EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF); Scientific Opinion on Flavouring Group Evaluation 08, Revision 5 (FGE.08Rev5): Aliphatic and alicyclic mono-, di-, tri-, and polysulphides with or without additional oxygenated functional groups from chemical groups 20 and 30

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SCIENTIFIC OPINION

Scientific Opinion on Flavouring Group Evaluation 08, Revision 5 (FGE.08Rev5):

Aliphatic and alicyclic mono-, di-, tri-, and polysulphides with or without additional oxygenated functional groups from chemical groups 20 and 30[1]

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

ABSTRACT

The Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids of the European Food Safety Authority was requested to evaluate 80 flavouring substances in the Flavouring Group Evaluation 08, Revision 4, using the Procedure in Commission Regulation (EC) No 1565/2000. Since the publication of the last revision of this FGE, the EFSA has been requested to evaluate additional toxicological data submitted for two flavouring substances, one on supporting substance 2,5-dihydroxy-2,5-dimethyl-1,4-dithiane [FL-no: 15.006], which support the evaluation of the candidate substance 2,5-dihydroxy-1,4-dithiane [FL-no: 15.134] and one on the candidate substance 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) [FL-no: 15.007], which have been included in the present revision of FGE.08. For the substances methyl methanethiosulphonate [FL-no: 12.159], 2-methylbutane-2-thiol [FL-no: 12.172], 2-methylpropane-2-thiol [FL-no: 12.174], ethyl-2-mercapto-2-methyl propanoate [FL-no: 12.304] and 2,4,4-trimethyl-1,3-oxathiane [FL-no: 16.057] there is an indication of a genotoxic potential *in vitro*. Therefore, in the absence of further genotoxicity data, the Panel concluded that the Procedure could not be applied to these five substances. For four substances, 3-mercaptooctanal [FL-no: 12.268], 3-mercaptodecanal [FL-no: 12.269], methanedithiol diacetate [FL-no: 12.271] and 3,5-dimethyl-1,2-dithiolane-4-one [FL-no: 12.295] no data on use as flavouring substances in Europe are available and no intake figures could be calculated, which is a preclude for evaluation of the four substances using the Procedure. The remaining 71 substances were evaluated through a stepwise approach that integrates information

[1] On request from the European Commission, Questions No EFSA-Q-2012-00623, EFSA-Q-2012-00622, EFSA-Q-2012-00511, adopted on 4 July 2012.

[2] Panel members: Ulla Beckman Sundh, Mona-Lise Binderup, Leon Brimer, Laurence Castle, Karl-Heinz Engel, Roland Franz, Nathalie Gontard, Rainer Gürtler, Trine Husøy, Klaus-Dieter Jany, Catherine Leclercq, Jean Claude Lhuguenot, Wim Mennes, Maria Rosaria Milana, Iona Pratt, Kettil Svensson, Maria de Fatima Tavares Pocas, Fidel Toldra, Detlef Wölfle. Correspondence: cef@efsa.europa.eu.

[3] Acknowledgement: The Panel wishes to thank the members of the Working Group on Flavourings for the preparation of this scientific opinion: Ulla Beckman Sundh, Vibe Beltoft, Leon Brimer, Wilfried Bursch, Angelo Carere, Karl-Heinz Engel, Henrik Frandsen, Rainer Gürtler, Frances Hill, Trine Husøy, John Christian Larsen, Pia Lund, Wim Mennes, Gerard Mulder, Karin Nørby, Gerrit Speijers, Harriet Wallin and EFSA's staff member Kim Rygaard Nielsen for the preparatory work on this scientific opinion.

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on the structure-activity relationships, intake from current uses, toxicological threshold of concern, and available data on metabolism and toxicity. The Panel concluded that 59 substances do not give rise to safety concerns at their levels of dietary intake, estimated on the basis of the MSDI approach. For 12 substances [FL-no: 12.093, 12.094, 12.097, 12.100, 12.112, 12.116, 12.120, 12.164, 12.167, 12.199, 15.102 and 15.125], evaluated through the Procedure, no appropriate NOAEL was available and additional data are required. Besides the safety assessment of the flavouring substances, the specifications for the materials of commerce have also been considered and for three substances, evaluated through the Procedure, information on the specifications is lacking.

KEY WORDS

Flavourings, safety, aliphatic, alicyclic, monosulphides, disulphides, trisulphides, polysulphides, (mono)thiols, sulphoxides, sulphones, FGE.08.

SUMMARY

The European Food Safety Authority (EFSA) asked the Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (the Panel) to provide scientific advice to the Commission on the implications for human health of chemically defined flavouring substances used in or on foodstuffs in the Member States. In particular, the Panel was requested to evaluate 80 flavouring substances in the Flavouring Group Evaluation 8, Revision 5 (FGE.08Rev5), using the Procedure as referred to in the Commission Regulation (EC) No 1565/2000. These 80 flavouring substances belong to chemical groups 20 and 30, Annex I of the Commission Regulation (EC) No 1565/2000.

Compared to FGE.08Rev4, this FGE.08Rev5 includes toxicity data for one supporting substance 2,5-dihydroxy-2,5-dimethyl-1,4-dithiane [FL-no: 15.006], which support the evaluation of the candidate substance 2,5-dihydroxy-1,4-dithiane [FL-no: 15.134]. Furthermore, a 90-day study on the candidate substance 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) [FL-no: 15.007] has been reconsidered after submission of an explanatory note from EFFA.

Additional information on specifications and/or stereoisomeric/positional composition submitted for [FL-no: 12.098, 12.163, 12.164, 12.250, 12.266, 12.277, 12.278, 12.298, 12.300, 12.301, 12.302, 12.305, 12.306, 15.007, 15.056, 15.110, 15.134, 16.062, 16.114 and 16.122] has also been included.

The present FGE.08Rev5 deals with 80 flavouring substances in total. These are divided into 11 subgroups:

Subgroup I) Acyclic sulphides: [FL-no: 12.096, 12.099, 12.117, 12.124, 12.127, 12.129, 12.152, 12.158, 12.163, 12.166, 12.177, 12.178, 12.181, 12.182, 12.183, 12.214, 12.277, 12.298, 12.299 and 12.306]

Subgroup II) Cyclic sulphides: [FL-no: 12.120, 15.102 and 15.125]

Subgroup III) Monothiols: [FL-no: 12.104, 12.135, 12.136, 12.172, 12.174, 12.180, 12.191, 12.205, 12.250, 12.266, 12.268, 12.269, 12.302, 12.303, 12.304 and 12.305]

Subgroup IV) Dithiols: [FL-no: 12.103 and 12.300]

Subgroup V) Acyclic and cyclic disulphides: [12.098, 12.111, 12.151, 12.295 and 12.301]

Subgroup VI) Acyclic polysulphides: [FL-no: 12.093, 12.094, 12.097, 12.100, 12.112, 12.116, 12.164 and 12.167]

Subgroup VII) Mono-, di-, tri- and polysulphides with thioacetal structure: [FL-no: 12.200, 15.047, 15.048, 15.056, 15.081, 15.083, 15.103, 15.110, 15.111, 15.134, 16.057, 16.062, 16.114 and 16.122]

Subgroup VIII) Thioesters: [FL-no: 12.106, 12.125, 12.165, 12.189, 12.196, 12.221, 12.271, 12.278 and 12.282]

Subgroup IX) Thioic acids: [FL-no: 12.199]

Subgroup X) Sulphoxides/sulphones and sulphonates: [FL-no: 12.159]

Subgroup XI) Cyclic thioketal with fused oxolane ring: [FL-no: 15.007].

Twenty-nine flavouring substances possess one or more chiral centres [FL-no: 12.104, 12.106, 12.120, 12.135, 12.177, 12.178, 12.180, 12.182, 12.214, 12.250, 12.266, 12.268, 12.269, 12.278,

12.295, 12.302, 12.305, 12.306, 15.007, 15.047, 15.048, 15.056, 15.083, 15.110, 15.134, 16.057, 16.062, 16.114 and 16.122]. The stereoisomeric composition has not been specified sufficiently for [FL-no: 12.268 and 12.269].

Due to the presence and the position of double bonds, four substances [FL-no: 12.098, 12.163, 12.164 and 12.298] can exist as geometrical isomers and due to the ring structure additional two substances [FL-no: 15.056 and 15.110] can exist as geometrical isomers. All information regarding the mixture and ratio of geometrical isomers has been given by the Industry.

Of the in total 80 candidate substances, 48 belong to structural class I, 21 belong to structural class II and 11 belong to structural class III.

Forty-eight of the flavouring substances in the present group have been reported to occur naturally in a wide range of food items.

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

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

According to the default MSDI approach, Flavour Industry have submitted data for 76 candidate substances, which have intakes in Europe ranging from 0.0012 to 6.1 microgram/capita/day. These intakes are below the threshold of concern value for structural class I (1800 microgram/person/day), structural class II (540 microgram/person/day) and structural class III (90 microgram/person/day) substances.

For three substances in structural class I, 3-mercaptooctanal, 3-mercaptodecanal, methanedithiol diacetate [FL-no: 12.268, 12.269 and 12.271] and for one substance in structural class II, 3,5-dimethyl-1,2-dithiolane-4-one [FL-no: 12.295], no data on use as flavouring substances in Europe are available, therefore no intakes can be estimated and accordingly these substances cannot be evaluated through the Procedure.

On the basis of the reported annual production volumes in Europe (MSDI approach), the combined intake of the 42 candidate substances belonging to class I and which have been evaluated through the Procedure, the 19 candidate substances belonging to class II which have been evaluated through the Procedure, and the 10 candidate substances belonging to class III which have been evaluated through the Procedure, would result in total intakes of approximately 11, 6 and 18 microgram/capita/day, respectively, which do not exceed the thresholds of concern for structural class I, II or III. Based on reported production volumes, European per capita intakes (MSDI) could be estimated for 70 of the 127 supporting substances. The total combined intakes of the candidate and supporting substances (for which there are European intake data) are approximately 648, 115 and 18 microgram/capita/day

for structural class I, II and III, respectively, which do not exceed the thresholds of concern for structural class I, II or III of 1800, 540 or 90 microgram/person/day, respectively.

Data on genotoxicity of the candidate substances are limited and the genotoxicity could not be adequately assessed. The data available, however, give rise to some concern of a genotoxic potential of two of the candidate substances, 2-methylpropane-2-thiol [FL-no: 12.174] and methyl methanethiosulphonate [FL-no: 12.159]. The Panel, therefore, concluded that the Procedure could not be applied to these two substances, nor to the structurally related candidate substances, 2-methylbutane-2-thiol [FL-no: 12.172], ethyl-2-mercapto-2-methyl propanoate [FL-no: 12.304] and 2,4,4-trimethyl-1,3-oxathiane [FL-no: 16.057], until adequate *in vivo* genotoxicity data become available. 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 considered by EFSA in the FGE.91 (EFSA, 2010q).

The genotoxicity data available for the remaining candidate substances do not preclude their evaluation through the Procedure.

The candidate substances and supporting substances are expected to share common routes of absorption, distribution and metabolism, and exhibit similar toxicological properties. These metabolic pathways are unlikely to be saturated, given the low levels of exposure from their use as flavouring substances. However, due to the reactivity of the metabolites, the candidate substances cannot be predicted to be metabolised to innocuous products.

It is considered that on the basis of the default MSDI approach 59 of the 71 candidate substances evaluated through the Procedure would not give rise to safety concerns at the estimated levels of intake arising from their use as flavouring substances. Additional toxicity data are required for the remaining candidate substances, three candidate substances in subgroup II [FL-no: 12.120, 15.102 and 15.125], eight candidate substances in subgroup VI [FL-no: 12.093, 12.094, 12.097, 12.100, 12.112, 12.116, 12.164 and 12.167], and the candidate substance in subgroup IX [FL-no: 12.199].

When the estimated intakes were based on the mTAMDI approach for the substances evaluated through the Procedure and for which information on use levels have been submitted, they ranged from 3.5 to 8000 microgram/person/day for the 40 candidate substances from structural class I, from 46 to 78 microgram/person/day for the 18 candidate substances assigned to structural class II and from 78 to 500 microgram/person/day for the eight candidate substances assigned to structural class III. For two substances from structural class I [FL-no: 12.250 and 12.282] and six substances from structural class III [FL-no: 12.120, 12.136, 12.301, 15.134, 16.114 and 16.122], the mTAMDI values are above the thresholds of concern for structural class I or III of 1800 or 90 microgram/person/day, respectively.

For the eight flavouring substances for which the intakes, estimated on the basis of the mTAMDI, exceed the relevant threshold for their structural class more reliable exposure data are required. For the nine substances [FL-no: 12.266, 12.268, 12.269, 12.271, 12.278, 12.295, 15.007, 15.125 and 16.062] for which use levels have not been provided exposure data are required. On the basis of such additional data, these flavouring substances should be re-evaluated using the Procedure. Subsequently, additional toxicological data might become necessary.

In order to determine whether the conclusion for the candidate substances evaluated through the Procedure can be applied to the material of commerce, it is necessary to consider the available specifications. Specifications including purity criteria and identity for the materials of commerce have been provided for 78 of the 80 candidate substances.. For the substances evaluated using the Procedure, specifications are missing for two of the substances [FL-no: 12.226 and 15.125] and are incomplete for one substance [FL-no: 12.282].

Thus, the final evaluation of the materials of commerce cannot be performed for three substances [FL-no: 12.266, 12.282 and 15.125], pending further information on specifications.

For 12 candidate substances [FL-no: 12.093, 12.094, 12.097, 12.100, 12.112, 12.116, 12.120, 12.164, 12.167, 12.199, 15.102 and 15.125] the Panel considered that additional toxicity data are needed. Furthermore, the Panel concluded that for five substances [FL-no: 12.159, 12.172, 12.174, 12.304 and 16.057], additional genotoxicity data are required and for four candidate substances [FL-no: 12.268, 12.269, 12.271 and 12.295], data on use as flavouring substances in Europe are required.

Accordingly, the final evaluation of the materials of commerce cannot be performed for 23 substances (including the nine substances not evaluated through the Procedure) [FL-no: 12.093, 12.094, 12.097, 12.100, 12.112, 12.116, 12.120, 12.159, 12.164, 12.167, 12.172, 12.174, 12.199, 12.266, 12.268, 12.269, 12.271, 12.282, 12.295, 12.304, 15.102, 15.125 and 16.057], pending further information (see Table 9.1).

The remaining 57 flavouring substances evaluated through the Procedure [FL-no: 12.096, 12.098, 12.099, 12.103, 12.104, 12.106, 12.111, 12.117, 12.124, 12.125, 12.127, 12.129, 12.135, 12.136, 12.151, 12.152, 12.158, 12.163, 12.165, 12.166, 12.177, 12.178, 12.180, 12.181, 12.182, 12.183, 12.189, 12.191, 12.196, 12.200, 12.205, 12.214, 12.221, 12.250, 12.277, 12.278, 12.298, 12.299, 12.300, 12.301, 12.302, 12.303, 12.305, 12.306, 15.007, 15.047, 15.048, 15.056, 15.081, 15.083, 15.103, 15.110, 15.111, 15.134, 16.062, 16.114 and 16.122] would present no safety concern at the estimated levels of intake based on the MSDI approach.

TABLE OF CONTENTS

BACKGROUND

Regulation (EC) No 2232/96 of the European Parliament and the Council (EC, 1996a) lays down a Procedure for the establishment of a list of flavouring substances the use of which will be authorised to the exclusion of all other substances in the EU. In application of that Regulation, a Register of flavouring substances used in or on foodstuffs in the Member States was adopted by Commission Decision 1999/217/EC (EC, 1999a), as last amended by Commission Decision 2009/163/EC (EC, 2009a). Each flavouring substance is attributed a FLAVIS-number (FL-number) and all substances are divided into 34 chemical groups. Substances within a group should have some metabolic and biological behaviour in common.

Substances which are listed in the Register are to be evaluated according to the evaluation programme laid down in Commission Regulation (EC) No 1565/2000 (EC, 2000a), which is broadly based on the Opinion of the Scientific Committee on Food (SCF, 1999a). For the submission of data by the manufacturer, deadlines have been established by Commission Regulation (EC) No 622/2002 (EC, 2002b).

The FGE is revised to include substances for which data were submitted after the deadline as laid down in Commission Regulation (EC) No 622/2002 and to take into account additional information that has been made available since the previous Opinion on this FGE.

The Revisions also includes newly notified substances belonging to the same chemical groups evaluated in this FGE.

After the completion of the evaluation programme the Union List of flavouring substances for use in or on foods in the EU shall be adopted (Article 5 (1) of Regulation (EC) No 2232/96) (EC, 1996a).

HISTORY OF THE EVALUATION

The first version of the Flavouring Group Evaluation 08 (FGE.08) dealt with 52 straight- and branched-chain or heterogeneous ring aliphatic hydrocarbons containing one or more sulphur atoms. The sulphur atoms are present as thiols, sulphides or sulphones.

The first Revision of FGE.08 (FGE.08Rev1) included the assessment of 14 additional candidate substances [FL-no: 12.093, 12.094, 12.182, 12.205, 12.266, 12.268, 12.269, 12.271, 12.277, 12.278, 12.282, 12.295, 12.298 and 15.125]. No metabolism data were available for these 14 candidate substances. For one new candidate substance [FL-no: 12.298] *in vitro* data on genotoxicity were submitted. No data on toxicity or genotoxicity were made available for the other 13 new candidate substances. In addition in FGE.08Rev1, the sub-grouping has been revised as tri- and poly-sulphides have been moved to a separate subgroup (acyclic polysulphides).

The second Revision of FGE.08 (FGE.08Rev2) included the assessment of one additional candidate substance [FL-no: 12.250]. No toxicity and/or metabolism data were provided for this substance. A search in open literature for this substance did not provide any further data on toxicity or metabolism.

The third Revision of FGE.08 (FGE.08Rev3) included the assessment of three additional candidate substances [FL-no: 15.007, 15.134 and 16.114]. No metabolism data were provided for these substances. A 90-day toxicity study has been provided for the candidate substance [FL-no: 15.007], but no toxicity studies have been provided for the other two candidate substances. A search in open literature for these substances did not provide any further data on toxicity or metabolism. The JECFA evaluated spiro(2,4-dithia-1-methyl-8-oxabicyclo[3.3.0]octane-3,3'-(1'-oxa-2'-methyl)-cyclopentane) (JECFA no 1296, not in the database and not in use in Europe), which is one of the two isomers of 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) [FL-no:

15.007]. Relevant information on the JECFA evaluated substance has been included in relevant sections of this revision of FGE.08. Furthermore, in the FGE.08, a need for additional data was identified for the cyclic sulphides in subgroup II. These data were submitted by Industry and included in Revision 3 of FGE.08. In Revision 3 additional information on stereoisomeric composition submitted for [FL-no: 12.098, 12.104, 12.106, 12.120, 12.135, 12.163, 12.164, 12.177, 12.178, 12.180, 12.182, 12.214, 12.295, 15.047, 15.048, 15.056, 15.083, 15.110 and 16.057] was included.

The fourth Revision of FGE.08 (FGE.08Rev4) included the assessment of 10 additional candidate substances [FL-no: 12.299, 12.300, 12.301, 12.302, 12.303, 12.304, 12.305, 12.306, 16.062 and 16.122]. Nine of these 10 substances are newly notified substances. For one of the substances, [FL-no: 16.062], which is a substance in the original Register, information on identity has only recently been submitted and accepted. No toxicity or metabolism data were provided for these 10 substances. A search in open literature for these substances did not provide any further data on toxicity or metabolism. Industry has informed that for 16 substances [FL-no: 12.093, 12.094, 12.097, 12.100, 12.112, 12.116, 12.164, 12.167, 12.172, 12.174, 12.199, 12.268, 12.269, 12.271, 12.295 and 15.125], for which there was a request for additional data, they do no longer support these substances for use as flavouring in the EU (EFSA, 2011aj).

FGE	Opinion adopted by EFSA	Link	No. of candidate substances
FGE.08	February 2007	http://www.efsa.europa.eu/en/efsajournal/pub/986.htm	52
FGE.08Rev1	March 2009	http://www.efsa.europa.eu/en/scdocs/scdoc/1021.htm	66
FGE.08Rev2	November 2009	http://www.efsa.europa.eu/en/scdocs/scdoc/1408.htm	67
FGE.08Rev3	February 2011	http://www.efsa.europa.eu/en/efsajournal/pub/1988.htm	70
FGE.08Rev4	November 2011	http://www.efsa.europa.eu/en/efsajournal/doc/2455.pdf	80
FGE.08Rev5	July 2012		80

The present Revision of FGE.08, FGE.08Rev5, considers the re-evaluation of two substances [FL-no: 15.134 and 15.007]. In revision 4 these two substances were put on hold due to lack of toxicity data. Additional information was provided to the Panel, a 90-day study on one supporting substance, 2,5-dihydroxy-2,5-dimethyl-1,4-dithiane [FL-no: 15.006], structurally related to the candidate substance 2,5-dihydroxy-1,4-dithiane [FL-no: 15.134] (subgroup VII). In addition, after submission of an explanatory note from (EFFA, 2012l), a 90-day study on candidate substance 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) [FL-no: 15.007] has been reconsidered.

Additional information from EFFA (EFFA, 2012j) on specifications and/or stereoisomeric/positional composition submitted for [FL-no: 12.098, 12.163, 12.164, 12.250, 12.266, 12.277, 12.278, 12.298, 12.300, 12.301, 12.302, 12.305, 12.306, 15.007, 15.056, 15.110, 15.134, 16.062, 16.114 and 16.122] has been included.

TERMS OF REFERENCE AS PROVIDED BY THE COMMISSION

The European Food Safety Authority (EFSA) is requested to carry out a risk assessment on flavouring substances in the Register (Commission decision 1999/217/EC), according to Commission Regulation (EC) No 1565/2000 (EC, 2000a), prior to their authorisation and inclusion in the Union list (Regulation (EC) No 1334/2008). In addition, the Commission requested EFSA to evaluate newly notified flavouring substances, where possible, before finalising the evaluation programme. The evaluation programme was finalised at the end of 2009.

In addition, in letter of 11 May 2009 the Commission requested EFSA to carry out a risk assessment on 2,5-dihydroxy-1,4-dithiane [FL-no: 15.134] and 2-pentyl-4-propyl-1,3-oxathiane [FL-no: 16.114] in accordance with Commission Regulation (EC) No 1565/2000 (EC, 2000a).

After the finalisation of the evaluation programme, in their letters of the 12th April 2010, 23rd May 2011 and 10th April 2012, the Commission requested EFSA to carry out an evaluation of the flavouring substances 3-(methylthio)propyl hexanoate [FL-no: 12.299], 1,1-propanedithiol [FL-no: 12.300], methyl-2-oxo-propyl disulphide [FL-no: 12.301], 2-butanol, 4-mercapto-3-methyl [FL-no: 12.302], 3-pentanethiol [FL-no: 12.303] and 4-methyl, 2-propyl, 1-3-oxathiane [FL-no: 16.122], of the flavouring substances ethyl-2-mercapto-2-methyl propanoate [FL-no: 12.304], 2-mercapto-4-heptanol [FL-no: 12.305] and 3-(methylthio)-decanal [FL-no: 12.306], and of the flavouring substance 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) [FL-no: 15.007], also according to Commission Regulation (EC) No 1565/2000 (EC, 2000a).

