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

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SCIENTIFIC OPINION

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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 Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF), Vittorio Silano, Claudia Bolognesi, Laurence Castle, Kevin Chipman, Jean-Pierre Cravedi, Karl-Heinz Engel, Paul Fowler, Roland Franz, Konrad Grob, Rainer Gürtler, Trine Husøy, Sirpa Kärenlampi, Maria Rosaria Milana, Karla Pfaff, Gilles Riviere, Jannavi Srinivasan, Maria de Fátima Tavares Poças, Christina Tlustos, Detlef Wölfle, Holger Zorn, Romualdo Benigni, Leon Brimer, Gerard Mulder, Agneta Oskarsson, Camilla Svendsen, Jan van Benthem, Maria Anastassiadou, Siiri Saarma and Wim Mennes

Abstract

The Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids of the European Food Safety Authority was requested to consider evaluations of flavouring substances assessed since 2000 by the Joint FAO/WHO Expert Committee on Food Additives (JECFA), and to decide whether further evaluation is necessary, as laid down in Commission Regulation (EC) No 1565/2000. The present revision of this FGE is on the assessment of recently submitted toxicity data on methyl propyl trisulfide [FL-no: 12.020], being the representative for a group of seven additional flavouring substances: diallyl trisulfide [FL-no: 12.009], dimethyl trisulfide [FL-no: 12.013], dipropyl trisulfide [FL-no: 12.023], methyl allyl trisulfide [FL-no: 12.045], diallyl polysulfides [FL-no: 12.074], methyl ethyl trisulfide [FL-no: 12.155] and diisopropyl trisulphide [FL-no: 12.280]. Specifications have been provided for all substances. The Panel decided that the 90-day study submitted for [FL-no: 12.020] can be considered only once it is clearly demonstrated that the material tested is representative of the material of commerce and that potential reaction products of the components are not of safety concern. Therefore, no conclusion on the safety of the eight flavouring substances [FL-no: 12.009, 12.013, 12.020, 12.023, 12.045, 12.074, 12.155 and 12.280] can be reached. For 2-methyl-4-oxopentane-2-thiol [FL-no: 12.169] and 2-mercapto-2-methylpentan-1-ol [FL-no: 12.241], additional subchronic toxicity data are required. The remaining nine substances [FL-no: 12.088, 12.179, 12.198, 12.212, 12.238, 12.239, 12.255, 12.257 and 12.291] in this FGE are not considered of safety concern under the intended conditions of use.

Keywords: flavourings, FGE.08, FGE.74, JECFA, sulphides, thiols

Requestor: European Commission

Question numbers: EFSA-Q-2016-00720, 00721, 00722, 00723, 00724, 00725, 00726, 00727

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1. Introduction

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

1.1.1. Background

The use of flavourings is regulated under Regulation (EC) No 1334/2008 of the European Parliament and Council of 16 December 2008[1] on flavourings and certain food ingredients with flavouring properties for use in and on foods. On the basis of Article 9(a) of this Regulation, an evaluation and approval are required for flavouring substances.

The Union list of flavourings and source materials was established by Commission Implementing Regulation (EC) No 872/2012[2]. The list contains flavouring substances for which the scientific evaluation should be completed in accordance with Commission Regulation (EC) No 1565/2000[3].

In May 2014 the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids adopted the opinion on the Flavouring Group Evaluation 74 Revision.3, (FGE.74rev.3) and concluded that further data are required for the tri- and polysulphides substances with Flavis numbers: [FL-nos: 12.009, 12.013, 12.020, 12.023, 12.045, 12.074, 12.155 and 12.280] belonging to subgroup VI of this Group.

It also indicated that for these eight substances in subgroup VI (acyclic tri- and polysulphides) [FL-no: 12.009, 12.013, 12.020, 12.023, 12.045, 12.074, 12.155 and 12.280], 90-day studies were available on [FL-no: 12.009 and 12.023], but the studies were not considered adequate for deriving a NOAEL (Morgareidge and Oser, 1970a,b). There were no data on stability of test substances and no results reported from histopathological examinations. The Panel also concluded that tri- and poly-sulphides cannot be covered by NOAELs for disulphides, due to the formation of more reactive metabolites than is the case for the disulphides.

On 19 July 2016 the Industry submitted a new dossier with safety studies concerning the substances in this subgroup addressing this issue.

1.1.2. Terms of Reference

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

1.2. Interpretation of the Terms of Reference

The European Commission requested EFSA to carry out a safety assessment on the substances diallyl trisulfide [FL-no: 12.009], dimethyl trisulfide [FL-no: 12.013], methyl propyl trisulfide [FL-no: 12.020], dipropyl trisulfide [FL-no: 12.023], methyl allyl trisulfide [FL-no: 12.045], diallyl polysulfides [FL-no: 12.074] and methyl ethyl trisulfide [FL-no: 12.155] and diisopropyl trisulphide [FL-no: 12.280] in accordance with Commission Regulation (EC) No 1565/2000.

2. Assessment

The approach used by EFSA for safety evaluation of flavouring substances is referred to in Commission Regulation (EC) No 1565/2000, hereafter named the 'EFSA Procedure'. This Procedure is based on the opinion of the Scientific Committee on Food (SCF, 1999), which has been derived from the evaluation procedure developed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 1995, 1996, 1997, 1999a), hereafter named the 'JECFA Procedure'. The CEF Panel 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

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

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

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

data. The evaluations 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 put through the EFSA Procedure.

The following issues are of special importance.

Intake

In its evaluation, the Panel as a default uses the 'maximised survey-derived daily intake' (MSDI) 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 List of flavouring substances[2] for which this is the case, the Panel will need the 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 level reported by 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).

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.

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.

Threshold of 1.5 μg/person per day (Step B5) used by JECFA

JECFA uses the threshold of concern of 1.5 μg/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 μg/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 46th meeting be amended to include the last step on the right-hand side of the original Procedure ('Do the condition of use result in an intake greater than 1.5 μg per day?') (JECFA, 1999a).

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 μg/person per day.

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.

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.

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 Flavouring Group Evaluation (FGE).

2.1. History of the evaluation of the substances in the present FGE

At its 61st meeting, JECFA evaluated a group of 12 flavouring substances consisting of aliphatic sulphides and thiols (JECFA, 2004a). One substance was not in the Register. The remaining 11 flavouring substances have originally been considered by EFSA in FGE.74 (EFSA, 2008). The Panel concluded that for two substances [FL-no: 12.169 and 12.241], the Procedure should not be applied until adequate genotoxicity data become available and for three substances [FL-no: 12.179, 12.198 and 12.212] additional toxicity data were required.

In the first revision of Flavouring Group Evaluation 74 (FGE.74Rev1), there was a reassessment of four candidate substances due to subgrouping of the substances based on the type of sulphur-containing functional groups. This is in accordance with what has been done in FGE.08Rev1 (EFSA CEF Panel, 2010a) and in FGE.91 (EFSA CEF Panel, 2010b), which also consider substances with sulphur-containing functional groups. The candidate substances in FGE.74Rev1 that have been reassessed due to this are [FL-no: 12.179, 12.198, 12.212 and 12.280]. The outcome of the evaluation is explained in Section 7. Furthermore, the FGE.74Rev1 included the assessment of seven additional substances [FL-no: 12.009, 12.013, 12.020, 12.023, 12.045, 12.074 and 12.155] evaluated by JECFA at the 53rd meeting (JECFA, 2000). The reason for the inclusion of these seven substances is explained in Section 2.2.1.

In the second revision of FGE.74, FGE.74Rev2, one candidate substance was added, diallyl sulfide [FL-no: 12.088]. This substance has been evaluated by JECFA at the 53rd meeting (JECFA, 2000). The reason for the inclusion of this substance is explained in Section 2.2.1. For four substances [FL-no: 12.009, 12.020, 12.045 and 12.169] additional information on specifications received after publication of FGE.74Rev1 has been included.

The third revision of FGE.74, FGE.74Rev3, concerned the evaluation of a group of ten substances for which the concern for genotoxicity was alleviated by the Panel. The Panel concluded that the evaluation for [FL-no: 12.009, 12.013, 12.020, 12.023, 12.045, 12.074, 12.155, 12.169, 12.241 and 12.280] could not be finalised at Step B4 of the Procedure due to the lack of a no observed adverse effect level (NOAEL) and that additional toxicity data would, therefore, be required.

FGE	Adopted by EFSA	Link	No. of substances
FGE.74	January 2008	http://www.efsa.europa.eu/en/efsajournal/pub/987.htm	11
FGE.74Rev1	September 2010	http://www.efsa.europa.eu/en/efsajournal/pub/1842.htm	18
FGE.74Rev2	November 2011	http://www.efsa.europa.eu/en/efsajournal/pub/2458.htm	19
FGE.74Rev3	May 2014	https://www.efsa.europa.eu/it/efsajournal/pub/3710.htm	19
FGE.74Rev4	January 2018	http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2018.5167/full	19

FGE: Flavouring Group Evaluation; EFSA: European Food Safety Authority.

The present revision of FGE.74, FGE.74Rev4, concerns the consideration of seven candidate substances [FL-no: 12.009, 12.013, 12.020, 12.023, 12.045, 12.074 and 12.155] from the 53rd meeting of JECFA and one candidate substance [FL-no: 12.280] from the 61st meeting of JECFA based on new toxicity data submitted on the representative substance methyl propyl trisulfide [FL-no: 12.020]. Additionally, normal and maximum use levels and updated production volumes have become available by the Flavour Industry for 18 substances: [FL-no: 12.009, 12.013, 12.020, 12.023, 12.045, 12.074, 12.088, 12.155, 12.169, 12.179, 12.198, 12.212, 12.238, 12.239, 12.241, 12.555, 12.257 and 12.280] (EFFA, 2017). The updated production volumes have been used for calculation of MSDI values.

2.2. Presentation of the substances in the JECFA flavouring group

2.2.1. Description

JECFA status

JECFA has evaluated a group of 12 flavouring substances consisting of aliphatic sulphides and thiols at the 61st meeting (JECFA, 2004a,b).

JECFA has at the 53rd meeting (JECFA, 2000), before 2000, evaluated a group of 137 flavouring substances consisting of aliphatic and aromatic sulphides and thiols with and without an additional oxygenated functional group.

EFSA considerations

This FGE deals with 19 JECFA-evaluated substances. Eleven substances from the 61st meeting, 2003, and eight substances from the 53rd meeting, 1999, because:

- Of the 12 aliphatic sulphides and thiols evaluated by JECFA at the 61st meeting, one is not in the Register (spiro[2,4-dithia-1-methyl-8-oxabicyclo(3.3.0)octane-3,3′-(1′-oxa-2′-methyl)-cyclopentane], JECFA-no: 1296). From the 61st JECFA meeting, 11 substances remain to be evaluated by EFSA.
- Of the 137 aliphatic and aromatic sulphides and thiols with and without an additional oxygenated functional group evaluated by JECFA at the 53rd meeting, seven are acyclic polysulphides [FL-no: 12.009, 12.013, 12.020, 12.023, 12.045, 12.074 and 12.155]. These seven substances were evaluated by JECFA before the year 2000 and have been used as supporting substances in FGE.08 and following revisions. For flavouring substances evaluated by JECFA before 2000, it is laid down in Commission Regulation (EC) No 1565/2000[3] that if they are considered acceptable at the current estimated intake by JECFA and comply with the general use criteria, they could be included in the list of authorised substances without undergoing a separate evaluation for the time being. In the FGE.08Rev1 (EFSA CEF Panel, 2010a), it was recognised that tri- and polysulphides may form reactive metabolites and accordingly in FGE.74Rev1, the Panel decided to reconsider these seven polysulphides previously evaluated by JECFA (see Comment on subgroup VI (acyclic tri- and polysulphides) below). Further, for diallyl sulfide [FL-no: 12.088], which JECFA evaluated at Step B5, no NOAEL exists to provide a margin of safety (MoS). However, as the estimated intake in the USA of 0.4 μg/capita per day is below the threshold of concern of 1.5 μg/person per day, the JECFA Committee noted that intakes below this value would not be expected to present a safety concern. In line with the opinion expressed by the SCF (1999), the Panel does not make use of this threshold of 1.5 μg/person per day. From the 53rd JECFA meeting, eight substances remain to be evaluated by EFSA. In addition, in FGE.08Rev1, the genotoxicity issues that were noted for candidate tertiary thiols are obviously also of relevance for two candidate JECFA-evaluated tertiary thiols [FL no: 12.169 and 12.241] in this consideration.

The Panel concluded that the substances in the JECFA flavouring group of aliphatic sulphides and thiols are structurally related to the group of aliphatic and alicyclic mono-, di-, tri- and polysulphides with or without additional oxygenated functional groups evaluated by EFSA in the Flavouring Group Evaluation 08, Revision 5 (FGE.08Rev5). Depending on the type of sulfur-containing functional groups, the substances in FGE.08Rev5 were subdivided into 11 subgroups:

I *Acyclic sulphides*
II *Cyclic sulphides*
III *Monothiols, including tertiary monothiols*
IV *Dithiols*
V *Acyclic and cyclic disulphides*
VI *Acyclic polysulphides*
VII *Mono-, di-, tri- and polysulphides with thioacetal structure*
VIII *Thioesters*
IX *Thioic acid*
X *Sulphoxides/sulphones and sulphonates*
XI *Cyclic thioketal fused with an oxolane ring.*

In the following part of this fourth revision of FGE.74 (FGE.74Rev4), there will be reference to the fifth revision of FGE.08 (FGE.08Rev5) (EFSA CEF Panel, 2012b). It is also the fifth revision of FGE.08 that is used in the application of the Procedure by EFSA (Section 7.2 of this FGE.74Rev4).

The 19 JECFA-evaluated substances in the present FGE will be considered in compliance with these EFSA defined subgroups.

Comment on Subgroup VI (acyclic tri- and polysulphides)

During the evaluation of the candidate substances in the FGE.08Rev1 (EFSA CEF Panel, 2010a), it was recognised that tri- and polysulphides (subgroup VI) may form reactive metabolites through reaction with endogenous thiols forming a thiol and a hydropersulphide or perthiol. Compared to thiols, perthiols may be strong reducing agents, forming reactive products when exposed to oxidants. Based on the above information it was concluded that tri- and polysulphides could not be covered by NOAELs for disulphides, due to the formation of more reactive metabolites.

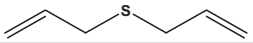
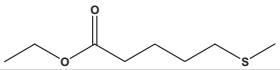
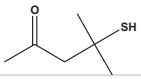
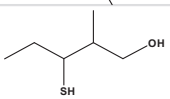
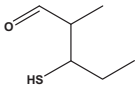
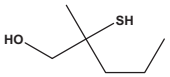
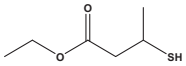
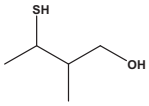
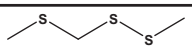
The Panel noted that in FGE.08Rev1 seven supporting substances are tri- or polysulphides [FL-no: 12.009, 12.013, 12.020, 12.023, 12.045, 12.074 and 12.155]. These substances were evaluated by JECFA before the year 2000[4] (accepted at Step B4 based on NOAELs derived from studies with disulphides), and therefore at first not included in the consideration performed by EFSA on the JECFA-evaluated substances in FGE.74.

Accordingly, the decision taken in FGE.08Rev1 has had an impact on the tri- and polysulphides in FGE.74 (one substance [FL-no: 12.280]) as well as those evaluated by JECFA at its 53rd meeting, before 2000 (seven substances [FL-no: 12.009, 12.013, 12.020, 12.023, 12.045, 12.074 and 12.155]), which were therefore included in the first revision of FGE.74 (FGE.74Rev1) (EFSA CEF Panel, 2010c).

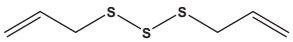
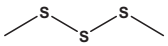
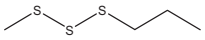
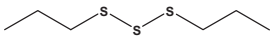
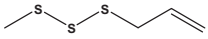
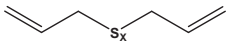
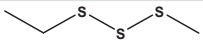
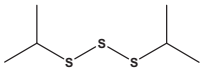
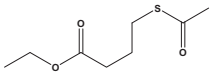
Distribution of the FGE.74Rev4 substances into subgroups

The 19 JECFA-evaluated substances in this FGE have been assigned to five subgroups, in accordance with the subdivision in FGE.08Rev5. This subdivision is shown in Table 1 below.

Table 1: Allocation of the 19 JECFA-evaluated substances considered in FGE.74 into subgroups according to subdivision in FGE.08Rev5

FL-no:	Register name	Structural formula
I Acyclic sulphides		
12.088	Diallyl sulfide	
12.179	2-(Methylthio)ethan-1-ol	
12.212	Ethyl-5-(methylthio)valerate	
III Monothiols		
12.169	2-Methyl-4-oxopentane-2-thiol	
12.238	3-Mercapto-2-methylpentan-1-ol	
12.239	3-Mercapto-2-methylpentanal	
12.241	2-Mercapto-2-methylpentan-1-ol	
12.255	Ethyl 3-mercaptobutyrate	
12.291	3-Mercapto-2-methyl-1-butanol	
V Acyclic and cyclic disulphides		
12.198	2,3,5-Trithiahexane	

[4] For flavouring substances evaluated by the JECFA before 2000 it is laid down in Commission Regulation (EC) 1565/2000 that if they are considered acceptable at the current estimated intake by the JECFA and comply with the general use criteria, they could be included in the list of authorised substances without undergoing a separate evaluation for the time being.

