were available, meaning that certain flavouring substances have been evaluated by JECFA only on the basis of these figures. For substances in the Union List¹³ of flavouring substances for which this is the case, the Panel will need European Union (EU) production figures in order to finalise the evaluation.

When the Panel examined the information provided by the European Flavour Industry on the use levels in various foods, it appeared obvious that the MSDI approach in a number of cases would grossly underestimate the intake by regular consumers of products flavoured at the use levels reported by the Industry, especially in those cases where the annual production values were reported to be small. In consequence, the Panel had reservations about the data on use and use levels provided and the intake estimates obtained by the MSDI approach. It is noted that JECFA, at its 65th meeting, considered 'how to improve the identification and assessment of flavouring agents, for which the MSDI estimates may be substantially lower than the dietary exposures that would be estimated from the anticipated average use levels in foods' (JECFA, 2006).

¹² EU MSDI: Amount added to food as flavour in (kg/year) × 10⁹/ (0.1 × population in Europe (= 375 × 10⁶) × 0.6 × 365) = µg/capita per day.

¹³ Commission Implementing Regulation (EU) No 872/2012 of 1 October 2012 adopting the list of flavouring substances provided for by Regulation (EC) No 2232/96 of the European Parliament and of the Council, introducing it in Annex I to Regulation (EC) No 1334/2008 of the European Parliament and of the Council and repealing Commission Regulation (EC) No 1565/2000 and Commission Decision 1999/217/EC. OJ L 267, 2.10.2012, p. 1–161.

In the absence of more accurate information that would enable the Panel to make a more realistic estimate of the intakes of the flavouring substances, the Panel has decided also to perform an estimate of the daily intakes per person using a modified Theoretical Added Maximum Daily Intake (mTAMDI) approach based on the normal use levels reported by Industry (see Appendix C.2).

As information on use levels for the flavouring substances has not been requested by JECFA or has not otherwise been provided to the Panel, it is not possible to estimate the daily intakes using the mTAMDI approach for many of the substances evaluated by JECFA. The Panel will need information on use levels in order to finalise the evaluation.

b) Threshold of 1.5 microgram/person per day (step B5) used by JECFA

JECFA uses the threshold of concern of 1.5 j.tg/person per day as part of the evaluation procedure:

'The Committee noted that this value was based on a risk analysis of known carcinogens which involved several conservative assumptions. The use of this value was supported by additional information on developmental toxicity, neurotoxicity and immunotoxicity. In the judgement of the Committee, flavouring substances for which insufficient data are available for them to be evaluated using earlier steps in the Procedure, but for which the intake would not exceed 1.5 j.tg/person per day would not be expected to present a safety concern. The Committee recommended that the Procedure for the Safety Evaluation of Flavouring Agents, used at the forty-sixth meeting, should be amended to include the last step on the right-hand side of the original procedure ('Do the conditions of use result in an intake greater than 1.5 j.tg per day?')' (JECFA, 1999).

In line with the opinion expressed by the Scientific Committee on Food (SCF, 1999), the Panel does not make use of this threshold of 1.5 j.tg per person per day.

c) Genotoxicity

As reflected in the opinion of 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.

d) Specifications

Regarding specifications, the evaluation by the Panel could lead to a different opinion than that of JECFA, since the Panel requests information on e.g. isomerism.

e) Structural Relationship

In the consideration of the JECFA-evaluated substances, the Panel will examine the structural relationship and metabolism features of the substances within the flavouring group and compare this with the corresponding FGE.

Appendix B – Specifications

Table B.1: Summary table on specifications data for flavouring substances in FGE.91Rev3 (for chemical structures see Appendix D)

Information included in the EU Union list Regulation No (EU) 1334/2008 as amended			Most recent available specifications data ^(a)				EFSA comments
FL-no JECFA-no FEMA no CoE no CAS no	Chemical name	Purity of the named compound	Phys. form Mol. formula Mol. weight	Solubility ^(c) Solubility in ethanol ^(d)	Boiling point, °C ^(e) Melting point, °C ID test Assay minimum (isomers distribution/SC ^(h))	Refrac. Index ^(f) Spec. gravity ^(g)	
12.012 1699 - 533 110-81-6	Diethyl disulfide	(b)	Liquid C ₄ H ₁₀ S ₂ 122.24	Practically insoluble or insoluble Soluble	152 IR MS 95%	1.502–1.508 0.990–0.996	
12.017 1659 546 75-08-1	Ethanethiol	(b)	Liquid C ₂ H ₆ S 62.13	Slightly soluble Soluble	35 IR NMR MS 95%	1.425–1.431 0.833–0.839	
12.021 1700 4073 600 2179-59-1	Allyl propyl disulfide	(b)	Liquid C ₆ H ₁₂ S ₂ 148.28	Practically insoluble or insoluble Soluble	66 (13 hPa) NMR MS 95%	1.497–1.517 0.999–1.005	
12.038 561 3177 11789 38462-22-5	8-Mercapto- <i>p</i> -menthan-3-one	(b)	Liquid C ₁₀ H ₁₈ OS 186.31	Insoluble Soluble	120 (13 hPa) IR 97% Mixture of four diastereoisomers, each about 25%	1.492–1.509 0.995–1.010	
12.065 471 3483 11904 59902-01-1	2,8-Dithianon-4-en-4-carboxaldehyde	(b)	Liquid C ₈ H ₁₄ OS ₂ 190.32	Slightly soluble	104–105 (13 hPa) IR NMR 40–70% (<i>E</i>)-isomer and 30–60% (<i>Z</i>)-isomer	1.557–1.567 1.105–1.107	Documentation provided to EFSA nr: 1

Information included in the EU Union list Regulation No (EU) 1334/2008 as amended			Most recent available specifications data ^(a)				EFSA comments
FL-no JECFA-no FEMA no CoE no CAS no	Chemical name	Purity of the named compound	Phys. form Mol. formula Mol. weight	Solubility ^(c) Solubility in ethanol ^(d)	Boiling point, °C ^(e) Melting point, °C ID test Assay minimum (isomers distribution/SC ^(h))	Refrac. Index ^(f) Spec. gravity ^(g)	
12.077 460 3597 - 766-92-7	Benzyl methyl sulfide	(b)	Liquid C ₈ H ₁₀ S 138.23	Slightly soluble Soluble	197 IR 98%	1.563–1.573 1.015–1.020	
12.079 470 3601 11549 40878-72-6	2-(Methylthiomethyl)but-2-enal	(b)	Liquid C ₆ H ₁₀ OS 130.21	Insoluble	77 (7 hPa) IR 40%-70% (<i>E</i>)-isomer and 30-60% (<i>Z</i>)-isomer	1.5228-1.5328 0.982-0.987	Documentation provided to EFSA nr: 1
12.085 523 3700 - 71159-90-5	<i>p</i> -Menth-1-ene-8-thiol	(b)	Liquid C ₁₀ H ₁₈ S 170.31	Slightly soluble Soluble	58 (0.4 hPa) IR 98% (racemate)	1.504 0.948 (20°)	
12.108 1672 - 11454 68084-03-7	Di-isopentyl thiomalate	(b)	Solid C ₁₄ H ₂₆ O ₄ S 290.42	Practically insoluble or insoluble Soluble	425 50 NMR MS 95% (racemate)	n.a. n.a.	
12.114 1701 4029 11451 3600-24-6	Diethyl trisulfide	(b)	Liquid C ₄ H ₁₀ S ₃ 154.3	Practically insoluble or insoluble Soluble	217 NMR MS 95%	1.556–1.560 1.121–1.231	No longer supported by Industry (DG SANCO, 2013)
12.126 1694 4041 11478 30453-31-7	Ethyl propyl disulfide	(b)	Liquid C ₅ H ₁₂ S ₂ 136.27	Practically insoluble or insoluble Soluble	180 IR NMR MS 95%	1.483–1.493 0.943–0.953	