ASSESSMENT

1. Presentation of the Substances in Flavouring Group Evaluation 08, Revision 5

1.1. Description

The present revision of Flavouring Group Evaluation 8, (FGE.08Rev5), using the Procedure as referred to in the Commission Regulation (EC) No 1565/2000 (the Procedure – shown in schematic form in Annex I of this FGE), deals with 80 flavouring substances (candidate substances) from chemical groups 20 and 30 of Annex I of Commission Regulation (EC) No 1565/2000 (EC, 2000a).

The 80 candidate substances under consideration in the present evaluation, with their chemical Register name, FLAVIS- (FL-), Chemical Abstract Service- (CAS-), Council of Europe- (CoE-) and Flavor and Extract Manufacturers Association- (FEMA-) numbers, structures and specifications are listed in Table 1.

The outcome of the Safety Evaluation is summarised in Table 2a.

The 80 candidate substances are straight or branched chain or heterogeneous ring aliphatic hydrocarbons containing one or more sulphur atoms. The sulphur-containing functional groups are present as thiols, sulphides or sulphones. Based on their structures, i.e. the position of the sulphur atoms or the sulphur containing functional groups, the candidate substances can be divided into 11 subgroups (see Table 4.1 in Section 4):

Subgroup I) Acyclic sulphides: [FL-no: 12.096, 12.099, 12.117, 12.124, 12.127, 12.129, 12.152, 12.158, 12.163, 12.166, 12.177, 12.178, 12.181, 12.182, 12.183, 12.214, 12.277, 12.298, 12.299 and 12.306]

Subgroup II) Cyclic sulphides: [FL-no: 12.120, 15.102 and 15.125]

Subgroup III) Monothiols: [FL-no: 12.104, 12.135, 12.136, 12.172, 12.174, 12.180 12.191, 12.205, 12.250, 12.266, 12.268, 12.269, 12.302, 12.303, 12.304 and 12.305]

Subgroup IV) Dithiols: [FL-no: 12.103 and 12.300]

Subgroup V) Acyclic and cyclic disulphides: [12.098, 12.111, 12.151, 12.295 and 12.301]

Subgroup VI) Acyclic polysulphides: [FL-no: 12.093, 12.094, 12.097, 12.100, 12.112, 12.116, 12.164 and 12.167]

Subgroup VII) Mono-, di-, tri- and polysulphides with thioacetal structure: [FL-no: 12.200, 15.047, 15.048, 15.056, 15.081, 15.083, 15.103, 15.110, 15.111, 15.134, 16.057, 16.062, 16.114 and 16.122]

Subgroup VIII) Thioesters: [FL-no: 12.106, 12.125, 12.165, 12.189, 12.196, 12.221, 12.271, 12.278 and 12.282]

Subgroup IX) Thioic acids: [FL-no: 12.199]

Subgroup X) Sulphoxides/sulphones and sulphonates: [FL-no: 12.159]

Subgroup XI) Cyclic thioketal with fused oxolane ring: [FL-no: 15.007].

The hydrolysis products of the candidate esters and thioesters are listed in Table 2b. In addition, the following hydrolysis products may theoretically be formed from the candidate thioacetals in an acid environment: Acetaldehyde, formaldehyde, butanal, hexanal, thioacetic acid, 2-methylpropanal, 3-methylbutanal, 3-mercaptobutanol, 3-mercaptohexan-1-ol, 3-mercapto-3-methylbutan-1-ol, mercaptoacetaldehyde, ethanethiol and hydrogen sulphide.

The 80 candidate substances are closely structurally related to 127 flavouring substances (supporting substances) evaluated at the 53rd meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in the groups "Simple aliphatic and aromatic sulphides and thiols" (JECFA, 2000b; JECFA, 2000c). The names and structures of the 127 supporting substances are listed in Table 3, together with their evaluation status. In table III.1 in Annex III the structures of candidate substances and supporting substances are listed together in their respective subgroups.

1.2. Stereoisomers

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

Twenty-nine flavouring substances possess one or more chiral centres [FL-no: 12.104, 12.106, 12.120, 12.135, 12.177, 12.178, 12.180, 12.182, 12.214, 12.250, 12.266, 12.268, 12.269, 12.278, 12.295, 12.302, 12.305, 12.306, 15.007, 15.047, 15.048, 15.056, 15.083, 15.110, 15.134, 16.057, 16.062, 16.114 and 16.122]. The stereoisomeric composition has not been specified sufficiently for two substances [FL-no: 12.268 and 12.269] (see Table 1).

Due to the presence and the position of double bonds, four substances [FL-no: 12.098, 12.163, 12.164 and 12.298] can exist as geometrical isomers and due to the ring structure, two substances [FL-no: 15.056 and 15.110] can exist as geometrical isomers. Industry has given the actual ratio of the compostion of the isomeric mixtures (EFFA, 2012j). For [FL-no: 15.007] the stereoisomeric composition and the composition of the positional isomeric mixture has been given by Industry (Flavour Industry, 2012c) (see Table 1).

1.3. Natural Occurrence in Food

Forty-eight flavouring substances have been reported to occur in one or more of the following food items, boiled or cooked meat (beef, pork, chicken, mutton), liver (pork), vegetables (onion, garlic, shallot, caucas, scallion, nira, leek, kohlrabi, radish, asparagus, potatoes, tomato), fruits and fruit juices (guava, passionfruit, durian, grapefruit juice), cheese, egg, clam, mushroom (shiitake and Agaricus), tea (black), beer, wine (red, white), rum, spices, peanuts and (roasted) sesame seed. Quantitative data on the natural occurrence in food have been reported for 13 out of these 48 substances:

Table 1.3.1 Candidate Substances Reported to Occur in Food (TNO, 2000; Idstein and Schreier, 1985)

FL-no:	Name:	Quantitative data reported
12.096	Allyl methyl sulphide	up to 12 mg/kg in garlic
12.104	Butane-2-thiol	up to 0.0002 mg/kg in beer
12.116	Dimethyl tetrasulphide	up to 0.001 mg/kg in beer, 2.8 mg/kg in nira
12.129	3-(Ethylthio)propan-1-ol	up to 0.06 mg/kg in white wine
12.135	3-Mercapto-2-methylpropionic acid	0.2 mg/kg in asparagus
12.152	Methyl butyl sulphide	0.001 mg/kg in beer
12.166	Methyl propyl sulphide	0.08 mg/kg in kohlrabi, 0.001 mg/kg in Guinea hen
12.167	Methyl propyl tetrasulphide	up to 6.7 mg/kg in onion
12.181	1-(Methylthio)pentan-3-one	0.1 mg/kg in kohlrabi
12.183	3-(Methylthio)propionic acid	up to 0.05 mg/kg in asparagus, up to 0.03 mg/kg in beer
12.191	Pentane-1-thiol	up to 0.008 mg/kg in beer
12.303	3-Pentanethiol	up to 0.05 mg/kg in guava
15.111	1,2,4-Trithiolane	1.6 mg/kg in shiitake mushroom

According to TNO the remaining 32 candidate substances have not been reported in any food items:

Table 1.3.2 Candidate Substances Not Reported to Occur in Food (TNO, 2000; TNO, 2010; TNO, 2011)

FL-no:	Name:
12.093	Diallyl hexasulphide
12.094	Diallyl heptasulphide
12.097	Allyl methyl tetrasulphide
12.106	S-2-Butyl 3-methylbutanethioate
12.136	3-Mercapto-2-oxopropionic acid
12.178	3-(Methylthio)butyric acid
12.182	2-(Methylthio)propionic acid
12.196	S-Prenyl thioisobutyrate
12.199	Ethanethionic acid
12.200	1,1-bis(Ethylthio)-ethane
12.205	Mercaptoacetaldehyde
12.214	Isobutyl-3-(methylthio)butyrate
12.221	S-Prenyl thioisopentanoate
12.250	3-Mercaptohexanal
12.266	Methyl-2-mercaptopropionate
12.268	3-Mercaptooctanal
12.269	3-Mercaptodecanal
12.271	Methanedithiol diacetate
12.277	3-(Methylthio)propyl butyrate
12.278	3-Acetyl-mercaptohexyl acetate
12.300	1,1-Propanedithiol

Table 1.3.2 Candidate Substances Not Reported to Occur in Food (TNO, 2000; TNO, 2010; TNO, 2011)

FL-no:	Name:
12.302	4-Mercapto-3-methyl 2-butanol
12.304	Ethyl-2-mercapto-2-methyl propanoate
12.305	2-Mercapto-4-heptanol
12.306	3-(Methylthio)-decanal
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)
15.083	3-Methyl-1,2,4-trithiolane
15.125	4-Tetrahydrothiopyranone
15.134	2,5-Dihydroxy-1,4-dithiane
16.057	2,4,4-Trimethyl-1,3-oxathiane
16.062	trans-2-Methyl-4-propyl-1,3-oxathiane
16.122	4-Methyl-2-propyl-1,3-oxathiane

2. Specifications

Purity criteria for 78 substances have been provided by the Flavour Industry (EFFA, 2002g; EFFA, 2004ak; EFFA, 2011e; Flavour Industry, 2006q; Flavour Industry, 2006r; Flavour Industry, 2009e; Flavour Industry, 2009o; Flavour Industry, 2009s; Flavour Industry, 2010h; Flavour Industry, 2011d). For two substances [FL-no: 12.266 and 15.125], specifications have not been provided.

Judged against the requirements in Annex II of Commission Regulation (EC) No 1565/2000 (EC, 2000a), the specifications submitted for 78 candidate substances is adequate for 74 of the substances. For two substances no specifications have been submitted [FL-no: 12.266 and 15.125]. The specifications are incomplete for four substances [FL-no: 12.268, 12.269, 12.271 and 12.282] (see Table 1). Additionally, the stereoisomeric composition has not been specified sufficiently for two substances [FL-no: 12.268 and 12.269].

3. Intake Data

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

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

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

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

One of the alternatives is the "Theoretical Added Maximum Daily Intake" (TAMDI) approach, which is calculated on the basis of standard portions and upper use levels (SCF, 1995) for flavourable

beverages and foods in general, with exceptional levels for particular foods. This method is regarded as a conservative estimate of the actual intake by most consumers because it is based on the assumption that the consumer regularly eats and drinks several food products containing the same flavouring substance at the upper use level.

One option to modify the TAMDI approach is to base the calculation on normal rather than upper use levels of the flavouring substances. This modified approach is less conservative (e.g., it may underestimate the intake of consumers being loyal to products flavoured at the maximum use levels reported) (EC, 2000a). However, it is considered as a suitable tool to screen and prioritise the flavouring substances according to the need for refined intake data (EFSA, 2004a).

3.1. Estimated Daily *per Capita* Intake (MSDI Approach)

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

Average *per capita* intake (MSDI) is estimated on the assumption that the amount added to food is consumed by 10 % of the population[4] (Eurostat, 1998). This is derived for candidate substances from estimates of annual volume of production provided by Industry and incorporates a correction factor of 0.6 to allow for incomplete reporting (60 %) in the Industry surveys (SCF, 1999a).

In the present revision of Flavouring Group Evaluation 8 (FGE.08Rev5) the total annual volume of production of the 76 candidate substances for use as flavouring substances in Europe, for which Industry has submitted production figures, has been reported to be approximately 280 kg[5]. For the remaining four candidate substances [FL-no: 12.268, 12.269, 12.271 and 12.295] data are not available. For 70 of the 127 supporting substances the annual volume of production is 740 kg (JECFA, 2000b). The annual volumes of production in Europe for 57 of the supporting substances were not reported.

On the basis of the annual volumes of production reported for the 76 candidate substances the MSDI values for each of these flavourings have been estimated (see Table 2a).

Eighty-six percent of the total annual volumes of production for the candidate substances is accounted for by 10 flavouring substances: allyl methyl sulphide [FL-no: 12.096], allyl propyl sulphide [FL-no: 12.099], S-2-butyl 3-methylbutanethioate [FL-no: 12.106], 2,8-epithio-p-menthane [FL-no: 12.120], 3-(methylthio)propyl butyrate [FL-no: 12.277], 3-acetyl-mercaptohexyl acetate [FL-no: 12.278], 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) [FL-no: 15.007], 1,2,4-trithiolane [FL-no: 15.111], 2,5-dihydroxy-1,4-dithiane [FL-no: 15.134] and trans-2-methyl-4-propyl-1,3-oxathiane [FL-no: 16.062].

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

[5] EFFA, 2002g; EFFA, 2002h; EFFA, 2002i; EFFA, 2004ak; Flavour Industry, 2006q; Flavour Industry, 2006r; Flavour Industry, 2009e; Flavour Industry, 2009o; Flavour Industry, 2009s; Flavour Industry, 2010h; Flavour Industry, 2011d; EFFA, 2011e.

The combined daily *per capita* intake of those 10 candidate substances from use as flavouring substance is estimated to be 30 microgram. The daily *per capita* intakes for the remaining substances are for each less than 0.37 microgram, and in total less than 5.2 microgram (see Table 2a).

3.2. Intake Estimated on the Basis of the Modified TAMDI (mTAMDI)

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

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

For the present evaluation of 80 candidate substances, information on food categories and normal and maximum use levels[6,7,8] were submitted by the Flavour Industry for 71 candidate substances (EFFA, 2002g; EFFA, 2002h; EFFA, 2002i; EFFA, 2004ak; EFFA, 2007a; Flavour Industry, 2006q; Flavour Industry, 2006r; Flavour Industry, 2009e; Flavour Industry, 2009o; Flavour Industry, 2010h; Flavour Industry, 2011d). No information on use levels have been submitted for the remaining nine candidate substances [FL-no: 12.266, 12.268, 12.269, 12.271, 12.278, 12.295, 15.007, 15.125 and 16.062].

The 71 candidate substances, for which normal and maximum use levels were submitted by Industry, are used in flavoured food products divided into the food categories, outlined in Annex III of the Commission Regulation (EC) No 1565/2000 (EC, 2000a), as shown in Table 3.1. For the present calculation of mTAMDI, the reported normal use levels were used. In the case where different use levels were reported for different food categories the highest reported normal use level was used.

According to the Flavour Industry the normal use levels for the 71 candidate substances, are in the range of 0.01 – 25 mg/kg food, and maximum use levels are in the range of 0.05 – 250 mg/kg (EFFA, 2002g; EFFA, 2002h; EFFA, 2002i; EFFA, 2004ak; EFFA, 2007a; Flavour Industry, 2006q; Flavour Industry, 2006r; Flavour Industry, 2009e; Flavour Industry, 2009o; Flavour Industry, 2010h; Flavour Industry, 2011d) (see Table II.1.2, Annex II).

For those candidate substances for which Industry has submitted normal and maximum use levels, and that have been evaluated through the Procedure the mTAMDI values range from 3.5 to 8000 microgram/person/day for 40 candidate substances from structural class I (see Section 5), from 46 to 78 microgram/person/day for 18 candidate substances from structural class II and from 7.5 to 500 microgram/person/day for the eight candidate substances from structural class III.

For detailed information on use levels and intake estimations based on the mTAMDI approach, see Section 6 and Annex II.

[6] ”Normal use” is defined as the average of reported usages and ”maximum use” is defined as the 95th percentile of reported usages (EFFA, 2002i).
[7] The normal and maximum use levels in different food categories (EC, 2000) have been extrapolated from figures derived from 12 model flavouring substances (EFFA, 2004e).
[8] The use levels from food category 5 “Confectionery” have been inserted as default values for food category 14.2 “Alcoholic beverages” for substances for which no data have been given for food category 14.2 (EFFA, 2007a).

Table 3.1 Use of Candidate Substances in Various Food Categories for 71 Candidate Substances for which Data on Use have been provided.

Food category	Description	Flavourings used*
01.0	Dairy products, excluding products of category 2	71 except [FL-no: 12.301, 12.302, 12.303, 12.305, 15.134, 16.114, 16.122]
02.0	Fats and oils, and fat emulsions (type water-in-oil)	71 except [FL-no: 12.299, 12.301, 12.302, 12.303, 12.304, 15.134, 16.114, 16.122]
03.0	Edible ices, including sherbet and sorbet	71 except [FL-no: 12.298, 12.300, 12.301, 12.302, 12.303, 12.305, 12.306, 15.134]
04.1	Processed fruits	71 except [FL-no: 15.134, 16.114]
04.2	Processed vegetables (incl. mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds	[FL-no: 12.250, 12.282, 12.298, 12.299, 12.300, 12.302, 12.303, 12.304, 12.305, 12.306, 16.122]
05.0	Confectionery	71 except [FL-no: 12.298, 12.300, 12.301, 12.302, 12.303, 12.306, 15.134]
06.0	Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery	71 except [FL-no: 12.221, 12.250, 12.298, 12.301, 12.302, 12.303, 12.305, 12.306, 15.134, 16.114]
07.0	Bakery wares	71 except [FL-no: 12.127, 16.114]
08.0	Meat and meat products, including poultry and game	71 except [FL-no: 12.304, 16.114]
09.0	Fish and fish products, including molluscs, crustaceans and echinoderms	71 except [FL-no: 12.298, 12.299, 12.300, 12.301, 12.302, 12.303, 12.304, 12.305, 15.134, 16.114, 16.122]
10.0	Eggs and egg products	Only [FL-no: 12.306]
11.0	Sweeteners, including honey	None
12.0	Salts, spices, soups, sauces, salads, protein products etc.	71 except [FL-no: 16.114]
13.0	Foodstuffs intended for particular nutritional uses	71 except [FL-no: 12.250, 12.277, 12.282, 12.298, 12.299, 12.300, 12.301, 12.302, 12.303, 12.304, 12.305, 12.306, 15.081, 15.134, 16.114, 16.122]
14.1	Non-alcoholic ("soft") beverages, excl. dairy products	71 except [FL-no: 12.116, 12.200, 12.298, 15.047, 15.048, 15.134]
14.2	Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts	71 except [FL-no: 12.298, 15.134]
15.0	Ready-to-eat savouries	71 except [FL-no: 12.165, 12.181, 12.277, 12.298, 12.304, 12.305]
16.0	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 1 – 15	71 except [FL-no: 12.298, 12.299, 12.300, 12.301, 12.302, 12.303, 12.306, 15.111, 15.134]

*No use levels have been submitted for [FL-no: 12.266, 12.268, 12.269, 12.271, 12.278, 12.295, 15.007, 15.125 and 16.062]

4. Absorption, Distribution, Metabolism and Elimination

There are lack of data demonstrating to what degree the candidate substances may be absorbed from the gastro-intestinal tract. According to available data on lipophilicity and solubility it is presumed that relevant supporting substances may be absorbed to the same degree as the candidate substances.

Depending on the type of sulphur-containing functional groups, the candidate substances can be subdivided into 11 subgroups, which are illustrated by representative structures shown in Table 4.1.

Table 4.1 Subgroups. The supporting substances are listed in brackets.

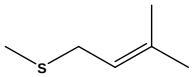
	FL-no	EU Register name
I: ACYCLIC SULPHIDES		
 and	12.096	Allyl methyl sulphide
	12.099	Allyl propyl sulphide
	12.117	Dipentyl sulphide
	12.124	Ethyl butyl sulphide

Table 4.1 Subgroups. The supporting substances are listed in brackets.

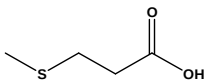
	FL-no	EU Register name
	12.127	Ethyl propyl sulphide
	12.129	3-(Ethylthio)propan-1-ol
	12.152	Methyl butyl sulphide
	12.158	Methyl isoprenyl sulphide
	12.163	Methyl prop-1-enyl sulfide
	12.166	Methyl propyl sulphide
	12.177	8-(Methylthio)-p-menthan-3-one
	12.178	3-(Methylthio)butyric acid
	12.181	1-(Methylthio)pentan-3-one
	12.182	2-(Methylthio)propionic acid
	12.183	3-(Methylthio)propionic acid
	12.214	Isobutyl-3-(methylthio)butyrate
	12.277	3-(Methylthio)propyl butyrate
	12.298	Di-(1-propenyl)-sulfid (mixture)
	12.299	3-(Methylthio)propyl hexanoate
	12.306	3-(methylthio)-decanal
	(12.001)	3-(Methylthio)propionaldehyde
	(12.002)	Methyl 3-(methylthio)propionate
	(12.006)	Dimethyl sulphide
	(12.007)	Dibutyl sulphide
	(12.040)	2-Methylthioacetaldehyde
	(12.041)	1-(Methylthio)butan-2-one
	(12.042)	2-(Methylthio)phenol
	(12.052)	Di-(3-oxobutyl) sulphide
	(12.053)	Ethyl 3-(methylthio)propionate
	(12.056)	3-(Methylthio)butanal
	(12.057)	4-(Methylthio)butan-2-one
	(12.058)	4-(Methylthio)-4-methylpentan-2-one
	(12.060)	Methyl 4-(methylthio)butyrate
	(12.061)	4-(Methylthio)butanal
	(12.062)	3-(Methylthio)propan-1-ol
	(12.063)	3-(Methylthio)hexan-1-ol
	(12.065)	2,8-Dithianon-4-en-4-carboxaldehyde
	(12.077)	Benzyl methyl sulphide
	(12.078)	4-(Methylthio)butan-1-ol
	(12.084)	Ethyl 4-(methylthio)butyrate
	(12.086)	Methyl 2-(methylthio)butyrate
	(12.088)	Diallyl sulphide
	(12.089)	Ethyl 3-(methylthio)butyrate
	(12.113)	Diethyl sulphide
	(12.118)	2,4-Dithiapentane
	(12.122)	Ethyl 2-(methylthio)acetate
	(12.154)	Methyl ethyl sulphide
	(12.162)	Methyl phenyl sulphide
	(12.176)	4-(Methylthio)-2-oxobutyric acid
	(12.187)	Methylthiomethyl butyrate
	(12.188)	Methylthiomethyl hexanoate
	(12.211)	But-1-enyl methyl sulphide
	(12.236)	3-(Methylthio)hexyl acetate
	(12.237)	3-(Methylthio)propyl acetate
II: CYCLIC SULPHIDES		

Table 4.1 Subgroups. The supporting substances are listed in brackets.