FL-no:	Register name	Structural formula
VI Acyclic tri- and polysulphides		
12.009	Diallyl trisulfide	
12.013	Dimethyl trisulfide	
12.020	Methyl propyl trisulfide	
12.023	Dipropyl trisulfide	
12.045	Methyl allyl trisulfide	
12.074	Diallyl polysulfides	 X=2,3,4 or 5
12.155	Methyl ethyl trisulfide	
12.280	Diisopropyl trisulphide	
VIII Thioesters		
12.257	Ethyl 4-(acetylthio)butyrate	

2.2.2. Isomers

JECFA status

Two substances have one chiral centre [FL-no: 12.241 and 12.255] and three substances have two chiral centres [FL-no: 12.238, 12.239 and 12.291] in the group of the JECFA-evaluated sulphides and thiols.

EFSA considerations

Adequate information on isomeric composition is available for all the substances in FGE.74Rev4. For the two stereoisomeric substances [FL-no: 12.241 and 12.255] with one chiral centre, the CAS register number (CASrn) is considered to cover the stereoisomeric composition as a racemate.

2.2.3. Specifications

JECFA status

The JECFA specifications are available for all 19 substances (JECFA, 1999b, 2003). See Table 2.

EFSA considerations

The available specifications are considered adequate for 19 substances.

3. Intake estimation

3.1. Status

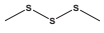
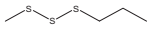
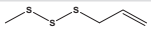
For all substances evaluated through the JECFA Procedure production volumes (JECFA, 2000, 2004b), EFSA has received updated production volumes for the EU (EFFA, 2017), based on which MSDI values have been calculated (see Appendix B, Table B.2).

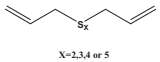
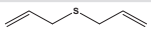
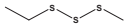
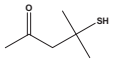
3.2. EFSA considerations

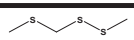
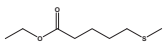
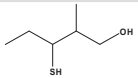
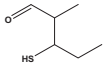
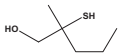
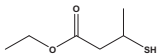
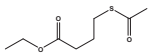
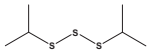
For all 19 substances, the flavour industry submitted normal and maximum use levels (Flavour Industry, 2008; EFFA, 2017). Based on the normal use levels, the mTAMDI figures have been calculated. For 18 substances [FL-no: 12.009, 12.013, 12.020, 12.023, 12.045, 12.074, 12.155, 12.169, 12.179, 12.198, 12.212, 12.238, 12.239, 12.241, 12.555, 12.257, 12.280 and 12.291], the mTAMDI intake estimates are below the threshold of toxicological concern (TTC) for their structural class. For one substance [FL-no: 12.088] the mTAMDI intake estimate is above the TTC for its structural class.

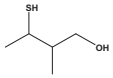
Use levels and mTAMDI values are presented in Appendix B, Tables B.1 and B.2.

Table 2: Specification summary of the substances in FGE.74 Revision 4

FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys. form Mol. formula Mol. weight	Solubility[a] Solubility in ethanol[b]	Boiling point, °C[c] Melting point, °C ID test Assay minimum	Refrac. Index[d] Spec. gravity[e]	EFSA comments
12.009 587	Diallyl trisulfide		3265 486 2050-87-5	Liquid $C_6H_{10}S_3$ 178.33	Insoluble Insoluble	112–120 (21 hPa) IR 65%	1.600–1.620 1.135–1.170	Min. assay value 65% (min. 95% allyl di-, tri- and tetrasulfides) (EFFA, 2017); secondary components 20–25% allyl disulfide; 5–7% allylsulfide; 5–7% allyl tetrasulfide (DG SANCO, 2011)
12.013 582	Dimethyl trisulfide		3275 539 3658-80-8	Liquid $C_2H_6S_3$ 126.26	Very slightly soluble Soluble	165–170 IR 97%	1.595–1.605 1.195–1.210	EFFA (2017)
12.020 584	Methyl propyl trisulfide		3308 586 17619-36-2	Liquid $C_4H_{10}S_3$ 154.30	Very slightly soluble Soluble	52 (1.6 hPa) IR 45%	1.558–1.570 1.095–1.101	Also contains 25% dipropyl trisulfide, 12% dipropyl disulfide, 14% dimethyl disulfide, 3% methyl propyl sulfide (EFFA, 2017). More than 95% identified components
12.023 585	Dipropyl trisulfide		3276 726 6028-61-1	Liquid $C_6H_{14}S_3$ 182.36	Almost insoluble Soluble	98 (5 hPa) IR 99%	1.542–1.590 0.952	Including up to 15% dipropyl disulfide (EFFA, 2017)
12.045 586	Methyl allyl trisulfide		3253 11867 34135-85-8	Liquid $C_4H_8S_3$ 152.29	Very slightly soluble Soluble	47 (1 hPa) NMR 80%	1.593–1.603 0.975–0.985	Min. 10% dimethyl trisulfide; 6–8% allyl trisulfide (EFFA, 2017). More than 95% identified components

FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys. form Mol. formula Mol. weight	Solubility[a] Solubility in ethanol[b]	Boiling point, °C[c] Melting point, °C ID test Assay minimum	Refrac. Index[d] Spec. gravity[e]	EFSA comments
12.074 588	Diallyl polysulfides		3533 11912 72869-75-1	Liquid $C_6H_{10}S_2$ 146.30	Insoluble Slightly soluble	68 (20 hPa) IR NMR 95%	1.643–1.653 1.220 (20°)	Mixture of allyl disulfides (2–10%), allyl trisulfides (20–30%), allyl tetrasulfides (30–40% and allyl pentasulfides (30–40%). The number of S atoms in the chemical formula varies from 2 to 5 (EFFA, 2017)
12.088 458	Diallyl sulfide		2042 11846 592-88-1	Liquid $C_6H_{10}S$ 114.21	Insoluble Sparingly soluble	138–139 IR 97%	1.488–1.492 0.887–0.892	Solubility in ethanol (EFFA, 2011)
12.155 583	Methyl ethyl trisulfide		3861 31499-71-5	Liquid $C_3H_8S_3$ 140.28	Very slightly soluble Soluble	46–47 (5 hPa) NMR 97%	1.510–1.520 0.955–0.965	
12.169 1293	2-Methyl-4-oxopentane-2-thiol		3997 11500 19872-52-7	Liquid $C_6H_{12}OS$ 132.23	Soluble Very slightly soluble	47–49 (20 hPa) IR NMR MS 48%	1.431–1.437 1.032–1.037	The Register name to be changed to 4-mercapto-4-methyl-2-pentanone. Min. assay value is 48% and secondary component 4-methyl-3-penten-2-one [FL-no: 07.101] 48-50% (DG SANCO, 2011); supplied as a 1% solution in propylene glycol. More than 95% identified components
12.179 1297	2-(Methylthio)ethan-1-ol		4004 11545 5271-38-5	Liquid C_3H_8OS 92.16	Insoluble Soluble	169–171 IR NMR MS 98%	1.490–1.498 1.055–1.065 (20°)	

FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys. form Mol. formula Mol. weight	Solubility[a] Solubility in ethanol[b]	Boiling point, °C[c] Melting point, °C ID test Assay minimum	Refrac. Index[d] Spec. gravity[e]	EFSA comments
12.198 1299	2,3,5-Trithiahexane		4021 42474-44-2	Liquid $C_3H_8S_3$ 140.30	Insoluble Soluble	56–58 (10 hPa) MS 95%	1.436–1.444 1.157–1.163	
12.212 1298	Ethyl-5-(methylthio) valerate		3978 233665-98-0	Liquid $C_8H_{16}O_2S$ 176.27	Insoluble Soluble	227 IR NMR MS 96%	1.460–1.464 0.993–1.003 (20°)	Register name to be changed to ethyl 5-(methylthio)valerate
12.238 1291	3-Mercapto-2-methylpentan-1-ol		3996 227456-27-1	Liquid $C_6H_{14}OS$ 134.24	Slightly soluble Soluble	50 (0.7 hPa) IR NMR 99%	1.480–1.490 0.985–0.995	Mixture of four diastereoisomers, each about 25% (EFFA, 2014)
12.239 1292	3-Mercapto-2-methylpentanal		3994 227456-28-2	Liquid $C_6H_{12}OS$ 132.23	Insoluble Soluble	98–100 (13 hPa) IR 96%	1.523–1.529 1.095–1.103	Mixture of four diastereoisomers, each about 25% (EFFA, 2014)
12.241 1290	2-Mercapto-2-methylpentan-1-ol		3995 258823-39-1	Liquid $C_6H_{14}OS$ 134.24	Slightly soluble Soluble	57–59 (0.8 hPa) IR NMR 99%	1.476–1.483 0.968–0.974 (20°)	Racemate. CASrn is considered to cover the stereoisomeric composition as racemate
12.255 1294	Ethyl 3-mercaptobutyrate		3977 156472-94-5	Liquid $C_6H_{12}O_2S$ 148.22	Insoluble Soluble	188 IR NMR MS 97%	1.448–1.453 1.011–1.021 (20°)	Racemate. CASrn is considered to cover the stereoisomeric composition as racemate
12.257 1295	Ethyl 4-(acetylthio)-butyrate		3974 104228-51-5	Liquid $C_8H_{14}O_3S$ 190.26	Insoluble Soluble	262 IR NMR MS 96%	1.468–1.472 1.073–1.083 (20°)	
12.280 1300	Diisopropyl trisulphide		 5943-34-0	Liquid $C_6H_{14}S_3$ 182.40	Insoluble Soluble	107–108 (13 hPa) NMR MS 95%	1.441–1.445 1.134–1.140	EFFA (2017)

FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys. form Mol. formula Mol. weight	Solubility[a] Solubility in ethanol[b]	Boiling point, °C[c] Melting point, °C ID test Assay minimum	Refrac. Index[d] Spec. gravity[e]	EFSA comments
12.291 1289	3-Mercapto-2-methyl-1-butanol		3993 227456-33-9	Liquid $C_5H_{12}OS$ 120.21	Slightly soluble Freely soluble	98 (at 2.7 hPa) IR NMR MS 98%	1.482–1.490 1.002–1.008	Mixture of four diastereoisomers, each about 25% (EFFA, 2014)

FL-no.: Flavour Information System number; JECFA: The 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; NMR: nuclear magnetic resonance; MS: mass spectrometry; CASrn: Chemical Abstract Service register number.
(a): Solubility in water, if not otherwise stated.
(b): Solubility in 95% ethanol, if not otherwise stated.
(c): At 1,013.25 hPa (1 atm), if not otherwise stated.
(d): At 20°C, if not otherwise stated.
(e): At 25°C, if not otherwise stated.

4. Genotoxicity

4.1. Genotoxicity studies – text taken[5] from the JECFA Report (JECFA, 2000, 2004b)

The reverse mutation test was performed for diallyl sulfide [FL-no: 12.088] (0.004–0.44 μg/mL), using *Salmonella* Typhimurium strain TA100. No genotoxicity was observed (Eder et al., 1982).[6]

Groups of male ICR mice were given two doses 48 h apart of a mixture containing diallyl sulfide [FL-no: 12.088], allyl disulfide (JECFA-no: 572) or diallyl trisulfide [FL-no: 12.009] in corn oil at doses of 10 or 20 mg/mL by gavage. The doses were estimated to provide 0.33 or 0.67 mmol/kg bw or 50 or 100 mg/kg bw on the basis of the composition of the mixture. No increase in the frequency of micronucleated polychromatic erythrocytes was seen in bone marrow cells (Marks et al., 1992).

Erythro- and *threo*-3-mercapto-2-methylbutanol [FL-no: 12.291 (3-mercapto-2-methyl-1-butanol)] (50–5,000 μg/plate) was evaluated for mutagenic activity in the modified Ames test with pre-incubation in the presence and absence of metabolic activation in *S.* Typhimurium strains TA97, TA98, TA100, TA102 and TA1535. No genotoxic effects were observed (Gocke, 1997).

For a summary of *in vitro*/*in vivo* genotoxicity data considered by JECFA, see Appendix C, Table C.1.

4.2. Genotoxicity studies – text taken[7] from EFSA FGE.08Rev5 (EFSA CEF Panel, 2012b)

In vitro/*in vivo*

Genotoxicity *in vitro* data are available for three candidate substances: di-(1-propenyl)-sulfide (mixture) [FL-no: 12.298] (subgroup I), 2-methylpropane-2-thiol [FL-no: 12.174] (subgroup III) and dibutyl disulfide [FL-no: 12.111] (subgroup V). In addition studies are available on 11 supporting substances from subgroups I (1), III (4), V (4) and VIII (2).

In vivo data are available for three supporting substances from subgroups I (1), III (1) and V (1).

Subgroup I (Acyclic sulphides)

In vitro data are available for the candidate substance, di-(1-propenyl)-sulfide [FL-no: 12.298]; Ames test: *S.* Typhimurium TA98, TA100, TA102, TA1535, TA1537, 1–100 μg/plate. Result was negative with and without metabolic activation (Stien, 2005).

For supporting substances, only data on diallyl sulfide [FL-no: 12.088] are available. Diallyl sulfide was negative in a limited bacterial reversion assay using one strain only (TA100) and provided equivocal results in an *in vitro* cytogenetic test in which increased incidences of cells with chromosomal aberrations and sister chromatid exchanges (SCEs), statistically significant but not dose related, were observed. *In vivo* diallyl sulfide was evaluated as negative in a micronucleus test in mouse bone marrow, which was, however, not designed to evaluate the genotoxicity of the substance itself as it was tested in a mixture. Overall the data available do not allow evaluation of the genotoxicity of the substances of this subgroup.

Subgroup III (Monothiols)

2-Methylpropane-2-thiol [FL-no: 12.174] is reported to be negative in an Ames test. It is reported to be positive in a mouse lymphoma assay without metabolic activation and negative in the test with metabolic activation, and it is reported to be negative in an *in vitro* SCE assay. However, these studies are reported only as summaries (Phillips Petroleum Company, 1990a). Some details are available for methods but not for the results. Although the validity of these studies cannot be fully evaluated, the positive result in the mouse lymphoma assay raises concern with respect to the potential for genotoxicity of this tertiary thiol and structurally related compounds, i.e. candidate substance 2-methylbutane-2-thiol [FL-no: 12.172] and ethyl-2-mercapto-2-methyl propanoate [FL-no: 12.304] and the five supporting substances [FL-no: 12.038, 12.085, 12.137, 12.138 and 12.145].

[5] The text is taken verbatim from the indicated reference source, but text related to substances not included in the present FGE has been removed.

[6] The Panel noted that the publication of Eder et al., 1982 is not the correct paper to quote from. It has not been possible for EFSA to identify the correct paper.

[7] The text is taken from the indicated reference source, but text related to subgroups not included in the present FGE has been removed.

The *in vitro* data available for the other substances in this subgroup do not provide indication of concern for genotoxicity.

Subgroup V (Acyclic and Cyclic disulphides)

Dibutyl disulfide [FL-no: 12.111] is reported to be negative in a mouse lymphoma assay (Dooley et al., 1987). However, the study is reported only as an abstract, and thus, the validity cannot be evaluated.

Further data are available for the supporting substances diallyl disulfide [FL-no: 12.008], dimethyldisulfide [FL-no: 12.026], phenyl disulfide [FL-no: 12.043] and benzyl disulfide [FL-no: 12.081]. All substances were reported to be negative in the Ames test. In addition, diallyl disulfide was reported to be positive in a chromosomal aberration assay *in vitro*, with and without metabolic activation, and weakly positive in a SCE assay. However, the validity of these findings is doubtful as chromosomal aberrations were only increased in conditions associated with extensive (> 90%) lethality, and because of the limitation of SCE in genotoxic hazard identification.

Subgroup VI (Acyclic tri- and polysulphides)

No genotoxicity information of sufficient quality is available.

Subgroup VIII (Thioesters)

The *in vitro* data available on supporting substances provide no indication of concern for genotoxicity.

Conclusion on genotoxicity

Most *in vitro* and *in vivo* studies are of limited or insufficient quality and provide only limited information.

The available data raise concern with respect to genotoxicity of three tertiary thiols [FL-no: 12.172, 12.174 and 12.304], included as candidate substances in subgroup III. Hydrolysis of the candidate substance 2,4,4-trimethyl-1,3-oxathiane [FL-no: 16.057], included in subgroup VII, leads to the formation of a tertiary thiol structurally related to the above-mentioned compounds. Therefore, there is also concern with respect to genotoxicity of this candidate substance. The Panel noted that in FGE.08 five of the supporting substances were tertiary thiols [FL-no: 12.038, 12.085, 12.137, 12.138 and 12.145] for which a concern for genotoxicity has been raised in the FGE.08Rev1. These supporting substances have been evaluated by JECFA at the 53rd meeting (JECFA, 2000JECFA, 2000, 2004b). These supporting substances have been considered by EFSA in FGE.91 (EFSA CEF Panel, 2010b).