Information included in the EU Union list Regulation No (EU) 1334/2008 as amended			Most recent available specifications data ^(a)				EFSA comments
FL-no JECFA-no FEMA no CoE no CAS no	Chemical name	Purity of the named compound	Phys. form Mol. formula Mol. weight	Solubility ^(c) Solubility in ethanol ^(d)	Boiling point, °C ^(e) Melting point, °C ID test Assay minimum (isomers distribution/SC ^(h))	Refrac. Index ^(f) Spec. gravity ^(g)	
12.130 1663 4259 11485 1639-09-4	Heptane-1-thiol	(b)	Liquid C ₇ H ₁₆ S 132.26	Practically insoluble or insoluble Soluble	175 IR NMR MS 95%	1.497–1.503 0.840–0.846	
12.134 1679 4260 - 34365-79-2	S-Isopropyl 3-methylbut-2-enethioate	(b)	Liquid C ₈ H ₁₄ OS 158.26	Practically insoluble or insoluble Soluble	236 NMR 95%	1.486–1.492 1.006–1.012	
12.137 544 3854 - 34300-94-2	3-Mercapto-3-methylbutan-1-ol	(b)	Liquid C ₅ H ₁₂ OS 120.2	Soluble Soluble	186 (950 hPa) NMR MS 96%	1.480–1.490 0.989 (20°)	
12.138 549 3855 - 50746-10-6	3-Mercapto-3-methylbutyl formate	(b)	Liquid C ₆ H ₁₂ O ₂ S 148.22	Soluble Soluble	181 IR 95%	1.462–1.472 1.03	
12.139 1666 4159 11880 7217-59-6	2-Mercaptoanisole	(b)	Liquid C ₇ H ₈ OS 140.2	Practically insoluble or insoluble Soluble	227 IR NMR MS 95%	1.589–1.595 1.137–1.149	
12.145 548 3785 94087-83-9	4-Methoxy-2-methylbutane-2-thiol	(b)	Liquid C ₆ H ₁₄ OS 134.24	Insoluble Soluble	59 IR 98%	1.445–1.455 0.907–0.923	

Information included in the EU Union list Regulation No (EU) 1334/2008 as amended			Most recent available specifications data ^(a)				EFSA comments
FL-no JECFA-no FEMA no CoE no CAS no	Chemical name	Purity of the named compound	Phys. form Mol. formula Mol. weight	Solubility ^(c) Solubility in ethanol ^(d)	Boiling point, °C ^(e) Melting point, °C ID test Assay minimum (isomers distribution/SC ^(h))	Refrac. Index ^(f) Spec. gravity ^(g)	
12.146 1691 4003 11525 16630-66-3	Methyl (methylthio)acetate	(b)	Liquid C ₄ H ₈ O ₂ S 120.2	Practically insoluble or insoluble Soluble	145 IR NMR MS 98%	1.464–1.466 1.105–1.115	
12.153 1693 4040 11470 20333-39-5	Methyl ethyl disulfide	(b)	Liquid C ₃ H ₈ S ₂ 108.22	Practically insoluble or insoluble Soluble	137 IR NMR MS 80% (SC: 7–8% diethyl sulfide and 8–10% dimethyl sulfide)	1.410–1.418 1.015–1.029	
12.162 459 3873 11533 100-68-5	Methyl phenyl sulfide	(b)	Liquid C ₇ H ₈ S 124.21	Insoluble Soluble	188–193 IR 98%	1.532–1.551 0.958–0.968	
12.169 1293 3997 11500 19872-52-7	2-Methyl-4-oxopentane-2-thiol	48% and 48–50% 4-methyl-3-penten-2-one	Liquid C ₆ H ₁₂ OS 132.23	Soluble Very slightly soluble	47–49 (20 hPa) IR NMR MS 1% solution in propylene glycol of a mixture of 2-methyl-4-oxopentane-2-thiol (48%) and 4-methyl-3-penten-2-one (48–50%)	1.431–1.437 1.032–1.037	The chemical name should be changed to 4-mercapto-4-methyl-2-pentanone
12.240 1684 4214 - 6540-86-9	2,4,6-Trithiaheptane	(b)	Liquid C ₄ H ₁₀ S ₃ 154.32	Slightly soluble Soluble	255 IR NMR MS 95%	1.444–1.445 1.540–1.550	

Information included in the EU Union list Regulation No (EU) 1334/2008 as amended			Most recent available specifications data ^(a)				EFSA comments
FL-no JECFA-no FEMA no CoE no CAS no	Chemical name	Purity of the named compound	Phys. form Mol. formula Mol. weight	Solubility ^(c) Solubility in ethanol ^(d)	Boiling point, °C ^(e) Melting point, °C ID test Assay minimum (isomers distribution/SC ^(h))	Refrac. Index ^(f) Spec. gravity ^(g)	
12.241 1290 3995 - 258823-39-1	2-Mercapto-2-methylpentan-1-ol	(b)	Liquid C ₆ H ₁₄ OS 134.24	Slightly soluble Soluble	57–59 (0.8 hPa) IR NMR 99% (racemate)	1.476–1.483 0.968–0.974 (20°)	
12.242 1675 4185 - 29414-47-9	Methylthiomethylmercaptan	(b)	Liquid C ₂ H ₆ S ₂ 94.2	Soluble Soluble	40 (2.7 hPa) NMR 97%	1.552–1.556 1.040–1.046	
12.243 1661 4097 - 6725-64-0	Dimercaptomethane	(b)	Liquid CH ₄ S ₂ 80.17	Soluble Soluble	118 NMR 95%	1.578–1.584 0.827–0.831	
12.252 1669 4158 - 31539-84-1	4-Mercapto-4-methyl-2-pentanol	(b)	Liquid C ₆ H ₁₄ OS 134.26	Soluble Soluble	51 (0.1 hPa) NMR 98% (racemate)	1.463–1.468 1.154–1.158	
12.253 1697 4025 - 72437-68-4	Amyl methyl disulfide	(b)	Liquid C ₆ H ₁₄ S ₂ 150.31	Practically insoluble or insoluble Soluble	198–202 IR NMR MS 97%	1.485–1.495 0.943–0.953	

Information included in the EU Union list Regulation No (EU) 1334/2008 as amended			Most recent available specifications data ^(a)				EFSA comments
FL-no JECFA-no FEMA no CoE no CAS no	Chemical name	Purity of the named compound	Phys. form Mol. formula Mol. weight	Solubility ^(c) Solubility in ethanol ^(d)	Boiling point, °C ^(e) Melting point, °C ID test Assay minimum (isomers distribution/SC ^(h))	Refrac. Index ^(f) Spec. gravity ^(g)	
12.254 1698 4027 - 63986-03-8	Butyl ethyl disulfide	At least 90%, secondary components 2–3% diethyl disulfide and 5–6% dibutyl disulfide	Liquid C ₆ H ₁₄ S ₂ 150.31	Practically insoluble or insoluble Soluble	202 IR NMR MS 90% (SC: 2–3% diethyl disulfide and 5–6% dibutyl disulfide)	1.492–1.502 0.950–0.968	
12.256 1695 4042 - 31499-70-4	Ethyl propyl trisulfide	(b)	Liquid C ₅ H ₁₂ S ₃ 168.34	Practically insoluble or insoluble Soluble	234–237 IR NMR MS 98% (SC: 20–30% diethyl trisulfide and 20–30% dipropyl trisulfide)	1.549–1.559 1.070–1.087	No longer supported by industry (DG SANCO, 2013)
12.259 1673 4300 - 29725-66-4	1-Mercapto- <i>p</i> -menthan-3-one	At least 89%, secondary components 8–9% piperitone and 1–2% alpha-terpineol	Liquid C ₁₀ H ₁₈ OS 186.31	Practically insoluble or insoluble Soluble	122 (1.3 hPa) NMR 89% (<i>cis</i> - and <i>trans</i> -Mercapto- <i>p</i> -menthan-3-one, two <i>cis</i> - and two <i>trans</i> -enantiomers (each 25%)/ SC: 8–9% piperitone and 1–2% alpha-terpineol)	1.487–1.497 0.989–0.999	
12.264 1670	4,2-Thiopentanone	(b)	Liquid C ₅ H ₁₀ OS 118.00	Insoluble Soluble	258–260 IR NMR MS 95% (racemate)	1.437–1.443 1.154–1.158	
12.265 1683 4157 - 92585-08-5	(<i>E</i>)-2-Methyl-1-methylthio-2-butene	(b)	Liquid C ₆ H ₁₂ S 116.23	Very slightly soluble Soluble	78 (100hPa) IR NMR MS 99.3% (<i>E</i>)-isomer (0.2% (<i>Z</i>)-isomer, SC: 0.2% 1-methylthio-2-propanone, 0.1% methyl 2-methyl-2-butenolate, 0.2% unknown)	1.471 0.861	