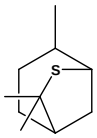
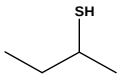
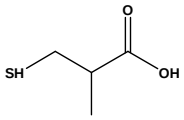
	FL-no	EU Register name
	12.120	2,8-Epithio-p-menthane
	15.102	Tetrahydrothiophene
	15.125	4-Tetrahydrothiopyranone
	(15.012)	4,5-Dihydrothiophen-3(2H)-one
	(15.023)	4,5-Dihydro-2-methylthiophene-3(2H)-one
	(15.066)	1,4-Dithiane
III: MONOTHIOLS		
 and 	12.104	Butane-2-thiol
	12.135	3-Mercapto-2-methylpropionic acid
	12.136	3-Mercapto-2-oxopropionic acid
	12.172	2-Methylbutane-2-thiol
	12.174	2-Methylpropane-2-thiol
	12.180	1-(Methylthio)ethane-1-thiol
	12.191	Pentane-1-thiol
	12.205	Mercaptoacetaldehyde
	12.250	3-Mercaptohexanal
	12.266	Methyl-2-mercaptopropionate
	12.268	3-Mercaptooctanal
	12.269	3-Mercaptodecanal
	12.302	2-Butanol, 4-mercapto-3-methyl
	12.303	3-Pentanethiol
	12.304	Ethyl-2-mercapto-2-methyl propanoate
	12.305	2-Mercapto-4-heptanol
	(12.003)	Methanethiol
	(12.004)	Allylthiol
	(12.005)	Phenylmethanethiol
	(12.010)	Butane-1-thiol
	(12.024)	3-Mercaptobutan-2-ol
	(12.027)	2-Methylbenzene-1-thiol
	(12.029)	Cyclopentanethiol
	(12.031)	3-Mercaptopentan-2-one
	(12.035)	2-,3- and 10-Mercaptopinane
	(12.036)	3-[(2-Mercapto-1-methylpropyl)thio]butan-2-ol
	(12.038)	8-Mercapto-p-menthan-3-one
	(12.039)	2-Mercaptopropionic acid
	(12.046)	Ethyl 2-mercaptopropionate
	(12.047)	3-Mercaptobutan-2-one
	(12.048)	2-Methylbutane-1-thiol
	(12.049)	3-Methylbutane-2-thiol
	(12.054)	2-(Ethylthio)phenol
	(12.055)	4-Mercaptobutan-2-one
	(12.064)	Thiogeraniol
	(12.071)	1-Propane-1-thiol
	(12.080)	Thiophenol
	(12.082)	2,6-(Dimethyl)thiophenol
	(12.083)	Ethyl 3-mercaptopropionate
	(12.085)	p-Menth-1-ene-8-thiol
	(12.128)	2-Ethylhexane-1-thiol
	(12.132)	Hexane-1-thiol
	(12.137)	3-Mercapto-3-methylbutan-1-ol
	(12.138)	3-Mercapto-3-methylbutyl formate

Table 4.1 Subgroups. The supporting substances are listed in brackets.

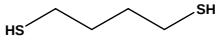
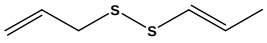
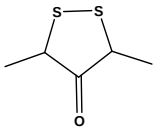
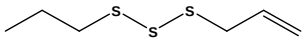
	FL-no	EU Register name
	(12.143)	1-Mercaptopropan-2-one
	(12.145)	4-Methoxy-2-methylbutane-2-thiol
	(12.170)	3-Methylbut-2-ene-1-thiol
	(12.171)	3-Methylbutane-1-thiol
	(12.173)	2-Methylpropane-1-thiol
	(12.192)	Pentane-2-thiol
	(12.194)	2-Phenylethane-1-thiol
	(12.197)	Propane-2-thiol
	(12.217)	3-Mercaptohexan-1-ol
	(12.234)	3-Mercaptohexyl acetate
	(12.235)	3-Mercaptohexyl butyrate
IV: DITHIOLS		
	12.103	Butane-1,4-dithiol
	12.300	1,1-Propanedithiol
	(12.022)	Butane-2,3-dithiol
	(12.034)	Octane-1,8-dithiol
	(12.066)	Ethane-1,2-dithiol
	(12.067)	Hexane-1,6-dithiol
	(12.069)	Nonane-1,9-dithiol
	(12.070)	Propane-1,2-dithiol
	(12.072)	Butane-1,2-dithiol
	(12.073)	Butane-1,3-dithiol
	(12.076)	Propane-1,3-dithiol
V: ACYCLIC AND CYCLIC DISULPHIDES		
 and	12.098	Allyl prop-1-enyl disulfide
	12.111	Dibutyl disulfide
	12.151	Methyl butyl disulfide
	12.295	3,5-dimethyl-1.2-dithiolane-4-one
	12.301	Methyl-2-oxo-propyl disulfide
	(12.008)	Diallyl disulfide
	(12.014)	Dipropyl disulfide
	(12.019)	Methyl propyl disulfide
	(12.026)	Dimethyl disulfide
	(12.028)	Dicyclohexyl disulfide
	(12.037)	Allyl methyl disulfide
	(12.043)	Diphenyl disulfide
	(12.044)	Prop-1-enyl propyl disulfide
	(12.068)	Benzyl methyl disulfide
	(12.075)	Methyl prop-1-enyl disulfide
	(12.081)	Dibenzyl disulfide
	(12.109)	Di-isopropyl disulfide
	(12.121)	Ethyl 2-(methyldithio)propionate
	(12.161)	Methyl phenyl disulfide
	(12.168)	2-Methyl-2-(methyldithio)propanal
	(12.218)	Methyl-3-methyl-1-butenyl disulphide
VI: ACYCLIC POLYSULPHIDES		
	12.093	Diallyl hexasulfide
	12.094	Diallyl heptasulfide
	12.097	Allyl methyl tetrasulfide
	12.100	Allyl propyl trisulfide
	12.112	Dibutyl trisulfide

Table 4.1 Subgroups. The supporting substances are listed in brackets.

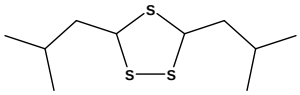
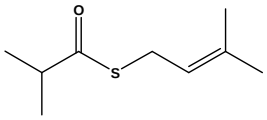
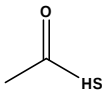
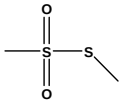
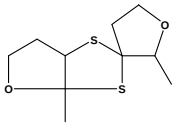
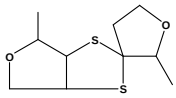
	FL-no	EU Register name
	12.116	Dimethyl tetrasulfide
	12.164	Methyl prop-1-enyl trisulfide
	12.167	Methyl propyl tetrasulfide
	(12.009)	Diallyl trisulfide
	(12.013)	Dimethyl trisulfide
	(12.020)	Methyl propyl trisulfide
	(12.023)	Dipropyl trisulfide
	(12.045)	Methyl allyl trisulfide
	(12.074)	Diallyl polysulfides
	(12.155)	Methyl ethyl trisulfide
VII : MONO-, DI-, TRI- AND POLYSULPHIDES WITH THIOACETAL STRUCTURE		
	12.200	1,1-bis(Ethylthio)-ethane
	15.047	3,5-Di-isobutyl-1,2,4-trithiolane
	15.048	3,5-Di-isopropyl-1,2,4-trithiolane
	15.056	3,6-Dimethyl-1,2,4,5-tetrathiane
	15.081	Lenthionine
	15.083	3-Methyl-1,2,4-trithiolane
	15.103	1,2,4,5-Tetrathiane
	15.110	2,4,6-Trimethyl-1,3,5-trithiane
	15.111	1,2,4-Trithiolane
	15.134	2,5-Dihydroxy-1,4-dithiane
	16.057	2,4,4-Trimethyl-1,3-oxathiane
	16.062	Trans-2-Methyl-4-propyl-1,3-oxathiane
	16.114	2-Pentyl-4-propyl-1,3-oxathiane
	16.122	4-Methyl, 2-propyl, 1-3-oxathiane
	(15.006)	2,5-Dihydroxy-2,5-dimethyl-1,4-dithiane
	(15.009)	Trithioacetone
	(15.025)	3,5-Dimethyl-1,2,4-trithiolane
	(15.034)	2-Methyl-1,3-dithiolane
	(15.036)	3-Methyl-1,2,4-trithiane
	(16.030)	2-Methyl-4-propyl-1,3-oxathiane
VIII: THIOESTERS		
	12.106	S-2-Butyl 3-methylbutanethioate
	12.125	Ethyl propanethioate
	12.165	S-Methyl propanethioate
	12.189	S-(Methylthiomethyl) 2-methylpropanethioate
	12.196	S-Prenyl thioisobutyrate
	12.221	S-Prenyl thioisopentanoate
	12.271	Methanedithiol diacetate
	12.278	3-Acetyl-mercaptohexyl acetate
	12.282	(S)-Methyl octanethioate
	(12.018)	S-Ethyl acetothioate
	(12.032)	S-Methyl butanethioate
	(12.059)	Propyl thioacetate
	(12.101)	Allyl thiopropionate
	(12.148)	S-Methyl 4-methylpentanethioate
	(12.149)	S-Methyl acetothioate
	(12.150)	S-Methyl benzothioate
	(12.156)	S-Methyl hexanethioate
	(12.157)	S-Methyl isopentanethioate
	(12.195)	S-Prenyl thioacetate

Table 4.1 Subgroups. The supporting substances are listed in brackets.

	FL-no	EU Register name
	(12.203)	Methylthio 2-(acetyloxy)propionate
	(12.227)	Methylthio-2-(propionyloxy)propionate
IX: THIOIC ACIDS		
	12.199	Ethanethioic acid
X: SULPHOXIDES/SULPHONES AND SULPHONATES		
	12.159	Methyl methanethiosulfonate
	(12.175)	Methylsulfinylmethane
XI: CYCLIC THIOKETAL WITH FUSED OXOLANE RING		
 	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)

Subgroups I (Acyclic sulphides), II (Cyclic sulphides), IX (thiocic acids) and X (Sulphoxides/sulphones and sulphonates)

Acyclic and cyclic monosulphides (thioethers) primarily undergo S-oxidation, catalyzed by cytochrome P450 and flavin-containing monoxygenases (FMO), leading to the formation of sulphoxides, which can be further oxidised, at least partially, to sulphones. Sulphoxides and sulphones are hydrophilic and usually chemically stable. Sulphoxides are the major urinary excretion products in mammals exposed to thioethers, whereas the amount of sulphones is generally low. The S-oxidation of sulphoxides to sulphones is an irreversible reaction, whereas reduction of the sulphoxides back to sulphides is a common route of metabolism (see Figure III.1, Annex III).

The oxygenated derivatives of sulphides, in addition to the above-described pathways, may be detoxified via the well-recognised biotransformations of alcohol, aldehyde, acid and ketone functional groups. Even, if also oxygen-containing functional groups are present in the organosulphur compounds, the S-oxidation is generally reported as the major metabolic pathway.

Three of the candidate substances from subgroup I are esters, isobutyl-3-(methylthio)butyrate [FL-no: 12.214], 3-(methylthio)propyl butyrate [FL-no: 12.277] and 3-(methylthio)propyl hexanoate [FL-no: 12.299], which are anticipated to be hydrolysed, respectively, to 2-methylpropanol [FL-no: 02.001] and 3-(methylthio)butyric acid [FL-no: 12.178], to 3-(methylthio)propan-1-ol [FL-no: 12.062] and butyric acid [FL-no: 08.005] and to 3-(methylthio)propan-1-ol [FL-no: 12.062] and hexanoic acid [FL-no: 08.009]. The substance from subgroup IX, ethanethioic acid [FL-no: 12.199], converts to acetic acid [FL-no: 08.002]. The candidate substance methyl methanethiosulphonate [FL-no: 12.159] from subgroup X is anticipated to be hydrolysed to methanesulphonic acid and methanethiol and/or methanethiosulphonic acid and methanol (See Table 2b).

Subgroups III (Monothiols) and IV (Dithiols)

Thiols may follow a combination of pathways including S-oxidation, oxidative desulphuration and dealkylation, alkylation and conjugation with glutathione (GSH) and/or glucuronic acid. The majority

of thiols are readily ionised at physiological pH to the nucleophilic thiolate anion giving rise to their reactivity. Thiols may form mixed disulphides, reacting with endogenous thiols present either in small hydrophilic molecules (i.e. GSH or cysteine, leading to products easily excreted in the urine) or in cellular macromolecules, as for instance in the catalytic site of many enzymes, resulting in adverse effect induction. Among conjugating reactions, thiol S-methylation catalysed by thiol-S-methyl-transferases, is a quite common pathway of biotransformation for simple aliphatic and aromatic thiols, followed by S-oxygenation to water-soluble methyl-sulphoxides and/or sulphones. Alternatively, thiols are enzymatically oxidised to reactive unstable sulphenic (R-S-OH) acid, which can be further oxidised to sulphinic ($R\text{-}SO_2H$) acid or react with excess thiol (preferentially GSH), yielding the corresponding disulphide. These latter can be either reduced back to thiols (enzymatically by thiol-transferase or chemically by exchange with GSH or endogenous thiols), or be oxidised to thio-sulphinic acid, which is hydrolysed to sulphinic acid and further oxidised to sulphonic ($R\text{-}SO_3H$) acid. This oxidation cycle followed by reduction could eventually deplete glycogen, due to NADPH production, deplete GSH and alter the cellular redox status. This condition has been associated, at least partially, with toxic effects induced by some sulphur-containing compounds. The metabolism of dithiols usually involves the same pathways described for thiols.

The oxygenated derivatives of thiols, in addition to the above-described pathways, may be detoxified via the well-recognized biotransformations of alcohol, aldehyde, acid and ketone functional groups. However, even in the presence of oxygenated functional groups in the organosulphur compounds, the S-oxidation is generally reported as the major metabolic pathway.

One of the substances in subgroup III, 1-(methylthio)ethane-1-thiol [FL-no: 12.180] is a thioacetal, which can be hydrolysed to acetaldehyde [FL-no: 05.001], methanethiol [FL-no: 12.003] and hydrogensulphide [not a Register substance]. Two candidate substances from subgroup III, methyl 2-mercaptopropionate [FL-no: 12.266] and ethyl-2-mercapto-2-methyl propanoate [FL-no: 12.304] are carboxylic acid esters, which are anticipated to be hydrolysed, respectively, to methanol and 2-mercaptopropionic acid [FL-no: 12.039] and to ethanol and to 2-mercapto-2-methyl propionic acid. The hydrolysis products are shown in Table 2b.

Subgroup V (Acyclic and cyclic disulphides)

Disulphides may be reduced to the respective thiols. Consequently, metabolic options available for thiols may also be available for disulphides. Disulphides may also be oxidised to thiosulphinates or thiosulphonates and hydrolysed to sulphinates or sulphonates. Thiosulphonates are readily hydrolysed to the corresponding sulphonic acid.

Cyclic disulphides may be metabolised through ringopening and disulphide reduction with consecutive formation of a dithiol, and then further metabolism following the scheme suggested for thiols.

Subgroup VI (Acyclic polysuphides)

The acyclic polysulphides may react with endogenous thiols such as reduced glutathione (GSH) or cysteine forming a thiol and a hydropersulphide or perthiol (RSH + R'SSH or R'SSSH or R'SxH, respectively). Compared to thiols, perthiols may be strong reducing agents, reacting rapidly with oxidants to form reactive products.

Subgroup VII (Mono-, di-, tri- and polysulphides with thioacetal structure)

The thioacetals and oxy-thioacetals may be subject to acid-hydrolysis in the stomach, similar to oxygen-containing acetals. However, thioacetals are more resistant to hydrolysis than oxygen-acetals (Satchell and Satchell, 1990; Smith and March, 2001). It is thus to be anticipated that these substances primarily may reach the intestinal lumen primarily intact and may be absorbed as such. Otherwise, the

flavouring substances in subgroup VII are anticipated to be metabolised like the cyclic sulphides in subgroup II.

Subgroup VIII (Thioesters)

Thioesters are hydrolysed by lipase and esterases to the corresponding thiocarboxylic acids and alcohols, or to the thiols and carboxylic acids. The rate of the enzymatic reaction increases with the length of the carboxylic acid carbon chain, whereas it is negatively affected by the level of oxygenation of the thiol moiety. When the hydrolysis products are carboxylic acids or alcohols, they follow the usual metabolic pathways for this kind of molecules (mainly conjugation and excretion), whereas the thiols undergo the above-mentioned metabolic reactions.

S-Thioesters are rapidly hydrolysed by lipases and esterases forming primarily the corresponding carboxylic acids and thiols. The rate of hydrolysis of thioesters increases as the C-chain length of the carboxylic acid fragment increases and decreases as oxygenation of the carbon chain in the thiol moiety increases. The hydrolysis products of the candidate thioesters are shown in Table 2b.

Subgroup XI (Cyclic thioketal with fused oxolane ring)

No data have been submitted for the candidate substance [FL-no: 15.007] allocated to subgroup XI. A search in open literature did not reveal any further information on the candidate substance; however some data on structurally related substances was available.

The candidate substance in subgroup XI, a cyclic thioketal with fused oxolane rings, is expected to be resistant to hydrolysis, and to be mainly absorbed as such. The sulphur atoms of the molecule are expected to be the main target for metabolic activity. The proposed preferred pathway of metabolism is sulphoxidation to yield the corresponding sulphoxide.

Conclusion

In conclusion, due to the reactivity of certain of the anticipated sulphur-containing metabolites, none of the candidate substances can be predicted to be metabolised to innocuous products.

More detailed information on the metabolism of candidate substances is given in Annex III.

5. Application of the Procedure for the Safety Evaluation of Flavouring Substances

The application of the Procedure is based on intakes estimated on the basis of the MSDI approach. Where the mTAMDI approach indicates that the intake of a flavouring substance might exceed its corresponding threshold of concern, a formal safety assessment is not carried out using the Procedure. In these cases the Panel requires more precise data on use and use levels. For comparison of the intake estimations based on the MSDI approach and the mTAMDI approach, see Section 6.

For the candidate substance methyl methanethiosulphonate [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 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 2a.

Step 1

The candidate substances were classified following the procedure established by Cramer et al. (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 (Annex I).

Step B3

The 42 substances in structural class I have estimated European daily per capita intakes ranging from 0.0012 to 6.1 microgram, which is below the threshold of concern of 1800 microgram/person/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 microgram, which is below the threshold of concern for class II of 540 microgram/person/day. The 10 substances evaluated through the Procedure in structural class III have estimated European daily per capita intakes ranging from 0.012 to 6.1 microgram, which is below the threshold of concern for class III of 90 microgram/person/day. Accordingly, the 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 adequate studies on supporting substances show a relatively high degree of variability in the reported No Observed Adverse Effect Levels (NOAELs), ranging from 0.06 to 250 mg/kg bw/day.

Subgroup I: The 20 candidate substances in subgroup I can be represented by the supporting substance dimethyl sulphide [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 body weight (bw)/day), which can be considered a NOAEL. The combined estimated daily per capita intake of 10 microgram for the 20 candidate substances in subgroup I corresponds to 0.17 microgram/kg bw/day at a body weight of 60 kg. Thus, a margin of safety 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.

Subgroup II: Within subgroup II, 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 required for the three cyclic sulphides in subgroup II [FL-no: 12.120, 15.102 and 15.125].

Subgroup III: Within subgroup III, adequate 90-day subchronic studies are available for four supporting substances, 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 only oral dose tested ranging from 0.06 up to 0.7 mg/kg bw/day. By adopting a conservative approach the lowest value (0.06 mg/kg bw/day) can be considered a NOAEL. The combined estimated daily per capita intake of 1.13 microgram for the 11 candidate substances evaluated through the Procedure in subgroup III corresponds to 0.019 microgram/kg bw/day at a body weight of 60 kg. Thus, a margin of safety of 3 x 10^3 can be calculated. The 11 candidate substances in subgroup III evaluated through the Procedure are accordingly not expected to be of safety concern at the estimated levels of intake.

Subgroup IV: The two candidate substances in subgroup IV can be represented by two supporting substances, butane-2,3-dithiol [FL-no: 12.022] and octane-1,2-dithiol [FL-no: 12.034], for which adequate 90-day subchronic studies are available. In the two studies, no adverse effects were produced by the almost identical only oral doses tested, that is 0.7 mg/kg bw/day, which can be considered a NOAEL. The estimated daily per capita intake of 0.42 microgram for the two candidate substances in subgroup IV corresponds to 0.007 microgram/kg bw/day at a body weight of 60 kg. Thus, a margin of safety of 1.0 x 10^5 can be calculated. The candidate substances in subgroup IV is accordingly not expected to be of safety concern at the estimated level of intake.

Subgroup V: Within subgroup V, adequate 90-day subchronic studies are available for two supporting substances, dicyclohexyl disulphide [FL-no: 12.028] and benzyl methyl disulphide [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 only oral dose tested, 0.23 and 1.15 mg/kg bw/day. By adopting a conservative approach, the lowest value (0.23 mg/kg bw/day) can be considered a NOAEL. The combined estimated daily per capita intake of 0.6 microgram for the four candidate substances evaluated through the Procedure in subgroup V corresponds to 0.01 microgram/kg bw/day at a body weight of 60 kg. Thus, a margin of safety of 2.3 x 10^5 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.

Subgroup VI: 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 required for the eight tri-, tetra- and polysulphides in subgroup VI [FL-no: 12.093, 12.094, 12.097, 12.100, 12.112, 12.116, 12.164 and 12.167].

Subgroup VII: This subgroup comprises 13 substances, which can be evaluated through the Procedure. Within subgroup VII, adequate 90-day subchronic studies are available for five supporting substances, trithioacetone [FL-no: 15.009], 3,5-dimethyl-1,2,4-trithiolane [FL-no: 15.025], 2-methyl-4-propyl-1,3-oxathiane [FL-no:16.030], 2-methyl-1,3-dithiolane [FL-no: 15.034] and 3-methyl-1,2,4-trithiane [FL-no: 15.036], which can be considered representative for 12 of the candidate substances in this subgroup. In the 90-day studies, no adverse effects were produced by the only oral dose tested, 0.2, 1.88, 0.44, 7 and 0.3 mg/kg bw/day, respectively. By adopting a conservative approach, the lowest value (0.2 mg/kg bw/day) can be considered a NOAEL. The combined estimated daily per capita intake of 3.9 microgram for these 12 candidate substances in subgroup VII corresponds to 0.065 microgram/kg bw/day at a body weight of 60 kg. Thus, a margin of safety of 3.1 x 10^3 can be calculated. These 12 substances are not expected to be of safety concern at the estimated levels of intake.

For the remaining candidate substance in subgroup VII, 2,5-dihydroxy-1,4-dithiane [FL-no: 15.134], a 90-day study is available for the supporting substance 2,5-dihydroxy-2,5-dimethyl-1,4-dithiane [FL-no: 15.006] that can be considered to be structurally related to this candidate substance. In the study no adverse effects were produced by the only oral dose tested, 3.14 mg/kg bw/day. Therefore the NOAEL is concluded to be 3.14 mg/kg bw/day for this supporting substance. The estimated daily *per capita* intake for the candidate substance 2,5-dihydroxy-1,4-dithiane [FL-no: 15.134] is 6.1 µg, which corresponds to 0.10 µg/kg bw/day at a body weight of 60 kg. Thus, a margin of safety of 3.1 x 10^4 may be calculated. The candidate substance is accordingly not expected to be of safety concern at the estimated level of intake.

Subgroup VIII: 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 only oral dose tested, 6.63 mg/kg bw/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 microgram/kg bw/day at a body weight of 60 kg. Thus, a margin of safety of 1.7 x 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.

Subgroup IX: Within subgroup IX, no data are available for the candidate substance ethanethioic acid [FL-no: 12.199]. Therefore, the Panel concluded that additional data are required for the candidate substance in subgroup IX.

Subgroup X: The only substance in subgroup X, [FL-no: 12.159], is not evaluated through the Procedure.

Subgroup XI: Within subgroup XI, a 90-day subchronic study from which a NOAEL may be derived is available for the candidate substance 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) [FL-no: 15.007]. In this study no adverse effects were produced by the dosage of 500 ppm in feed, calculated to correspond to 25 mg/kg bw/day, which is the NOAEL that can be derived from this study. The estimated daily per capita intake for the candidate substance is 6.1 µg/capita/day, which corresponds to 0.10 µg/kg bw/day. Thus, a margin of safety of 2.5 x 10^5 may be calculated. The candidate substance is accordingly not expected to be of safety concern at the estimated level of intake.

The conclusion from step B4 is that for the 59 candidate substances belonging to subgroups I, III, IV, V, VII, VIII and XI, 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 three candidate substances belonging to subgroup II [FL-no: 12.120, 15.102 and 15.125], 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], and the one candidate substance of subgroup IX [FL-no: 12.199], additional toxicity data are required.