In addition, genotoxicity of the candidate substance methyl methanethiosulfonate [FL-no: 12.159], included in subgroup X, could not be assessed from the data available. However, due to the similarity with methyl methanesulfonate, a direct acting mutagen and carcinogen, there is concern with respect to genotoxic potential of this candidate substance.

Therefore, the Panel decided that the Procedure could not be applied to the candidate substances [FL-no: 12.159, 12.172, 12.174, 12.304 and 16.057] until adequate *in vivo* genotoxicity data become available.

The other *in vitro*/*in vivo* genotoxicity data available, often from limited or poorly reported studies do not provide clear indication of concern for genotoxicity for the remaining candidate substances included in the present evaluation.

For a summary of *in vitro*/*in vivo* genotoxicity data considered by EFSA, see Appendix C, Tables C.2 and C.3 of this FGE.

4.3. Genotoxicity study on 2-methyl-4-oxopentane-2-thiol [FL-no: 12.169] from FGE.91Rev2 (EFSA CEF Panel, 2014)

In vitro

2-Methyl-4-oxopentane-2-thiol [FL-no: 12.169] was tested in *S.* Typhimurium strains TA98, TA100, TA102, TA1535 and TA1537 in the presence or absence of S9-mix (Mc Garry, 2012). In the first experiment, the concentrations tested were 5.0, 15.8, 50.0, 158.1, 500.0, 1,581 and 5,000 μg/plate, and the plate incorporation method was used. No evidence of toxicity was observed in the absence or presence of S9-mix in any tester strains. In the second experiment, the concentrations were 156.3, 312.5, 625.0, 1,250, 2,500 and 5,000 μg/plate of 2-methyl-4-oxopentane-2-thiol, and treatments in the presence of S9-mix used the pre-incubation method. Evidence of toxicity was observed through slight thinning of the background lawn and/or marked reduction in revertant numbers in all strains at

2,500 and/or 5,000 μg/plate in the presence of S9-mix and in TA1537 in the absence of S9-mix. Thus, the study design complied with current recommendations and an acceptable top concentration was achieved. There was no evidence of any mutagenic effect induced by 2-methyl-4-oxopentane-2-thiol in any of the strains, either in the absence or presence of S9-mix.

For a summary of the genotoxicity data on 2-methyl-4-oxopentane-2-thiol, see Appendix C, Table C.4.

4.4. EFSA Considerations

Subgroup III includes the tertiary thiols for which a genotoxicity concern was established based on data from a limited gene mutation assay for candidate substances in FGE.08Rev1 [FL-no: 12.174] and additional genotoxicity data were requested for this group of substances. Since the publication of the latest revision of FGE.08, FGE.08Rev5, the Industry has submitted a new bacterial mutation assay for the tertiary thiol [FL-no: 12.169] included in FGE.74. This substance is considered by the Panel to be representative for the whole group of tertiary thiols (in FGE.08, FGE.74 and FGE.91). Based on the new genotoxicity data, the Panel concluded that 2-methyl-4-oxopentane-2-thiol [FL-no: 12.169] was not genotoxic in the assay and that 2-methyl-4-oxopentane-2-thiol [FL-no: 12.169] and 2-mercapto-2-methylpentan-1-ol [FL-no: 12.241] do no longer give rise to concern with respect to gene mutations. Therefore, these two substances can be evaluated using the Procedure in the present FGE. The Panel noted that of the material of commerce for [FL no: 12.169], approximately half consists of the α, β-unsaturated carbonyl, 4-methyl-3-penten-2-one [FL-no: 07.101], for which concern for genotoxicity was ruled out in FGE.204 (EFSA CEF Panel, 2012a) and evaluated using the Procedure in FGE.63Rev2 (EFSA CEF Panel, 2013).

Although the available data are limited[8] the Panel considered that for the 19 substances in FGE.74Rev4 the genotoxicity data do not preclude evaluating these substances through the Procedure.

5. Evaluation of the additional data submitted for the tri- and polysulphides in FGE.74

In the earlier EFSA-evaluations of acyclic tri- and polysulphides (subgroup VI in FGE.08), the evaluation stopped at Step B4 due to lack of NOAEL for a representative substance. The available 90-day feeding studies on dipropyl trisulfide and diallyl trisulfide in rats by Morgareidge and Oser (1970a, b) were not considered sufficient to derive a NOAEL.

The shortcomings of the Morgareidge and Oser studies are the following:

- No data on the stability of the test substance in feed are given.
- There are no histopathology data.
- Nearly all animals, including control animals, were affected by inflammatory changes in respiratory tract, and in other organs (mainly liver). These changes (probably caused by infections) prohibit adequate interpretation of the study results.
- The data on haematology, clinical chemistry and urine analysis (performed for eight animals in the test-substance groups respectively, and eight animals in the control group at weeks 6 and 12) are only shown as a mean for the three groups, and without any indication of variation between the individuals (e.g. no SD, etc.)

Data submitted on the metabolism and structure–activity relationships of sulphides were mainly in accordance with the information provided in FGE.08 and FGE.74.

To support a potential read-across, the flavour industry submitted a discussion with the aim to show that NOAELs from toxicity studies for sulphides, disulphides and for tri- and polysulphides are of the same magnitude. In this discussion, route to route extrapolation from inhalatory to oral was applied, but the technique to accomplish this has been shown to be inadequate (Rennen et al., 2004). Additionally, the oral long-term studies presented were one-dose-level-only and consequently of limited relevance to judge whether substances are equipotent or not. The discussion on magnitude of NOAELs was, therefore, not considered relevant. The Panel concluded that the extrapolations made by the applicant cannot be used to support derivation of a NOAEL for the oral route.

In FGE.74Rev3, the Panel concluded that the additional information as submitted by the flavour industry (IOFI, 2013) was insufficient to evaluate the safety of the tri- and polysulphides and that a

[8] The Panel noted that an *in vivo* micronucleus study is available on [FL-no: 12.169], which will be considered in the next revision of this Opinion.

90-day study from which a NOAEL can be derived was needed for the safety evaluation of these substances.

In this revision of FGE.74, FGE.74Rev4 the Panel evaluated a new dossier addressing this issue. The dossier contains repeated-dose subchronic toxicity studies on the representative substance, methyl propyl trisulfide [FL-no: 12.020] to cover the evaluation of the substances in the tri- and polysulphides subgroup (subgroup VI).

The Panel noted that [FL-no: 12.020] is reported as a mixture of 45% purity, with 54% mono-, di- and tri-sulphides as secondary components (see Table 2). The material tested in the repeated-dose toxicity studies has a higher purity (~ 70%). Thirty per cent of the tested preparation has not been identified. Regarding the higher purity of the substance tested, the Panel considers that the potential risk associated with the secondary components present in commercial preparations might have been underestimated in the performed toxicity study.

In the 14-day dose-range finding repeated-dose toxicity study (see Section 6.1), the recovery of the administered substance [FL-no: 12.020] in the feed decreased from 75% to 50% within a week (Bauter, 2015a). Therefore, the applicant decided to perform the 90-day study by gavage (see Section 6.3). It is unknown whether this disappearance of the substance from feed is due to volatilisation or reaction to potentially more toxic products in food. Without knowing the fate of the missing fraction, it cannot be assessed whether these products would be covered by a gavage study.

6. Short-term and subchronic toxicity

6.1. A 14-day range-finding study with methyl propyl trisulfide [FL-no: 12.020] mixed in the diet (Bauter, 2015a)

In an initial experiment, the candidate substance was administered via the feed to male and female rats during 14 days (Bauter, 2015a) at levels targeting 150, 300 and 600 mg/kg bw per day. However, the compound was not stable in the diets so that the recovery of the administered substance [FL-no: 12.020] in the feed decreased from 75% to 50% within a week. Although none of the rats died, after autopsy enlarged spleens with dark discoloration at all dose levels was observed. This indicates haemolysis, a known effect of sulphides. Because of the low stability of the compound in the feed, as well as the poor palatability, the company decided to administer it by oral gavage in a follow up experiment, at lower doses.

For a summary of the additional toxicity studies considered by EFSA, see Appendix D, Table D.1.

6.2. A 14-day range finding study with methyl propyl trisulfide [FL-no: 12.020] by oral gavage (Bauter, 2015b)

A 14 day range-finding study by oral gavage was performed in male and female Sprague–Dawley rats (5/sex per dose) at dose levels 12.5 (Group 2), 50 (group 3) and 100 (group 4) mg/kg bw per day (Bauter 2015b). The test substance was mixed with corn oil.

There were no test substance-related mortalities. No clinical observations or changes in body weight, body weight gain, mean daily food consumption or food efficiency associated with test substance administration were observed.

Blood cytology evaluation revealed the presence of Heinz bodies in all Groups 3 and 4 animals; these were present with a lower incidence and severity in Group 2 animals. Additionally, Group 4 animals had evidence of polychromasia. Splenic enlargement and/or dark red discoloration of the spleen were observed in all Groups 3 and 4 animals and in individual Group 2 males and females. Spleen weights were increased. Microscopic findings of increased spleen iron deposits and evidence of increased splenic erythropoiesis were observed in Groups 3 and 4 and/or individual Group 2 males and females. For a study of longer duration, by means of oral gavage administration of the test substance animals were expected to tolerate dose levels up to 12.5 mg/kg bw per day.

For a summary of the additional toxicity studies considered by EFSA, see Appendix D, Table D.1.

6.3. A 90-day study by oral gavage with [FL-no: 12.020]

During a 90-day subchronic oral gavage study according to OECD guideline 408 and Good laboratory practice (GLP), Sprague–Dawley CD® rats (10/sex per Group) received 0.5, 2 and 6 mg/kg bw per day of the test substance mixed with corn oil (0.1, 0.4, and 1.2 mg/mL, w/v, respectively) by

oral gavage; vehicle controls received corn oil (5 mL/kg bw per day) (Koetzner, 2016). The test substance was shown to be stable in the solutions for gavage for the whole period.

There were no mortalities or changes in clinical/ophthalmological parameters, body weight, body weight gain, food consumption, or food efficiency attributable to methyl propyl trisulfide administration. There were no test substance-related changes in most male and female haematology, coagulation, clinical chemistry and urinalysis values. Small decreases in red blood cell count, haemoglobin and haematocrit observed in the high-dose groups, in males and females. These effects are consistent indications of haematotoxic effects and are considered adverse.

There were no macroscopic or microscopic changes attributable to administration of the test substance. No differences in organ weights, organ-to-body weight ratios or organ-to-brain weight ratios were observed in female rats. Decreases in absolute thymus weights in high-dose male rats were without histologic correlates and were not directly correlated with any other study parameters and are, therefore, not considered adverse.

Under the conditions of the study, the NOAEL of the test compound was determined to be 2 mg/kg bw per day for both males and females based on haematotoxic effects.

For a summary of the additional toxicity studies considered by EFSA, see Appendix D, Table D.1.

6.4. EFSA considerations

The Panel noted the uncertainties regarding the suitability of the flavouring substance [FL-no: 12.020] administered in the toxicity study to represent the material of commerce, and the potential formation of reaction products in feed, as outlined in Sections 5 and 6.1. Despite EFSA's request, the applicant did not provide the respective information.

Therefore, the Panel decided that the 90-day study can be considered only once it is clearly demonstrated that the material tested is representative of the material of commerce and that potential reaction products are not of safety concern.

7. Application of the Procedure

7.1. Application of the Procedure to 19 aliphatic sulphides and thiols evaluated by the JECFA[9] (JECFA, 2000, 2004b)

According to JECFA, 15 substances belong to structural class I and 4 to structural class II using the decision tree approach presented (Cramer et al., 1978).

None of the substances could be anticipated to be metabolised to innocuous products and all were evaluated via the B-side of the Procedure. The estimated daily per capita intakes of the 19 flavouring substances are below the threshold of concern for structural class I and II, and a NOAEL exists to provide an adequate MoS to the estimated intake as flavouring substances (Step B4).

Step B4

For *erythro*- and *threo*-3-mercapto-2-methylbutanol [FL-no: 12.291] (3-mercapto-2-methyl-1-butanol)], the no observed effect level (NOEL) of 0.7 mg/kg bw per day for the structurally related substance 2-mercapto-3-butanol [FL-no: 12.024] from a 92-day study in rats fed by gavage (Cox et al., 1974) provides an adequate MoS (> 10,000) in relation to known levels of intake of this agent.

This NOEL is also appropriate for the structurally related agents (±)-2-mercapto-2-methylpentan-1-ol [FL-no: 12.241], 3-mercapto-2-methylpentan-1-ol (racemic) [FL-no: 12.238], 3-mercapto-2-methylpentanal [FL-no: 12.239] and (±)-ethyl 3-mercaptobutyrate [FL-no: 12.255], because they are all acyclic thiols with oxidised side-chains that are anticipated to undergo oxidation or hydrolysis and subsequent metabolism via similar metabolic pathways.

For 4-mercapto-4-methyl-2-pentanone [FL-no: 12.169], the NOEL of 1.9 mg/kg bw per day for the structurally related substance 3-mercapto-2-pentanone [FL-no: 12.031] administered to rats by gavage in a 92-day study (Morgareidge, 1971) provides an adequate MoS (> 10,000) in relation to known levels of intake of this agent.

For ethyl 4-(acetylthio)butyrate [FL-no: 12.257], the NOEL of 6.5 mg/kg bw per day reported in a 13-week study in rats (Shellenberger, 1970) fed with the structurally related substance ethylthioacetate [FL-no: 12.018] provides an adequate MoS (> 10,000) in relation to known levels of intake of this agent.

[9] The text from the indicated reference sources was taken verbatim with the only difference that the JECFA numbers were replaced by the corresponding [FL-no:].

For 2-(methylthio)ethanol [FL-no: 12.179], the NOEL of 1.4 mg/kg bw per day reported in a 13-week study in rats (Cox et al., 1979) fed by gavage with the structurally related substance 2-(methylthiomethyl)-3-phenylpropenal [FL-no: 12.087] provides an adequate MoS (> 10,000) in relation to known levels of intake of this agent. This NOEL is also appropriate for the structurally related agent ethyl-5-(methylthio)valerate [FL-no: 12.212], which is also an acyclic sulfide with an oxidised side-chain that is anticipated to undergo oxidation and subsequent metabolism via similar pathways.

For 2,3,5-trithiahexane [FL-no: 12.198], the NOEL of 0.3 mg/kg bw per day reported in a 13-week study (Mondino, 1981) in rats fed with the structurally related substance 3-methyl-1,2,4-trithiane [FL-no: 15.036] provides an adequate MoS (> 10,000) in relation to known levels of intake of this agent.

For diisopropyl trisulphide [FL-no: 12.280], the NOEL of 4.8 mg/kg bw per day reported in a 13-week study (Morgareidge and Oser, 1970a) in rats fed by gavage with the structurally related substance dipropyl trisulfide [FL-no: 12.023] provides an adequate MoS (> 100,000) in relation to known levels of intake of this agent.

For diallyl trisulfide [FL-no: 12.009] and dipropyl trisulfide [FL-no: 12.023], the NOELs of 4.6 and 4.8 mg/kg bw per day, respectively, were reported in a 90 days study (Morgareidge and Oser, 1970a, b) at a single dose, which gave adequate margins of safety for [FL-no: 12.013, 12.020, 12.045, 12.074 and 12.155]. The dose that had no effect is more than 10,000 times greater than the estimated per capita intake in Europe and more than 100,000 times higher than the estimated per capita intake in the United States.

No adequate NOEL was available for diallyl sulfide [FL-no: 12.088] or a related substance, therefore no adequate MoS can be provided. Accordingly, the evaluation of the substance proceeds to Step B5.

Step B5

For diallyl sulfide [FL-no: 12.088], the intake is estimated to be 0.4 μg/capita per day in the USA, which is lower than 1.5 μg/day, therefore, JECFA has concluded that there is no safety concern based on the intake data.

In conclusion, JECFA evaluated all substances as to be of no safety concern at the estimated levels of intake as flavouring substances based on the MSDI approach.

The evaluations of the 19 aliphatic sulfides and thiols with the outcome of the JECFA evaluations are summarised in Appendix E, Table E.1 of this FGE.

7.2. Application of the Procedure to aliphatic and alicyclic mono-, di-, tri-, and polysulphides with or without additional oxygenated functional groups evaluated by EFSA in FGE.08Rev5 (EFSA CEF Panel, 2012b)[10]

The application of the Procedure is based on intakes estimated on the basis of the MSDI approach.