Information included in the EU Union list Regulation No (EU) 1334/2008 as amended			Most recent available specifications data ^(a)				EFSA comments
FL-no JECFA-no FEMA no CoE no CAS no	Chemical name	Purity of the named compound	Phys. form Mol. formula Mol. weight	Solubility ^(c) Solubility in ethanol ^(d)	Boiling point, °C ^(e) Melting point, °C ID test Assay minimum (isomers distribution/SC ^(h))	Refrac. Index ^(f) Spec. gravity ^(g)	
12.267 1667 4207 - 19788-50-2	Propyl-2-mercaptopropionate	(b)	Liquid C ₆ H ₁₂ O ₂ S 148.23	Very slightly soluble Soluble	193 IR NMR MS 97.3% (racemate)	1.4497 1.018	
12.272 1702 4263 - 1113-13-9	Propyl propanethiosulfonate	(b)	Liquid C ₆ H ₁₄ O ₂ S ₂ 182.31	Sparingly soluble Soluble	113 IR NMR MS 95%	1.485 1.121	No longer supported by Industry (DG SANCO, 2013)
12.273 1692 4183 - 51755-70-5	3-(Methylthio)heptanal	At least 92%; secondary component 5–7% (<i>E</i>)-hept-2-enal	Liquid C ₈ H ₁₆ OS 160.28	Insoluble Soluble	95–96 IR NMR MS 92% (racemate) (SC: 5–7% 2-(<i>E</i>)-heptenal)	1.469–1.475 0.943–0.947	
12.274 1687 4094 - 54644-28-9, 54717-12-3	3,6-Diethyl-1,2,4,5-tetrathiane and 3,5-diethyl-1,2,4-trithiolane mix in vegetable oil triglycerides	0.18% 3,6-diethyl-1,2,4,5-tetrathiane isomer I+ II; 0.05% 3,5-diethyl-1,2,4-trithiolane isomer I; 0.1% 3,5-diethyl-1,2,4-trithiolane isomer II; 99% vegetable oil triglyceride	Liquid C ₆ H ₁₂ S ₄ / C ₆ H ₁₂ S ₃ 212.43/ 180.36	Insoluble Soluble	64–70 (1.3 hPa) NMR MS 95% (1% sol.) Mixture of three diastereo-isomers. Due to the symmetry there is one meso-form (<i>cis</i> -form) and two <i>trans</i> -forms.	1.447–1.453 0.948–0.952	

Information included in the EU Union list Regulation No (EU) 1334/2008 as amended			Most recent available specifications data ^(a)				EFSA comments
FL-no JECFA-no FEMA no CoE no CAS no	Chemical name	Purity of the named compound	Phys. form Mol. formula Mol. weight	Solubility ^(c) Solubility in ethanol ^(d)	Boiling point, °C ^(e) Melting point, °C ID test Assay minimum (isomers distribution/SC ^(h))	Refrac. Index ^(f) Spec. gravity ^(g)	
12.275 1681 4076 - 156420-69-8	Allylthio hexanoate	(b)	Liquid C ₉ H ₁₆ OS 172.29	Insoluble Soluble	195–196 IR NMR MS 98%	1.473–1.479 0.930–0.934	
12.276 1671 4162 - 400052-49-5	(S)-1-Methoxy-3-heptanethiol	(b)	Liquid C ₈ H ₁₈ OS 162.30	Slightly soluble Soluble	203.8 IR NMR MS 99%	1.456–1.457 0.908–0.908	
12.284 1709 - - 53897-60-2	bis(1-Mercaptopropyl)sulfide	(b)	Liquid C ₆ H ₁₄ S ₃ 182	Insoluble Soluble	225–226(101 hPa) IR NMR > 98% (mixture of diastereo-isomers. Due to the symmetry, there is one meso-form (50%) and two other diastereoisomers (25% each)	1.542–1.552 1.077–1.087	
12.285 1688 - - 53475-15-3	3-Methylthio-2-butanone	(b)	Liquid C ₅ H ₁₀ OS 118.2	Slightly soluble Soluble	160 IR NMR 97% (racemate)	1.468–1.4774 0.992–0.998	
12.286 1689 - - 143764-28-7	4-Methylthio-2-pentanone	(b)	Liquid C ₆ H ₁₂ OS 132.22	Very slightly soluble Soluble	183 NMR MS 98% (racemate)	1.468–1.472 0.969–0.979	
12.287 1690 4166 - 207983-28-6	Methyl 3-(methylthio)butanoate	(b)	Liquid C ₆ H ₁₂ O ₂ S 148.22	Very slightly soluble Soluble	193 NMR 98% (racemate)	1.459–1.465 1.034–1.040	

Information included in the EU Union list Regulation No (EU) 1334/2008 as amended			Most recent available specifications data ^(a)				EFSA comments
FL-no JECFA-no FEMA no CoE no CAS no	Chemical name	Purity of the named compound	Phys. form Mol. formula Mol. weight	Solubility ^(c) Solubility in ethanol ^(d)	Boiling point, °C ^(e) Melting point, °C ID test Assay minimum (isomers distribution/SC ^(h))	Refrac. Index ^(f) Spec. gravity ^(g)	
12.288 1664 - - 628-00-2	Heptan-2-thiol	(b)	Liquid C ₇ H ₁₆ S 132.27	Slightly soluble Soluble	164 NMR 98% (racemate)	1.442–1.448 0.832–0.838	
12.289 1665 - - 6263-65-6	1-Phenylethylmercaptan	(b)	Liquid C ₈ H ₁₀ S 138.23	Practically insoluble or insoluble Soluble	199 NMR MS 98% (racemate)	1.552–1.558 1.001–1.007	
12.290 1674 4167 - 54051-19-3	Methyl-3-mercaptopbutanoate	(b)	Liquid C ₅ H ₁₀ O ₂ S 134.20	Practically insoluble or insoluble Soluble	172 NMR 98.5% (racemate)	1.451–1.461 1.052–1.058	
12.292 1704 4136 - 796857-79-9	Hexyl 3-mercaptopbutanoate	(b)	Liquid C ₁₀ H ₂₀ O ₂ S 204.33	Practically insoluble or insoluble Soluble	268 NMR 98%(racemate)	1.459–1.465 0.949–0.955	
12.293 1660 - - 69382-62-3	Ethane-1,1-dithiol	1% solution of ethane-1,1-dithiol, solvent 95% ethanol	Liquid C ₂ H ₆ S ₂ 94.2	Soluble Soluble	Distils: 71–78 NMR 99% (1% solution of ethane-1,1-dithiol, purity 99% min, in ethanol)	1.369–1.375 0.829–0.833	
12.294 1696 4168 - 72437-56-0	Isopentyl methyl disulfide	(b)	Liquid C ₆ H ₁₄ S ₂ 150.31	Practically insoluble or insoluble Soluble	184–200 NMR 95%	1.516–1.522 0.995–1.001	

Information included in the EU Union list Regulation No (EU) 1334/2008 as amended			Most recent available specifications data ^(a)				EFSA comments
FL-no JECFA-no FEMA no CoE no CAS no	Chemical name	Purity of the named compound	Phys. form Mol. formula Mol. weight	Solubility ^(c) Solubility in ethanol ^(d)	Boiling point, °C ^(e) Melting point, °C ID test Assay minimum (isomers distribution/SC ^(h))	Refrac. Index ^(f) Spec. gravity ^(g)	
12.297 1708 4289 - 548774-80-7	3-Mercaptoheptyl acetate	(b)	Liquid C ₉ H ₁₈ O ₂ S 190.30	Slightly soluble Soluble	242 IR NMR MS 99.9% (racemate)	1.4605–1.4607 0.9826–0.9830	
12.304 2085 4714 33441-50-8	Ethyl-2-mercapto-2-methyl propanoate	(b)	Liquid C ₆ H ₁₂ O ₂ S 148.06	Slightly soluble Soluble	186 IR NMR MS > 95%	1.4245–1.4645 0.961–1.081	
15.049 1686 4030 - 54644-28-9	3,5-Diethyl-1,2,4-trithiolane	(b)	Liquid C ₆ H ₁₂ S ₃ 180.35	Practically insoluble or insoluble Soluble	77 (0.1 hPa) NMR 95% (mixture of three diastereoisomers. Due to the symmetry there is one meso-form (<i>cis</i> -form (50%) and two <i>trans</i> -forms (25% each)	1.558–1.570 1.147–1.160	
17.036 1710 4322 - 21593-77-1	S-allyl-L-cysteine	(b)	Solid C ₆ H ₁₁ NOS 161.22	Moderate soluble Slightly soluble	214–216 IR NMR MS 95%	1.542 1.191	

FL-No: FLAVIS number; JECFA: Joint FAO/WHO Expert Committee on Food Additives; FEMA: Flavor and Extract Manufacturers Association; CoE: Council of Europe; CAS: Chemical Abstract Service; ID: Identity; IR: infrared spectroscopy; MS: mass spectrometry; NMR: nuclear magnetic resonance.