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] due to concern for genotoxicity (see Section 8.4) or lack of tonnage data to provide an MSDI.

6. Comparison of the Intake Estimations Based on the MSDI- and the mTAMDI Approach

For 76 candidate substances in this FGE, the intake estimates based on the MSDI approach have been presented in Table 6.1. For 71 candidate substances, the intake estimates based on mTAMDI approach have been presented in Table 6.1 as well. The candidate substances not evaluated through the Procedure due to concern for genotoxicity [FL-no: 12.159, 12.172, 12.174, 12.304 and 16.057] are not included in the following calculations.

The estimated intakes for the 40 candidate substances in structural class I, based on the mTAMDI approach, range from 3.5 to 8000 microgram/person/day. For two of these substances [FL-no: 12.250 and 12.282] the mTAMDI values are above the threshold of concern for structural class I substances of 1800 microgram/person/day.

The estimated intakes for the 18 candidate substances assigned to structural class II, based on the mTAMDI approach, range from 46 to 78 microgram/person/day, which is below the threshold of concern for structural class II substances of 540 microgram/person/day.

The estimated intakes for the eight candidate substances assigned to structural class III, based on the mTAMDI approach, range from 7.5 to 500 microgram/person/day. For six of these substances [FL-no: 12.120, 12.136, 12.301, 15.134, 16.114 and 16.122] the mTAMDI values are above the threshold of concern for structural class III substances of 90 microgram/person/day.

Thus, for the eight candidate substances [FL-no: 12.120, 12.136, 12.250, 12.282, 12.301, 15.134, 16.114 and 16.122] for which the mTAMDI is above the threshold for their structural class, as well as for the nine substances [FL-no: 12.266, 12.268, 12.269, 12.271, 12.278, 12.295, 15.007, 15.125, 16.062] for which use levels have not been provided, further information is required. This would include more reliable intake data and then, if required, additional toxicological data.

For comparison of the intake estimates based on the MSDI approach and the mTAMDI approach, see Table 6.1.

Table 6.1 Estimated intakes based on the MSDI approach and the mTAMDI approach

FL-no	EU Register name	MSDI (µg/*capita*/day)	mTAMDI (µg/person/day)	Structural class	Threshold of concern (µg/person/day)
12.103	Butane-1,4-dithiol	0.3	78	Class I	1800
12.104	Butane-2-thiol	0.18	78	Class I	1800
12.106	S-2-Butyl 3-methylbutanethioate	0.8	240	Class I	1800
12.111	Dibutyl disulfide	0.37	78	Class I	1800
12.112	Dibutyl trisulfide	0.12	78	Class I	1800
12.116	Dimethyl tetrasulfide	0.016	46	Class I	1800
12.117	Dipentyl sulfide	0.0037	74	Class I	1800
12.124	Ethyl butyl sulfide	0.037	190	Class I	1800
12.125	Ethyl propanethioate	0.012	160	Class I	1800
12.127	Ethyl propyl sulfide	0.085	78	Class I	1800
12.129	3-(Ethylthio)propan-1-ol	0.12	190	Class I	1800
12.135	3-Mercapto-2-methylpropionic acid	0.12	78	Class I	1800
12.151	Methyl butyl disulfide	0.0061	78	Class I	1800
12.152	Methyl butyl sulfide	0.0024	78	Class I	1800
12.158	Methyl isoprenyl sulfide	0.0012	78	Class I	1800
12.163	Methyl prop-1-enyl sulfide	0.0097	78	Class I	1800
12.164	Methyl prop-1-enyl trisulfide	0.0061	78	Class I	1800
12.165	S-Methyl propanethioate	0.012	110	Class I	1800
12.166	Methyl propyl sulfide	0.0024	78	Class I	1800
12.167	Methyl propyl tetrasulfide	0.0037	78	Class I	1800
12.178	3-(Methylthio)butyric acid	0.12	160	Class I	1800
12.180	1-(Methylthio)ethane-1-thiol	0.12	78	Class I	1800
12.181	1-(Methylthio)pentan-3-one	0.12	70	Class I	1800
12.182	2-(Methylthio)propionic acid	0.011	160	Class I	1800
12.183	3-(Methylthio)propionic acid	0.21	160	Class I	1800
12.189	S-(Methylthiomethyl) 2-methylpropanethioate	0.061	160	Class I	1800
12.191	Pentane-1-thiol	0.12	78	Class I	1800
12.196	S-Prenyl thioisobutyrate	0.012	160	Class I	1800

Table 6.1 Estimated intakes based on the MSDI approach and the mTAMDI approach

FL-no	EU Register name	MSDI (µg/*capita*/day)	mTAMDI (µg/person/day)	Structural class	Threshold of concern (µg/person/day)
12.199	Ethanethioic acid	0.0012	160	Class I	1800
12.200	1,1-bis(Ethylthio)-ethane	0.0012	46	Class I	1800
12.205	Mercaptoacetaldehyde	0.011	160	Class I	1800
12.214	Isobutyl-3-(methylthio)butyrate	0.12	160	Class I	1800
12.221	S-Prenyl thioisopentanoate	0.012	150	Class I	1800
12.250	3-Mercaptohexanal	0.012	1900	Class I	1800
12.266	Methyl-2-mercaptopropionate	0.12		Class I	1800
12.277	3-(Methylthio)propyl butyrate	6.1	1400	Class I	1800
12.278	3-Acetyl-mercaptohexyl acetate	1.2		Class I	1800
12.282	(S)-Methyl octanethioate	0.24	8000	Class I	1800
12.298	Di-(1-propenyl)-sulfid (mixture)	0.12	28	Class I	1800
12.299	3-(Methylthio)propyl hexanoate	0.061	1100	Class I	1800
12.303	3-Pentanethiol	0.03	3.5	Class I	1800
12.306	3-(Methylthio)-decanal	0.12	17	Class I	1800
12.304	Ethyl-2-mercapto-2-methyl propanoate	0.012	110	Class I	1800
12.172	2-Methylbutane-2-thiol	0.15	78	Class I	1800
12.174	2-Methylpropane-2-thiol	0.0012	78	Class I	1800
12.268	3-Mercaptooctanal			Class I	1800
12.269	3-Mercaptodecanal			Class I	1800
12.271	Methanedithiol diacetate			Class I	1800
12.093	Diallyl hexasulfide	0.011	78	Class II	540
12.094	Diallyl heptasulfide	0.011	78	Class II	540
12.096	Allyl methyl sulfide	0.99	78	Class II	540
12.097	Allyl methyl tetrasulfide	0.012	78	Class II	540
12.098	Allyl prop-1-enyl disulfide	0.17	78	Class II	540
12.099	Allyl propyl sulfide	1.6	78	Class II	540
12.100	Allyl propyl trisulfide	0.12	78	Class II	540
12.177	8-(Methylthio)-p-menthan-3-one	0.37	78	Class II	540
12.302	2-Butanol, 4-mercapto-3-methyl	0.061	69	Class II	540
12.305	2-Mercapto-4-heptanol	0.12	56	Class II	540
15.047	3,5-Di-isobutyl-1,2,4-trithiolane	0.024	46	Class II	540
15.048	3,5-Di-isopropyl-1,2,4-trithiolane	0.0061	46	Class II	540
15.056	3,6-Dimethyl-1,2,4,5-tetrathiane	0.0024	78	Class II	540
15.083	3-Methyl-1,2,4-trithiolane	0.0024	78	Class II	540
15.102	Tetrahydrothiophene	0.024	78	Class II	540
15.103	1,2,4,5-Tetrathiane	0.073	78	Class II	540
15.110	2,4,6-Trimethyl-1,3,5-trithiane	0.0061	78	Class II	540
15.111	1,2,4-Trithiolane	2.4	78	Class II	540
15.125	4-Tetrahydrothiopyranone	0.12		Class II	540
12.295	3,5-Dimethyl-1,2-dithiolane-4-one			Class II	540
16.057	2,4,4-Trimethyl-1,3-oxathiane	0.0012	78	Class II	540
12.120	2,8-Epithio-p-menthane	3.7	370	Class III	90
12.136	3-Mercapto-2-oxopropionic acid	0.24	160	Class III	90
12.300	1,1-Propanedithiol	0.12	7.5	Class III	90
12.301	Methyl-2-oxo-propyl disulfide	0.061	170	Class III	90
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)	6.1		Class III	90
15.081	Lenthionine	0.012	78	Class III	90
15.134	2,5-Dihydroxy-1,4-dithiane	6.1	500	Class III	90
16.062	trans-2-Methyl-4-propyl-1,3-oxathiane	1.0		Class III	90
16.114	2-Pentyl-4-propyl-1,3-oxathiane	0.12	290	Class III	90
16.122	4-Methyl, 2-propyl, 1-3-oxathiane	0.24	230	Class III	90
12.159	Methyl methanethiosulfonate	0.061	160	Class III	90

7. Considerations of Combined Intakes from Use as Flavouring Substances

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

this may lead to underestimation of exposure. After completion of all FGEs, this issue should be readdressed.

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

On the basis of the reported annual production volumes in Europe (EFFA, 2002g; EFFA, 2002h; EFFA, 2002i; EFFA, 2004ak; EFFA, 2007a; Flavour Industry, 2006q; Flavour Industry, 2006r; Flavour Industry, 2009e; Flavour Industry, 2009o; Flavour Industry, 2010h; Flavour Industry, 2011d), the combined estimated daily per capita intakes as flavourings of the candidate substances evaluated using the Procedure and assigned to structural class I (42 substances), structural class II (19 substances) and structural class III (10 substances) are 11, 6 and 18 microgram, respectively. These values do not exceed the thresholds of concern for a substance belonging to structural class I, II or III of 1800, 540 or 90 microgram/person/day, respectively.

The 71 candidate substances, to which the Procedure has been applied, are structurally related to 127 supporting substances evaluated by the JEFCA at its 53th JECFA meetings (JECFA, 2000b). Based on reported production volumes, European per capita intakes (MSDI) could be estimated for 70 of the 127 supporting substances (distributed as 43 supporting substances in structural class I, 24 supporting substances in structural class II and one supporting substance in structural class III). Production volumes in Europe were not reported for 57 of the supporting substances.

The total combined estimated daily per capita intake as flavourings of the candidate substances evaluated using the Procedure and the supporting substances (for which there are European intake data) assigned to structural class I, II and III are 648, 115 and 18 microgram, respectively. These values do not exceed the thresholds of concern for substances belonging to structural class I, II or III of 1800, 540 or 90 microgram/person/day, respectively.

8. Toxicity

8.1. Acute Toxicity

Data are available on four candidate substances butane-2-thiol [FL-no: 12.104], 2-methylbutane-2-thiol [FL-no: 12.172] and 2-methylpropane-2-thiol [FL-no: 12.174], belonging to subgroup III, and tetrahydrothiophene [FL-no: 15.102], included in subgroup II. In addition data are available on 35 supporting substances. The LD_{50} values varied from 100 to more than 20000 mg/kg body weight (bw).

The acute toxicity data are summarised in Annex IV, Table IV.1.

8.2. Subacute, Subchronic, Chronic and Carcinogenicity Studies

Data from repeated-dose toxicity studies were available for three candidate substances. For 3-(Methylthio)propionic acid [FL-no: 12.183], included in subgroup I, for 2,8-Epithio-p-menthane [FL-no: 12.120], included in subgroup II, and for the candidate substance [FL-no: 15.007], in subgroup XI, and for 33 supporting substances included in subgroup I (2), II (1), III (11), IV (2), V (5), VI (2), VII (5), VIII (4), X (1) (see Annex IV, Table IV.2). In most of the subchronic studies no effects were observed at the highest dose tested, which in the majority of cases was the only tested dose. Due to different kinds of limitations (see Table IV.2) several of these studies could not be used for derivation of a No Observed Adverse Effect Level (NOAEL).

Subgroup I (Acyclic sulphides); For the candidate substance 3-(methylthio)propionic acid [FL-no: 12.183] included in subgroup I, a two-week oral study is available. Only one dose is tested at which effects were reported. The study could not be used for derivation of a NOAEL.

Subgroup II (Cyclic sulphides); For the candidate substance 2,8-epithio-p-menthane [FL-no: 12.120], included in subgroup II, a 28-day oral study in rats has been made available since the adoption of FGE.08Rev2. Only one dose (10 mg/kg bw) is tested at which effects were reported for male rats. No NOAEL could be allocated for male rats, based on urine casts, increased kidney weights and the presence of renal focal tubular degeneration/regeneration. These effects were not seen in female rats. It is not considered appropriate to use this study for derivation of a NOAEL. Otherwise there is only a study on a supporting substance from subgroup II [FL-no: 15.012]. This is an unpublished and uncompleted report in which histopathology results are not available (Morgareidge and Oser, 1970a). The study could not be used for derivation of a NOAEL. No NOAEL is available for subgroup II.

Subgroup VI (Acyclic polysulphides); For two supporting substances in subgroup VI, diallyl trisulphide [FL-no: 12.009] and dipropyl trisulphide [FL-no: 12.023], 90-day studies are available as unpublished reports (Morgareidge and Oser, 1970c; Morgareidge and Oser, 1970d). In these studies 15 male and 15 female rats were given the test substances in feed, at one dose level, control rats, 15 male and 15 female, received basal diet. Diets were blended with test substances prepared as 1 % solutions in acetone. Weekly supplies of feed were stored in sealed jars in a "cool, dark place". There is, however, no data on stability of test substances during storage. Nominal dose was 4.16 mg/kg bw per day for both test substances, but actual dose was 4.6 mg/kg bw per day for diallyl trisulphide and 4.8 mg/kg bw per day for dipropyl sulphide. No abnormal or remarkable findings were made concerning weight gain, food utilization or on the haematological, biochemical or urinary parameters that were measured. However, there are no data on stability of test substances; and since no results are reported from histopathological examinations it is not possible to derive NOAELs from these studies. No NOAEL is available for subgroup VI.

Subgroup IX (Thioic acid); There are no supporting substances in subgroup IX, and there are no studies available for the one candidate substance [FL-no: 12.199] in this subgroup. No NOAEL is available for subgroup IX.

Subgroup X (Sulphoxides/sulphones and sulphonates); There are three long term studies available for one supporting substance [FL-no: 12.175] in subgroup X. Due to limitations of these studies, such as lack of report on histopathology or confounding effects of solvents, no NOAELs could be derived. There are no NOAELs available for subgroup X.

Subgroup XI (Cyclic thioketal with fused oxolane rings); There is a 90-day study in rats available for the candidate substance [FL-no: 15.007] (Wheldon et al., 1970). The Panel noted that the JECFA (JECFA, 2005c) has evaluated the same study and derived a NOAEL. However, some of the study details that are described in the JECFA report are not in accordance with the data that have been submitted to the Panel, e.g. the JECFA refers to 15 male rats per dosage group whereas the study by Wheldon et al (1970) reports 10 male rats per dosage group. The study is presented below:

In a 13 week study, groups of 10 male CFY rats were fed diets containing 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.e. [FL-no: 15.007] (Wheldon et al., 1970). Rats were housed 5 per cage with free access to water and rat feed. Test compound was mixed directly into the rat feed.

Dosage groups: Diets held a concentration of 0 (control), 500 mg/kg feed calculated to correspond to 25 mg/kg bw/day(hereafter called low-dose group), 5000 mg/kg feed calculated to correspond to 250 mg/kg bw/day (hereafter called medium-dose group), or 5000 mg/kg feed raised to 10000 mg/kg feed after week 1 and to 20000 mg/kg feed at week 6 calculated to correspond to 250 mg/kg bw/day, raised to 500 mg/kg bw/day and further to 1000 mg/kg bw/day (hereafter called high-dose group).

Feed consumption: A decrease in food consumption was reported in all treated groups throughout the study. Mean daily food consumption was for the low-dose group ca. 91 % of control, for the medium-dose group ca. 78 % of control and for the high-dose group ca. 78 % of control. For the low dose group food intake was depressed only during the first 8 weeks.

Weight gain: At 13 weeks mean weight in the low-dose group was 91 % of control, in the medium-dose group 74 % of control and in the high-dose group 54 % of control. In the low-dose group body weights lagged behind the controls only in the final 4 weeks.

Feed conversion ratio: Group mean feed conversion ratios were for months 1, 2 and 3 as follows, for control 3.6, 5.6, 6.4; for the low-dose group 3.4, 5.4, 9.6; for the medium-dose group 3.9, 5.9, 13.9; and for the high-dose group 4.9, 13.1, 25.5. The feed conversion ratio was affected in all dosage groups in a dose dependent manner, and seems also to be related to the time of exposure.

Haematology: At 13 weeks samples of blood were drawn from 5 rats of control and dosage groups. In the high-dose group statistically significant decreases in PCV, Hb, RBC, MCHC, MCV and increase in total white cell count, and neutrophile and lymphocyte counts were observed. The medium-dose group exhibited a similar pattern, statistically significant for all parameters except for PCV and MCHC. Animals in the low-dose group did not show significant haematological changes. An abnormal blood pigmentation, identified as methaemoglobin was found in all animals in the high-dose group.

Biochemistry: No blood chemistry determinations or urine analyses were performed in the study.

Macroscopic pathology: All spleens of the high-dose group were enlarged. In a 4 weeks pre-test with doses of 5000 mg/kg feed (250 mg/kg bw) and 10000 mg/kg feed (500 mg/kg bw) (this dose was lowered to 2500 mg/kg feed and then raised again) dark coloration of spleens were seen in all treated animals This is not discussed when the results of the 90-day study are presented, and presence or absence of such an effect is not mentioned.

Histopathology: Histopathology was only performed on 5 animals from the high-dose group and on 5 animals from the control group. No specific findings are mentioned.

Overall the study by Wheldon et al. (1970) does not follow current standard (OECD guidelines). Main limitations of the study are that only male rats were tested and that no blood chemistry or urine analyses were performed in the study. The low-dose group (500 mg/kg feed level), however, does not show toxic effects other than that growth rate was slightly reduced during the final weeks of the study. The Panel concludes that this level of intake may be used as a NOAEL, which corresponds to an intake of 25 mg/kg bw/day.

Consequently no NOAELs are available for subgroups II, VI, IX and X.

Studies on supporting substances used for NOAEL derivation for the application of the Procedure

Subgroup I (Acyclic sulphides)

Dimethyl sulphide [FL-no: 12.006]

Four groups of 15 Wistar rats per sex were given dimethyl sulphide by daily oral gavage in corn oil at dose levels of 2.5, 25 or 250 mg/kg bw for 14 weeks; the control group received the same volume of corn oil only. An additional two groups (five/sex/dose) were given daily doses of 0.25 or 250 mg/kg bw for two or six weeks, respectively. The animals were weighed on day 0 and then weekly throughout the study. Food and water consumption were measured over a 24 hours period preceding the day of weighing. Urine samples were collected during weeks 2, 6 and 14, and examined for volume, appearance, specific gravity, microscopic constituents, and content of glucose, ketones, bile

salts and blood. At sacrifice, blood was taken for haematological examinations. Gross abnormalities were noted and organ weights taken. Histological examinations were also performed. There was no adverse effect at any level in dosed rats and therefore, 250 mg/kg bw/day was considered as the NOAEL derived from the study (Butterworth et al., 1975b).

Subgroup III (Monothiols)

2,6-Dimethylthiophenol [FL-no: 12.082]

2,6-Dimethylthiophenol was administered in corn oil by gavage to Sprague-Dawley rats (16/sex/group) at an average daily intake of 0.43 mg/kg bw for 13 weeks. Control animals received the same volume of corn oil only. Weekly measurements of body weight and food intake were taken. Haematological examination and blood chemical determinations as well as urine analysis were performed at weeks 4 and 13. Organ weights, gross pathology and histological examinations were performed at the time of necropsy. There were no significant differences between the treated animals and the control group. The NOAEL derived from the study is concluded to be 0.43 mg/kg bw/day (Peano et al., 1981).

Cyclopentanethiol [FL-no: 12.029]

Cyclopentanethiol, dissolved in acetone and blended into a basal laboratory diet to yield an actual daily dose of 0.56 mg/kg, was administered to Sprague-Dawley rats (15/sex/group) for 90 days. Control animals received basal laboratory diet admixed with acetone. Dietary acetone was fully evaporated before presentation to the animals. Samples of each treatment diet were taken weekly for assessment of stability and concentration of the test substance. Clinical signs and mortality were recorded daily. Body weights and food consumption were measured weekly. Haematological examinations, blood chemistry determinations and urine analyses were performed at weeks 6 and 12 on 8 males and 8 females from each group. At necropsy, organ weights were recorded and histopathological examinations were performed. No differences between control and treated animals were observed in any of the tested parameters. The NOAEL derived from the study is concluded to be 0.56 mg/kg bw per day (Morgareidge and Oser, 1970b).

2,3- and 10-mercaptopinane [FL-no: 12.035]

2,3- and 10-mercaptopinane, blended into a basal laboratory diet to yield an actual daily dose of 0.06 mg/kg, was administered to Sprague-Dawley rats (17/sex/group) for 90 days. Control animals received basal laboratory diet. Samples of each treatment diet were taken weekly for assessment of stability and concentration of the test substance. Clinical signs and mortality were recorded daily. Body weights and food consumption were controlled weekly. Haematological examinations, blood chemistry determinations and urine analyses were performed at weeks 6 and 12. At necropsy, organ weights were recorded and histopathological examinations were performed. No differences between control and treated animals were observed in any of the tested parameters. The NOAEL derived from the study is concluded to be 0.06 mg/kg bw per day (Oser, 1966).

2-Mercapto-3-butanol [FL-no: 12.024]

2-Mercapto-3-butanol was administered to Sprague-Dawley rats (15/sex/group) for 90 days, dissolved in acetone and blended into a basal laboratory diet to yield an actual daily dose of 0.705 mg/kg. Control animals received basal laboratory diet admixed with acetone. Dietary acetone was fully evaporated before presentation to the animals. Samples of each treatment diet were taken weekly for assessment of stability and concentration of the test substance. Clinical signs and mortality were recorded daily. Body weights and food consumption were measured weekly. Haematological examinations, blood chemistry determinations and urine analyses were performed at weeks 6 and 12. At necropsy, organ weights were recorded and histopathological examinations were performed. No

differences between control and treated animals were observed in any of the tested parameters. The NOAEL for 2-mercapto-3-butanol is concluded to be 0.705 mg/kg bw per day (Cox et al., 1974a).

Subgroup IV (Dithiols)

2,3-Butanedithiol [FL-no: 12.022] and 1,8-Octanedithiol [FL-no: 12.034]

2,3-Butanedithiol and 1,8-octanedithiol were administered to Sprague-Dawley rats (15/sex/group) for 90 days, following the study design used by Cox et al. (Cox et al., 1974a). The test item was dissolved in acetone and blended into a basal laboratory diet to yield an actual daily dose of 0.703 and 0.705 mg/kg, respectively. Control animals received basal laboratory diet admixed with acetone. Dietary acetone was fully evaporated before presentation to the animals. Samples of each treatment diet were taken weekly for assessment of stability and concentration of the test substance. Clinical signs and mortality were recorded daily. Body weights and food consumption were measured weekly. Haematological examinations, blood chemistry determinations and urine analyses were performed at weeks 6 and 12. At necropsy, organ weights were recorded and histopathological examinations were performed. No differences between control and treated animals were observed in any of the tested parameters. The NOAEL for 2,3-butanedithiol and for 1,8-octanedithiol is concluded to be 0.705 mg/kg bw per day (Cox et al., 1974c; Cox et al., 1974d).