For the candidate substance methyl methanethiosulfonate [FL-no: 12.159] (the only substance in subgroup X), there is an indication of a genotoxic potential *in vitro*. Furthermore, for three candidate substances (in subgroup III), 2-methylbutane-2-thiol [FL-no: 12.172], 2-methylpropane-2-thiol [FL-no: 12.174] and ethyl-2-mercapto-2-methyl propanoate [FL-no: 12.304] and one candidate substance (in subgroup VII), 2,4,4-trimethyl-1,3-oxathiane [FL-no: 16.057], a concern for genotoxicity was also identified based on experimental evidence for [FL-no: 12.174] and the structural similarity among these four substances. Therefore, in the absence of further genotoxicity data, the Panel concluded that the Procedure could not be applied to these five substances.

For four candidate substances, 3-mercaptooctanal [FL-no: 12.268] (subgroup III), 3-mercaptodecanal [FL-no: 12.269] (subgroup III), methanedithiol diacetate [FL-no: 12.271] (subgroup VIII) and 3,5-dimethyl-1,2-dithiolane-4-one [FL-no: 12.295] (subgroup V), no data on use as flavouring substances in Europe are available. Therefore, no intakes in Europe can be estimated and accordingly the Panel concluded that the Procedure could not be applied to these four substances.

Thus, for in total nine candidate substances, the Procedure could not be applied: [FL-no: 12.159, 12.172, 12.174, 12.268, 12.269, 12.271, 12.295, 12.304 and 16.057].

For the safety evaluation of the remaining 71 candidate substances from chemical groups 20 and 30 the Procedure as outlined in Annex I was applied, based on the MSDI approach. The stepwise evaluations of the 71 substances evaluated through the Procedure are summarised in Table E.1.

[10] The text is taken verbatim from the indicated reference source, but text related to subgroups not included in the present FGE has been removed.

Step 1

The candidate substances were classified following the procedure established by Cramer et al. (1978). For the 71 candidate substances evaluated through the Procedure, 42 substances were classified into structural class I, 19 substances were classified into structural class II and 10 substances were classified into structural class III.

Step 2

Step 2 requires consideration of whether metabolic pathways exist to metabolise the candidate substances to innocuous products at the expected levels of intake. The candidate substances may be biotransformed to reactive metabolites, such as thiols, sulphoxides and sulphones and, in consequence, they are not predicted to be metabolised to innocuous products. Therefore, the evaluation of all 71 candidate substances proceeds via the B-side of the Procedure scheme.

Step B3

The 42 substances in structural class I have estimated European daily per capita intakes ranging from 0.0012 to 6.1 μg, which is below the threshold of concern of 1,800 μg/person per day. The 19 substances evaluated through the Procedure in structural class II have estimated European daily per capita intakes ranging from 0.0024 to 2.4 μg, which is below the threshold of concern for class II of 540 μg/person per day. The 10 substances in structural class III have estimated European daily per capita intakes from 0.012 to 6.1 μg, which is below the threshold of concern for class III of 90 μg/person per day. Accordingly, all 71 candidate substances proceed to Step B4 of the Procedure.

Step B4

No adequate studies on candidate substances are available. Repeated-dose toxicity studies are available on some supporting substances, which, with very few exceptions, have been carried out testing only one dose, giving rise to no observed adverse effects. The results of the adequate studies on supporting substances show a relatively high degree of variability in the reported NOAELs, ranging from 0.06 to 250 mg/kg bw per day.

The 20 candidate substances in subgroup I can be represented by the supporting substance dimethyl sulfide [FL-no: 12.006], for which an adequate 90-day subchronic study is available, indicating that no adverse effects were produced by the highest oral dose tested (250 mg/kg bw per day), which can be considered a NOAEL. The combined estimated daily per capita intake of 10 μg for the 18 candidate substances in subgroup I corresponds to 0.17 μg/kg bw per day at a body weight of 60 kg. Thus, a MoS of 1.5×10^6 can be calculated. The 20 candidate substances in subgroup I are accordingly not expected to be of safety concern at the estimated levels of intake.

Within subgroup III, adequate 90-day subchronic studies are available for four supporting secondary thiols, 2-mercapto-3-butanol [FL-no: 12.024], cyclopentanethiol [FL-no: 12.029], 2,3- and 10-mercaptopinane [FL-no: 12.035] and 2,6-(dimethyl)thiophenol [FL-no: 12.082], which can be considered representative of the 11 candidate substances evaluated through the Procedure in this subgroup. In the four studies, no adverse effects were produced by the highest oral dose tested ranging from 0.06 up to 0.7 mg/kg bw per day. By adopting a conservative approach, the lowest value (0.06 mg/kg bw per day) can be considered a NOAEL. The combined estimated daily per capita intake of 1.13 μg for the 11 candidate substances evaluated through the Procedure in subgroup III corresponds to 0.019 μg/kg bw per day at a body weight of 60 kg. Thus, a MoS of 3×10^3 can be calculated. The 11 candidate substances in subgroup III are accordingly not expected to be of safety concern at the estimated levels of intake.

Within subgroup V, adequate 90-day subchronic studies are available for two supporting substances dicyclohexyl disulfide [FL-no: 12.028] and benzyl methyl disulfide [FL-no: 12.068], which can be considered representative of the four candidate substances in this subgroup evaluated through the Procedure. In the two studies, no adverse effects were produced by the highest oral dose tested: 0.23 and 1.15 mg/kg bw per day. By adopting a conservative approach, the lowest value (0.23 mg/kg bw per day) can be considered a NOAEL. The combined estimated daily per capita intake of 0.6 μg for the four candidate substances evaluated through the Procedure in subgroup V corresponds to 0.01 μg/kg bw per day at a body weight of 60 kg. Thus, a MoS of 2.3×10^4 can be calculated. The four candidate substances in subgroup V are accordingly not expected to be of safety concern at the estimated levels of intake.

Within subgroup VI, no adequate toxicity study from which a NOAEL could be established was available, neither on the candidate substances nor on supporting substances. Therefore, the

Panel concluded that additional data are still required for the eight tri-, tetra- and polysulphides in subgroup VI of FGE.08 [FL-no: 12.093, 12.094, 12.097, 12.100, 12.112, 12.116, 12.164 and 12.167].[11]

Within subgroup VIII, an adequate 90-day subchronic study is available for one supporting substance, ethyl thioacetate [FL-no: 12.018], which can be considered representative of the eight candidate substances evaluated through the Procedure in this subgroup. In the study, no adverse effects were produced by the highest oral dose tested: 6.63 mg/kg bw per day. Therefore, the NOAEL is concluded to be 6.63 mg/kg bw per day for ethyl thioacetate. The combined estimated daily per capita intake of 2.4 μg for the eight candidate substances in subgroup VIII corresponds to 0.04 μg/kg bw per day at a body weight of 60 kg. Thus, a MoS of 1.7×10^5 can be calculated. The eight candidate substances in subgroup VIII are accordingly not expected to be of safety concern at the estimated levels of intake.

The conclusion from Step B4 is that for the 43 candidate substances belonging to subgroups I, III, V and VIII, and evaluated through the Procedure, adequate NOAELs exist for the candidate substance or for structurally related substances providing adequate margins of safety at the estimated levels of intake. Therefore, these candidate substances are not expected to be of safety concern at the levels of exposure estimated by the MSDI approach.

For the eight candidate substances belonging to subgroup VI [FL-no: FL-no: 12.093, 12.094, 12.097, 12.100, 12.112, 12.116, 12.164 and 12.167], additional toxicity data are required.

The evaluations of the aliphatic and alicyclic mono-, di-, tri- and polysulphides are summarised in Appendix E, Table E.2.

7.3. EFSA Considerations relevant for FGE.74

The 19 JECFA-evaluated aliphatic sulphides and thiols are distributed into five subgroups of structurally related substances. The subgrouping is in compliance with the one used in FGE.08Rev5 (see Section 2.2.1 and Table 1).

Although the available data are limited, the Panel considered that for the remaining 19 substances in FGE.74Rev4 the genotoxicity data do not preclude evaluating these substances through the Procedure.

The Panel agrees with the application of the Procedure as performed by JECFA for five aliphatic sulphides and thiols, namely [FL-no: 12.238, 12.239, 12.255, 12.257 and 12.291].

For 14 substances [FL-no: 12.009, 12.013, 12.020, 12.023, 12.045, 12.074, 12.088, 12.155, 12.169, 12.179, 12.198, 12.212, 12.241 and 12.280], the Panel did not agree with the application of the Procedure by JECFA for the following reasons:

JECFA derives a NOAEL of 1.4 mg/kg bw per day reported in a 13-week study in rats (Cox et al., 1979) fed by gavage with 2-(methylthiomethyl)-3-phenylpropenal [FL-no: 12.087]. The Panel did not agree with JECFA that 2-(methylthiomethyl)-3-phenylpropenal [FL-no: 12.087] is structurally related to 2-(methylthio)ethan-1-ol [FL-no: 12.179] or ethyl-5-(methylthio)-valerate [FL-no: 12.212]. JECFA derived a NOAEL of 0.3 mg/kg bw per day reported in a 13-week study (Mondino, 1981) in rats fed with 3-methyl-1,2,4-trithiane [FL-no: 15.036]. The Panel does not agree with JECFA that 2,3,5-trithiahexane [FL-no: 12.198] is structurally related to 3-methyl-1,2,4-trithiane [FL-no: 15.036].

However, in the first revision of FGE.74, FGE.74Rev1, all substances have been distributed to subgroups with respect to sulphur-containing functional groups, according to FGE.08 and following revisions. The JECFA-evaluated substances 2-(methylthio)ethan-1-ol and ethyl-5-(methylthio)valerate [FL-no: 12.179 and 12.212] have been allocated to subgroup I, *Acyclic sulphides*, and 2,3,5-trithiahexane [FL-no: 12.198] has been allocated to subgroup V, *Acyclic and cyclic disulphides*. Appropriate NOAELs exist for these two subgroups, as is argued in FGE.08Rev5. Since based on these NOAELs adequate margins of safety can be calculated for [FL no: 12.179, 12.198 and 12.212], in line with JECFA, the Panel also concludes that these substances are not expected to be of safety concern at the estimated levels of intake.

For the diallyl sulfide [FL-no: 12.088], JECFA evaluated this substance at Step B5 of the Procedure to be of no safety concern as the estimated intake in the USA of 0.4 μg/capita per day is below 1.5 μg/person per day. 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 μg/person per day. However, this substance is allocated into subgroup I, for which an appropriate NOAEL (reported for dimethylsulfide [FL-no: 12.006]) exist, as is demonstrated in FGE.08Rev5. The NOAEL of 250 mg/kg bw per day

[11] A number of these substances are no longer supported by industry (see Table E.2).

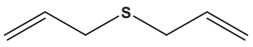
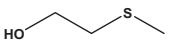
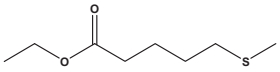
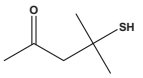
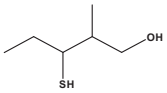
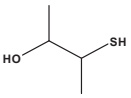
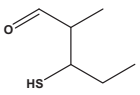
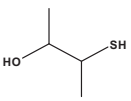
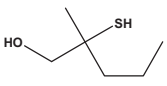
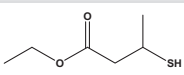
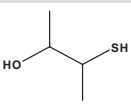
provides a MoS of 9.7 × 10^5 based on a European MSDI of 15.44 μg/capita per day and accordingly the Panel concludes that this substance is not expected to be of safety concern at the estimated level of intake based on the MSDI approach. The mTAMDI intake estimate for [FL-no: 12.088] is 1,413 μg/person per day which is above the TTC for structural class II for which the TTC is 540 μg/person per day. Industry indicated that for the two tertiary thiols, 2-methyl-4-oxopentane-2-thiol [FL-no: 12.169] and 2-mercapto-2-methylpentan-1-ol [FL-no: 12.241], both from subgroup III, JECFA derives a NOAEL from 90-day studies performed with secondary thiols (3-mercapto-3-butanol [FL-no: 12.031] (Morgareidge, 1971) and 2-mercapto-3-butanol [FL-no: 12.024] (Cox et al., 1974), respectively. The Panel did not agree with JECFA that the tertiary and secondary thiols are sufficiently structurally related for a read-across with respect to deriving a NOAEL. Accordingly, the Panel concluded at Step B4 that further data are required for the evaluation of [FL-no: 12.169 and 12.241].

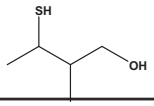
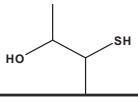
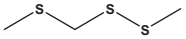
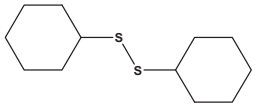
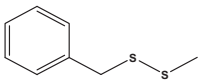
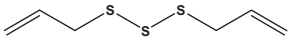
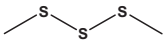
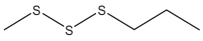
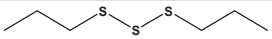
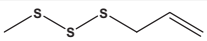
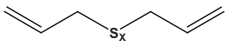
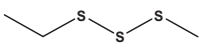
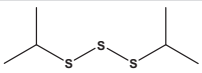
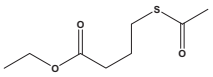
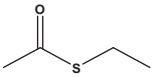
For the eight substances in subgroup VI (acyclic tri- and polysulphides) [FL-no: 12.009, 12.013, 12.020, 12.023, 12.045, 12.074, 12.155 and 12.280], 90-day studies were available on [FL-no: 12.009 and 12.023], but the studies were not considered adequate for deriving a NOAEL (Morgareidge and Oser, 1970a; Morgareidge and Oser, 1970b) (see Section 5). It has also been concluded that tri- and polysulphides cannot be covered by NOAELs for disulphides, due to the formation of more reactive metabolites than is the case for the disulphides. Accordingly, the Panel concluded at Step B4 (contrary to JECFA) that further data are required for the tri- and polysulphides [FL-no: 12.009, 12.013, 12.020, 12.023, 12.045, 12.074, 12.155 and 12.280].The applicant has recently provided a 90-day study on methyl propyl trisulfide [FL-no: 12.020] to support the safety evaluation of subgroup VI. In Sections 5 and 6.4, it has been explained that this 90-day study cannot be used for the evaluation of the eight substances in subgroup VI without further information. Therefore, no conclusion on the safety of these eight flavouring substances can be reached.

In summary, no safety concern was identified for the following substances: [FL-no: 12.088, 12.179, 12.198, 12.212, 12.238, 12.239, 12.255, 12.257 and 12.291]. For the remaining substances [FL-no: 12.009, 12.013, 12.020, 12.023, 12.045, 12.074, 12.155, 12.169, 12.241 and 12.280], additional data are needed to finalise the evaluation.

An overview of the EFSA considerations is given in Table 3 below.

Table 3: Overview of supporting substances providing adequate NOAEL for the procedure Step B4

FL-no	Register name	Structural formula	Supporting substances providing NOAEL
I Acyclic sulphides			
12.088	Diallyl sulfide		
12.179	2-(Methylthio)ethan-1-ol		
12.212	Ethyl-5-(methylthio)valerate		
III Monothiols			
12.169	2-Methyl-4-oxopentane-2-thiol		No adequate NOAEL available for Step B4 in the Procedure – additional data required
12.238	3-Mercapto-2-methylpentan-1-ol		
12.239	3-Mercapto-2-methylpentanal		
12.241	2-Mercapto-2-methylpentan-1-ol		No adequate NOAEL available for Step B4 in the Procedure – additional data required
12.255	Ethyl 3-mercaptobutyrate		

FL-no	Register name	Structural formula	Supporting substances providing NOAEL
12.291	3-Mercapto-2-methyl-1-butanol		
V Acyclic and cyclic disulphides			
12.198	2,3,5-Trithiahexane		 and 
VI Acyclic tri- and polysulphides			
12.009	Diallyl trisulfide		No adequate NOAEL available for Step B4 in the Procedure – additional data required
12.013	Dimethyl trisulfide		No adequate NOAEL available for Step B4 in the Procedure – additional data required
12.020	Methyl propyl trisulfide		No adequate NOAEL available for step B4 in the Procedure – additional data required
12.023	Dipropyl trisulfide		No adequate NOAEL available for step B4 in the Procedure – additional data required
12.045	Methyl allyl trisulfide		No adequate NOAEL available for step B4 in the Procedure – additional data required
12.074	Diallyl polysulfides	 X=2,3,4 or 5	No adequate NOAEL available for step B4 in the Procedure – additional data required
12.155	Methyl ethyl trisulfide		No adequate NOAEL available for step B4 in the Procedure – additional data required
12.280	Diisopropyl trisulphide		No adequate NOAEL available for step B4 in the Procedure – additional data required
VIII Thioesters			
12.257	Ethyl 4-(acetylthio) butyrate		

The results of the evaluation of the 19 candidate substances in this FGE have been included in Appendix E, Table E.1.

8. Conclusions

In FGE.74Rev4, the EFSA considered 11 aliphatic sulphides and thiols evaluated by JECFA at its 61st meeting and seven trisulphides and one monosulphide in a group of aliphatic and aromatic sulphides and thiols evaluated at its 53rd meeting. Accordingly, the consideration dealt with the 19 JECFA-evaluated substances.