(a): JECFA (2000, 2003, 2005); EFSA CEF Panel (2012a, 2014a, 2018); Documentation provided to EFSA nr: 1.

(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 1,013.25 hPa, if not otherwise stated.

(f): At 20°C, if not otherwise stated.

(g): At 25°C, if not otherwise stated.

(h): Secondary components.

Appendix C – Exposure estimates

C.1. Normal and Maximum Use Levels

Table C.1: Normal and maximum use levels (mg/kg) of JECFA-evaluated flavouring substances in FGE.91Rev3 in food categories listed in Annex III of Reg. (EC) 1565/2000 (EFSA CEF Panel, 2012a and Documentation provided to EFSA n. 3 and 4)

FL-no	Food categories																		
	Normal use levels ^(a) (mg/kg)																		
	Maximum use levels (mg/kg)																		
	01.0	02.0	03.0	04.1	04.2	05.0	05.3 ^b	06.0	07.0	08.0	09.0	10.0	11.0	12.0	13.0	14.1	14.2	15.0	16.0
12.012	0.2 1	0.1 0.5	0.2 1	0.2 1	– –	0.2 1	NI	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
12.017	0.2 1	0.1 0.5	0.2 1	0.2 1	– –	0.2 1	NI	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
12.021	0.2 1	0.1 0.5	0.2 1	0.2 1	– –	0.2 1	NI	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
12.038	0.9 1	– –	– –	– –	– –	0.62 1.41	1 5	0.62 6.21	0.62 1.41	0.1 0.2	– –	– –	– –	– –	– –	0.4 1	0.1 0.2	– –	– –
12.065	0.35 1	0.04 0.4	0.5 2	0.2 0	0.2 0.5	0.5 1	1 –	0.61 1	0.73 1.1	0.55 3.32	0.06 0.1	– –	– –	0.15 1	– –	0.5 1	0.15 0.5	0.1 0.28	0.35 1
12.079	0.35 1	0.04 0.4	0.5 2	0.2 0	0.2 0.5	0.5 1	1 –	0.61 1	0.73 1.1	0.55 3.32	0.06 0.1	– –	– –	0.15 1	– –	0.5 1	0.15 0.5	0.1 0.28	0.35 1
12.085	0.0003 0.001	– –	0.0003 0.001	– –	0.0003 0.001	0.0003 0.002	0.0003 0.004	0.0003 0.001	0.0003 0.001	0.0003 0.001	– –	– –	– –	– –	– –	0.0003 0.001	0.00015 0.00015	– –	– –
12.108	0.4 2	0.2 1	0.4 2	0.3 1.5	– –	0.4 2	NI	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
12.126	0.2 1	0.1 0.5	0.2 1	0.2 1	– –	0.2 1	NI	0.1 0.5	0.2 1	0.1 0.2	0.1 0.2	– –	– –	0.1 0.5	0.2 1	0.1 0.3	0.2 1	0.4 2	0.1 0.5
12.130	0.2 1	0.1 0.5	0.2 1	0.2 1	– –	0.2 1	NI	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
12.134	0.4 2	0.2 1	0.4 2	0.3 1.5	– –	0.4 2	NI	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	2 1
12.137	0.01 0.02	– –	– –	– –	– –	0.02 0.05	0.1 0.5	0.01 0.02	0.02 0.05	0.02 0.05	– –	– –	– –	0.02 0.05	– –	0.01 0.02	0 0	0.02 0.05	– –
12.138	0.03 1	– –	– –	– –	0.0001 0.001	0.0001 0.001	0.001 0.1	0.02 1	0.0001 1	0.1 1	– –	– –	– –	0.1 1	– –	0.00001 1	0.00001 0.08	– –	– –

FL-no	Food categories																		
	Normal use levels ^(a) (mg/kg)																		
	Maximum use levels (mg/kg)																		
	01.0	02.0	03.0	04.1	04.2	05.0	05.3 ^b	06.0	07.0	08.0	09.0	10.0	11.0	12.0	13.0	14.1	14.2	15.0	16.0
12.139	0.4 2	0.2 1	0.4 2	0.3 1.5	– –	0.4 2	NI	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
12.145	0.001 0.01	– –	0.02 0.05	– –	0.01 0.03	0.02 0.05	0.02 0.1	0.0001 0.01	0.0001 0.01	0.02 0.05	0.001 0.01	– –	– –	0.02 0.03	– –	0.01 0.02	0.01 0.03	– –	– –
12.146	0.4 2	0.2 1	0.4 2	0.3 1.5	– –	0.4 2	NI	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
12.153	0.2 1	0.1 0.5	0.2 1	0.2 1	– –	0.2 1	NI	0.1 0.5	0.2 1	0.1 0.2	0.1 0.2	– –	– –	0.1 0.5	0.2 1	0.1 0.3	0.2 1	0.4 2	0.1 0.5
12.169	0.002 0.017	– –	0.003 0.034	– –	0.003 0.017	0.017 0.085	0.034 0.1	0.0003 0.002	0.017 0.034	0.0003 0.002	– –	– –	– –	– –	– –	0.0003 0.003	0.002 0.017	– –	– –
12.240	0.2 1	0.1 0.5	0.2 1	0.2 1	– –	0.2 1	NI	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
12.241	0.004 0.04	0.005 0.05	0.004 0.04	– –	– –	0.005 0.1	0.01 0.2	0.004 0.04	0.005 0.05	0.01 0.1	– –	– –	– –	0.01 0.2	– –	0.002 0.05	0.002 0.05	– –	– –
12.242	0.2 1	0.1 0.5	0.2 1	0.2 1	– –	0.2 1	NI	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
12.243	0.2 1	0.1 0.5	0.2 1	0.2 1	– –	0.2 1	NI	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
12.252	0.01 0.05	– –	0.01 0.05	– –	0.01 0.05	0.02 0.1	0.02 0.1	0.02 0.1	0.02 0.1	0.01 0.05	– –	– –	0.1 0.5	10 100	– –	0.01 0.05	0.02 0.1	0.02 0.1	– –
12.253	0.2 1	0.1 0.5	0.2 1	0.2 1	– –	0.2 1	NI	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
12.254	0.2 1	0.1 0.5	0.2 1	0.2 1	– –	0.2 1	NI	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
12.259	0.001 0.01	– –	0.02 0.05	– –	0.01 0.03	0.02 0.05	0.02 0.1	0.001 0.01	0.001 0.01	0.02 0.05	0.001 0.01	– –	– –	0.02 0.03	– –	0.01 0.02	0.01 0.03	– –	– –
12.264	5 10	5 20	– –	2 10	2 10	– –	NI	– –	10 30	3 20	– –	– –	– –	5 30	– –	1 5	5 10	5 30	– –
12.273	1 10	0.1 1	– –	2 10	2 10	– –	NI	– –	10 50	3 20	– –	– –	– –	2 10	– –	1 10	1 10	5 30	– –