Subgroup V (Acyclic and cyclic disulphides)

Dicyclohexyl disulphide [FL-no: 12.028] and benzyl methyl disulphide [FL-no: 12.068]

The two supporting substances were administered to Sprague-Dawley rats (15/sex/group) for 90 days, following the study design used by Cox et al., 1974a.

The test item was dissolved in acetone and blended into a basal laboratory diet to yield an actual daily dose of 0.232 mg/kg and 1.15 mg/kg dicyclohexyl disulphide and benzyl methyl disulphide, respectively. Control animals received basal laboratory diet admixed with acetone. Dietary acetone was fully evaporated before presentation to the animals. Samples of each treatment diet were taken weekly for assessment of stability and concentration of the test substance. Clinical signs and mortality were recorded daily. Body weights and food consumption were measured weekly. Haematological examinations, blood chemistry determinations and urine analyses were performed at weeks 6 and 12. At necropsy, organ weights were recorded and histopathological examinations were performed. No differences between control and treated animals were observed in any of the tested parameters. The NOAEL for dicyclohexyl disulphide and for benzyl methyl disulphide is concluded to be 0.232 and 1.15 mg/kg bw per day, respectively (Cox et al., 1974e; Gallo et al., 1976a).

NOAELs for dithiols (subgroup IV) may be utilised for the evaluation of the cyclic disulphide 3,5-dimethyl-1,2-dithiolane-4-one [FL-no: 12.295] which is proposed to be ring opened and oxidised to a dithiol; there are however no intake data for this candidate substance, and it is consequently not taken through the Procedure, as is stated in Section 5.

Subgroup VII (Mono-, di-,tri- and poly-sulphides with thioacetal structure)

2,5-dihydroxy-2,5-dimethyl-1,4-dithiane [FL-no: 15.006]

2,5-dimethyl-2,5-dihydroxy-1,4-dithiane was administered to Sprague-Dawley rats (15/sex) for 90 days. The substance was dissolved in acetone and blended into a basal laboratory diet to yield an actual daily dose of 3.14 mg/kg bw. Control animals (15/sex) received basal laboratory diet admixed with acetone. Dietary acetone was fully evaporated before presentation to the animals. Clinical signs and mortality were recorded daily. Body weights and food consumption were measured weekly. Haematological examinations, blood chemistry determinations and urine analyses were performed at weeks 6 and 12 on 8 rats from each group. At necropsy, organ weights were recorded for livers and

kidneys, and pathological examinations were performed. Histopathology was performed extensively on 8 rats per group, and for the remaining rats histopathological examination was performed on kidneys and livers only. No differences between control and treated animals were observed in any of the tested parameters. The NOAEL is concluded to be 3.14 mg/kg bw per day for 2,5-dimethyl-2,5-dihydroxy-1,4-dithiane (Cox et al., 1973a).

3,5-Dimethyl-1,2,4-trithiolane [FL-no: 15.025] and 2-methyl-4-propyl-1,3-oxathiane [FL-no: 16.030]

3,5-dimethyl-1,2,4-trithiolane and 2-methyl-4-propyl-1,3-oxathiane dissolved in corn oil were administered by oral intubation to Wistar rats (15/sex/group) for 90 days, following the same study design. The daily dose was 1.88 mg/kg bw and 0.44 mg/kg bw for 3,5-dimethyl-1,2,4-trithiolane and 2-methyl-4-propyl-1,3-oxathiane, respectively. Control rats were given corn oil alone. Body weight and food intake were regularly recorded throughout the study. Blood was collected at 6 and 12 weeks, for haemoglobin concentration, packed cell volume and erythrocyte plus leukocyte counts analysis. Urea concentration was also measured. At study termination, organ weights were recorded, gross necropsy observations and histological evaluations were conducted. Although a slight increase in food intake was noted, there were no significant differences between treated and control rats for body weight. Some sporadic differences between control and treated animals were observed but none was statistically significant. The NOAEL for 3,5-dimethyl-1,2,4-trithiolane and for 2-methyl-4-propyl-1,3-oxathiane is concluded to be 1.88 and 0.44 mg/kg bw per day, respectively (BIBRA, 1976).

3-Methyl-1,2,4-trithiane [FL-no: 15.036]

3-Methyl-1,2,4-trithiane was administered in corn oil orally to Sprague-Dawley rats (16/sex/group) at a dose of 0.3 mg/kg bw/day for 13 weeks. Weekly body weight and food intake measurements were taken. Haematological examinations and blood urea determinations were conducted at weeks 4 and 13. At necropsy, organ weights were taken and histopathology was performed. No adverse effects were observed. The NOAEL is concluded to be 0.3 mg/kg bw per day for 3-methyl-1,2,4-trithiane (Mondino, 1981a).

2-Methyl-1,3-dithiolane [FL-no: 15.034]

Thirty-two (16/sex) Sprague-Dawley rats received an aqueous propylene glycol solution (0.2 % w/w) containing 7 mg/kg bw of 2-methyl-1,3-dithiolane daily by oral intubation for 91 days. Control animals received 0.02 % propylene glycol only. Body weight and food consumption were regularly recorded during the study. Haematological examinations and blood chemical determinations were performed at weeks 4 and 13. At study termination gross pathology, organ weights and histological examinations were carried out. There were no differences between the control and treatment groups for any parameters, except for a slight non-significant reduction in haemoglobin levels in the treated females only. The NOAEL was therefore concluded to be 7 mg/kg bw/day (Griffiths et al., 1979a).

Trithioacetone [FL-no: 15.009]

Trithioacetone was administered to Sprague-Dawley rats (15/sex/group) for 90 days, dissolved in acetone and blended into a basal laboratory diet to yield an actual daily dose of 0.2 mg/kg. Control animals received basal laboratory diet admixed with acetone. Dietary acetone was fully evaporated before presentation to the animals. Samples of each treatment diet were taken weekly for assessment of stability and concentration of the test substance. Clinical signs and mortality were recorded daily. Body weights and food consumption were measured weekly. Haematological examinations, blood chemistry determinations and urine analyses were performed at weeks 6 and 12. At necropsy, organ weights were recorded and histopathological examinations were performed. No differences between control and treated animals were observed in any of the tested parameters. The NOAEL is concluded to be 0.2 mg/kg bw per day for trithioacetone (Cox et al., 1973b).

Subgroup VIII (Thioesters)

Ethyl thioacetate [FL-no: 12.018]

Ethyl thioacetate was administered to rats (12/sex/group) in the diet for 90 days at a daily actual dose of 6.63 mg/kg bw/day. A control group received basal diet alone. The animals were observed daily for clinical signs. Body weights and food consumption were recorded weekly. During weeks 6 and 13, urine samples were collected for complete analysis. Haematological analysis was carried out at 6 weeks (on 8 animals/group) and at 13 weeks. At study termination animals were necropsied and their tissues examined for gross pathological changes. Organs were weighed and tissues retained for histological evaluations. There were no significant differences between treated and control animals in any of the tested parameters. The NOAEL is concluded to be 6.63 mg/kg bw per day for ethyl thioacetate (Shellenberger, 1970b).

Consequently no NOAELs are available for subgroups II, VI, IX, X and XI.

The repeated-dose toxicity data are summarised in Annex IV, Table IV.2.

8.3. Developmental / Reproductive Toxicity Studies

Data were available on two supporting substances included in subgroup III. However, for one of them, 1-butanethiol [FL-no: 12.010], data were obtained after inhalation, a route of exposure with limited value for flavouring substances. For the available data it may be concluded that effects on development or reproduction were only observed at exposure levels associated with maternal toxicity.

The developmental/reproductive toxicity data are summarised in Annex IV, Table IV.3.

8.4. Genotoxicity Studies

Genotoxicity *in vitro* data are available for five of the 70 candidate substances, di-(1-propenyl)-sulphid (mixture) [FL-no: 12.298] (subgroup I), tetrahydrothiophene [FL-no: 15.102] (subgroup II); 2-methylpropane-2-thiol [FL-no: 12.174] (subgroup III), dibutyl disulphide [FL-no: 12.111] (subgroup V) and methyl methanethiosulphonate [FL-no: 12.159] (subgroup X). In addition studies are available on 14 supporting substances from subgroups I (1), II (1), III (4), IV (1), V (4), VIII (2) and IX (1).

In vivo data are available for one candidate substance [FL-no: 12.159] (subgroup X) and for four supporting substances from subgroups I (1), III (1), V (1) and VI (1).

Subgroup I (Acyclic sulphides)

In vitro data are available for the candidate substance, di-(1-propenyl)-sulphide [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, 2005c).

For supporting substances, only data on diallyl sulphide [FL-no: 12.088] are available. Diallyl sulphide 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 sulphide 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 II (Cyclic sulphides)

For this subgroup, data on only one candidate substance, tetrahydrothiophene [FL-no: 15.102], are available. The substance is reported to be negative in an Ames test, a cytogenetic assay in human lymphocytes, a gene mutation (HPRT) assay in Chinese hamster ovary (CHO) cells, a SCE assay in CHO cells and an unscheduled DNA synthesis (UDS) test in human epithelial cells. It is stated that the Ames test, the cytogenetic assay and the HPRT assay were performed according to OECD protocols. These studies are reported as abstracts in the IUCLID dataset (Pennwalt Corporation, 1987a-d; Pennwalt Corporation, 1987e).

In addition, limited *in vitro* data on the supporting substance 1,4-dithiane [FL-no: 15.066] provide some indication of concern for genotoxicity. The substance was shown to be mutagenic in *S. typhimurium* strains TA98 and TA100, however, the mutagenic activity was completely abolished in the presence of S9. In the same study the substance was reported to be negative in a SCE assay, with and without S9.

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].

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

Subgroup IV (Dithiols)

Equivocal results were reported for the only supporting substance tested, 1,2-ethanedithiol [FL-no: 12.066]. It was evaluated positive for induction of gene mutations and SCEs *in vitro* in a poorly reported study. However, increased mutation frequencies were associated with unacceptably high toxicity, and the relevance of SCEs for genotoxicity assessment is unclear. Moreover, the validity of the latter data set is questionable, as the distinct effect of S9 on toxicity observed in the other mammalian cell mutation study was not replicated. 1,2-Ethanedithiol [FL-no: 12.066] was reported in an abstract to be negative in the Ames test.

Subgroup V (Acyclic and cyclic disulphides)

Dibutyl disulphide [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 abstract, and thus, the validity cannot be evaluated.

Further data are available for the supporting substances diallyl disulphide [FL-no: 12.008], dimethyldisulphide [FL-no: 12.026], phenyl disulphide [FL-no: 12.043] and benzyl disulphide [FL-no: 12.081]. All substances were reported to be negative in the Ames test. In addition, diallyl disulphide 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 VII (Mono-, di-, tri- and polysulphides with thioacetal structure)

There are no data available on genotoxicity for the substances in this group. However, one of the hydrolysis products of the candidate substance 2,4,4-trimethyl-1,3-oxathiane [FL-no: 16.057] is structurally related to the above-mentioned tertiary thiols, raising concern with respect to the genotoxicity of this candidate. Therefore, in the absence of further genotoxicity data, the Panel concluded that the Procedure could not be applied to 2,4,4-trimethyl-1,3-oxathiane [FL-no: 16.057].

Subgroup VIII (Thioesters)

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

Subgroup IX (Thioic acids)

No data are available for the candidate substance of this group. Moreover, there are no supporting substances.

Subgroup X (Sulphoxides/sulphones and sulphonates)

Methyl methanethiosulphonate (MMTS) [FL-no: 12.159] is structurally similar to methyl methanesulphonate (MMS), a direct acting genotoxic carcinogen. However, the presence of an additional sulphur is expected to decrease the electrophilicity and therefore the possible genotoxicity of the candidate substance. MMTS is reported to be negative in an Ames test and in a mitotic recombination/mutagenicity assay with *Saccharomyces cerevisiae* (Dorange et al., 1983). However, as pointed out by the authors, thiosulphonates in general, and MMTS in particular, are non-specific antimicrobial agents that are active at low concentrations on bacteria, as well as on yeast and other fungi. Therefore, bacterial test systems and yeast assays are not appropriate to evaluate genotoxicity of thiosulphonates. MMTS [FL-no: 12.159] has also been shown to be negative in an assay performed with *Nicotiana tabacum* seeds (Dorange et al., 1983), but the relevance of this test is unknown.

Antimutagenic activity has been shown for MMTS, which occurs naturally in some vegetables from Cruciferae and Liliaceae species (Marks et al., 1993; Nakamura et al., 1993; Nakamura et al., 1996; Ito et al., 1997; Nakamura et al., 1997a). However, antimutagenicity studies per se are not specifically designed to evaluate the genotoxic potential of chemicals.

In conclusion, the limited relevance of the tests carried out so far in bacteria and yeasts and the lack of tests on mammalian cells do not allow an adequate evaluation of the genotoxic potential of MMTS. In addition, the similarity with MMS raises concern with respect to the genotoxicity of this candidate substance.

Methylsulphinyl methane [FL-no: 12.175] (synonym: dimethylsulphoxide, DMSO) was reported to be positive in an Ames test at high doses, which resulted in reduced bacterial survival. The validity of this finding is highly questionable compared to the overwhelming evidence on absence of genotoxic properties provided by the wide use of DMSO as solvent for test material in genotoxicity assays including controls for solvent activity. Further data on other supporting substances are of limited or insufficient quality and cannot be evaluated.

Subgroup XI Cyclic thioketal with fused oxolane ring

No data are available for the candidate substance of this group. Moreover, there are no supporting substances.

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 the JECFA at the 53rd meeting (JECFA, 2000b; JECFA, 2000c). These supporting substances have been considered by EFSA in FGE.91 (EFSA, 2010q).

In addition, genotoxicity of the candidate substance methyl methanethiosulphonate [FL-no: 12.159], included in subgroup X, could not be assessed from the data available. However, due to the similarity with methyl methanesulphonate, 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.

Genotoxicity data are summaries in Annex IV, Table IV.4 and Table IV.5.

9. Conclusions

Compared to FGE.08Rev4, this FGE.08Rev5 includes toxicity data for one supporting substance, 2,5-dihydroxy-2,5-dimethyl-1,4-dithiane [FL-no: 15.006], which support the evaluation of the candidate substance 2,5-dihydroxy-1,4-dithiane [FL-no: 15.134]. After submission of an explanatory note from EFFA, a 90-day study on candidate substance 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) [FL-no: 15.007] has been reconsidered after submission of an explanatory note from EFFA.

Additional information on specifications and/or stereoisomeric/positional composition submitted for [FL-no: 12.098, 12.163, 12.164, 12.250, 12.266, 12.277, 12.278, 12.298, 12.300, 12.301, 12.302, 12.305, 12.306, 15.007, 15.056, 15.110, 15.134, 16.062, 16.114 and 16.122] has also been included.

The total 80 candidate substances in FGE.08Rev5 are divided into 11 subgroups:

Subgroup I) Acyclic sulphides: [FL-no: 12.096, 12.099, 12.117, 12.124, 12.127, 12.129, 12.152, 12.158, 12.163, 12.166, 12.177, 12.178, 12.181, 12.182, 12.183, 12.214, 12.277, 12.298, 12.299 and 12.306]

Subgroup II) Cyclic sulphides: [FL-no: 12.120, 15.102 and 15.125]

Subgroup III) Monothiols: [FL-no: 12.104, 12.135, 12.136, 12.172, 12.174, 12.180, 12.191, 12.205, 12.250, 12.266, 12.268, 12.269, 12.302, 12.303, 12.304 and 12.305]

Subgroup IV) Dithiols: [FL-no: 12.103 and 12.300]

Subgroup V) Acyclic and cyclic disulphides: [12.098, 12.111, 12.151, 12.295 and 12.301]

Subgroup VI) Acyclic polysulphides: [FL-no: 12.093, 12.094, 12.097, 12.100, 12.112, 12.116, 12.164 and 12.167]

Subgroup VII) Mono-, di-, tri- and polysulphides with thioacetal structure: [FL-no: 12.200, 15.047, 15.048, 15.056, 15.081, 15.083, 15.103, 15.110, 15.111, 15.134, 16.057, 16.062, 16.114 and 16.122]

Subgroup VIII) Thioesters: [FL-no: 12.106, 12.125, 12.165, 12.189, 12.196, 12.221, 12.271, 12.278 and 12.282]

Subgroup IX) Thioic acids: [FL-no: 12.199]

Subgroup X) Sulphoxides/sulphones and sulphonates: [FL-no: 12.159]

Subgroup XI) Cyclic thioketal with fused oxolane ring: [FL-no: 15.007].

Twenty-nine flavouring substances possess one or more chiral centres [FL-no: 12.104, 12.106, 12.120, 12.135, 12.177, 12.178, 12.180, 12.182, 12.214, 12.250, 12.266, 12.268, 12.269, 12.278, 12.295, 12.302, 12.305, 12.306, 15.007, 15.047, 15.048, 15.056, 15.083, 15.110, 15.134, 16.057, 16.062, 16.114 and 16.122]. The stereoisomeric composition has not been specified sufficiently for two substances [FL-no: 12.268 and 12.269].

Due to the presence and the position of double bonds, four substances [FL-no: 12.098, 12.163, 12.164 and 12.298] can exist as geometrical isomers and due to the ring structure additional two substances [FL-no: 15.056 and 15.110] can exist as geometrical isomers. Industry has stated that these substances occur as mixtures of geometrical isomers, and given the actual ratio of the composition of the mixtures. For [FL-no: 15.007] the stereoisomeric composition and the composition of the positional isomeric mixture has been given by Industry.

Of the in total 80 candidate substances, 48 belong to structural class I, 21 belong to structural class II and 11 belong to structural class III.

Forty-eight of the flavouring substances in the present group have been reported to occur naturally in a wide range of food items.

According to the default MSDI approach, Flavour Industry have submitted data for 76 candidate substances, which have intakes in Europe ranging from 0.0012 to 6.1 microgram/capita/day, which are below the threshold of concern value for structural class I (1800 microgram/person/day), structural class II (540 microgram/person/day) and structural class III (90 microgram/person/day) substances.

For three substances in structural class I, 3-mercaptooctanal, 3-mercaptodecanal, methanedithiol diacetate [FL-no: 12.268, 12.269 and 12.271] and for one substance in structural class II, 3,5-dimethyl-1,2-dithiolane-4-one [FL-no: 12.295], no data on use as flavouring substances in Europe are available, therefore no intakes can be estimated and accordingly these substances cannot be evaluated through the Procedure.

On the basis of the reported annual production volumes in Europe (MSDI approach), the combined intake of the 42 candidate substances belonging to class I, the 19 candidate substances belonging to class II and the 10 candidate substances belonging to class III, evaluated through the Procedure, would result in total intakes of approximately 11, 6 and 18 microgram/*capita*/day, respectively, which do not exceed the thresholds of concern for structural class I, II or III. Based on reported production volumes, European per *capita* intakes (MSDI) could be estimated for 70 of the 127 supporting substances. The total combined intakes of the candidate and supporting substances (for which there are European intake data) are approximately 648, 115 and 18 microgram/*capita*/day for structural class I, II and III,

respectively, which do not exceed the thresholds of concern for structural class I, II or III of 1800, 540 or 90 microgram/person/day, respectively.

Data on genotoxicity of the candidate substances are limited and the genotoxicity could not be adequately assessed. The data available, however, give rise to some concern of a genotoxic potential of two of the candidate substances, 2-methylpropane-2-thiol [FL-no: 12.174] and methyl methanethiosulphonate [FL-no: 12.159]. The Panel, therefore, concluded that the Procedure could not be applied to these two substances, nor to the structurally related candidate substances, 2-methylbutane-2-thiol [FL-no: 12.172], ethyl-2-mercapto-2-methyl propanoate [FL-no: 12.304] and 2,4,4-trimethyl-1,3-oxathiane [FL-no: 16.057], until adequate *in vivo* genotoxicity data become available. 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 considered by EFSA in the FGE.91 (EFSA, 2010q).

The genotoxicity data available for the remaining candidate substances do not preclude their evaluation through the Procedure.

The candidate substances and supporting substances are expected to share common routes of absorption, distribution and metabolism, and exhibit similar toxicological properties. These metabolic pathways are unlikely to be saturated, given the low levels of exposure from their use as flavouring substances. However, due to the reactivity of the metabolites, the candidate substances cannot be predicted to be metabolised to innocuous products.

It is considered that on the basis of the default MSDI approach 59 of the 71 candidate substances evaluated through the Procedure would not give rise to safety concerns at the estimated levels of intake arising from their use as flavouring substances. Additional toxicity data are required for the remaining 12 candidate substances, three candidate substances in subgroup II [FL-no: 12.120, 15.102 and 15.125], eight candidate substances in subgroup VI [FL-no: 12.093, 12.094, 12.097, 12.100, 12.112, 12.116, 12.164 and 12.167], and the candidate substance in subgroup IX [FL-no: 12.199].

When the estimated intakes were based on the mTAMDI for the substances evaluated through the Procedure and for which intake data have been submitted, they ranged from 3.5 to 8000 microgram/person/day for the 40 candidate substances from structural class I, from 46 to 78 microgram/person/day for the 18 candidate substances assigned to structural class II and from 78 to 500 microgram/person/day for the eight candidate substances assigned to structural class III. For two substances from structural class I [FL-no: 12.250 and 12.282] and six substances from structural class III [FL-no: 12.120, 12.136, 12.301, 15.134, 16.114 and 16.122], the mTAMDI values are above the thresholds of concern for structural class I or III of 1800 or 90 microgram/person/day, respectively.

For the eight flavouring substances for which the intakes, estimated on the basis of the mTAMDI, exceed the relevant threshold for their structural class more reliable exposure data are required. For the nine substances [FL-no: 12.266, 12.268, 12.269, 12.271, 12.278, 12.295, 15.007, 15.125 and 16.062] for which use levels have not been provided exposure data are required. On the basis of such additional data, these flavouring substances should be re-evaluated using the Procedure. Subsequently, additional toxicological data might become necessary.

In order to determine whether the conclusion for the candidate substances evaluated through the Procedure can be applied to the material of commerce, it is necessary to consider the available specifications. Specifications including purity criteria and identity for the materials of commerce have been provided for 78 of the 80 candidate substances. For the substances evaluated using the Procedure, specifications are missing for the two substances [FL-no: 12.226 and 15.125] and are incomplete for one substance [FL-no: 12.282].

Thus, the final evaluation of the materials of commerce cannot be performed for three substances [FL-no: 12.266, 12.282 and 15.125], pending further information on stereoisomeric/positional composition and/or specifications.

For 12 candidate substances [FL-no: 12.093, 12.094, 12.097, 12.100, 12.112, 12.116, 12.120, 12.164, 12.167, 12.199, 15.102 and 15.125] the Panel considered that additional toxicity data are needed. Furthermore, the Panel concluded that for five substances [FL-no: 12.159, 12.172, 12.174, 12.304, and 16.057], additional genotoxicity data are required and for four candidate substances [FL-no: 12.268, 12.269, 12.271 and 12.295], data on use as flavouring substances in Europe are required.

Accordingly, the final evaluation of the materials of commerce cannot be performed for 23 substances (including the nine substances not evaluated through the Procedure) [FL-no: 12.093, 12.094, 12.097, 12.100, 12.112, 12.116, 12.120, 12.159, 12.164, 12.167, 12.172, 12.174, 12.199, 12.266, 12.268, 12.269, 12.271, 12.282, 12.295, 12.304, 15.102, 15.125 and 16.057], pending further information (see Table 9.1).