The Panel concluded that the 19 substances in the JECFA flavouring group of aliphatic sulphides and thiols are structurally related to a group of aliphatic and alicyclic mono-, di- and polysulphides with or without additional oxygenated functional groups evaluated by EFSA in the FGE.08Rev5.

In previous versions of this FGE, a concern with respect to genotoxicity was identified for two candidate substances [FL-no: 12.169 and 12.241]. Additional genotoxicity data was evaluated for

2-methyl-4-oxopentane-2-thiol [FL-no: 12.169], which is considered to be supporting for [FL-no: 12.241]. Although the available data were limited, the Panel considered that for the remaining 19 substances in FGE.74Rev4, the genotoxicity data do not preclude evaluating these substances through the Procedure.

The Panel agrees with the application of the Procedure as performed by JECFA for five of the 19 aliphatic sulphides and thiols [FL-no: 12.238, 12.239, 12.255, 12.257 and 12.291]. For these five substances, the Panel concluded, similar to JECFA that these would not pose any safety concern at the current levels of exposure based on the MSDI approach. For three substances [FL-no: 12.179, 12.198 and 12.212], the Panel reached the same conclusion, but used a NOAEL from a different study as the one used by JECFA.

For diallyl sulphide [FL-no: 12.088], JECFA evaluated this substance at step B5 to be of no safety concern as the estimated intake in the USA is 0.4 μg/capita per day, which is below 1.5 μg/day. In line with the opinion expressed by the SCF (1999), the Panel does not make use of this threshold of 1.5 μg/person per day. However, the Panel decided that this substance could be allocated to subgroup I, for which a supporting substance [FL-no: 12.006] provides a NOAEL. Based on the intake estimate (MSDI) for diallyl sulfide [FL-no: 12.088] and this NOAEL, an adequate MoS of 9.7×10^5 could be calculated.

For the two tertiary thiols [FL-no: 12.169 and 12.241], the Panel did not agree with JECFA that appropriate studies were available for deriving NOAELs, and accordingly the Panel concluded that additional data are required for these two substances.

Within subgroup VI, a 90-day study on methyl propyl trisulfide [FL-no: 12.020] has recently become available. The Panel noted the uncertainties regarding the suitability of the flavouring substance administered in the toxicity study to represent the material of commerce, and the potential formation of reaction products in feed, as outlined in Section 6. Despite EFSA's request, the applicant did not provide the respective information.

Therefore, the Panel decided that the 90-day study can be considered only once it is clearly demonstrated that the material tested is representative of the material of commerce and that potential reaction products are not of safety concern. Therefore, no conclusion on the safety of these eight flavouring substances [FL-no: FL-no: 12.009, 12.013, 12.020, 12.023, 12.045, 12.074, 12.155 and 12.280] can be reached.

For all 19 substances, EU production volumes and use levels have been provided by the flavour industry. For 18 substances [FL-no: 12.009, 12.013, 12.020, 12.023, 12.045, 12.074, 12.155, 12.169, 12.179, 12.198, 12.212, 12.238, 12.239, 12.241, 12.555, 12.257, 12.280 and 12.291] the mTAMDI intake estimates are below the TTC for their structural class.

For one substance [FL-no: 12.088], the mTAMDI intake estimate is above the TTC for its structural class, Therefore, more reliable exposure data are required in order to finalise its evaluation. On the basis of such additional data, [FL no: 12.088] should be reconsidered using the Procedure. Following this, additional toxicological data might become necessary.

In order to determine whether the conclusion for the JECFA-evaluated substances can be applied to the materials of commerce, it is necessary to consider the available specifications. Specifications including purity criteria and identity are available for all the 19 JECFA-evaluated substances. These have been considered adequate for all substances. Thus, for 10 candidate substances in FGE.74Rev4 [FL-no: 12.009, 12.013, 12.020, 12.023, 12.045, 12.074, 12.155, 12.169, 12.241 and 12.280], the Panel concluded that additional data are required to finalise their evaluation.

For the remaining nine JECFA-evaluated aliphatic sulphides and thiols [FL-no: 12.088, 12.179, 12.198, 12.212, 12.238, 12.239, 12.255, 12.257 and 12.291], the Panel agrees with the JECFA conclusion 'No safety concern at estimated levels of intake as flavouring substances' based on the MSDI approach.

Documentation provided to EFSA

1) Bauter, 2015a. Methyl propyl trisulfide: palatability/toxicity study. A 14-day dietary study in rats. Product Safety Labs, PLS Study Number 40136. Unpublished report submitted by EFFA to EFSA.
2) Bauter, 2015b. Methyl propyl trisulfide. A repeat dose oral gavage range-finding study in rats. Product Safety Labs, PLS Study Number 41058. Unpublished report submitted by EFFA to EFSA.
3) Cox GE, Bailey DE and Morgareidge K, 1974. 90-day feeding study in rats with compound 14935 (2-mercapto-3-butanol). Food and Drug Research Laboratories, Inc. Lab. no. 2107d. December 30, 1974. Unpublished report submitted by EFFA to SCF.

4) Cox GE, Rucci G and Babish JG, 1979. 90-day subacute dietary toxicity study of IFF code no. 78-002-2 (2-(methylthiomethyl)-3-phenyl-propenal) in Sprague-Dawley rats. Food and Drug Research Laboratories, Inc. Lab. no. 5732b. Unpublished report submitted by EFFA to SCF.
5) DG SANCO (Directorate General for Health and Consumer Affairs), 2011. Information from DG SANCO 29/11/2011, on the chemical specifications of the five flavouring substances [FL-no: 12.009, 12.020, 12.045 and 12.169] from FGE.74.
6) DG SANCO (Directorate General for Health and Consumer Affairs), 2012. Information from DG SANCO 07/02 2012, concerning two lists of 85 and 15 non-supported substances and one list of 30 substances for which no data have been submitted or which are duplicates. FLAVIS.2.23rev1.
7) DG SANCO (Directorate General for Health and Consumer Affairs), 2013. Information from DG SANCO 30/10 2013, concerning a list of 19 non-supported substances. FLAVIS.2.27.
8) EFFA (European Flavour Association), 2011. Information on solubility on selected substances in FGE.74Rev2 and FGE.91Rev1. Private communication from EFFA to the FLAVIS secretariat. 21 and 24 October 2011. FLAVIS/8.129.
9) EFFA (European Flavour Association), 2013. E-mail from EFFA to FLAVIS Secretariat, Danish Food Institute, Technical University of Denmark, dated 9 and 24 September 2013. Information on substances in FGE.33Rev1 [FL-no: 13.119], FGE.74Rev3 [FL-no: 12.009, 12.020, 12.045, 12.155, 12.238, 12.239, 12.169 and 12.291], FGE.91Rev2 [FL-no: 12.038, 12.085, 12.145, 12.162, 12.252, 12.259, 12.274, 12.284 and 15.049]. FLAVIS/8.206.
10) EFFA (European Flavour Association), 2014. E-mail from EFFA to FLAVIS Secretariat, Danish Food Institute, Technical University of Denmark, dated 13 January 2014. Information on substances in FGE.74Rev3 [FL-no: 12.238, 12.239 and 12.291], FGE.78Rev2 [FL-no: 01.004, 01.018, 01.019, 01.040, 01.045 and 01.061] and FGE.91Rev2 [FL-no: 12.038 and 12.085]. FLAVIS/8.219.
11) EFFA (European Flavour Association), 2017. Information on stability, specifications and use levels on selected substances in FGE.74Rev4 submitted from EFFA to EFSA.
12) Flavour Industry, 2008. Unpublished information submitted by Flavour Industry to FLAVIS Secretariat. A-40/A-94.
13) IOFI (International Organization of the Flavor Industry), 2012. Addendum of Additional Data Relevant to the Flavouring Group Evaluation of the Chemical Group 20 (Annex I of 1565/2000/EC) 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.08 (2008). Addendum to FGE.74 and FGE.91. 16/12-2012. FLAVIS/8.193.
14) IOFI (International Organization of the Flavor Industry), 2013. Addendum of Additional Data Relevant to the Flavouring Group Evaluation of the Chemical Group 20 (Annex I of 1565/2000/EC) Simple Aliphatic Sulphides and Thiols Structurally Related to Aliphatic and aromatic mono-, di-, tri- and polysulphides with or without additional oxygenated functional groups in FGE.74Rev2. Addendum to FGE.74Rev2. 11/06/2013. FLAVIS/8.211.
15) IOFI (International Organization of the Flavor Industry), 2016. Addendum of Additional Data Relevant to the Flavouring Group Evaluation of the Chemical Group 20 (Annex I of 1565/2000/EC) from Flavouring Group Evaluation 74Rev2, Consideration of Aliphatic and aromatic mono- and di- thiols and mono-, di-, tri-, and polysulfides with or without additional oxygenated functional groups used as Flavouring Agents Evaluated by JECFA (53rd meeting), as evaluated by EFSA in FGE.08Rev3 (2011) and FGE.74Rev3 (2014). Addendum to FGE.74Rev3. 15/6/2016.
16) Koetzner, 2016. A 90-day oral gavage study in rats. Product Safety Labs, PLS Study Number 40137. Unpublished report submitted by EFFA to EFSA.
17) Mc Garry S, 2012. Audited Draft Report. Reverse mutation in five histidine-requiring strains of *Salmonella typhimurium*. 2-Mercapto-4-methylpentan-2-one. Covance Laboratories Ltd. Study no. 8261928. September 2012. Unpublished report submitted by EFFA to FLAVIS Secretariat.
18) Mondino A, 1981. Thirteen week repeated dose study of the test article TT 191 (3-methyl-1,2,4-trithiane) orally administered to Sprague Dawley Charles River CD (SD) BR rats at the

dosage of 0.3 mg/Kg/day. Istituto di Ricerche Biomediche, Antoine Marxer, S.p.A. Exp. no. 1196/191. May 15, 1981. Unpublished report submitted by EFFA to SCF.

19) Morgareidge K and Oser BL, 1970a. 90-Day feeding studies in rats with dipropyltrisulfide (30204). Food and Drug Research Laboratories, Inc. Lab. no. 0030. August 24, 1970. Unpublished report submitted by EFFA to FLAVIS Secretariat.
20) Morgareidge K and Oser BL, 1970b. 90-Day feeding studies in rats with diallyltrisulfide (30404). Food and Drug Research Laboratories, Inc. Lab. no. 0029. August 24, 1970. Unpublished report submitted by EFFA to FLAVIS Secretariat.
21) Morgareidge K, 1971. 90-Day feeding study with 2-keto-3-pentanethiol in rats. Food and Drug Research Laboratories, Inc. Lab. no. 0249. January 22, 1971. Unpublished report submitted by EFFA to SCF.
22) Pence DH, Farrow MG and Draus MA, 1982. Initial Submission: Letter from Phillips Petroleum Co. to USEPA regarding genotoxicity studies with 1,2-ethanedithiol, with cover letter dated 082492. Hazleton Laboratories America, Inc. EPA Doc 88-920009153, microfiche no. OTS0546435. Unpublished report submitted by EFFA to SCF.
23) Phillips Petroleum Company, 1990a. Toxicity study summary of tertiary butyl mercaptan (TOX1-6). Unpublished report submitted by Flavour Industry to FLAVIS Secretariat.
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Abbreviations

BW	body weight
CAS	Chemical Abstract Service
CASrn	CAS register number
CEF	Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CHO	Chinese hamster ovary (cells)
CoE	Council of Europe
EFFA	European Flavour and Fragrance Association
EPA	United States Environmental Protection Agency
FAO	Food and Agriculture Organization of the United Nations
FEMA	Flavor and Extract Manufacturers Association
FGE	Flavouring Group Evaluation
FLAVIS	(FL) Flavour Information System (database)
GLP	Good laboratory practice
HPRT	hypoxanthine phosphoribosyl transferase
ID	Identity
IP	intraperitoneal
IR	infrared spectroscopy
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
MoS	Margin of Safety
MS	mass spectrometry
MSDI	maximised survey-derived daily intake
mTAMDI	modified theoretical added maximum daily intake
NMR	nuclear magnetic resonance
No	number
NOAEL	no observed adverse effect level
NOEL	no observed effect level

OECD	Organisation for Economic Cooperation and Development
SCE	chromatic exchange
SCF	Scientific Committee on Food
TTC	threshold of toxicological concern
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, 1999a).

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, III) for which thresholds of concern (human exposure thresholds) have been specified. Exposures below these thresholds are not considered to present a safety concern.

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

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

- can the flavourings be predicted to be metabolised to innocuous products[12] (Step 2)?
- do their exposures exceed the threshold of concern for the structural class (Step A3 and B3)?
- are the flavourings or their metabolites endogenous[13] (Step A4)?
- does a NOAEL exist on the flavourings or on structurally related substances (Step 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.

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

[13] '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).

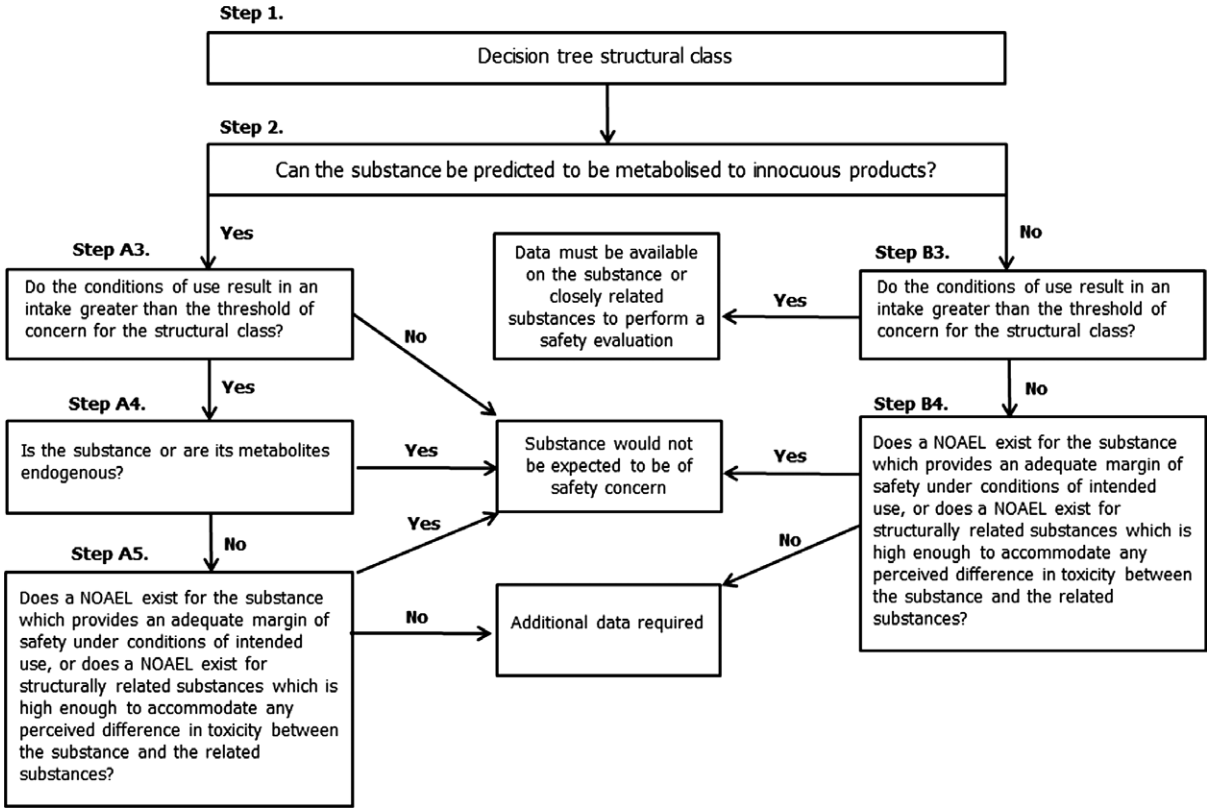


Figure A.1: Procedure for the safety evaluation of chemically defined flavouring substances

Appendix B – Exposure estimate

Table B.1: Normal and maximum use levels (mg/kg food) for the JECFA-evaluated substances in FGE.74Rev4