FL-no	Food categories																		
	Normal use levels ^(a) (mg/kg)																		
	Maximum use levels (mg/kg)																		
	01.0	02.0	03.0	04.1	04.2	05.0	05.3 ^b	06.0	07.0	08.0	09.0	10.0	11.0	12.0	13.0	14.1	14.2	15.0	16.0
12.274	10 30	5 20	– –	5 20	5 20	– –	NI	– –	10 30	10 20	– –	– –	– –	10 30	– –	1 5	5 10	10 20	– –
12.275	0.4 2	0.4 2	– –	0.2 10	0.2 10	2 10	NI	– –	2.5 12.5	0.3 1.5	0.3 1.5	– –	– –	0.5 5	– –	0.05 0.5	0.25 1.3	0.5 2.5	– –
12.276	0.001 0.01	0.01 0.1	0.001 0.01	0.001 0.01	– –	0.001 0.01	NI	0.002 0.02	0.002 0.02	0.002 0.02	0.002 0.02	– –	– –	0.003 0.03	0.003 0.03	0.001 0.005	0.005 0.05	0.001 0.01	0.001 0.01
12.284	0.05 1.25	0.05 1.25	0.5 12.5	0.05 1.25	0.05 1.25	5 125	NI	1 25	0.5 12.5	0.05 1.25	0.05 1.25	– –	– –	0.05 1.25	– –	5 125	5 125	0.05 1.25	0.05 1.25
12.285	1 5	– –	0.5 0.7	– –	– –	– –	NI	– –	0.8 1	0.2 0.5	– –	– –	– –	1 5	– –	0.5 6	– –	– –	– –
12.286	0.5 0.7	– –	0.5 0.7	– –	– –	– –	NI	– –	0.8 1	2 0.5	– –	– –	– –	– –	– –	0.5 0.6	– –	– –	0.5 1
12.287	0.01 10	0.001 10	0.01 10	0.05 10	– –	– –	NI	– –	– –	0.05 10	– –	0.001 10	– –	0.05 10	– –	0.001 5	– –	0.05 10	0.05 10
12.288	– –	– –	– –	– –	– –	– –	NI	– –	– –	1 5	– –	– –	– –	1 5	– –	4 10	– –	– –	– –
12.289	0.001 0.008	– –	– –	0.001 0.008	– –	0.002 0.025	NI	– –	0.002 0.04	0.002 0.025	– –	– –	– –	0.005 1	– –	0.001 0.02	0.002 0.025	– –	0.1 0.5
12.290	– –	– –	– –	– –	– –	– –	NI	– –	– –	1 5	– –	– –	– –	1 5	– –	0.5 5	– –	– –	– –
12.292	– –	– –	– –	– –	– –	– –	NI	– –	– –	1 5	– –	– –	– –	1 5	– –	0.5 5	– –	– –	– –
12.293	– –	0.2 2	– –	0.2 2	– –	– –	NI	– –	– –	1 5	– –	– –	– –	0.6 5	– –	0.2 2	– –	1 5	– –
12.294	– –	– –	0.25 0.5	– –	– –	– –	NI	– –	0.25 0.5	1 1.5	– –	– –	– –	– –	– –	0.5 1	– –	– –	0.25 0.5
12.297	0.01 0.1	0.1 1	0.01 0.1	0.01 0.1	– –	0.01 0.1	NI	0.02 0.2	0.02 0.2	0.02 0.2	0.02 0.2	– –	– –	0.03 0.3	0.03 0.3	0.01 0.05	0.05 0.5	0.01 0.1	0.01 0.1
12.304	0.2 1	– –	0.1 0.5	0.2 1	0.2 1	0.3 1.5	– –	0.1 0.5	0.1 0.5	– –	– –	– –	– –	0.4 2	– –	0.2 1	0.1 0.5	– –	0.1 0.5

FL-no	Food categories																		
	Normal use levels ^(a) (mg/kg)																		
	Maximum use levels (mg/kg)																		
	01.0	02.0	03.0	04.1	04.2	05.0	05.3 ^b	06.0	07.0	08.0	09.0	10.0	11.0	12.0	13.0	14.1	14.2	15.0	16.0
15.049	0.2	0.1	0.2	0.2	–	0.2	NI	0.1	0.2	0.1	0.1	–	–	0.1	0.2	0.1	0.2	0.4	0.1
	1	0.5	1	1	–	1		0.5	1	0.2	0.2	–	–	0.5	1	0.3	1	2	0.5

NI: no information provided.

(a): 'Normal use' is defined as the average of reported usages and 'maximum use' is defined as the 95th percentile of reported usages.

(b): Additional food category 05.3 (chewing-gum as per Annex II part D of Reg. (EC) 1333/2008) for which industry submitted use levels (Documentation provided to EFSA n. 3 and 4) These data have been considered in the calculation of mTAMDI.

C.2. mTAMDI calculations

The method for calculation of modified Theoretical Added Maximum Daily Intake (mTAMDI) values is based on the approach used by the SCF up to 1995 (SCF, 1995). The assumption is that a person may consume the amount of flavourable foods and beverages listed in Table C.2. These consumption estimates are then multiplied by the reported use levels in the different food categories and summed up.

Table C.2: Estimated amount of flavourable foods, beverages, and exceptions assumed to be consumed per person per day (SCF, 1995)

Class of product category	Intake estimate (g/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.3):

- Beverages (SCF, 1995) correspond to food category 14.1
- Foods (SCF, 1995) correspond to the food categories 1, 2, 3, 4.1, 4.2, 6, 7, 8, 9, 10, 13, and/or 16
- Exception a (SCF, 1995) corresponds to food category 5 and 11
- Exception b (SCF, 1995) corresponds to food category 15
- Exception c (SCF, 1995) corresponds to food category 14.2
- Exception d (SCF, 1995) corresponds to food category 12
- Exception e (SCF, 1995) corresponds to others, e.g. chewing gum.

Table C.3: Distribution of the 18 food categories listed in Commission Regulation (EC) No 1565/2000 into the seven SCF food categories used for mTAMDI calculations (SCF, 1995)

Key	Food categories according to Commission Regulation 1565/2000	Distribution of the seven SCF food categories		
	Food category	Foods	Beverages	Exceptions
01.0	Dairy products, excluding products of category 02.0	Foods		
02.0	Fats and oils, and fat emulsions (type water-in-oil)	Foods		
03.0	Edible ices, including sherbet and sorbet	Foods		
04.1	Processed fruit	Foods		
04.2	Processed vegetables (incl. mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds	Foods		
05.0	Confectionery			Exception a
06.0	Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery	Foods		
07.0	Bakery wares	Foods		
08.0	Meat and meat products, including poultry and game	Foods		
09.0	Fish and fish products, including molluscs, crustaceans and echinoderms	Foods		
10.0	Eggs and egg products	Foods		
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	Foods		
14.1	Non-alcoholic ('soft') beverages, excl. dairy products		Beverages	
14.2	Alcoholic beverages, incl. 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	Foods		

mTAMDI: modified Theoretical Added Maximum Daily Intake.