The remaining 57 flavouring substances evaluated through the Procedure [FL-no: 12.096, 12.098, 12.099, 12.103, 12.104, 12.106, 12.111, 12.117, 12.124, 12.125, 12.127, 12.129, 12.135, 12.136, 12.151, 12.152, 12.158, 12.163, 12.165, 12.166, 12.177, 12.178, 12.180, 12.181, 12.182, 12.183, 12.189, 12.191, 12.196, 12.200, 12.205, 12.214, 12.221, 12.250, 12.277, 12.278, 12.298, 12.299, 12.300, 12.301, 12.302, 12.303, 12.305, 12.306, 15.007, 15.047, 15.048, 15.056, 15.081, 15.083, 15.103, 15.110, 15.111, 15.134, 16.062, 16.114 and 16.122] would present no safety concern at the levels of intake estimated on the basis of the MSDI approach.

Table 9.1: Candidate substances (n=23) in FGE.08Rev5 with inadequate data

Substances evaluated through the Procedure							Substances not evaluated through the Procedure		
Substance	Specifications not provided	Missing information on stereoisomerism	Missing information on composition of the mixture	Missing ID test	Specification not adequate (e.g. missing BP, RI, SG, SE, SW)	Request for additional toxicity data	Request for additional genotoxicity data	Missing tonnage data. No MSDI can be calculated	Substance
12.093						X			12.093
12.094						X			12.094
12.097						X			12.097
12.100						X			12.100
12.112						X			12.112
12.116						X			12.116
12.120						X			12.120
12.159							X		12.159
12.164						X			12.164
12.167						X			12.167
12.172							X		12.172
12.174							X		12.174
12.199						X			12.199
12.266	X								12.266
12.268		X		X				X	12.268
12.269		X		X				X	12.269
12.271				X				X	12.271
12.282				X					12.282
12.295								X	12.295
12.304							X		12.304
15.102						X			15.102
15.125	X					X			15.125
16.057							X		16.057

TABLE 1: SPECIFICATION SUMMARY OF THE SUBSTANCES IN THE FGE.08REV5

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 08, Revision 5

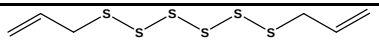
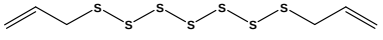
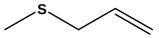
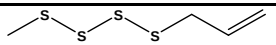
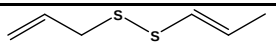
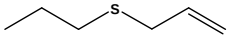
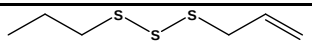
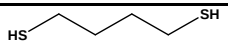
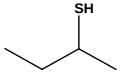
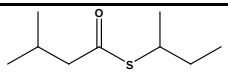
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
12.093	Diallyl hexasulfide		3533 11912	Solid $C_6H_{10}S_6$ 274.50	Practically insoluble or insoluble Freely soluble	470 76 NMR 95 %	n.a. n.a.	CASrn 137443-18-6 to be introduced in the Register. Substance no longer supported by Industry (EFSA, 2011aj).
12.094	Diallyl heptasulfide		3533 11912	Solid $C_6H_{10}S_7$ 306.60	Practically insoluble or insoluble Freely soluble	539 121 NMR 95 %	n.a. n.a.	CASrn 139693-24-6 to be introduced in the Register. Substance no longer supported by Industry (EFSA, 2011aj).
12.096	Allyl methyl sulfide		11429 10152-76-8	Liquid C_4H_8S 88.17	Practically insoluble or insoluble Freely soluble	93 MS 95 %	1.468-1.474 0.874-0.880	
12.097	Allyl methyl tetrasulfide		90195-83-8	Solid $C_4H_8S_4$ 184.37	Practically insoluble or insoluble Freely soluble	267 23 MS 95 %	n.a. n.a.	Substance no longer supported by Industry (EFSA, 2011aj).
12.098	Allyl prop-1-enyl disulfide		11433 33368-82-0	Liquid $C_6H_{10}S_2$ 146.28	Practically insoluble or insoluble Freely soluble	205 NMR 95 %	1.541-1.547 1.004-1.010	Mixture of E/Z stereoisomers: 50-70 % (E) (EFFA, 2012j).
12.099	Allyl propyl sulfide		11434 27817-67-0	Liquid $C_6H_{12}S$ 148.29	Practically insoluble or insoluble Freely soluble	144 MS 95 %	1.474-1.480 0.860-0.866	
12.100	Allyl propyl trisulfide		11435 33922-73-5	Liquid $C_6H_{12}S_3$ 180.36	Practically insoluble or insoluble Freely soluble	253 MS 95 %	1.584-1.590 1.050-1.056	Substance no longer supported by Industry (EFSA, 2011aj).
12.103	Butane-1,4-dithiol		1191-08-8	Liquid $C_4H_{10}S_2$ 122.24	Slightly soluble Freely soluble	73 (13 hPa) MS 95 %	1.524-1.530 1.041-1.047	
12.104	Butane-2-thiol		513-53-1	Liquid $C_4H_{10}S$ 90.18	Slightly soluble Freely soluble	85 MS 95 %	1.431-1.437 0.826-0.832	Racemate (EFFA, 2010a).
12.106	S-2-Butyl 3-methylbutanethioate		2432-91-9	Liquid $C_9H_{18}OS$ 174.30	Practically insoluble or insoluble Freely soluble	181 MS	1.452-1.459 0.898-0.906	Racemate (EFFA, 2010a).

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 08, Revision 5

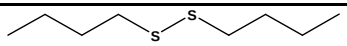
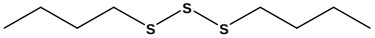
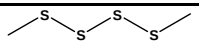
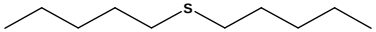
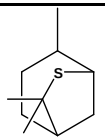
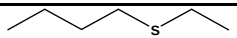
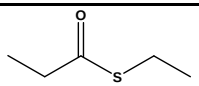
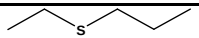
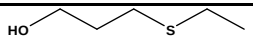
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
						98 %		
12.111	Dibutyl disulfide		 629-45-8	Liquid $C_8H_{18}S_2$ 178.35	Practically insoluble or insoluble Freely soluble	101 (13 hPa) MS 95 %	1.488-1.494 0.934-0.940	
12.112	Dibutyl trisulfide		 5943-31-7	Liquid $C_8H_{18}S_3$ 210.41	Practically insoluble or insoluble Freely soluble	139 (16 hPa) NMR 95 %	1.525-1.531 1.015-1.021	Substance no longer supported by Industry (EFSA, 2011aj).
12.116	Dimethyl tetrasulfide		 11459 5756-24-1	Liquid $C_2H_6S_4$ 158.31	Practically insoluble or insoluble Freely soluble	60 (1.3 hPa) MS 95 %	1.658-1.664 1.303-1.309	Substance no longer supported by Industry (EFSA, 2011aj).
12.117	Dipentyl sulfide		 872-10-6	Liquid $C_{10}H_{22}S$ 174.34	Practically insoluble or insoluble Freely soluble	108 (20 hPa) MS 95 %	1.450-1.456 0.836-0.842	
12.120	2,8-Epithio-p-menthane		4108 68398-18-5	Liquid $C_{10}H_{18}S$ 170.31	Practically insoluble or insoluble Freely soluble	114 (31 hPa) MS 95 %	1.511-1.517 0.999-1.005	Occurs as the mixture of diastereoisomers where the methyl group and S-containing bridge are in cis- and trans-position relative to each other, hence a mixture of cis- and trans- of the substituents on the six membered ring (equal ratio) (EFFA, 2010a).
12.124	Ethyl butyl sulfide		 638-46-0	Liquid $C_6H_{14}S$ 118.24	Practically insoluble or insoluble Freely soluble	144 MS 95 %	1.443-1.449 0.834-0.840	
12.125	Ethyl propanethioate		 2432-42-0	Liquid $C_5H_{10}OS$ 118.19	Practically insoluble or insoluble Freely soluble	136 MS 95 %	1.452-1.458 0.957-0.963	
12.127	Ethyl propyl sulfide		 11479 4110-50-3	Liquid $C_5H_{12}S$ 104.21	Practically insoluble or insoluble Freely soluble	118 MS 95 %	1.440-1.446 0.836-0.842	
12.129	3-(Ethylthio)propan-1-ol		 18721-61-4	Liquid $C_5H_{12}OS$ 120.21	Slightly soluble Freely soluble	99 (13 hPa) NMR 95 %	1.480-1.486 0.989-0.995	

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 08, Revision 5

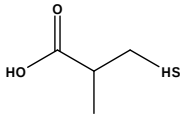
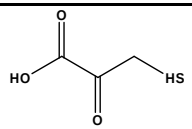
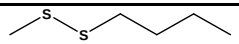
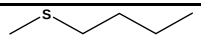
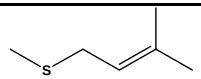
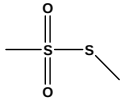
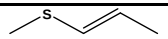
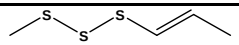
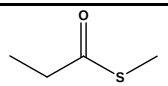
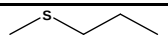
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
12.135	3-Mercapto-2-methylpropionic acid		 26473-47-2	Solid $C_4H_8O_2S$ 120.17	Soluble Freely soluble	113 (13 hPa) 43 NMR 95 %	n.a. n.a.	Racemate (EFFA, 2010a).
12.136	3-Mercapto-2-oxopropionic acid		3901 2464-23-5	Solid $C_3H_4O_3S$ 120.12	Soluble Freely soluble	253 97 NMR 95 %	n.a. n.a.	
12.151	Methyl butyl disulfide		 60779-24-0	Liquid $C_5H_{12}S_2$ 136.27	Practically insoluble or insoluble Freely soluble	58 (13 hPa) MS 95 %	1.497-1.503 0.984-0.990	
12.152	Methyl butyl sulfide		 628-29-5	Liquid $C_5H_{12}S$ 104.21	Practically insoluble or insoluble Freely soluble	123 MS 95 %	1.442-1.448 0.839-0.845	
12.158	Methyl isoprenyl sulfide		 5897-45-0	Liquid $C_6H_{12}S$ 116.22	Practically insoluble or insoluble Freely soluble	145 NMR 95 %	1.478-1.484 0.862-0.868	Register name to be changed to Methyl 3-methyl-2-butenylsulphide.
12.159	Methyl methanethiosulfonate		 11520 2949-92-0	Liquid $C_2H_6O_2S_2$ 126.19	Slightly soluble Freely soluble	104 (13 hPa) MS 95 %	1.507-1.513 1.315-1.321	
12.163	Methyl prop-1-enyl sulfide		 11538 10152-77-9	Liquid C_4H_8S 88.17	Practically insoluble or insoluble Freely soluble	103 NMR 95 %	1.487-1.493 0.867-0.873	Mixture of E/Z stereoisomers: 50-70 % (E) (EFFA, 2012j).
12.164	Methyl prop-1-enyl trisulfide		 11539 33368-80-8	Liquid $C_4H_8S_3$ 152.17	Practically insoluble or insoluble Freely soluble	223 NMR 95 %	1.586-1.592 1.112-1.118	Substance no longer supported by Industry (EFSA, 2011aj). Mixture of E/Z stereoisomers: 50-70 % (E) (EFFA, 2012j).
12.165	S-Methyl propanethioate		4172 5925-75-7	Liquid C_4H_8OS 104.17	Practically insoluble or insoluble Freely soluble	120 MS 95 %	1.459-1.465 0.891-0.897	
12.166	Methyl propyl sulfide		 11541 3877-15-4	Liquid $C_4H_{10}S$ 90.18	Practically insoluble or insoluble Freely soluble	96 MS	1.438-1.444 0.834-0.840	

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 08, Revision 5

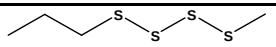
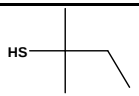
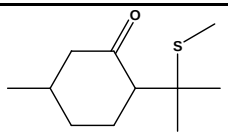
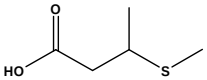
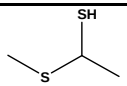
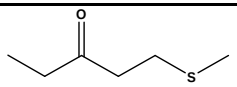
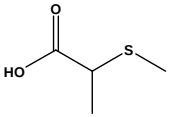
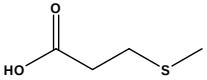
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
						95 %		
12.167	Methyl propyl tetrasulfide		87148-08-1	Liquid $C_4H_{10}S_4$ 186.18	Practically insoluble or insoluble Freely soluble	259 NMR 95 %	1.622-1.628 1.197-1.203	Substance no longer supported by Industry (EFSA, 2011aj).
12.172	2-Methylbutane-2-thiol		1679-09-0	Liquid $C_5H_{12}S$ 104.21	Practically insoluble or insoluble Freely soluble	99 MS 95 %	1.432-1.438 0.809-0.815	Substance no longer supported by Industry (EFSA, 2011aj).
12.174	2-Methylpropane-2-thiol		11537 75-66-1	Liquid $C_4H_{10}S$ 90.18	Slightly soluble Freely soluble	64 MS 95 %	1.417-1.423 0.797-0.803	Substance no longer supported by Industry (EFSA, 2011aj).
12.177	8-(Methylthio)-p-menthan-3-one		32637-94-8	Liquid $C_{11}H_{20}OS$ 200.34	Practically insoluble or insoluble Freely soluble	72 (0.1 hPa) NMR 95 %	1.495-1.501 0.951-0.957	Mixture of isomers ((R/R), (R/S), (S/R) & (S/S) at equal ratio, i.e. 25 % of each) (EFFA, 2010a). CASrn in Register refers to (Z) isomer. CASrn to be changed.
12.178	3-(Methylthio)butyric acid		16630-65-2	Liquid $C_5H_{10}O_2S$ 134.19	Soluble Freely soluble	127 (13 hPa) MS 95 %	1.479-1.486 1.102-1.108	Racemate (EFFA, 2010a).
12.180	1-(Methylthio)ethane-1-thiol		31331-53-0	Liquid $C_3H_8S_2$ 108.22	Slightly soluble Freely soluble	58 (35 hPa) NMR 95 %	1.522-1.528 0.879-0.885	Racemate (EFFA, 2010a).
12.181	1-(Methylthio)pentan-3-one		66735-69-1	Liquid $C_6H_{12}OS$ 132.22	Practically insoluble or insoluble Freely soluble	88 (16 hPa) MS 95 %	1.467-1.473 0.987-0.993	
12.182	2-(Methylthio)propionic acid		58809-73-7	Solid $C_4H_8O_2S$ 120.17	Practically insoluble or insoluble Freely soluble	110 (13 hPa) 48 MS 95 %	n.a. n.a.	Racemate (EFFA, 2010a).
12.183	3-(Methylthio)propionic acid		646-01-5	Liquid $C_4H_8O_2S$ 120.17	Soluble Freely soluble	125 (16 hPa) MS 95 %	1.485-1.491 1.155-1.161	

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 08, Revision 5

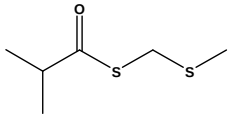
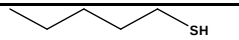
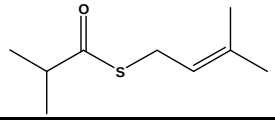
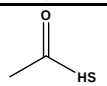
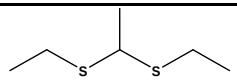
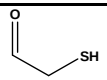
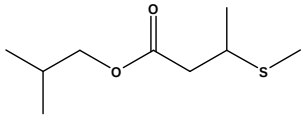
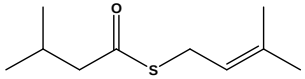
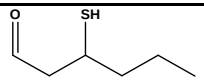
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
12.189	S-(Methylthiomethyl) 2-methylpropanethioate		 77974-85-7	Liquid $C_6H_{12}OS_2$ 164.03	Practically insoluble or insoluble Freely soluble	273 NMR 95 %	1.452-1.456 1.031-1.037	
12.191	Pentane-1-thiol		 110-66-7	Liquid $C_5H_{12}S$ 104.21	Slightly soluble Freely soluble	126 MS 95 %	1.441-1.450 0.831-0.844	
12.196	S-Prenyl thioisobutyrate		 53626-94-1	Liquid $C_9H_{16}OS$ 172.28	Practically insoluble or insoluble Freely soluble	100 (20 hPa) NMR 95 %	1.483-1.489 1.109-1.115	
12.199	Ethanethioic acid		4210 507-09-5	Liquid C_2H_4OS 76.11	Slightly soluble Freely soluble	88 MS 95 %	1.459-1.465 1.066-1.072	Substance no longer supported by Industry (EFSA, 2011aj).
12.200	1,1-bis(Ethylthio)-ethane		 14252-42-7	Liquid $C_6H_{14}S_2$ 150.30	Practically insoluble or insoluble Freely soluble	80 (13 hPa) MS 95 %	1.499-1.505 0.967-0.973	
12.205	Mercaptoacetaldehyde		 4124-63-4	Liquid C_2H_4OS 76.11	Slightly soluble Freely soluble	84 NMR 95 %	1.495-1.501 1.112-1.118	
12.214	Isobutyl-3-(methylthio)butyrate		4150 127931-21-9	Liquid $C_9H_{18}O_2S$ 190.30	Practically insoluble or insoluble Freely soluble	224 NMR 95 %	1.458-1.464 0.875-0.881	Racemate (EFFA, 2010a).
12.221	S-Prenyl thioisopentanoate		 75631-91-3	Liquid $C_{10}H_{18}OS$ 186.28	Practically insoluble or insoluble Freely soluble	248 MS 95 %	1.475-1.481 1.003-1.009	
12.250	3-Mercaptohexanal		4585 51755-72-7	Liquid $C_6H_{12}OS$ 132.22	Practically insoluble or insoluble Soluble	41-42 (2.7 hPa) MS 92 %	1.515-1.525 0.973-1.083	Racemate (EFFA, 2012j). Secondary components: 5 % Trans-2-hexenal, 1 % 3-Mercaptohexenal diethyl ether, 1 % dimers.

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 08, Revision 5

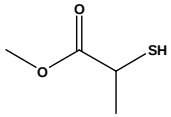
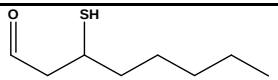
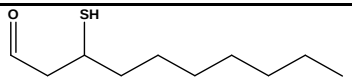
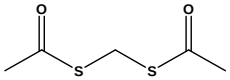
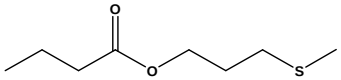
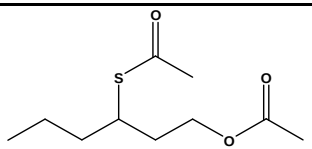
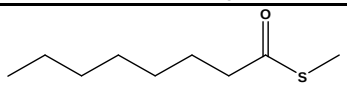
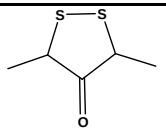
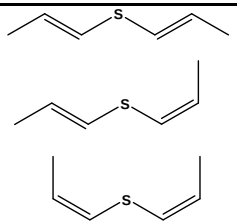
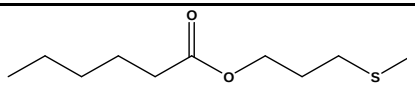
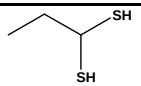
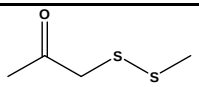
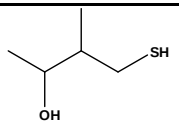
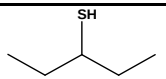
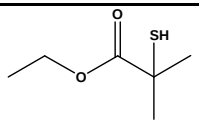
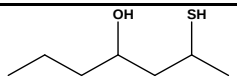
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
12.266	Methyl-2-mercaptopropionate		53907-46-3					AV 7), BP 8), ID 9), MP 10), PF 11), RI 12), SG 13), SE 14), SW 15) Racemate (EFFA, 2012j).
12.268	3-Mercaptooctanal 6)		473438-39-0	Liquid $C_8H_{16}OS$ 160.28	Slightly soluble Freely soluble	220 > 93 %	1.459 0.930	ID 9). Stereoisomeric composition to be specified. CASrn in Register refers to the racemate. Substance no longer supported by Industry (EFSA, 2011aj).
12.269	3-Mercaptodecanal 6)			Liquid $C_{10}H_{20}OS$ 188.33	Slightly soluble Freely soluble	260 95 %	1.460 0.917	ID 9). Stereoisomeric composition to be specified. CASrn is missing. Substance no longer supported by Industry (EFSA, 2011aj).
12.271	Methanedithiol diacetate		2506-35-6	Liquid $C_5H_8O_2S_2$ 164.25	Slightly soluble Freely soluble	211 > 95 %	1.530 1.232	ID 9). Substance no longer supported by Industry (EFSA, 2011aj).
12.277	3-(Methylthio)propyl butyrate		4160 16630-60-7	Liquid $C_8H_{16}O_2S$ 176.20	Slightly soluble Soluble	232 IR NMR MS 99.9 %	1.4609-1.4611 0.9076-0.9080	
12.278	3-Acetyl-mercaptohexyl acetate		136954-25-1	Liquid $C_8H_{18}O_3S$ 218.3	Insoluble Soluble	212 MS 98 %	1.4681 1.0352	Racemate (EFFA, 2012j).
12.282	(S)-Methyl octanethioate		2432-83-9	Liquid $C_9H_{18}OS$ 174	Insoluble Soluble	165 (35 hPa) > 97 %	1.464-1.465 0.922-0.924	ID 9).
12.295	3,5-Dimethyl-1,2-dithiolane-4-one		122152-29-8	Liquid $C_5H_8OS_2$ 148.25		235 NMR 95%	1.552 1.194	Mixture of isomers ((R/R), (R/S), (S/R) & (S/S) at equal ratio, i.e. 25 % of each) (EFFA, 2010a). Substance no longer supported by Industry (EFSA, 2011aj).

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 08, Revision 5

FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
12.298	Di-(1-propenyl)-sulfid (mixture)		4386	Liquid $C_6H_{10}S$ 114.211		137-140 MS 95 %	1.512-1.514 0.9048-0.9058	Mixture of isomers with CASrn: 65819-74-1, 37981-37-6, 37981-36-5. Mixture of E/Z stereoisomers: 20-40 % (E,E), 40-60 % (E,Z), 10-20 % (Z,Z) (EFFA, 2012j).
12.299	3-(Methylthio)propyl hexanoate		4436 906079-63-8	Liquid $C_{10}H_{20}O_2S$ 204.12	Insoluble Soluble	271 n.a. NMR MS 98 %	1.4584 0.9641	
12.300	1,1-Propanedithiol		4670 88497-17-0	Liquid $C_3H_8S_2$ 108.20	Slightly soluble Soluble	137 n.a. IR NMR MS >95 %	1.507 1.015	
12.301	Methyl-2-oxo-propyl disulfide		4696 122861-78-3	Liquid $C_4H_8OS_2$ 136.24	Soluble Soluble	189.2 n.a. IR NMR MS >90 %	1.524 1.132-1.145	At least 90%, secondary components 1-mercaptopropan-2-one (less than 8 %), 1,1-disfulanediyldipropan-2-one (less than 5 %) and 1,3-dimethyltrisulfane (less than 3 %) (Flavour Industry, 2012c).
12.302	2-Butanol, 4-mercapto-3-methyl		4698 33959-27-2	Liquid $C_5H_{12}OS$ 120.21	Soluble Soluble	190.1 n.a. IR NMR MS >95 %	1.472 0.968-0.980	Racemate (EFFA, 2012j).
12.303	3-Pentanethiol		4694 616-31-9	Liquid $C_5H_{12}S$ 104.21	Sparingly soluble Soluble	112-113 n.a. IR NMR MS >95 %	1.444-1.448 0.824-0.836	
12.304	Ethyl-2-mercapto-2-methyl propanoate		4714 33441-50-8	Liquid $C_6H_{12}O_2S$ 148.06	Slightly soluble Soluble	186 IR NMR MS >95 %	1.4245-1.4645 0.961-1.081	
12.305	2-Mercapto-4-heptanol		4733 1006684-20-	Liquid $C_7H_{16}OS$ 148.09	Slightly soluble Soluble	220 IR NMR MS	1.4705-1.4745 0.888-1.008	Mixture of diastereoisomers: 25 % of each (EFFA, 2012j).