FL-no	Food categories Normal use levels (mg/kg) Maximum use levels (mg/kg)																		
	01.0	**02.0**	**03.0**	**04.1**	**04.2**	**05.0**	**05.3**	**06.0**	**07.0**	**08.0**	**09.0**	**10.0**	**11.0**	**12.0**	**13.0**	**14.1**	**14.2**	**15.0**	**16.0**
12.009	0.60	0.04	0.5	–	0.2	0.75	12.8	0.62	0.80	0.73	0.1	0.08	–	0.45	–	0.5	0.1	1.10	–
	2	1	2	–	1.5	2	15	1	2	1	1	1	–	1	–	1	2	5	–
12.013	0.60	0.04	0.5	–	0.2	0.75	12.8	0.62	0.80	0.73	0.1	0.08	–	0.45	–	0.5	0.1	1.10	–
	2	1	2	–	1.5	2	15	1	2	1	1	1	–	1	–	1	2	5	–
12.020	0.60	0.04	0.5	–	0.2	0.75	12.8	0.62	0.80	0.73	0.1	0.08	–	0.45	–	0.5	0.1	1.10	–
	2	1	2	–	1.5	2	15	1	2	1	1	1	–	1	–	1	2	5	–
12.023	0.60	0.04	0.5	–	0.2	0.75	12.8	0.62	0.80	0.73	0.1	0.08	–	0.45	–	0.5	0.1	1.10	–
	2	1	2	–	1.5	2	15	1	2	1	1	1	–	1	–	1	2	5	–
12.045	0.60	0.04	0.5	–	0.2	0.75	12.8	0.62	0.80	0.73	0.1	0.08	–	0.45	–	0.5	0.1	1.10	–
	2	1	2	–	1.5	2	15	1	2	1	1	1	–	1	–	1	2	5	–
12.074	0.60	0.04	0.5	–	0.2	0.75	12.8	0.62	0.80	0.73	0.1	0.08	–	0.45	–	0.5	0.1	1.10	–
	2	1	2	–	1.5	2	15	1	2	1	1	1	–	1	–	1	2	5	–
12.088	0.01	0.01	0.01	–	0.01	0.04	–	0.01	7.67	9.22	0.01	0.01	0.01	8.9	–	0.01	0.01	–	0.01
	1	0.5	1	–	1	1	–	0.5	10	10	0.2	0.2	0.2	10	–	0.5	1	–	0.5
12.155	0.60	0.04	0.5	–	0.2	0.75	12.8	0.62	0.80	0.73	0.1	0.08	–	0.45	–	0.5	0.1	1.10	–
	2	1	2	–	1.5	2	15	1	2	1	1	1	–	1	–	1	2	5	–
12.169	0.60	0.04	0.5	–	0.2	0.75	12.8	0.62	0.80	0.73	0.1	0.08	–	0.45	–	0.5	0.1	1.10	–
	2	1	2	–	1.5	2	15	1	2	1	1	1	–	1	–	1	2	5	–
12.179	0.60	0.04	0.5	–	0.2	0.75	12.8	0.62	0.80	0.73	0.1	0.08	–	0.45	–	0.5	0.1	1.10	–
	2	1	2	–	1.5	2	15	1	2	1	1	1	–	1	–	1	2	5	–
12.198	0.60	0.04	0.5	–	0.2	0.75	12.8	0.62	0.80	0.73	0.1	0.08	–	0.45	–	0.5	0.1	1.10	–
	2	1	2	–	1.5	2	15	1	2	1	1	1	–	1	–	1	2	5	–
12.212	0.60	0.04	0.5	–	0.2	0.75	12.8	0.62	0.80	0.73	0.1	0.08	–	0.45	–	0.5	0.1	1.10	–
	2	1	2	–	1.5	2	15	1	2	1	1	1	–	1	–	1	2	5	–
12.238	0.60	0.04	0.5	–	0.2	0.75	12.8	0.62	0.80	0.73	0.1	0.08	–	0.45	–	0.5	0.1	1.10	–
	2	1	2	–	1.5	2	15	1	2	1	1	1	–	1	–	1	2	5	–

FL-no	Food categories Normal use levels (mg/kg) Maximum use levels (mg/kg)																	
	01.0	**02.0**	**03.0**	**04.1**	**04.2**	**05.0**	**05.3**	**06.0**	**07.0**	**08.0**	**09.0**	**10.0**	**11.0**	**12.0**	**13.0**	**14.1**	**14.2**	**15.0**	**16.0**
12.239	0.60	0.04	0.5	–	0.2	0.75	12.8	0.62	0.80	0.73	0.1	0.08	–	0.45	–	0.5	0.1	1.10	–
	2	1	2	–	1.5	2	15	1	2	1	1	1	–	1	–	1	2	5	–
12.241	0.60	0.04	0.5	–	0.2	0.75	12.8	0.62	0.80	0.73	0.1	0.08	–	0.45	–	0.5	0.1	1.10	–
	2	1	2	–	1.5	2	15	1	2	1	1	1	–	1	–	1	2	5	–
12.255	0.60	0.04	0.5	–	0.2	0.75	12.8	0.62	0.80	0.73	0.1	0.08	–	0.45	–	0.5	0.1	1.10	–
	2	1	2	–	1.5	2	15	1	2	1	1	1	–	1	–	1	2	5	–
12.257	0.60	0.04	0.5	–	0.2	0.75	12.8	0.62	0.80	0.73	0.1	0.08	–	0.45	–	0.5	0.1	1.10	–
	2	1	2	–	1.5	2	15	1	2	1	1	1	–	1	–	1	2	5	–
12.280	0.60	0.04	0.5	–	0.2	0.75	12.8	0.62	0.80	0.73	0.1	0.08	–	0.45	–	0.5	0.1	1.10	–
	2	1	2	–	1.5	2	15	1	2	1	1	1	–	1	–	1	2	5	–
12.291	–	0.1	–	0.01	–	–	–	–	0.1	0.1	–	–	–	0.1	–	–	–	0.1	0.1
	–	0.5	–	0.1	–	–	–	–	1	2	–	–	–	1	–	–	–	1	0.5

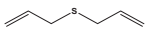
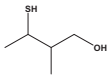
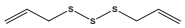
Table B.2: Estimated intakes based on the MSDI approach and the mTAMDI approach

FL-no	EU register name	MSDI – EU (μg/capita per day)	mTAMDI (μg/person per day)	Structural class	Threshold of concern (μg/person per day)
12.013	Dimethyl trisulfide	7.83	348	I	1,800
12.020	Methyl propyl trisulfide	4.89	348	I	1,800
12.023	Dipropyl trisulfide	11.28	348	I	1,800
12.155	Methyl ethyl trisulfide	0.001	348	I	1,800
12.169	2-Methyl-4-oxopentane-2-thiol	3.64	348	I	1,800
12.179	2-(Methylthio)ethan-1-ol	0.02	348	I	1,800
12.198	2,3,5-Trithiahexane	0.001	348	I	1,800
12.212	Ethyl-5-(methylthio)valerate	0.001	348	I	1,800
12.238	3-Mercapto-2-methylpentan-1-ol	0.01	348	I	1,800
12.239	3-Mercapto-2-methylpentanal	0.02	348	I	1,800
12.241	2-Mercapto-2-methylpentan-1-ol	0.012	348	I	1,800
12.255	Ethyl 3-mercaptobutyrate	3.4	348	I	1,800
12.257	Ethyl 4-(acetylthio)butyrate	3.4	348	I	1,800
12.280	Diisopropyl trisulphide	0.24	348	I	1,800
12.291	3-Mercapto-2-methyl-1-butanol	0.061	17	I	1,800
12.009	Diallyl trisulfide	0.05	348	II	540
12.045	Methyl allyl trisulfide	0.001	348	II	540
12.074	Diallyl polysulfide	3.19	348	II	540
12.088	Diallyl sulfide	15.44	1,413	II	540

MSDI: maximised survey-derived daily intake; mTAMDI: modified theoretical added maximum daily intake; ND: not derived.

Appendix C – Summary of the genotoxicity data

Table C.1: Genotoxicity data (*in vitro*/*in vivo*) evaluated by JECFA (2000, 2004b)

FL-no JECFA-no	EU register name JECFA name	Structural formula	End-point	Test system	Concentration	Results	Reference	Comments
In vitro								
12.088 458	Diallyl sulfide		Reverse mutation	*Salmonella* Typhimurium TA100	0.004–0.44 μg/mL	Negative[a]	Eder et al. (1982)	The Panel noted that the publication by Eder et al. (1982a) is not the correct paper to quote from. It has not been possible for EFSA to identify the correct paper
12.291 1289	3-Mercapto-2-methyl-1-butanol		Reverse mutation	*S.* Typhimurium TA1535, TA97, TA98, TA100, TA102	50–5,000 μg/plate	Negative[a]	Gocke (1997)	The racemate (*erythro*- and *threo*-3-Mercapto-2-methyl-1-butanol) was used in the toxicological evaluation
In vivo								
12.009 587	Diallyl trisulfide		*In vivo* mouse micronucleus test	Mouse	0.33–0.67 mM/kg (59–120 mg/kg)[b]	Negative	Marks et al. (1992)	Insufficient quality. Mixture of three substances was tested

(a): With and without metabolic activation from S9.
(b): Study used a mixture of allyl sulfide, allyl disulfide and allyl trisulfide in the respective ratio, 68:20:12.

Table C.2: Genotoxicity data (*in vitro*) EFSA/FGE.08Rev5 (EFSA CEF Panel, 2012b)

Chemical name [FL-no]	Test system	Test object	Concentration	Result	Reference	Comments
Subgroup I – Acyclic Sulphides						
(Diallyl sulfide [12.088])	Ames test	*Salmonella* Typhimurium TA100	0.004–0.44 μg/mL	Negative (± S9)	Eder et al. (1982)	Review. No details on method and results reported. Only TA100 used
	Sister chromatid exchange	Chinese hamster ovary cells	200–600 μg/mL	Positive[a]	Musk et al. (1997)	Limited quality of study. Insufficiently reported
	Chromosomal aberrations	Chinese hamster ovary cells	200–600 μg/mL	Positive[a]	Musk et al. (1997)	Limited quality of study. Insufficiently reported

Chemical name [FL-no]	Test system	Test object	Concentration	Result	Reference	Comments
Di-(1-propenyl)-sulfide (mixture) [12.298]	Ames test	*S.* Typhimurium TA98, TA100, TA102, TA1535, TA1537	1–100 μg/plate	Negative[(a)]	Stien (2005)	Un-published GLP study. Study considered valid
Subgroup II – Cyclic Sulphides						
Tetrahydrothiophene [15.102]	Ames test	*S.* Typhimurium TA98, TA100, TA1535, TA1537	50–5,000 μg/plate	Negative (± S9)	Pennwalt Corporation (1987a)	Validity of this study cannot be fully evaluated (only abstract provided)
	Cytogenetic assay	Human lymphocytes	12.5–125 μg/mL	Negative (± S9)	Pennwalt Corporation (1987a)	Validity of this study cannot be fully evaluated (only abstract provided)
	HPRT assay	Chinese hamster ovary cells	100–200 μg/mL	Negative (± S9)	Pennwalt Corporation (1987a)	Validity of this study cannot be fully evaluated (only abstract provided)
	Sister chromatid exchange	Chinese hamster ovary cells	15.63–125 μg/mL	Negative (± S9)	Pennwalt Corporation (1987b)	Validity of this study cannot be fully evaluated (only abstract provided)
	Unscheduled DNA synthesis	Human epithelial cells	2.5–5,120 μg/mL	Negative (± S9)	Pennwalt Corporation (1987a)	Validity of this study cannot be fully evaluated (only abstract provided)
(1,4-Dithiane [15.066])	Ames test	*S.* Typhimurium TA98, TA100	0.8–100 μ mol/plate (96.2–12,024 μg/plate)	Positive (−S9) Negative (+S9)	Lee et al. (1994)	Only two strains were tested, otherwise acceptable study
	Sister chromatid exchange	Chinese hamster ovary cells	2,000 μM (240 μg/mL)	Negative (± S9)	Lee et al. (1994)	Insufficient quality
Subgroup III – Monothiols						
2-Methylpropane-2-thiol [12.174]	Ames test	*S.* Typhimurium TA98, TA100, TA1535, TA1537, TA1538	10,000 μg/plate	Negative (±S9)	Phillips Petroleum Company (1990a)	Validity of this study cannot be fully evaluated (only abstract provided)
	Forward mutational MLTK assay	L5178Y/tk+/- mouse lymphoma cells	1,000 μg/mL	Positive (−S9) Negative (+S9)	Phillips Petroleum Company, (1990a)	Validity of this study cannot be fully evaluated (only abstract provided)
	Sister chromatid exchange	Chinese hamster ovary cells	1,350 μg/mL	Negative (+S9)[(b)]	Phillips Petroleum Company (1990a)	Validity of this study cannot be fully evaluated (only abstract provided)
(Allyl mercaptan [12.004])	Modified Ames test	*S.* Typhimurium TA98, TA100, TA1535, TA1537, TA1538	0.005–1.5 μL/mL (4.6–1,400 μg/mL)	Negative (±S9)	Eder et al. (1980)	Acceptable quality
(Benzyl mercaptan [12.005])	Ames test	*S.* Typhimurium TA98, TA100, TA1535, TA1537, TA1538	3.6 mg/plate (3,600 μg/plate)	Negative (±S9)	Wild et al. (1983)	Review. Methods and results insufficiently documented

Chemical name [FL-no]	Test system	Test object	Concentration	Result	Reference	Comments
(2-Mercaptopropionic acid [12.039])	Ames test	*S.* Typhimurium TA98, TA100, TA1535, TA1537, TA1538	3.6 mg/plate (3,600 μg/plate)	Negative (±S9)	Wild et al. (1983)	Review. Methods and results insufficiently documented
(Benzenethiol [12.080])	Ames test	*S.* Typhimurium TA98, TA100	25–500 μg/plate	Negative (±S9)	LaVoie et al. (1979)	Insufficient quality (only two strains were used, and all doses -except the lowest dose - were toxic)
Subgroup IV – Dithiols						
(1,2-Ethanedithiol [12.066])	Ames test	*S.* Typhimurium TA98, TA100, TA1535, TA1537, TA1538	5 doses up to 5,000 μg/plate	Negative (±S9)	Phillips Petroleum Company (1990b)	Validity cannot be fully evaluated (only abstract provided).
	Sister chromatid exchange	Chinese hamster ovary cells	0.5–50 μg/mL	Positive (±S9)	Pence et al. (1982)	Acceptable quality.
	Forward mutational assay	L5178Y/tk+/- mouse lymphoma cells	150 μg/mL	Positive (−S9)	Pence et al. (1982)	Positive only at cytotoxic concentrations.
	Forward mutational assay	L5178Y/tk+/- mouse lymphoma cells	1 μg/mL	Negative (+S9)	Pence et al. (1982)	Insufficiently documented.
Subgroup V – Acyclic Di-, Tri-, and Polysulphides						
(Diallyl disulfide [12.008])	Modified Ames test	*S.* Typhimurium TA98, TA100, TA1535, TA1537, TA1538	0.0015–0.15 μg/mL	Negative (±S9)	Eder et al. (1980)	Acceptable quality.
	Sister chromatid exchange	Chinese hamster ovary cells	2–25 μg/mL	Weakly positive (±S9)	Musk et al. (1997)	Limited quality. Insufficiently reported.
	Chromosomal aberrations	Chinese hamster ovary cells	2–25 μg/mL	Positive (±S9)	Musk et al. (1997)	Limited quality. Insufficiently reported.
(Dimethyl disulfide [12.026])	Ames test	*S.* Typhimurium TA98, TA100, TA102	0.000011–1.1 mmol/ plate(1.04–104,000 μg/plate)	Negative (±S9)	Aeschbacher et al. (1989)	Limited quality (only 3 strains used).
(Phenyl disulfide [12.043])	Ames test	*S.* Typhimurium TA98, TA100, TA1535, TA1537, TA1538	3.6 mg/plate (3,600 μg/plate)	Negative (±S9)	Wild et al. (1983)	Review. Methods and results insufficiently documented.
(Benzyl disulfide [12.081])	Ames test	*S.* Typhimurium TA98, TA100, TA1535, TA1537, TA1538	3.6 mg/plate (3,600 μg/plate)	Negative (±S9)	Wild et al. (1983)	Review. Methods and results insufficiently documented.
Dibutyl disulfide [12.111]	Forward mutational assay	Mouse lymphoma cells	NR	Negative (−S9)	Dooley et al. (1987)	Validity cannot be fully evaluated (only abstract provided).