Table C.4: Estimated intakes based on the MSDI and the mTAMDI approach for substances in FGE.91Rev3

FL-no	Union List chemical name	MSDI EU ^(a) (µg/capita per day)	MSDI US ^(b) (µg/capita per day)	mTAMDI ^(c) (µg/person per day)	Structural class ^(d)	Threshold of concern (µg/person per day)
12.012	Diethyl disulfide	0.012	ND	78	Class I	1,800
12.017	Ethanethiol	0.49	ND	78	Class I	1,800
12.085	<i>p</i> -Menth-1-ene-8-thiol	37	1	0.15	Class I	1,800
12.126	Ethyl propyl disulfide	0.012	ND	78	Class I	1,800
12.130	Heptane-1-thiol	0.037	ND	78	Class I	1,800
12.134	<i>S</i> -Isopropyl 3-methylbut-2-enethioate	0.012	ND	370	Class I	1,800
12.146	Methyl (methylthio)acetate	0.24	1	160	Class I	1,800
12.153	Methyl ethyl disulfide	0.012	ND	78	Class I	1,800
12.240	2,4,6-Trithiaheptane	0.012	ND	78	Class I	1,800
12.242	Methylthiomethylmercaptan	0.012	0.1	78	Class I	1,800
12.243	Dimercaptomethane	0.012	ND	78	Class I	1,800
12.253	Amyl methyl disulfide	0.012	ND	78	Class I	1,800
12.254	Butyl ethyl disulfide	0.012	ND	78	Class I	1,800
12.264	4,2-Thiopentanone	0.12	0.07	2,000	Class I	1,800
12.265	(<i>E</i>)-2-Methyl-1-methylthio-2-butene	0.012	0.1	ND	Class I	1,800
12.267	Propyl-2-mercaptopropionate	0.012	0.1	ND	Class I	1,800
12.273	3-(Methylthio)heptanal	0.012	ND	1,800	Class I	1,800
12.276	(<i>S</i>)-1-Methoxy-3-heptanethiol	0.012	2	1.9	Class I	1,800
12.284	bis(1-Mercaptopropyl)sulfide	0.12	0.6	2,000	Class I	1,800
12.285	3-Methylthio-2-butanone	0.012	ND	320	Class I	1,800
12.286	4-Methylthio-2-pentanone	0.012	0.01	430	Class I	1,800
12.287	Methyl 3-(methylthio)butanoate	0.012	0.01	9	Class I	1,800
12.288	Heptan-2-thiol	0.012	0.01	1,400	Class I	1,800
12.290	Methyl-3-mercaptobutanoate	0.012	0.01	320	Class I	1,800
12.292	Hexyl 3-mercaptobutanoate	0.012	0.01	320	Class I	1,800
12.293	Ethane-1,1-dithiol	0.012	0.01	230	Class I	1,800
12.294	Isopentyl methyl disulfide	0.012	ND	300	Class I	1,800
12.297	3-Mercaptoheptyl acetate	0.0012	0.01	19	Class I	1,800
12.021	Allyl propyl disulfide	0.037	ND	78	Class II	540

FL-no	Union List chemical name	MSDI EU ^(a) (µg/capita per day)	MSDI US ^(b) (µg/capita per day)	mTAMDI ^(c) (µg/person per day)	Structural class ^(d)	Threshold of concern (µg/person per day)
12.077	Benzyl methyl sulfide	0.09	0.02	ND	Class II	540
12.162	Methyl phenyl sulfide	0.012	0.4	ND	Class II	540
12.259	1-Mercapto- <i>p</i> -menthan-3-one	0.24	ND	7	Class II	540
12.274	3,6-Diethyl-1,2,4,5-tetrathiane and 3,5-diethyl-1,2,4-trithiolane mix in vegetable oil triglycerides	0.61	ND	2,200	Class II	540
12.275	Allylthio hexanoate	0.012	ND	430	Class II	540
12.289	1-Phenylethylmercaptan	0.012	ND	14	Class II	540
15.049	3,5-Diethyl-1,2,4-trithiolane	0.61	0.01	78	Class II	540
12.065	2,8-Dithianon-4-en-4-carboxaldehyde	0.012	0.1	280	Class III	90
12.079	2-(Methylthiomethyl)but-2-enal	0.024	0.1	280	Class III	90
12.108	Di-isopentyl thiomalate	0.012	ND	160	Class III	90
12.139	2-Mercaptoanisole	1.5	ND	160	Class III	90
12.304	Ethyl-2-mercapto-2-methyl propanoate	0.012	0.01	110	Class III	90
17.036	<i>S</i> -allyl-L-cysteine	30	2	ND	Class III	90
12.137	3-Mercapto-3-methylbutan-1-ol	6.8	2	7.2	Class III	90
12.138	3-Mercapto-3-methylbutyl formate	0.12	0.1	15	Class III	90
12.145	4-Methoxy-2-methylbutane-2-thiol	0.12	0.8	7	Class III	90
12.038	8-Mercapto- <i>p</i> -menthan-3-one	31	2	270	Class III	90
12.169	2-Methyl-4-oxopentane-2-thiol	3.7	0.02	2.9	Class III	90
12.241	2-Mercapto-2-methylpentan-1-ol	2.4	4	2.4	Class III	90
12.252	4-Mercapto-4-methyl-2-pentanol	0.012	0.1	210	Class III	90

MSDI: Maximised Survey-derived Daily Intake; mTAMDI: modified Theoretical Added Maximum Daily Intake.

ND: not determined, as no data on uses and use levels available.

(a): Based on EU production figures by JECFA (2000, 2003, 2005) and submitted by industry (Documentation provided to EFSA nr: 2, 3 and 4).

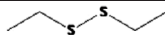

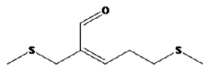
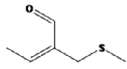
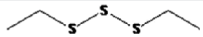
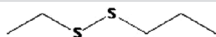
(b): Based on US production figures by JECFA (2000, 2003, 2005).

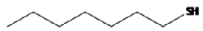
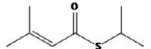
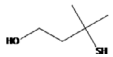
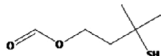
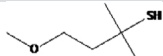
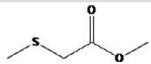
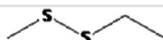
(c): Based on use levels submitted by industry (Documentation provided to EFSA nr. 3 and 4).

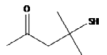
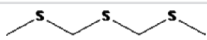
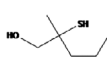

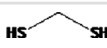
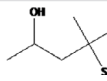

(d): Determined with OECD Toolbox (version 4.3.1 available at <https://www.oecd.org/chemicalsafety/risk-assessment/oecd-qsar-toolbox.htm>)

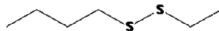

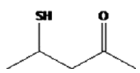
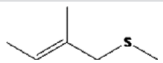
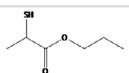
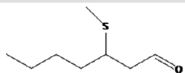
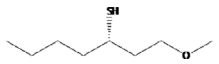
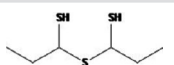
Appendix D – Summary of safety evaluations

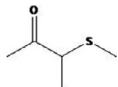
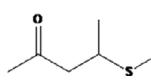
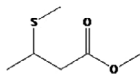
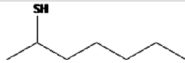
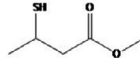
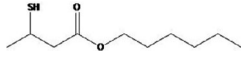

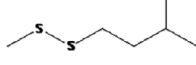
Table D.1: Summary of safety evaluations performed by JECFA (JECFA, 2000, 2004, 2012) and EFSA conclusions on flavouring substances in FGE.91 and its revisions

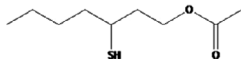

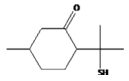
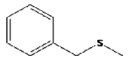
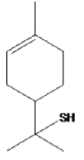
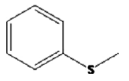
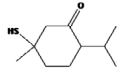
FL-no JECFA-no	EU Union List chemical name	Structural formula	JECFA conclusions Class ^(a) Evaluation procedure path ^(b) Outcome on the named compound based on the MSDI ^(c) approach	EFSA conclusion Procedural path if different from JECFA, Conclusion based on the MSDI ^(d) approach on the named compound and on the material of commerce
12.012 1699	Diethyl disulfide		Class I B3: Intake below threshold B4: Adequate NOAEL exists No safety concern	No safety concern at the estimated level of intake. Concluded in FGE.91.
12.017 1659	Ethanethiol		Class I B3: Intake below threshold B4: Adequate NOAEL exists No safety concern	No safety concern at the estimated level of intake. Concluded in FGE.91
12.065 471	2,8-Dithianon-4-en-4-carboxaldehyde		Class I B3: Intake below threshold B4: no adequate NOAEL exists B5: intake below 1,5 µg/person per day No safety concern	Class III B3: Intake below threshold B4: Adequate NOAEL exists No safety concern Concluded in FGE.91Rev3
12.079 470	2-(Methylthiomethyl)but-2-enal		Class I B3: Intake below threshold B4: Adequate NOAEL exists B5: intake below 1,5 µg/person per day No safety concern	Class III B3: Intake below threshold B4: Adequate NOAEL exists No safety concern Concluded in FGE.91Rev3
12.114 1701	Diethyl trisulfide		Class I B3: Intake below threshold B4: Adequate NOAEL exists No safety concern	No longer supported by Industry (DG SANCO, 2013)
12.126 1694	Ethyl propyl disulfide		Class I B3: Intake below threshold B4: Adequate NOAEL exists No safety concern	No safety concern at the estimated level of intake Concluded in FGE.91