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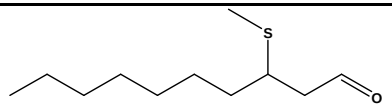
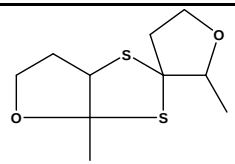
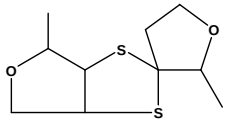
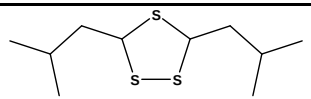
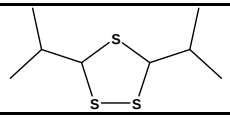
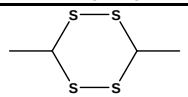
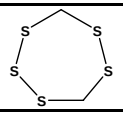
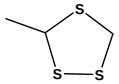
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
			3			>95 %		
12.306	3-(Methylthio)-decanal		4734 1256932-15-6	Liquid $C_{11}H_{22}OS$ 202.14	Slightly soluble Soluble	282 IR NMR MS >95 %	1.4765-1.4805 0.852-0.972	Racemate (EFFA, 2012j).
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**	3270 2325 38325-25-6	Liquid $C_{10}H_{16}O_2S_2$ 232.20	Insoluble Soluble	135-140 IR MS 95 %	1.559-1.565 1.200-1.208	Composition of structural isomers, I: 65 %, II: 35 %. Mixture of stereoisomers (Flavour Industry, 2012c).
15.047	3,5-Di-isobutyl-1,2,4-trithiolane		92900-67-9	Solid $C_{10}H_{20}S_3$ 236.40	Practically insoluble or insoluble Freely soluble	295 156 NMR 95 %	n.a. n.a.	Mixture of isomers ((R/R), (R/S), (S/R) & (S/S) at equal ratio, i.e. 25 % of each) (EFFA, 2010a).
15.048	3,5-Di-isopropyl-1,2,4-trithiolane		54934-99-5	Solid $C_8H_{16}S_3$ 208.39	Practically insoluble or insoluble Freely soluble	263 133 MS 95 %	n.a. n.a.	Mixture of isomers ((R/R), (R/S), (S/R) & (S/S) at equal ratio, i.e. 25 % of each) (EFFA, 2010a).
15.056	3,6-Dimethyl-1,2,4,5-tetrathiane		67411-27-2	Solid $C_4H_8S_4$ 184.35	Practically insoluble or insoluble Freely soluble	264 198 MS 95 %	n.a. n.a.	Mixture of diastereoisomers (EFFA., 2010a). Composition of stereoisomeric mixture: 50 % cis and 50 % trans (EFFA, 2012j).
15.081	Lenthionine		11619 292-46-6	Solid $C_2H_4S_5$ 188.35	Practically insoluble or insoluble Freely soluble	287 61 MS 95 %	n.a. n.a.	
15.083	3-Methyl-1,2,4-trithiolane		51647-38-2	Solid $C_3H_6S_3$ 138.28	Practically insoluble or insoluble Freely soluble	198 111 MS 95 %	n.a. n.a.	Racemate (EFFA, 2010a).

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 08, Revision 5

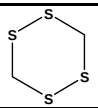
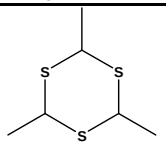
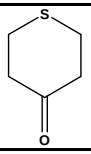
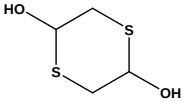
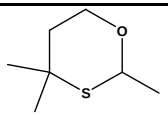
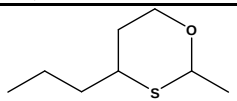
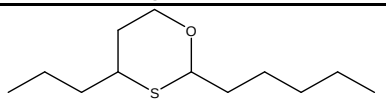
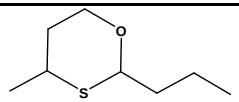
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
15.102	Tetrahydrothiophene		 110-01-0	Liquid C_4H_8S 88.17	Slightly soluble Freely soluble	120 MS 95 %	1.499-1.505 0.995-1.001	
15.103	1,2,4,5-Tetrathiane		 291-22-5	Solid $C_2H_4S_4$ 156.29	Practically insoluble or insoluble Freely soluble	239 126 MS 95 %	n.a. n.a.	
15.110	2,4,6-Trimethyl-1,3,5-trithiane		 2765-04-0	Solid $C_6H_{12}S_3$ 180.34	Practically insoluble or insoluble Freely soluble	246 125 MS 95 %	n.a. n.a.	Mixture of diastereoisomers (EFFA, 2010a). Composition of stereoisomeric mixture: 10-25 % cis and 75-90 % trans (EFFA, 2012j).
15.111	1,2,4-Trithiolane		 289-16-7	Solid $C_2H_4S_3$ 124.23	Practically insoluble or insoluble Freely soluble	102 (13 hPa) 104 MS 95 %	n.a. n.a.	
15.125	4-Tetrahydrothiopyranone		 1072-72-6					AV 7), BP 8), ID 9), MP 10), PF 11), RI 12), SG 13), SE 14), SW 15) Substance no longer supported by Industry (EFSA, 2011aj).
15.134	2,5-Dihydroxy-1,4-dithiane		3826 40018-26-6	Solid $C_4H_8O_2S_2$ 152.23	Slightly soluble Soluble	n.a. 130 IR NMR 97 %	n.a. n.a.	Mixture of diastereoisomers (25-30 % (2S,5S + 2R/5R) and 70-75 % (2S,5R + 2R/5S)) (Flavour Industry, 2012a).
16.057	2,4,4-Trimethyl-1,3-oxathiane		 72472-02-7	Solid $C_7H_{14}OS$ 146.25	Practically insoluble or insoluble Freely soluble	70 (25 hPa) 32 NMR 95 %	n.a. n.a.	Racemate (EFFA, 2010a).
16.062	trans-2-Methyl-4-propyl-1,3-oxathiane		 59324-17-3	Liquid $C_8H_{16}OS$ 160.28	Insoluble Soluble	89-90 (16 hPa) n.a. MS 98 %	1.475-1.485 0.970-0.982	Mixture of diastereoisomers: 25 % of each (EFFA, 2012j).
16.114	2-Pentyl-4-propyl-1,3-oxathiane 6)		4499 59323-81-8	Liquid $C_{12}H_{24}OS$ 216.38	Almost insoluble Soluble	299 n.a. NMR MS 97 %	1.475-1.481 0.936-0.942	Mixture of diastereoisomers: 25 % of each (EFFA, 2012j).

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 08, Revision 5

FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
16.122	4-Methyl, 2-propyl, 1-3-oxathiane 6)		4677 1064678-08-5	Liquid $C_8H_{16}OS$ 160.09	Slightly soluble Soluble	80 (13 hPa) n.a. IR NMR MS 97 %	1.4769 0.940-0.950	Mixture of diastereoisomers: 25 % of each (EFFA, 2012j).

1) Solubility in water, if not otherwise stated.

2) Solubility in 95 % ethanol, if not otherwise stated.

3) At 1013.25 hPa, if not otherwise stated.

4) At 20°C, if not otherwise stated.

5) At 25°C, if not otherwise stated.

6) Stereoisomeric composition not specified.

7) AV: Missing minimum assay value.

8) BP: Missing boiling point.

9) ID: Missing identification test.

10) MP: Missing melting point.

11) PF: Missing data on physical form.

12) RI: Missing refractive index.

13) SG: Missing specific gravity.

14) SE: Missing data on solubility in ethanol.

15) SW: Missing data on solubility.

TABLE 2A: SUMMARY OF SAFETY EVALUATION APPLYING THE PROCEDURE (BASED ON INTAKES CALCULATED BY THE MSDI APPROACH)

Table 2a: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)

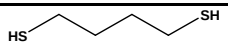
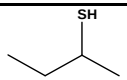
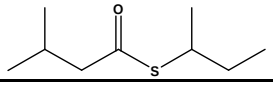
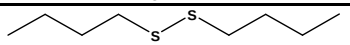
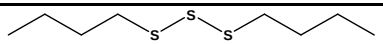
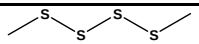
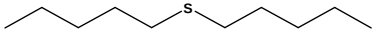
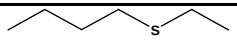
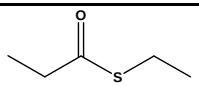
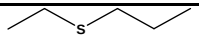
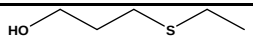
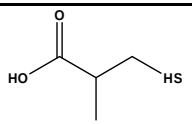
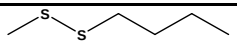
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
12.103	Butane-1,4-dithiol		0.3	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
12.104	Butane-2-thiol		0.18	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
12.106	S-2-Butyl 3-methylbutanethioate		0.8	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
12.111	Dibutyl disulfide		0.37	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
12.112	Dibutyl trisulfide		0.12	Class I B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		a)
12.116	Dimethyl tetrasulfide		0.016	Class I B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		a)
12.117	Dipentyl sulfide		0.0037	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
12.124	Ethyl butyl sulfide		0.037	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
12.125	Ethyl propanethioate		0.012	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
12.127	Ethyl propyl sulfide		0.085	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
12.129	3-(Ethylthio)propan-1-ol		0.12	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
12.135	3-Mercapto-2-methylpropionic acid		0.12	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
12.151	Methyl butyl disulfide		0.0061	Class I B3: Intake below threshold,	4)	6)	

Table 2a: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)

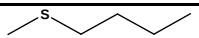
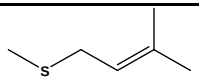
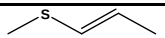
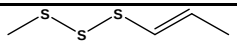
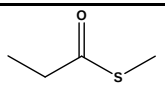
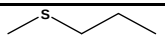
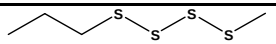
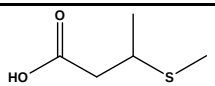
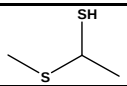
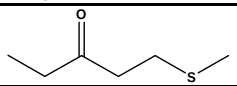
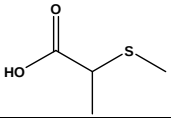
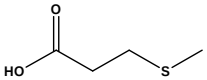
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
				B4: Adequate NOAEL exists			
12.152	Methyl butyl sulfide		0.0024	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
12.158	Methyl isoprenyl sulfide		0.0012	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
12.163	Methyl prop-1-enyl sulfide		0.0097	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
12.164	Methyl prop-1-enyl trisulfide		0.0061	Class I B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		a)
12.165	S-Methyl propanethioate		0.012	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
12.166	Methyl propyl sulfide		0.0024	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
12.167	Methyl propyl tetrasulfide		0.0037	Class I B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		a)
12.178	3-(Methylthio)butyric acid		0.12	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
12.180	1-(Methylthio)ethane-1-thiol		0.12	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
12.181	1-(Methylthio)pentan-3-one		0.12	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
12.182	2-(Methylthio)propionic acid		0.011	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
12.183	3-(Methylthio)propionic acid		0.21	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	

Table 2a: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)

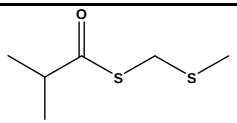
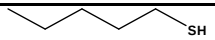
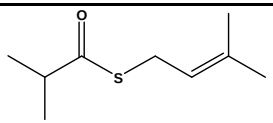
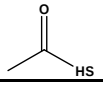
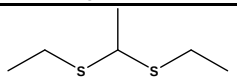
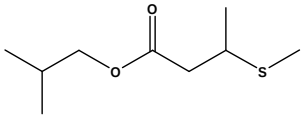
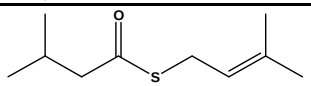
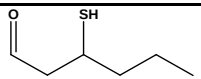
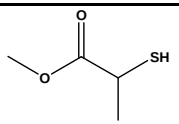
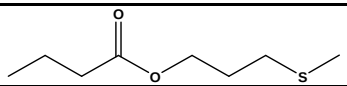
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
12.189	S-(Methylthiomethyl) 2-methylpropanethioate		0.061	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
12.191	Pentane-1-thiol		0.12	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
12.196	S-Prenyl thioisobutyrate		0.012	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
12.199	Ethanethioic acid		0.0012	Class I B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		a)
12.200	1,1-bis(Ethylthio)-ethane		0.0012	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
12.205	Mercaptoacetaldehyde		0.011	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
12.214	Isobutyl-3-(methylthio)butyrate		0.12	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
12.221	S-Prenyl thioisopentanoate		0.012	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
12.250	3-Mercaptohexanal		0.012	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
12.266	Methyl-2-mercaptopropionate		0.12	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	8)	
12.277	3-(Methylthio)propyl butyrate		6.1	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	

Table 2a: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)

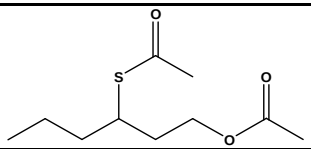
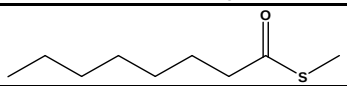
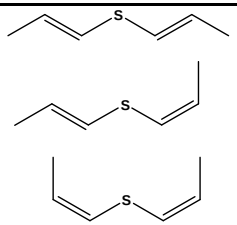
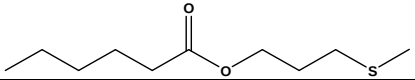
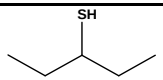
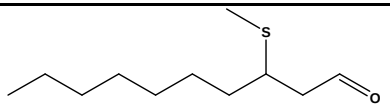
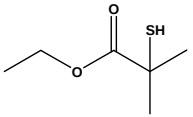
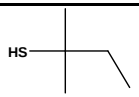
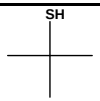
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
12.278	3-Acetyl-mercaptohexyl acetate		1.2	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
12.282	(S)-Methyl octanethioate		0.24	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	7)	
12.298	Di-(1-propenyl)-sulfid (mixture)		0.12	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
12.299	3-(Methylthio)propyl hexanoate		0.061	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
12.303	3-Pentanethiol		0.03	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
12.306	3-(Methylthio)-decanal		0.12	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
12.304	Ethyl-2-mercapto-2-methyl propanoate		0.012	Class I No evaluation			b)
12.172	2-Methylbutane-2-thiol		0.15	Class I No evaluation			c)
12.174	2-Methylpropane-2-thiol		0.0012	Class I No evaluation			c)

Table 2a: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)

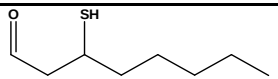
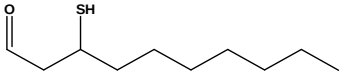
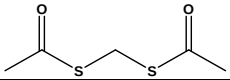
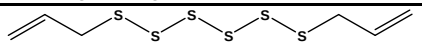
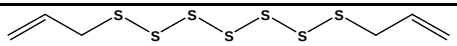
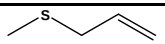
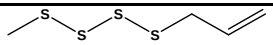
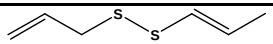
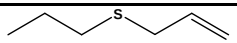
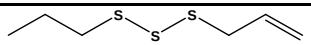
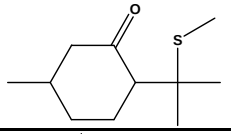
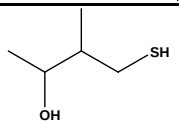
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
12.268	3-Mercaptooctanal			Class I No evaluation			d)
12.269	3-Mercaptodecanal			Class I No evaluation			d)
12.271	Methanedithiol diacetate			Class I No evaluation			d)
12.093	Diallyl hexasulfide		0.011	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		a)
12.094	Diallyl heptasulfide		0.011	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		a)
12.096	Allyl methyl sulfide		0.99	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
12.097	Allyl methyl tetrasulfide		0.012	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		a)
12.098	Allyl prop-1-enyl disulfide		0.17	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
12.099	Allyl propyl sulfide		1.6	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
12.100	Allyl propyl trisulfide		0.12	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		a)
12.177	8-(Methylthio)-p-menthan-3-one		0.37	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
12.302	2-Butanol, 4-mercapto-3-methyl		0.061	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	

Table 2a: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)

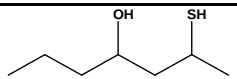
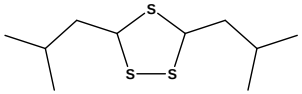
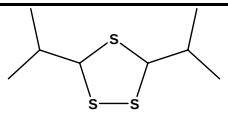
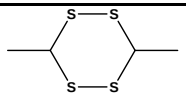
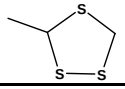
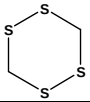
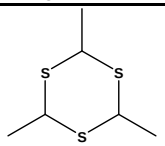
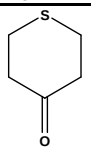
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
12.305	2-Mercapto-4-heptanol		0.12	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.047	3,5-Di-isobutyl-1,2,4-trithiolane		0.024	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.048	3,5-Di-isopropyl-1,2,4-trithiolane		0.0061	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.056	3,6-Dimethyl-1,2,4,5-tetrathiane		0.0024	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.083	3-Methyl-1,2,4-trithiolane		0.0024	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.102	Tetrahydrothiophene		0.024	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
15.103	1,2,4,5-Tetrathiane		0.073	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.110	2,4,6-Trimethyl-1,3,5-trithiane		0.0061	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.111	1,2,4-Trithiolane		2.4	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.125	4-Tetrahydrothiopyranone		0.12	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		a)

Table 2a: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)

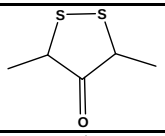
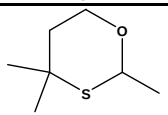
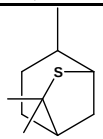
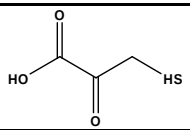
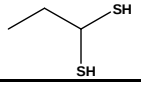
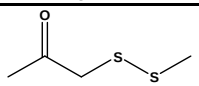
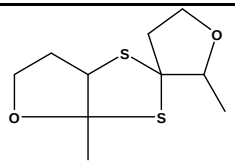
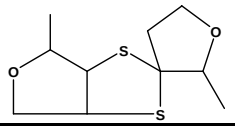
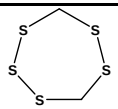
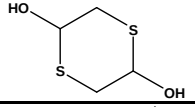
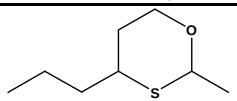
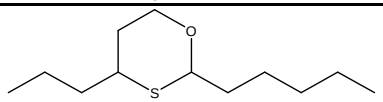
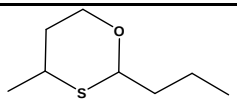
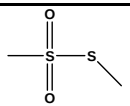
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
12.295	3,5-Dimethyl-1,2-dithiolane-4-one			Class II No evaluation			d)
16.057	2,4,4-Trimethyl-1,3-oxathiane		0.0012	Class II No evaluation			b)
12.120	2,8-Epithio-p-menthane		3.7	Class III B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
12.136	3-Mercapto-2-oxopropionic acid		0.24	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
12.300	1,1-Propanedithiol		0.12	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
12.301	Methyl-2-oxo-propyl disulfide		0.061	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
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	4)	6)	

Table 2a: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)

FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
15.081	Lenthionine		0.012	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.134	2,5-Dihydroxy-1,4-dithiane		6.1	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
16.062	trans-2-Methyl-4-propyl-1,3-oxathiane		1.0	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
16.114	2-Pentyl-4-propyl-1,3-oxathiane		0.12	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
16.122	4-Methyl, 2-propyl, 1-3-oxathiane		0.24	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
12.159	Methyl methanethiosulfonate		0.061	Class III No evaluation			b)

1) EU MSDI: Amount added to food as flavour in (kg / year) x 10E9 / (0.1 x population in Europe (= 375 x 10E6) x 0.6 x 365) = µg/capita/day.

2) Thresholds of concern: Class I = 1800 µg/person/day, Class II = 540 µg/person/day, Class III = 90 µg/person/day.

3) Procedure path A substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot.

4) No safety concern based on intake calculated by the MSDI approach of the named compound.

5) Data must be available on the substance or closely related substances to perform a safety evaluation.

6) No safety concern at estimated level of intake of the material of commerce meeting the specification of Table 1 (based on intake calculated by the MSDI approach).

7) 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.

8) No conclusion can be drawn due to lack of information on the purity of the material of commerce.

a) Substance no longer supported by Industry (EFSA, 2011aj).

b) Evaluation deferred pending in vivo genotoxicity data.

c) Evaluation deferred pending in vivo genotoxicity data. Substance no longer supported by Industry (EFSA, 2011aj).

d) Evaluation deferred pending EU tonnage data. Substance no longer supported by Industry (EFSA, 2011aj).