Chemical name [FL-no]	Test system	Test object	Concentration	Result	Reference	Comments
Subgroup VIII – Thioesters						
(Methylthio 2-(acetyloxy)propionate [12.203])	Ames test	*S.* Typhimurium TA98, TA100, TA1535, TA1537,*Escherichia coli* WP2uvrA	0.156–5.0 mg/plate (156–5,000 μg/plate	Negative (±S9)	Watanabe and Morimoto (1989a)	Acceptable quality.
(Methylthio 2-(propionyloxy) propionate [12.227])	Ames test	*S.* Typhimurium TA98, TA100, TA1535, TA1537,*E. coli* WP2uvrA	0.156–5.0 mg/plate (156–5,000 μg/plate)	Negative (±S9)	Watanabe and Morimoto (1989b)	Acceptable quality.
Subgroup X – Sulphoxides/Sulphones and Sulphonates						
Methyl methane-thiosulfonate [12.159]	Ames test	*S.* Typhimurium TA98, TA100, TA1535, TA1537, TA1538, TA2637	0.6–60 μg/plate	Negative (−S9)	Dorange et al. (1983)	Test is not appropriate for antimicrobial agents[(f)].
	Ames test	*S.* Typhimurium TA98, TA100, TA1535, TA1537, TA1538, TA2637	2–600 μg/plate	Negative (+S9)	Dorange et al. (1983)	Test is not appropriate for antimicrobial agents[(f)]
	Ames test	*S.* Typhimurium TA98, TA100, TA2637	0.6–60 μg/plate	Negative (−S9)	Dorange et al. (1983)	Test is not appropriate for antimicrobial agents[(f)]
	Ames test	*S.* Typhimurium TA98, TA100, TA2637	0.6–200 μg/plate	Negative (+S9)	Dorange et al. (1983)	Test is not appropriate for antimicrobial agents[(f)]
	Ames test	*S.* Typhimurium TA98, TA100, TA2637	NR	Negative[(c)]	Dorange et al. (1983)	Test is not appropriate for antimicrobial agents[(f)]
	Ames test	*S.* Typhimurium TA98, TA100, TA2637	0.6–200 μg/plate	Negative[(d)]	Dorange et al. (1983)	Test is not appropriate for antimicrobial agents[(f)]
	Yeast assay	*Saccharomyces cerevisiae* Strain D7	1–300 μg/mL	Negative (±S9)	Dorange et al. (1983)	Test is not appropriate for antimicrobial agents[(f)]
	Yeast assay	*S. cerevisiae* Haploid strain N123	1–100 μg/mL	Negative (±S9)	Dorange et al. (1983)	Test is not appropriate for antimicrobial agents[(f)]

Chemical name [FL-no]	Test system	Test object	Concentration	Result	Reference	Comments
(Methylsulfinyl methane [12.175]) (synonym: dimethylsulfoxide, DMSO)	Ames test	*S.* Typhimurium TA97, TA98, TA100	100,000–300,000 μg/plate	Negative (±S9)	Brams et al. (1987)	Insufficient method (3 strains and 3 concentrations only)
	Ames test	*S.* Typhimurium TA97, TA98, TA100, TA1535, TA1537	100–10,000 μg/plate	Negative (±S9)	Zeiger et al. (1992)	Acceptable quality
	Ames test	*S.* Typhimurium TA97, TA98, TA100, TA102, TA104, TA1535, TA1538, *E. coli* WP2	0.1–0.4 mL/plate (100,000–400,000 μg/plate)	Negative (-S9)	Hakura et al. (1993)	Good quality study
	Ames test	*S.* Typhimurium TA1537, TA2637, *E. coli* WP2uvrA	0.1–0.4 mL/plate (100,000–400,000 μg/plate)	Positive (-S9)[(e)]	Hakura et al. (1993)	Good quality study. Positive at high doses with reduced bacterial survival. Doses routinely used in Ames test were negative

HPRT: hypoxanthine phosphoribosyl transferase; NR: not reported.
(a): With and without metabolic activation at clearly cytotoxic concentrations.
(b): A statistically significant increase in the number of SCEs per chromosome was seen at 1,350 μg/mL and the 450 μg/mL dose level in the presence of metabolic activation; but no significant increase was seen in the remaining dose levels, and no dose level showed a two fold increase in SCEs; therefore, t-butyl mercaptan is not considered to be mutagenic.
(c): With 100 μL/plate fecalase.
(d): With 100 μL/plate S9 metabolic activation and 100 μL/plate fecalase. Negative results reported after 2 days of incubation. Results for TA98 test strain were positive after 5 days of incubation.
(e): Positive results obtained at doses where lethal toxicity was observed. Negative results obtained at doses routinely used in Ames test.
(f): Thiosulphonates in general, and methyl methane thiosulphonate in particular, are non-specific antimicrobial agents that are active at low concentrations on prokaryotic bacteria, as well as on yeast and other eukaryotic fungi. This was even pointed out by Dorange et al. (1983). Therefore bacterial test systems and yeast assays are not appropriate to evaluate genotoxicity of thiosulphonates.

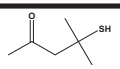
Table C.3: Genotoxicity data (*in vivo*) EFSA/FGE.08Rev5 (EFSA CEF Panel, 2012b)

Chemical name [FL-no]	Test system	Test object	Route	Dose	Result	Reference	Comments
Subgroup I – Acyclic Sulphides							
(Diallyl sulfide [12.088])	*In vivo* mouse micronucleus test	Mouse	Gavage	0.33–0.67 mM/kg (38–77 mg/kg)[(a)]	Negative	Marks et al. (1992)	Insufficient quality. Mixture of three substances was tested
Subgroup III – Monothiols							
(2-Mercaptopropionic acid [12.039])	*In vivo* BASC test	*Drosophila*	Dietary route	10 mM (1,061 μg/mL)	Negative	Wild et al. (1983)	Limited quality (insufficiently documented). The article compiles results obtained with 76 substances in 3 test systems

Chemical name [FL-no]	Test system	Test object	Route	Dose	Result	Reference	Comments
Subgroup V – Acyclic and cyclic Disulphides							
(Allyl disulfide [12.008])	*In vivo* mouse micronucleus test	Mouse	Gavage	0.33–0.67 mM/kg (48–98 mg/kg)[(a)]	Negative	Marks et al. (1992)	Insufficient quality. Mixture of three substances was tested
Subgroup VI – Acyclic Tri- and Polysulphides							
(Diallyl trisulfide [12.009])	*In vivo* mouse micronucleus test	Mouse	Gavage	0.33–0.67 mM/kg (59–120 mg/kg)[(a)]	Negative	Marks et al. (1992)	Insufficient quality. Mixture of three substances was tested
Subgroup X – Sulphoxides/Sulphones and Sulphonates							
Methyl methane-thiosulfonate [12.159]	*In vivo* genetic mutation	*Nicotiana tabacum* seeds	–	2–4 mg/mL (2,000–4,000 μg/mL)	Negative	Dorange et al. (1983)	Obscure test system[(b)]. This assay cannot be regarded as standard test
	In vivo genetic mutation	*Nicotiana tabacum* seeds	–	50–400 μg/mL	Negative	Dorange et al. (1983)	Obscure test system[(b)]. This assay cannot be regarded as standard test

(a): Study used a mixture of allyl sulfide, allyl disulfide and ally trisulfide in the respective ratio, 68:20:12.
(b): Heterozygotic seeds were used. After exposure, the seeds were blotted on filter paper and planted in earthenware pots in medium normally used for planting tobacco. The leaves were analysed for alterations indicating genotoxicity.

Table C.4: Summary of additional genotoxicity data on 2-methyl-4-oxopentane-2-thiol [FL-no: 12.169] (EFSA CEF Panel, 2014)

FL-no JECFA-no	EU Register name JECFA name	Structural formula	End-point	Test system	Concentration	Results	Reference	Comments
12.169	2-Methyl-4-oxopentane-2-thiol		Reverse mutation	*S.* Typhimurium TA98, TA100, TA1535, TA1537 and TA102	5, 15. 81, 50, 158.1, 500, 1,581 and 5,000 μg/plate[(a)]	Negative	Mc Garry (2012)	Valid GLP study, in compliance with OECD 471 Guideline
					156.3, 312.5, 625.0, 1,250, 2,500 and 5,000 μg/plate[(a),(b)]	Negative		

(a): In the absence and presence of S9-mix metabolic bioactivation.
(b): Assay modified with pre-incubation in presence of S9-mix.

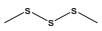
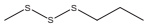
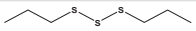
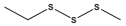
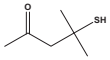
Appendix D – Summary of toxicity data

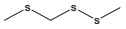
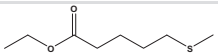
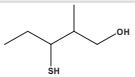
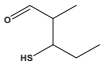
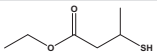
Table D.1: Summary of subacute, subchronic studies on methyl propyl trisulfide [FL-no: 12.020]

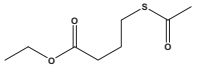
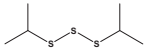
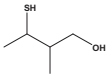
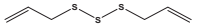
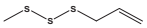
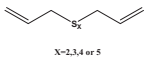
Chemical name [FL-no]	Species/sex No/group	Route	Dose levels (mg/kg bw per day)	Duration	NOAEL (mg/kg per day)	Reference	Comments
Methyl propyl trisulfide [12.020]	Rat/M,F 5	Diet	150, 300 and 600	14 days	–	Bauter (2015a)	The study was performed with a preparation of the substance of 69.9% purity Because of the low stability of the compound in the feed, as well as the poor palatability, the company decided to administer it by oral gavage in a follow up experiment, at lower doses
	Rat/M,F 5	Gavage	12.5, 50 and 100	14 days	–	Bauter (2015b)	The study was performed with a preparation of the substance of 69.9% purity
	Rat/M,F 10	Gavage	0.5, 2 and 6	90 days	2	Koetzner (2016)	The study was performed with a preparation of the substance of 69.9% purity

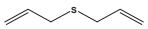
Appendix E – Summary of the safety evaluation

Table E.1: Summary of Safety Evaluation by the JECFA (2000, 2004b)

FL-no JECFA-no	EU Register name	Structural formula	EU MSDI[(a)] US MSDI (μg/capita per day)	Class[(b)] Evaluation procedure path as applied by JECFA[(c)]	Outcome on the named compound as concluded by JECFA [[(d)] or [(e)]]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
12.013 582	Dimethyl trisulfide		7.83 0.02	Class I B3: Intake below threshold B4: Adequate NOAEL exists	d	Toxicity data required	
12.020 584	Methyl propyl trisulfide		4.89 0.1	Class I B3: Intake below threshold B4: Adequate NOAEL exists	d	Toxicity data required	
12.023 585	Dipropyl trisulfide		11.28 1	Class I B3: Intake below threshold B4: Adequate NOAEL exists	d	Toxicity data required	
12.155 583	Methyl ethyl trisulfide		0.001 1	Class I B3: Intake below threshold B4: Adequate NOAEL exists	d	Toxicity data required	
12.169 1293	2-Methyl-4-oxopentane-2-thiol		3.64 0.02	Class I B3: Intake below threshold B4: Adequate NOAEL exists	d	Toxicity data required	
12.179 1297	2-(Methylthio)ethan-1-ol		0.02 0.9	Class I B3: Intake below threshold B4: Adequate NOAEL exists	d	No safety concern at the estimated level of intake based on the MSDI approach	No safety concern at the estimated level of intake based on the MSDI approach

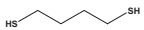
FL-no JECFA-no	EU Register name	Structural formula	EU MSDI[a] US MSDI (μg/capita per day)	Class[b] Evaluation procedure path as applied by JECFA[c]	Outcome on the named compound as concluded by JECFA [[d] or [e]]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
12.198 1299	2,3,5-Trithiahexane		0.001 0.04	Class I B3: Intake below threshold B4: Adequate NOAEL exists	d	No safety concern at the estimated level of intake based on the MSDI approach	No safety concern at the estimated level of intake based on the MSDI approach
12.212 1298	Ethyl-5-(methylthio) valerate		0.001 2	Class I B3: Intake below threshold B4: Adequate NOAEL exists	d	No safety concern at the estimated level of intake based on the MSDI approach	No safety concern at the estimated level of intake based on the MSDI approach
12.238 1291	3-Mercapto-2-methylpentan-1-ol		0.01 0.7	Class I B3: Intake below threshold B4: Adequate NOAEL exists	d	No safety concern at the estimated level of intake based on the MSDI approach	No safety concern at the estimated level of intake based on the MSDI approach
12.239 1292	3-Mercapto-2-methylpentanal		0.02 4	Class I B3: Intake below threshold B4: Adequate NOAEL exists	d	No safety concern at the estimated level of intake based on the MSDI approach	No safety concern at the estimated level of intake based on the MSDI approach
12.241 1290	2-Mercapto-2-methylpentan-1-ol		0.012 4	Class I B3: Intake below threshold B4: Adequate NOAEL exists	d	Toxicity data required	
12.255 1294	Ethyl 3-mercaptobutyrate		3.4 4	Class I B3: Intake below threshold B4: Adequate NOAEL exists	d	No safety concern at the estimated level of intake based on the MSDI approach	No safety concern at the estimated level of intake based on the MSDI approach

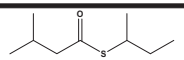
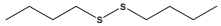
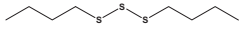
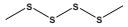
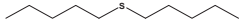
FL-no JECFA-no	EU Register name	Structural formula	EU MSDI[(a)] US MSDI (μg/capita per day)	Class[(b)] Evaluation procedure path as applied by JECFA[(c)]	Outcome on the named compound as concluded by JECFA [[(d)] or [(e)]]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
12.257 1295	Ethyl 4-(acetylthio)-butyrate		3.4 4	Class I B3: Intake below threshold B4: Adequate NOAEL exists	d	No safety concern at the estimated level of intake based on the MSDI approach	No safety concern at the estimated level of intake based on the MSDI approach
12.280 1300	Diisopropyl trisulphide		0.24 0.007	Class I B3: Intake below threshold B4: Adequate NOAEL exists	d	Toxicity data required	
12.291 1289	3-Mercapto-2-methyl-1-butanol		0.061 2	Class I B3: Intake below threshold B4: Adequate NOAEL exists	d	No safety concern at the estimated level of intake based on the MSDI approach	No safety concern at the estimated level of intake based on the MSDI approach
12.009 587	Diallyl trisulfide		0.05 0.02	Class II B3: Intake below threshold B4: Adequate NOAEL exists	d	Toxicity data required	
12.045 586	Methyl allyl trisulfide		0.001 0.9	Class II B3: Intake below threshold B4: Adequate NOAEL exists	d	Toxicity data required	
12.074 588	Diallyl polysulfides		3.19 0.02	Class II B3: Intake below threshold B4: Adequate NOAEL exists	d	Toxicity data required	

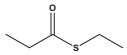
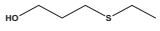
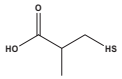
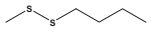
FL-no JECFA-no	EU Register name	Structural formula	EU MSDI[a] US MSDI (μg/capita per day)	Class[b] Evaluation procedure path as applied by JECFA[c]	Outcome on the named compound as concluded by JECFA [[d] or [e]]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
12.088 458	Diallyl sulfide		15.44 0.4	Class II B3: Intake below threshold B4: No adequate NOAEL B5: Intake below 1.5 μg/person per day	f	No safety concern at the estimated level of intake based on the MSDI approach	No safety concern at the estimated level of intake based on the MSDI approach

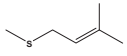
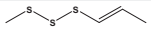
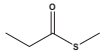
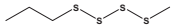
MSDI: maximised survey-derived daily intake; NOAEL: no observed adverse effect level.
(a): EU MSDI: Amount added to food as flavour in (kg/year) × 10^9/(0.1 × population in Europe (= 375 × 10^6) × 0.6 × 365) = μg/capita per day. EU MSDIs may deviate from those reported in the JECFA evaluation because for several substances new data were available.
(b): 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.
(c): Procedure path A substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot.
(d): No safety concern based on intake calculated by the MSDI approach of the named compound.
(e): Data must be available on the substance or closely related substances to perform a safety evaluation.
(f): Cleared by JECFA as intake below 1.5 μg/person per day.