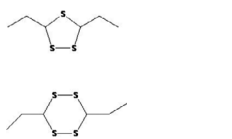
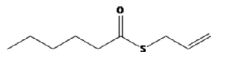
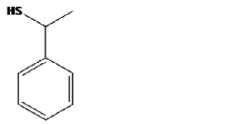
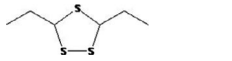
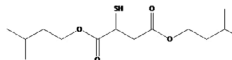
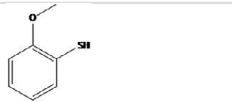
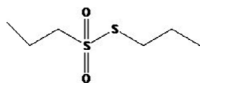
FL-no JECFA-no	EU Union List chemical name	Structural formula	JECFA conclusions Class ^(a) Evaluation procedure path ^(b) Outcome on the named compound based on the MSDI ^(c) approach	EFSA conclusion Procedural path if different from JECFA, Conclusion based on the MSDI ^(d) approach on the named compound and on the material of commerce
12.130 1663	Heptane-1-thiol		Class I B3: Intake below threshold, B4: Adequate NOAEL exists No safety concern	No safety concern at the estimated level of intake. Concluded in FGE.91.
12.134 1679	S-Isopropyl 3-methylbut-2-enethioate		Class I B3: Intake below threshold, B4: Adequate NOAEL exists No safety concern	No safety concern at the estimated level of intake. Concluded in FGE.91.
12.137 544	3-Mercapto-3-methylbutan-1-ol		Class I B3: Intake below threshold, B4: Adequate NOAEL exists No safety concern	Class III B3: Intake below threshold, B4: adequate NOAEL exists No safety concern. Concluded in FGE.91Rev3
12.138 549	3-Mercapto-3-methylbutyl formate		Class I B3: Intake below threshold, B4: Adequate NOAEL exists No safety concern	Class III B3: Intake below threshold, B4: adequate NOAEL exists No safety concern. Concluded in FGE.91Rev3
12.145 548	4-Methoxy-2-methylbutane-2-thiol		Class I B3: Intake below threshold, B4: Adequate NOAEL exists No safety concern	Class III B3: Intake below threshold, B4: adequate NOAEL exists No safety concern. Concluded in FGE.91Rev3
12.146 1691	Methyl (methylthio)acetate		Class I B3: Intake below threshold, B4: Adequate NOAEL exists No safety concern	No safety concern at the estimated level of intake. Concluded in FGE.91.
12.153 1693	Methyl ethyl disulfide		Class I B3: Intake below threshold, B4: Adequate NOAEL exists No safety concern	No safety concern at the estimated level of intake. Concluded in FGE.91.

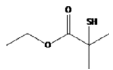
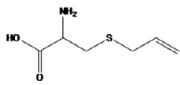
FL-no JECFA-no	EU Union List chemical name	Structural formula	JECFA conclusions Class ^(a) Evaluation procedure path ^(b) Outcome on the named compound based on the MSDI ^(c) approach	EFSA conclusion Procedural path if different from JECFA, Conclusion based on the MSDI ^(d) approach on the named compound and on the material of commerce
12.169 1293	2-Methyl-4-oxopentane-2-thiol		Class I B3: Intake below threshold, B4: Adequate NOAEL exists No safety concern	Class III B3: Intake below threshold, B4: adequate NOAEL exists No safety concern. Concluded in FGE.91Rev3 The chemical name should be changed to 4-mercapto-4-methyl-2-pentanone.
12.240 1684	2,4,6-Trithiaheptane		Class I B3: Intake below threshold, B4: Adequate NOAEL exists No safety concern	No safety concern at the estimated level of intake. Concluded in FGE.91.
12.241 1290	2-Mercapto-2-methylpentan-1-ol		Class I B3: Intake below threshold, B4: Adequate NOAEL exists No safety concern	Class III B3: Intake below threshold, B4: adequate NOAEL exists No safety concern. Concluded in FGE.91Rev3
12.242 1675	Methylthiomethylmercaptan		Class I B3: Intake below threshold, B4: Adequate NOAEL exists No safety concern	No safety concern at the estimated level of intake. Concluded in FGE.91.
12.243 1661	Dimercaptomethane		Class I B3: Intake below threshold, B4: Adequate NOAEL exists No safety concern	No safety concern at the estimated level of intake. Concluded in FGE.91.
12.252 1669	4-Mercapto-4-methyl-2-pentanol		Class I B3: Intake below threshold, B4: Adequate NOAEL exists No safety concern	Class III B3: Intake below threshold, B4: adequate NOAEL exists No safety concern. Concluded in FGE.91Rev3
12.253 1697	Amyl methyl disulfide		Class I B3: Intake below threshold, B4: Adequate NOAEL exists No safety concern	No safety concern at the estimated level of intake. Concluded in FGE.91.

FL-no JECFA-no	EU Union List chemical name	Structural formula	JECFA conclusions Class ^(a) Evaluation procedure path ^(b) Outcome on the named compound based on the MSDI ^(c) approach	EFSA conclusion Procedural path if different from JECFA, Conclusion based on the MSDI ^(d) approach on the named compound and on the material of commerce
12.254 1698	Butyl ethyl disulfide		Class I B3: Intake below threshold, B4: Adequate NOAEL exists No safety concern	No safety concern at the estimated level of intake. Concluded in FGE.91.
12.256 1695	Ethyl propyl trisulfide		Class I B3: Intake below threshold, B4: Adequate NOAEL exists No safety concern	Additional toxicity data required in FGE.91. No longer supported by Industry (DG SANCO, 2013).
12.264 1670	4,2-Thiopentanone		Class I B3: Intake below threshold, B4: Adequate NOAEL exists No safety concern	No safety concern at the estimated level of intake. Concluded in FGE.91.
12.265 1683	(E)-2-Methyl-1-methylthio-2- butene		Class I B3: Intake below threshold, B4: Adequate NOAEL exists No safety concern	No safety concern at the estimated level of intake. Concluded in FGE.91.
12.267 1667	Propyl-2-mercaptopropionate		Class I B3: Intake below threshold, B4: Adequate NOAEL exists No safety concern	No safety concern at the estimated level of intake. Concluded in FGE.91.
12.273 1692	3-(Methylthio)heptanal		Class I B3: Intake below threshold, B4: Adequate NOAEL exists No safety concern	No safety concern at the estimated level of intake. Concluded in FGE.91.
12.276 1671	(S)-1-Methoxy-3- heptanethiol		Class I B3: Intake below threshold, B4: Adequate NOAEL exists No safety concern	No safety concern at the estimated level of intake. Concluded in FGE.91.
12.284 1709	bis(1-Mercaptopropyl)sulfide		Class I B3: Intake below threshold, B4: Adequate NOAEL exists No safety concern	No safety concern at the estimated level of intake. Concluded in FGE.91.

FL-no JECFA-no	EU Union List chemical name	Structural formula	JECFA conclusions Class ^(a) Evaluation procedure path ^(b) Outcome on the named compound based on the MSDI ^(c) approach	EFSA conclusion Procedural path if different from JECFA, Conclusion based on the MSDI ^(d) approach on the named compound and on the material of commerce
12.285 1688	3-Methylthio-2-butanone		Class I B3: Intake below threshold, B4: Adequate NOAEL exists No safety concern	No safety concern at the estimated level of intake. Concluded in FGE.91.
12.286 1689	4-Methylthio-2-pentanone		Class I B3: Intake below threshold, B4: Adequate NOAEL exists No safety concern	No safety concern at the estimated level of intake. Concluded in FGE.91.
12.287 1690	Methyl 3-(methylthio) butanoate		Class I B3: Intake below threshold, B4: Adequate NOAEL exists No safety concern	No safety concern at the estimated level of intake. Concluded in FGE.91.
12.288 1664	Heptan-2-thiol		Class I B3: Intake below threshold, B4: Adequate NOAEL exists No safety concern	No safety concern at the estimated level of intake. Concluded in FGE.91.
12.290 1674	Methyl-3-mercaptobutanoate		Class I B3: Intake below threshold, B4: Adequate NOAEL exists No safety concern	No safety concern at the estimated level of intake. Concluded in FGE.91.
12.292 1704	Hexyl 3-mercaptobutanoate		Class I B3: Intake below threshold, B4: Adequate NOAEL exists No safety concern	No safety concern at the estimated level of intake. Concluded in FGE.91.
12.293 1660	Ethane-1,1-dithiol		Class I B3: Intake below threshold, B4: Adequate NOAEL exists No safety concern	No safety concern at the estimated level of intake. Concluded in FGE.91.
12.294 1696	Isopentyl methyl disulfide		Class I B3: Intake below threshold, B4: Adequate NOAEL exists No safety concern	No safety concern at the estimated level of intake. Concluded in FGE.91.