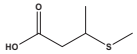
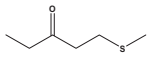
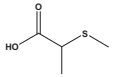
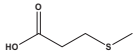
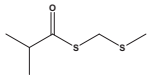
Table E.2: Summary of safety evaluation by EFSA (FGE.08Rev5) (EFSA CEF Panel, 2012b)

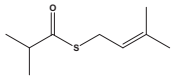
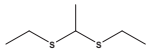
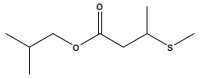
FL-no	EU Register name	Structural formula	MSDI[a] (μg/capita per day)	Class[b] Evaluation procedure path[c]	Outcome on the named compound [[d] or [e]]	Outcome on the material of commerce [[f], [g] or [h]]	Evaluation remarks
12.103	Butane-1,4-dithiol		0.3	Class I B3: Intake below threshold B4: Adequate NOAEL exists	d	f	
12.104	Butane-2-thiol		0.18	Class I B3: Intake below threshold B4: Adequate NOAEL exists	d	f	

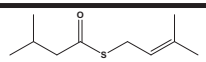
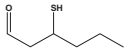
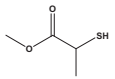
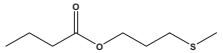
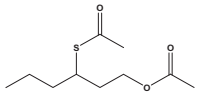
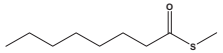
FL-no	EU Register name	Structural formula	MSDI[a] (μg/capita per day)	Class[b] Evaluation procedure path[c]	Outcome on the named compound [[d] or [e]]	Outcome on the material of commerce [[f], [g] or [h]]	Evaluation remarks
12.106	*S*-2-Butyl 3-methylbutanethioate		0.8	Class I B3: Intake below threshold B4: Adequate NOAEL exists	d	f	
12.111	Dibutyl disulfide		0.37	Class I B3: Intake below threshold B4: Adequate NOAEL exists	d	f	
12.112	Dibutyl trisulfide		0.12	Class I B3: Intake below threshold B4: No adequate NOAEL	Additional data required		Substance no longer supported by Industry (DG SANCO, 2012)
12.116	Dimethyl tetrasulfide		0.016	Class I B3: Intake below threshold B4: No adequate NOAEL	Additional data required		Substance no longer supported by Industry (DG SANCO, 2012)
12.117	Dipentyl sulfide		0.0037	Class I B3: Intake below threshold B4: Adequate NOAEL exists	d	f	
12.124	Ethyl butyl sulfide		0.037	Class I B3: Intake below threshold B4: Adequate NOAEL exists	d	f	

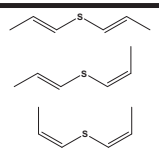
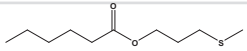
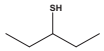
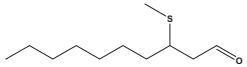
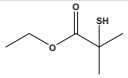
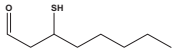
FL-no	EU Register name	Structural formula	MSDI[a] (μg/capita per day)	Class[b] Evaluation procedure path[c]	Outcome on the named compound [[d] or [e]]	Outcome on the material of commerce [[f], [g] or [h]]	Evaluation remarks
12.125	Ethyl propanethioate		0.012	Class I B3: Intake below threshold B4: Adequate NOAEL exists	d	f	
12.127	Ethyl propyl sulfide		0.085	Class I B3: Intake below threshold B4: Adequate NOAEL exists	d	f	
12.129	3-(Ethylthio)propan-1-ol		0.12	Class I B3: Intake below threshold B4: Adequate NOAEL exists	d	f	
12.135	3-Mercapto-2-methylpropionic acid		0.12	Class I B3: Intake below threshold B4: Adequate NOAEL exists	d	f	
12.151	Methyl butyl disulfide		0.0061	Class I B3: Intake below threshold B4: Adequate NOAEL exists	d	f	
12.152	Methyl butyl sulfide		0.0024	Class I B3: Intake below threshold B4: Adequate NOAEL exists	d	f	

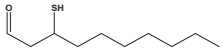
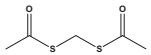
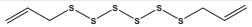
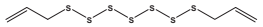
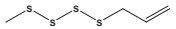
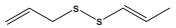
FL-no	EU Register name	Structural formula	MSDI[a] (μg/capita per day)	Class[b] Evaluation procedure path[c]	Outcome on the named compound [[d] or [e]]	Outcome on the material of commerce [[f], [g] or [h]]	Evaluation remarks
12.158	Methyl isoprenyl sulphide		0.0012	Class I B3: Intake below threshold B4: Adequate NOAEL exists	d	f	
12.163	Methyl prop-1-enyl sulfide		0.0097	Class I B3: Intake below threshold B4: Adequate NOAEL exists	d	f	
12.164	Methyl prop-1-enyl trisulfide		0.0061	Class I B3: Intake below threshold B4: No adequate NOAEL	Additional data required		Substance no longer supported by Industry (DG SANCO, 2012)
12.165	*S*-Methyl propanethioate		0.012	Class I B3: Intake below threshold B4: Adequate NOAEL exists	d	f	
12.166	Methyl propyl sulfide		0.0024	Class I B3: Intake below threshold B4: Adequate NOAEL exists	d	f	
12.167	Methyl propyl tetrasulfide		0.0037	Class I B3: Intake below threshold B4: No adequate NOAEL	Additional data required		Substance no longer supported by Industry (DG SANCO, 2012)

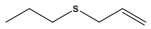
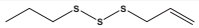
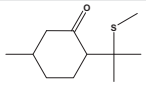
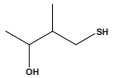
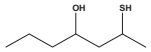
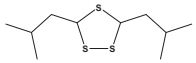
FL-no	EU Register name	Structural formula	MSDI[(a)] (μg/capita per day)	Class[(b)] Evaluation procedure path[(c)]	Outcome on the named compound [(d) or (e)]	Outcome on the material of commerce [(f), (g) or (h)]	Evaluation remarks
12.178	3-(Methylthio)butyric acid		0.12	Class I B3: Intake below threshold B4: Adequate NOAEL exists	d	f	
12.180	1-(Methylthio)ethane-1-thiol		0.12	Class I B3: Intake below threshold B4: Adequate NOAEL exists	d	f	
12.181	1-(Methylthio)pentan-3-one		0.12	Class I B3: Intake below threshold B4: Adequate NOAEL exists	d	f	
12.182	2-(Methylthio)propionic acid		0.011	Class I B3: Intake below threshold B4: Adequate NOAEL exists	d	f	
12.183	3-(Methylthio)propionic acid		0.21	Class I B3: Intake below threshold B4: Adequate NOAEL exists	d	f	
12.189	*S*-(Methylthiomethyl) 2-methylpropanethioate		0.061	Class I B3: Intake below threshold B4: Adequate NOAEL exists	d	f	

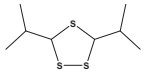
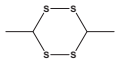
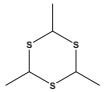
FL-no	EU Register name	Structural formula	MSDI[a] (μg/capita per day)	Class[b] Evaluation procedure path[c]	Outcome on the named compound [[d] or [e]]	Outcome on the material of commerce [[f], [g] or [h]]	Evaluation remarks
12.191	Pentane-1-thiol		0.12	Class I B3: Intake below threshold B4: Adequate NOAEL exists	d	f	
12.196	*S*-Prenyl thioisobutyrate		0.012	Class I B3: Intake below threshold B4: Adequate NOAEL exists	d	f	
12.199	Ethanethioic acid		0.0012	Class I B3: Intake below threshold B4: No adequate NOAEL	Additional data required		Substance no longer supported by Industry (DG SANCO, 2012)
12.200	1,1-bis(Ethylthio)-ethane		0.0012	Class I B3: Intake below threshold B4: Adequate NOAEL exists	d	f	
12.205	Mercaptoacetaldehyde		0.011	Class I B3: Intake below threshold B4: Adequate NOAEL exists	d	f	
12.214	Isobutyl-3-(methylthio) butyrate		0.12	Class I B3: Intake below threshold B4: Adequate NOAEL exists	d	f	

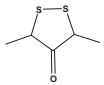
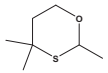
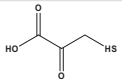
FL-no	EU Register name	Structural formula	MSDI[(a)] (μg/capita per day)	Class[(b)] Evaluation procedure path[(c)]	Outcome on the named compound [(d) or (e)]	Outcome on the material of commerce [(f), (g) or (h)]	Evaluation remarks
12.221	*S*-Prenyl thioisopentanoate		0.012	Class I B3: Intake below threshold B4: Adequate NOAEL exists	d	f	
12.250	3-Mercaptohexanal		0.012	Class I B3: Intake below threshold B4: Adequate NOAEL exists	d	f	
12.266	Methyl-2-mercaptopropionate		0.12	Class I B3: Intake below threshold B4: Adequate NOAEL exists	d	h	
12.277	3-(Methylthio)propyl butyrate		6.1	Class I B3: Intake below threshold B4: Adequate NOAEL exists	d	f	
12.278	3-Acetyl-mercaptohexyl acetate		1.2	Class I B3: Intake below threshold B4: Adequate NOAEL exists	d	f	
12.282	(*S*)-Methyl octanethioate		0.24	Class I B3: Intake below threshold B4: Adequate NOAEL exists	d	g	

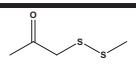
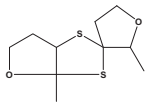
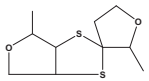
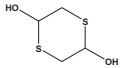
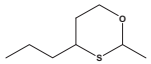
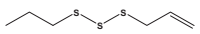
FL-no	EU Register name	Structural formula	MSDI[a] (μg/capita per day)	Class[b] Evaluation procedure path[c]	Outcome on the named compound [[d] or [e]]	Outcome on the material of commerce [[f], [g] or [h]]	Evaluation remarks
12.298	Di-(1-propenyl)-sulfide (mixture)		0.12	Class I B3: Intake below threshold B4: Adequate NOAEL exists	d	f	
12.299	3-(Methylthio)propyl hexanoate		0.061	Class I B3: Intake below threshold B4: Adequate NOAEL exists	d	f	
12.303	3-Pentanethiol		0.03	Class I B3: Intake below threshold B4: Adequate NOAEL exists	d	f	
12.306	3-(Methylthio)-decanal		0.12	Class I B3: Intake below threshold B4: Adequate NOAEL exists	d	f	
12.304	Ethyl-2-mercapto-2-methyl propanoate		0.012	Class I No evaluation			Pending update, as new genotoxicity data have become available.
12.172	2-Methylbutane-2-thiol		0.15	Class I No evaluation			Substance no longer supported by Industry (DG SANCO, 2012)
12.174	2-Methylpropane-2-thiol		0.0012	Class I No evaluation			Substance no longer supported by Industry (DG SANCO, 2012)
12.268	3-Mercaptooctanal			Class I No evaluation			Substance no longer supported by Industry (DG SANCO, 2012)

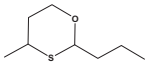
FL-no	EU Register name	Structural formula	MSDI[a] (μg/capita per day)	Class[b] Evaluation procedure path[c]	Outcome on the named compound [[d] or [e]]	Outcome on the material of commerce [[f], [g] or [h]]	Evaluation remarks
12.269	3-Mercaptodecanal			Class I No evaluation			Substance no longer supported by Industry (DG SANCO, 2012)
12.271	Methanedithiol diacetate			Class I No evaluation			Substance no longer supported by Industry (DG SANCO, 2012)
12.093	Diallyl hexasulfide		0.011	Class II B3: Intake below threshold B4: No adequate NOAEL	Additional data required		Substance no longer supported by Industry (DG SANCO, 2012)
12.094	Diallyl heptasulfide		0.011	Class II B3: Intake below threshold B4: No adequate NOAEL	Additional data required		Substance no longer supported by Industry (DG SANCO, 2012)
12.096	Allyl methyl sulfide		0.99	Class II B3: Intake below threshold B4: Adequate NOAEL exists	d	f	
12.097	Allyl methyl tetrasulfide		0.012	Class II B3: Intake below threshold B4: No adequate NOAEL	Additional data required		Substance no longer supported by Industry (DG SANCO, 2012)
12.098	Allyl prop-1-enyl disulfide		0.17	Class II B3: Intake below threshold B4: Adequate NOAEL exists	d	f	

FL-no	EU Register name	Structural formula	MSDI[a] (μg/capita per day)	Class[b] Evaluation procedure path[c]	Outcome on the named compound [[d] or [e]]	Outcome on the material of commerce [[f], [g] or [h]]	Evaluation remarks
12.099	Allyl propyl sulfide		1.6	Class II B3: Intake below threshold B4: Adequate NOAEL exists	d	f	
12.100	Allyl propyl trisulfide		0.12	Class II B3: Intake below threshold B4: No adequate NOAEL	Additional data required		Substance no longer supported by Industry (DG SANCO, 2012)
12.177	8-(Methylthio)-*p*-menthan-3-one		0.37	Class II B3: Intake below threshold B4: Adequate NOAEL exists	d	f	
12.302	2-Butanol, 4-mercapto-3-methyl		0.061	Class II B3: Intake below threshold B4: Adequate NOAEL exists	d	f	
12.305	2-Mercapto-4-heptanol		0.12	Class II B3: Intake below threshold B4: Adequate NOAEL exists	d	f	
15.047	3,5-Di-isobutyl-1,2,4-trithiolane		0.024	Class II B3: Intake below threshold B4: Adequate NOAEL exists	d	f	

FL-no	EU Register name	Structural formula	MSDI[a] (μg/capita per day)	Class[b] Evaluation procedure path[c]	Outcome on the named compound [[d] or [e]]	Outcome on the material of commerce [[f], [g] or [h]]	Evaluation remarks
15.048	3,5-Di-isopropyl-1,2,4-trithiolane		0.0061	Class II B3: Intake below threshold B4: Adequate NOAEL exists	d	f	
15.056	3,6-Dimethyl-1,2,4,5-tetrathiane		0.0024	Class II B3: Intake below threshold B4: Adequate NOAEL exists	d	f	
15.083	3-Methyl-1,2,4-trithiolane		0.0024	Class II B3: Intake below threshold B4: Adequate NOAEL exists	d	f	
15.102	Tetrahydrothiophene		0.024	Class II B3: Intake below threshold B4: No adequate NOAEL	Additional data required		Substance no longer supported by Industry (DG SANCO, 2012)
15.103	1,2,4,5-Tetrathiane		0.073	Class II B3: Intake below threshold B4: Adequate NOAEL exists	d	f	
15.110	2,4,6-Trimethyl-1,3,5-trithiane		0.0061	Class II B3: Intake below threshold B4: Adequate NOAEL exists	d	f	

FL-no	EU Register name	Structural formula	MSDI[a] (μg/capita per day)	Class[b] Evaluation procedure path[c]	Outcome on the named compound [[d] or [e]]	Outcome on the material of commerce [[f], [g] or [h]]	Evaluation remarks
15.111	1,2,4-Trithiolane		2.4	Class II B3: Intake below threshold B4: Adequate NOAEL exists	d	f	
15.125	4-Tetrahydrothiopyranone		0.12	Class II B3: Intake below threshold B4: No adequate NOAEL	Additional data required		Substance no longer supported by Industry (DG SANCO, 2012)
12.295	3,5-Dimethyl-1,2-dithiolane-4-one			Class II No evaluation			Substance no longer supported by Industry (DG SANCO, 2012)
16.057	2,4,4-Trimethyl-1,3-oxathiane		0.0012	Class II No evaluation			Substance no longer supported by Industry (DG SANCO, 2012)
12.120	2,8-Epithio-*p*-menthane		3.7	Class III B3: Intake below threshold B4: No adequate NOAEL	Additional data required		Substance no longer supported by Industry (DG SANCO, 2013)
12.136	3-Mercapto-2-oxopropionic acid		0.24	Class III B3: Intake below threshold B4: Adequate NOAEL exists	d	f	
12.300	1,1-Propanedithiol		0.12	Class III B3: Intake below threshold B4: Adequate NOAEL exists	d	f	

FL-no	EU Register name	Structural formula	MSDI[(a)] (μg/capita per day)	Class[(b)] Evaluation procedure path[(c)]	Outcome on the named compound [[(d)] or [(e)]]	Outcome on the material of commerce [[(f)], [(g)] or [(h)]]	Evaluation remarks
12.301	Methyl-2-oxo-propyl disulfide		0.061	Class III B3: Intake below threshold B4: Adequate NOAEL exists	d	f	
15.007	Spiro(2,4-dithia-1-methyl-8-oxabicyclo[3.3.0]octane-3,3′-(1′-oxa-2′-methyl)-cyclopentane) and Spiro(2,4-dithia-6-methyl-7-oxabicyclo [3.3.0]octane-3,3′-(1′-oxa-2′-methyl)-cyclopentane)	 I  II	6.1	Class III B3: Intake below threshold B4: Adequate NOAEL exists	d	f	
15.081	Lenthionine		0.012	Class III B3: Intake below threshold B4: Adequate NOAEL exists	d	f	
15.134	2,5-Dihydroxy-1,4-dithiane		6.1	Class III B3: Intake below threshold B4: Adequate NOAEL exists	d	f	
16.062	*trans*-2-Methyl-4-propyl-1,3-oxathiane		1.0	Class III B3: Intake below threshold B4: Adequate NOAEL exists	d	f	
16.114	2-Pentyl-4-propyl-1,3-oxathiane		0.12	Class III B3: Intake below threshold B4: Adequate NOAEL exists	d	f	

FL-no	EU Register name	Structural formula	MSDI[(a)] (μg/capita per day)	Class[(b)] Evaluation procedure path[(c)]	Outcome on the named compound [[(d)] or [(e)]]	Outcome on the material of commerce [[(f)], [(g)] or [(h)]]	Evaluation remarks
16.122	4-Methyl, 2-propyl, 1-3-oxathiane		0.24	Class III B3: Intake below threshold B4: Adequate NOAEL exists	d	f	
12.159	Methyl methanethiosulfonate		0.061	Class III No evaluation			Substance no longer supported by Industry (DG SANCO, 2013)

MSDI: maximised survey-derived daily intake; NOAEL: no observed adverse effect level.
(a): EU MSDI: Amount added to food as flavour in (kg/year) × 10^9/(0.1 × population in Europe (= 375 × 10^6) × 0.6 × 365) = μg/capita per day.
(b): Thresholds of concern: Class I = 1,800 μg/person per day, Class II = 540 μg/person per day, Class III = 90 μg/person per day.
(c): Procedure path A substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot.
(d): No safety concern based on intake calculated by the MSDI approach of the named compound.
(e): Data must be available on the substance or closely related substances to perform a safety evaluation.
(f): No safety concern at the estimated level of intake of the material of commerce meeting the specification requirement (based on intake calculated by the MSDI approach).
(g): Tentatively regarded as presenting no safety concern (based on intake calculated by the MSDI approach) pending further information on the purity of the material of commerce and/or information on stereoisomerism.
(h): No conclusion can be drawn due to lack of information on the purity of the material of commerce.