FL-no JECFA-no	EU Union List chemical name	Structural formula	JECFA conclusions Class ^(a) Evaluation procedure path ^(b) Outcome on the named compound based on the MSDI ^(c) approach	EFSA conclusion Procedural path if different from JECFA, Conclusion based on the MSDI ^(d) approach on the named compound and on the material of commerce
12.297 1708	3-Mercaptoheptyl acetate		Class I B3: Intake below threshold, B4: Adequate NOAEL exists No safety concern	No safety concern at the estimated level of intake. Concluded in FGE.91.
12.021 1700	Allyl propyl disulfide		Class I B3: Intake below threshold, B4: Adequate NOAEL exists No safety concern	No safety concern at the estimated level of intake. Concluded in FGE.91.
12.038 561	8-Mercapto- <i>p</i> -menthan-3-one		Class II B3: Intake below threshold, B4: Adequate NOAEL exists No safety concern	Class III B3: Intake below threshold, B4: adequate NOAEL exists No safety concern. Concluded in FGE.91Rev3
12.077 460	Benzyl methyl sulfide		Class II B3: Intake below threshold, B4: No adequate NOAEL	No safety concern at the estimated level of intake. Concluded in FGE.91Rev1.
12.085 523	<i>p</i> -Menth-1-ene-8-thiol		Class II B3: Intake below threshold, B4: Adequate NOAEL exists No safety concern.	Class I B3: Intake below threshold, B4: adequate NOAEL exists No safety concern. Concluded in FGE.91Rev3
12.162 459	Methyl phenyl sulfide		Class II B3: Intake below threshold, B4: No adequate NOAEL B5: intake below 1,5 µg/person per day No safety concern	Class II B3: Intake below threshold, B4: Adequate NOAEL exists. Concluded in FGE.91Rev1.
12.259 1673	1-Mercapto- <i>p</i> -menthan-3-one		Class II B3: Intake below threshold, B4: Adequate NOAEL exists No safety concern.	Class III B3: Intake below threshold, B4: adequate NOAEL exists No safety concern. Concluded in FGE.91Rev3

FL-no JECFA-no	EU Union List chemical name	Structural formula	JECFA conclusions Class ^(a) Evaluation procedure path ^(b) Outcome on the named compound based on the MSDI ^(c) approach	EFSA conclusion Procedural path if different from JECFA, Conclusion based on the MSDI ^(d) approach on the named compound and on the material of commerce
12.274 1687	3,6-Diethyl-1,2,4,5-tetrathiane and 3,5-diethyl-1,2,4-trithiolane mix in vegetable oil triglycerides		Class II B3: Intake below threshold, B B4: Adequate NOAEL exists No safety concern	No safety concern at the estimated level of intake. Concluded in FGE.91.
12.275 1681	Allylthio hexanoate		Class II B3: Intake below threshold, B B4: Adequate NOAEL exists No safety concern	No safety concern at the estimated level of intake. Concluded in FGE.91.
12.289 1665	1-Phenylethylmercaptan		Class II B3: Intake below threshold, B B4: Adequate NOAEL exists No safety concern	No safety concern at the estimated level of intake. Concluded in FGE.91.
15.049 1686	3,5-Diethyl-1,2,4-trithiolane		Class II B3: Intake below threshold, B B4: Adequate NOAEL exists No safety concern	No safety concern at the estimated level of intake. Concluded in FGE.91.
12.108 1672	Di-isopentyl thiomalate		Class III B3: Intake below threshold, B4: No adequate NOAEL B5: intake below 1,5 µg/person per day No safety concern	Class III B3: Intake below threshold, B4: Adequate NOAEL exists. Concluded in FGE.91Rev1.
12.139 1666	2-Mercaptoanisole		Class III B3: Intake below threshold, B4: Adequate NOAEL exists No safety concern	No safety concern at the estimated level of intake. Concluded in FGE.91.
12.272 1702	Propyl propanethiosulfonate		Class III B3: Intake below threshold, B4: No adequate NOAEL B5: intake below 1,5 µg/person per day No safety concern	Class III B3: Intake below threshold, B4: Adequate NOAEL exists. Concluded in FGE.91Rev1. No longer supported by Industry (DG SANCO, 2013).

FL-no JECFA-no	EU Union List chemical name	Structural formula	JECFA conclusions Class ^(a) Evaluation procedure path ^(b) Outcome on the named compound based on the MSDI ^(c) approach	EFSA conclusion Procedural path if different from JECFA, Conclusion based on the MSDI ^(d) approach on the named compound and on the material of commerce
12.304 2085	Ethyl-2-mercapto-2-methyl propanoate		Class I B3: Intake below threshold, B4: Adequate NOAEL exists No safety concern	Class III B3: Intake below threshold, B4: adequate NOAEL exists No safety concern. Concluded in FGE.91Rev3
17.036 1710	S-allyl-L-cysteine		Class III B3: Intake below threshold, B4: Adequate NOAEL exists No safety concern	No safety concern at the estimated level of intake. Concluded in FGE.91.

MSDI: Maximised Survey-derived Daily Intake; NOAEL: no observed adverse effect level.

(a): Toxicological thresholds of concern: Class I = 1,800 µg/person per day, Class II = 540 µg/person per day, Class III = 90 µg/person per day.

(b): Procedure path A substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot.

(c): EU MSDI: Amount added to food as flavour in (kg/year) × 10⁹ / (0.1 × population in Europe (= 375 × 10⁶) × 0.6 × 365) = µg/capita per day.

(d): Refer to Appendix C for MSDI values considered by EFSA based on EU production figures submitted by industry (Documentation provided to EFSA n.: 2, 3 and 4).

Appendix E – Repeated dose toxicity studies

Table E.1: Subacute and subchronic toxicity studies considered in FGE.91Rev3. The supporting substances are listed in brackets

UL chemical name [FL-no]	Species; Sex No./Group	Route	Dose levels (mg/kg bw per day) if not specified	Duration	NO(A)EL (mg/kg bw per day)	Reference	Comments
2,8-Dithianon-4-en-4-carboxaldehyde [FL-no: 12.065]	Rats; male, female 5/sex per group	Gavage	Daily administration of 0.33 and 3.3 mg/kg bw per day (corresponding to 500 and 5000 times the expected human intake, respectively)	28 days	–	Central Institute for Nutrition and Food Research; 1974 (EFSA CEF Panel, 2012a,b)	No treatment-related pathological changes identified in liver and kidneys
(Dimethyl sulfide [FL-no: 12.006])	Rats; male, female 15/sex per group	Gavage	2.5, 25 or 250	14 weeks	250	Butterworth et al. (1975)	No adverse effect at any level in dosed rats
	Rats; male, female 5/sex per group		Daily dose of 0.25 mg/kg bw	2 weeks			
	Rats; male, female 5/sex per group		Daily dose of 250 mg/kg bw	6 weeks			
(2-methyl-4-oxopentane-2-thiol)	Rats; male, female 5/sex per group	Gavage	0 (10% propylene glycol), 0.015, 0.065 and 0.130	14-days	–	Documentation provided to EFSA nr: 5	
	Rats; male, female 10/sex per group		0 (10% propylene glycol), 0.13, 0.20 and 0.26	90-days	0.26	Documentation provided to EFSA nr: 5	NOAEL is the highest dose tested

NOAEL: no observed adverse effect level; bw: body weight.