TABLE 2B: EVALUATION STATUS OF HYDROLYSIS PRODUCTS OF CANDIDATE ESTERS AND THIOACETALS (POTENTIAL)

Table 2b: Evaluation Status of Hydrolysis Products of Candidate Esters as well as thioacetals (potential)

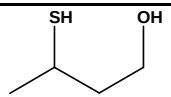
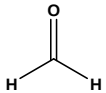
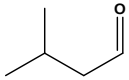
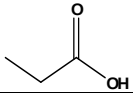
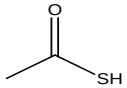
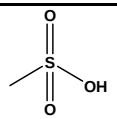
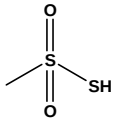
FL-no	EU Register name JECFA no	Structural formula	SCF status 1) JECFA status 2) CoE status 3) EFSA status	Structural class 4) Procedure path (JECFA) 5)	Comments
	3-Mercaptobutanol		Not evaluated as flavouring substance		Not in EU-Register
	Methanol		Not evaluated as flavouring substance		Not in EU-Register
	Hydrogensulphide		Not evaluated as flavouring substance		Not in EU-Register
	Formaldehyde		Not evaluated as flavouring substance		Not in EU-Register
	3-Methylbutanaldehyde		Not evaluated as flavouring substance		Not in EU-Register
	Proprionic acid		Not evaluated as flavouring substance		Not in EU-Register
	Thioacetic acid		Not evaluated as flavouring substance		Not in EU-Register
	Methanesulphonic acid		Not evaluated as flavouring substance		Not in EU-Register
	Methanethiosulphonic acid		Not evaluated as flavouring substance		Not in EU-Register

Table 2b: Evaluation Status of Hydrolysis Products of Candidate Esters as well as thioacetals (potential)

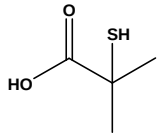
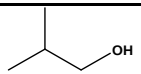
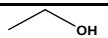
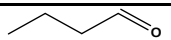
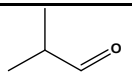
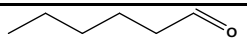
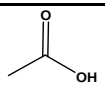
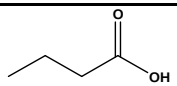
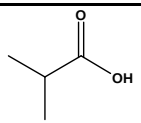
FL-no	EU Register name JECFA no	Structural formula	SCF status 1) JECFA status 2) CoE status 3) EFSA status	Structural class 4) Procedure path (JECFA) 5)	Comments
	2-Mercapto-2-methyl propanoic acid		Not evaluated as flavouring substance		Not in EU-Register
02.001	2-Methylpropan-1-ol 251		Category 1 a) Category A b)	Class I A3: Intake below threshold	
02.078	Ethanol 41		Category 1 a)	No evaluation	At the forty-sixth JECFA meeting (JECFA, 1997a), the Committee concluded that ethanol posed no safety concern at its current level of intake when ethyl esters are used as flavouring agents.
05.001	Acetaldehyde 80		Category 1 a) Category A b)	Class I A3: Intake above threshold, A4: Endogenous	
05.003	Butanal 86		Category 1 a) Category A b)	Class I A3: Intake below threshold	
05.004	2-Methylpropanal 252		Category 1 a) Category A b)	Class I A3: Intake below threshold	
05.008	Hexanal 92		Category 1 a) Category A b)	Class I A3: Intake below threshold	
08.002	Acetic acid 81		Category 1 a) Category A b)	Class I A3: Intake above threshold, A4: Endogenous	
08.005	Butyric acid 87		Category 1 a) Category A b)	Class I A3: Intake above threshold, A4: Endogenous	
08.006	2-Methylpropionic acid 253		Category 1 a) Category A b)	Class I A3: Intake below threshold	

Table 2b: Evaluation Status of Hydrolysis Products of Candidate Esters as well as thioacetals (potential)

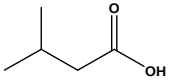
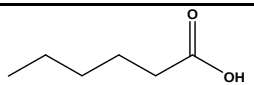
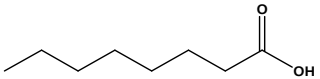
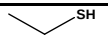
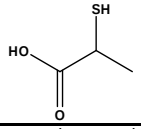
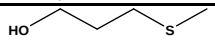
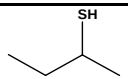
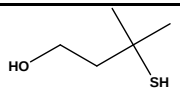
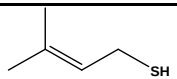
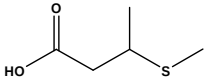
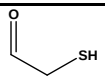
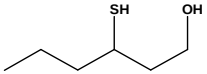
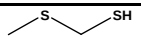
FL-no	EU Register name JECFA no	Structural formula	SCF status 1) JECFA status 2) CoE status 3) EFSA status	Structural class 4) Procedure path (JECFA) 5)	Comments
08.008	3-Methylbutyric acid 259		Category 1 a) Category A b)	Class I A3: Intake below threshold	
08.009	Hexanoic acid 93		Category 1 a) Category A b)	Class I A3: Intake above threshold, A4: Endogenous	
08.010	Octanoic acid 99		Category 1 a) Category A b)	Class I A3: Intake above threshold, A4: Endogenous	
12.003	Methanethiol 508		 Category B b)	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	
12.017	Ethanethiol 1659		 Category B b)	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	
12.039	2-Mercaptopropionic acid 551			Class I B3: Intake below threshold, B4: Adequate NOAEL exists	
12.062	3-(Methylthio)propan-1-ol 461			Class I B3: Intake below threshold, B4: Adequate NOAEL exists	
12.104	Butane-2-thiol		FGE.08	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	
12.137	3-Mercapto-3-methylbutan-1-ol 544			Class I B3: Intake below threshold, B4: Adequate NOAEL exists	
12.170	3-Methylbut-2-ene-1-thiol 522			Class I B3: Intake below threshold, B4: Adequate NOAEL exists	

Table 2b: Evaluation Status of Hydrolysis Products of Candidate Esters as well as thioacetals (potential)

FL-no	EU Register name JECFA no	Structural formula	SCF status 1) JECFA status 2) CoE status 3) EFSA status	Structural class 4) Procedure path (JECFA) 5)	Comments
12.178	3-(Methylthio)butyric acid		FGE.08	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	
12.205	Mercaptoacetaldehyde		FGE.08	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	
12.217	3-Mercaptohexan-1-ol 545			Class I B3: Intake below threshold, B4: Adequate NOAEL exists	
12.242	Methylthiomethylmercaptan 1675			Class I B3: Intake below threshold, B4: Adequate NOAEL exists	

1) Category 1: Considered safe in use Category 2: Temporarily considered safe in use Category 3: Insufficient data to provide assurance of safety in use Category 4): Not acceptable due to evidence of toxicity.

2) No safety concern at estimated levels of intake.

3) Category A: Flavouring substance, which may be used in foodstuffs Category B: Flavouring substance which can be used provisionally in foodstuffs.

4) Threshold of concern: Class I = 1800 µg/person/day, Class II = 540 µg/person/day, Class III = 90 µg/person/day.

5) Procedure path A substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot.

a) (SCF, 1995).

b) (CoE, 1992).

TABLE 3: SUPPORTING SUBSTANCES SUMMARY

Table 3: Supporting Substances Summary

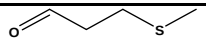
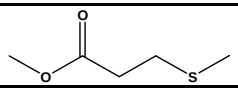
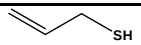
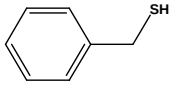
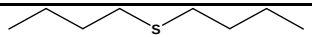
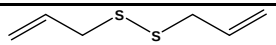
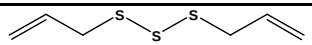
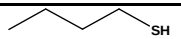
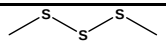
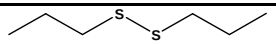
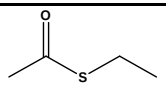
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
12.001	3-(Methylthio)propionaldehyde		2747 125 3268-49-3	466 JECFA specification (JECFA, 2000d)	28	Category B a)	
12.002	Methyl 3-(methylthio)propionate		2720 428 13532-18-8	472 JECFA specification (JECFA, 1999c)	94	Category B a)	
12.003	Methanethiol		2716 475 74-93-1	508 JECFA specification (JECFA, 2000d)	54	Category B a)	
12.004	Allylthiol		2035 476 870-23-5	521 JECFA specification (JECFA, 2000d)	0.16	Category B a)	
12.005	Phenylmethanethiol		2147 477 100-53-8	526 JECFA specification (JECFA, 1999c)	1.2	Deleted a)	
12.006	Dimethyl sulfide		2746 483 75-18-3	452 JECFA specification (JECFA, 1999c)	380	Category A a)	
12.007	Dibutyl sulfide		2215 484 544-40-1	455 JECFA specification (JECFA, 2002d)	2.3	Category A a)	
12.008	Diallyl disulfide		2028 485 2179-57-9	572 JECFA specification (JECFA, 2000d)	58	Category B a)	
12.009	Diallyl trisulfide		3265 486 2050-87-5	587 JECFA specification (JECFA, 2000d).	3.5	Category B a)	
12.010	Butane-1-thiol		3478 526 109-79-5	511 JECFA specification (JECFA, 1999c)	0.39	Category B a)	
12.013	Dimethyl trisulfide		3275 539 3658-80-8	582 JECFA specification (JECFA, 2000d).	1.1	Category A a)	
12.014	Dipropyl disulfide		3228 540 629-19-6	566 JECFA specification (JECFA, 2002d)	3.4	Category B a)	
12.018	S-Ethyl acetothioate		3282 11665 625-60-5	483 JECFA specification (JECFA, 2002d)	0.012	Deleted a)	

Table 3: Supporting Substances Summary

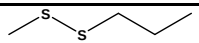
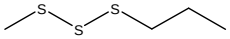
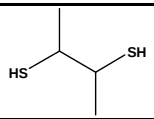
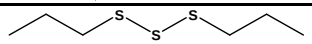
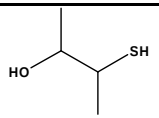
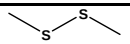
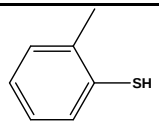
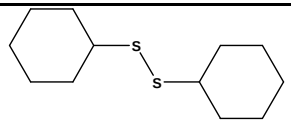
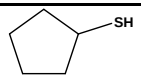
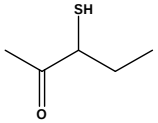
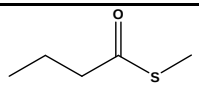
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
12.019	Methyl propyl disulfide		3201 585 2179-60-4	565 JECFA specification (JECFA, 2000d)	3.9	Category B a)	
12.020	Methyl propyl trisulfide		3308 586 17619-36-2	584 JECFA specification (JECFA, 2000d)	0.21	Category A a)	
12.022	Butane-2,3-dithiol		3477 725 4532-64-3	539 JECFA specification (JECFA, 1999c)	0.049	Category A a)	
12.023	Dipropyl trisulfide		3276 726 6028-61-1	585 JECFA specification (JECFA, 2000d).	7.3	Category A a)	
12.024	3-Mercaptobutan-2-ol		3502 760 37887-04-0	546 JECFA specification (JECFA, 2000d)	4.0	Category A a)	
12.026	Dimethyl disulfide		3536 2175 624-92-0	564 JECFA specification (JECFA, 2002d)	6.9	Category B a)	
12.027	2-Methylbenzene-1-thiol		3240 2272 137-06-4	528 JECFA specification (JECFA, 2000d)	17	Category A a)	
12.028	Dicyclohexyl disulfide		3448 2320 2550-40-5	575 JECFA specification (JECFA, 2000d)	0.012	Category A a)	
12.029	Cyclopentanethiol		3262 2321 1679-07-8	516 JECFA specification (JECFA, 2000d)	ND	Category B a)	
12.031	3-Mercaptopentan-2-one		3300 2327 67633-97-0	560 JECFA specification (JECFA, 2000d)	ND	Category A a)	
12.032	S-Methyl butanethioate		3310 2328 2432-51-1	484 JECFA specification (JECFA, 2000d)	2.9	Category A a)	

Table 3: Supporting Substances Summary

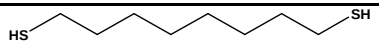
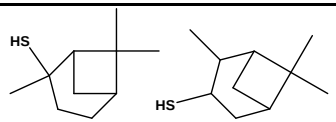
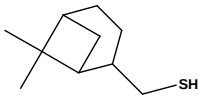
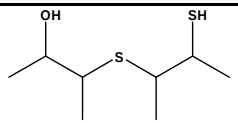
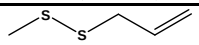
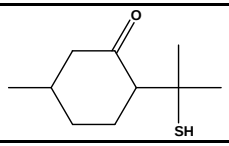
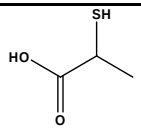
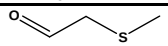
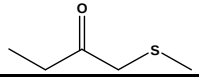
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
12.034	Octane-1,8-dithiol		3514 2331 1191-62-4	541 JECFA specification (JECFA, 1999c)	2.1	Category A a)	
12.035	2-,3- and 10-Mercaptopinane	 **2-Mercaptopinane** **3-Mercaptopinane**  **10-Mercaptopinane**	3503 2332	520 JECFA specification (JECFA, 2000d)	0.037	Category A a)	
12.036	3-[(2-Mercapto-1-methylpropyl)thio]butan-2-ol		3509 2353 54957-02-7	547 JECFA specification (JECFA, 1999c)	ND	Category A a)	
12.037	Allyl methyl disulfide		3127 11866 2179-58-0	568 JECFA specification (JECFA, 2003b)	0.0012		
12.038	8-Mercapto-p-menthan-3-one		3177 11789 38462-22-5	561 JECFA specification (JECFA, 2000d).	10		
12.039	2-Mercaptopropionic acid		3180 11790 79-42-5	551 JECFA specification (JECFA, 2002d)	2.1		
12.040	2-Methylthioacetaldehyde		3206 11686 23328-62-3	465 JECFA specification (JECFA, 2003b)	ND		
12.041	1-(Methylthio)butan-2-one		3207 11543 13678-58-5	496 JECFA specification (JECFA, 1999c)	0.0037		

Table 3: Supporting Substances Summary

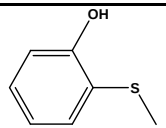
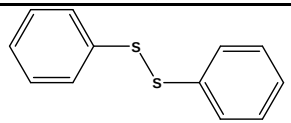
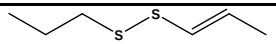
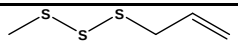
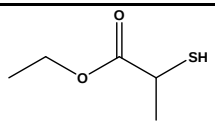
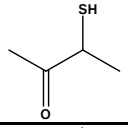
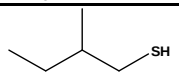
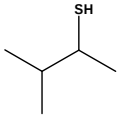
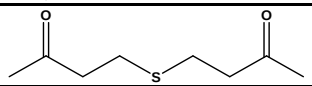
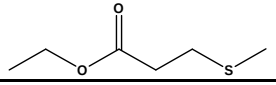
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
12.042	2-(Methylthio)phenol		3210 11553 1073-29-6	503 JECFA specification (JECFA, 2000d)	0.61		
12.043	Diphenyl disulfide		3225 11757 882-33-7	578 JECFA specification (JECFA, 1999c)	ND		
12.044	Prop-1-enyl propyl disulfide		3227 11699 5905-46-4	570 JECFA specification (JECFA, 2005b)	ND		
12.045	Methyl allyl trisulfide		3253 11867 34135-85-8	586 JECFA specification (JECFA, 2003b).	ND		
12.046	Ethyl 2-mercaptopropionate		3279 11469 19788-49-9	552 JECFA specification (JECFA, 2000d)	0.39		
12.047	3-Mercaptobutan-2-one		3298 11497 40789-98-8	558 JECFA specification (JECFA, 2000d)	3.2		
12.048	2-Methylbutane-1-thiol		3303 11509 1878-18-8	515 JECFA specification (JECFA, 1999c)	0.3		
12.049	3-Methylbutane-2-thiol		3304 11510 2084-18-6	517 JECFA specification (JECFA, 1999c)	0.012		
12.052	Di-(3-oxobutyl) sulfide		3335 11441 40790-04-3	502 JECFA specification (JECFA, 2003b)	ND		
12.053	Ethyl 3-(methylthio)propionate		3343 11476 13327-56-5	476 JECFA specification (JECFA, 2002d)	24		

Table 3: Supporting Substances Summary

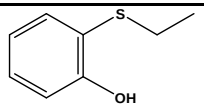
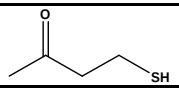
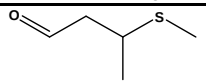
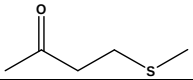
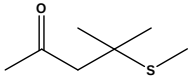
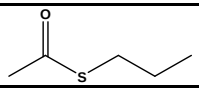
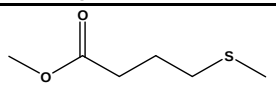
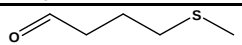
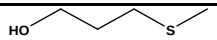
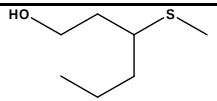
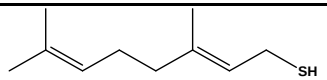
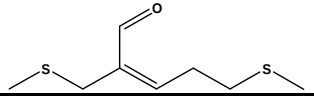
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
12.054	2-(Ethylthio)phenol		3345 11666 4500-58-7	529 JECFA specification (JECFA, 2000d)	0.00012		
12.055	4-Mercaptobutan-2-one		3357 11498 34619-12-0	559 JECFA specification (JECFA, 2003b)	ND		
12.056	3-(Methylthio)butanal		3374 11687 16630-52-7	467 JECFA specification (JECFA, 2000d)	0.085		
12.057	4-(Methylthio)butan-2-one		3375 11688 34047-39-7	497 JECFA specification (JECFA, 2000d)	0.012		
12.058	4-(Methylthio)-4-methylpentan-2-one		3376 11551 23550-40-5	500 JECFA specification (JECFA, 2000d)	0.024		
12.059	Propyl thioacetate		3385 11576 2307-10-0	485 JECFA specification (JECFA, 1999c)	0.27		
12.060	Methyl 4-(methylthio)butyrate		3412 11526 53053-51-3	474 JECFA specification (JECFA, 1999c)	0.061		
12.061	4-(Methylthio)butanal		3414 11542 42919-64-2	468 JECFA specification (JECFA, 2003b)	ND		
12.062	3-(Methylthio)propan-1-ol		3415 11554 505-10-2	461 JECFA specification (JECFA, 2001c)	2.8		
12.063	3-(Methylthio)hexan-1-ol		3438 11548 51755-66-9	463 JECFA specification (JECFA, 1999c)	3.2		
12.064	Thiogeraniol		3472 11583 39067-80-6	524 JECFA specification (JECFA, 2000d)	1.1		
12.065	2,8-Dithianon-4-en-4-carboxaldehyde		3483 11904 59902-01-1	471 JECFA specification (JECFA, 2005b).	0.012		JECFA adopted at step B5 (1.5 microgram/person/day) (JECFA, 2000b).

Table 3: Supporting Substances Summary

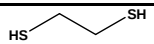
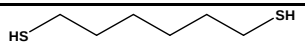
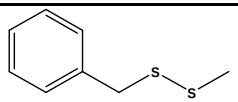
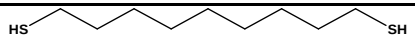
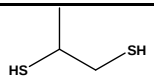
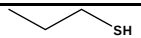
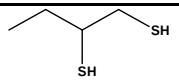
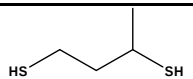
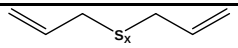
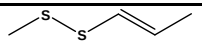
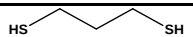
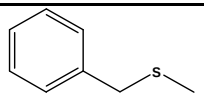
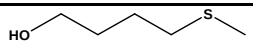
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
12.066	Ethane-1,2-dithiol		3484 11467 540-63-6	532 JECFA specification (JECFA, 1999c)	0.0012		
12.067	Hexane-1,6-dithiol		3495 11486 1191-43-1	540 JECFA specification (JECFA, 2002d)	1.6		
12.068	Benzyl methyl disulfide		3504 11508 699-10-5	577 JECFA specification (JECFA, 1999c)	0.012		
12.069	Nonane-1,9-dithiol		3513 11558 3489-28-9	542 JECFA specification (JECFA, 2002d)	0.0012		
12.070	Propane-1,2-dithiol		3520 11564 814-67-5	536 JECFA specification (JECFA, 2000d)	ND		
12.071	1-Propane-1-thiol		3521 11816 107-03-9	509 JECFA specification (JECFA, 2000d)	2.2		
12.072	Butane-1,2-dithiol		3528 11909 16128-68-0	537 JECFA specification (JECFA, 1999c)	ND		
12.073	Butane-1,3-dithiol		3529 11910 24330-52-7	538 JECFA specification (JECFA, 1999c)	ND		
12.074	Diallyl polysulfides	 X=2,3,4 or 5	3533 11912 72869-75-1	588 JECFA specification (JECFA, 2000d).	1.2		
12.075	Methyl prop-1-enyl disulfide		3576 11712 5905-47-5	569 JECFA specification (JECFA, 2003b)	ND		
12.076	Propane-1,3-dithiol		3588 11929 109-80-8	535 JECFA specification (JECFA, 1999c)	0.85		
12.077	Benzyl methyl sulfide		3597 766-92-7	460 JECFA specification (JECFA, 1999c).	0.09		JECFA adopted at step B5 (1.5 microgram/person/day) (JECFA, 2000b).
12.078	4-(Methylthio)butan-1-ol		3600 20582-85-8	462 JECFA specification (JECFA, 2000d)	0.012		

Table 3: Supporting Substances Summary

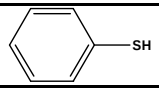
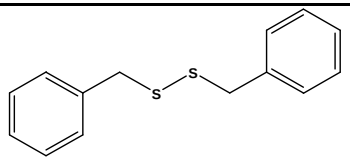
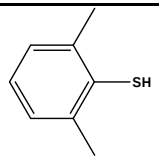
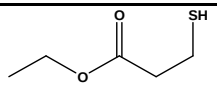
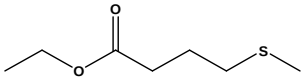
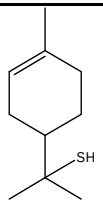
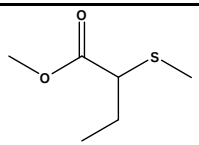
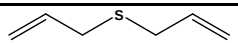
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
12.080	Thiophenol		3616 11585 108-98-5	525 JECFA specification (JECFA, 1999c)	0.73		
12.081	Dibenzyl disulfide		3617 150-60-7	579 JECFA specification (JECFA, 2000d)	0.012		
12.082	2,6-(Dimethyl)thiophenol		3666 118-72-9	530 JECFA specification (JECFA, 1999c)	1.3		
12.083	Ethyl 3-mercaptopropionate		3677 5466-06-8	553 JECFA specification (JECFA, 2002d)	0.073		
12.084	Ethyl 4-(methylthio)butyrate		3681 22014-48-8	477 JECFA specification (JECFA, 1999c)	ND		
12.085	p-Menth-1-ene-8-thiol		3700 71159-90-5	523 JECFA specification (JECFA, 2000d)	0.34		
12.086	Methyl 2-(methylthio)butyrate		3708 51534-66-8	486 JECFA specification (JECFA, 2000d).	0.097		JECFA evaluated S-methyl 2-methylbutanethioate (CASrn 42075-45-6).
12.088	Diallyl sulfide		2042 11846 592-88-1	458 JECFA specification (JECFA, 2000d). Solubility in ethanol (EFFA, 2011k).	3.5		JECFA adopted at step B5 (1.5 microgram/person/day) (JECFA, 2000b).

Table 3: Supporting Substances Summary

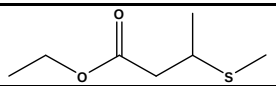
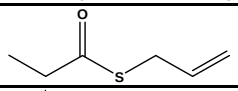
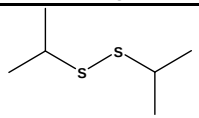
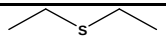
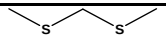
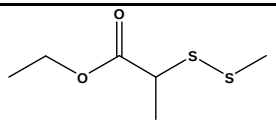
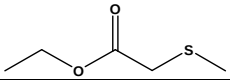
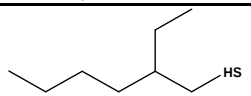
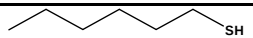
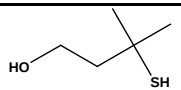
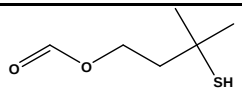
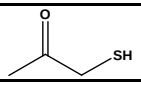
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
12.089	Ethyl 3-(methylthio)butyrate		3836 11475	480 JECFA specification (JECFA, 2003b)	ND		
12.101	Allyl thiopropionate		3329 11436 41820-22-8	490 JECFA specification (JECFA, 2002d)	ND		
12.109	Di-isopropyl disulfide		3827 11455 4253-89-8	567 JECFA specification (JECFA, 1999c)	ND		
12.113	Diethyl sulfide		3825 11450 352-93-2	454 JECFA specification (JECFA, 1999c)	ND		
12.118	2,4-Dithiapentane		3878 1618-26-4	533 JECFA specification (JECFA, 2000d)	ND		
12.121	Ethyl 2-(methyldithio)propionate		3834 11471 23747-43-5	581 JECFA specification (JECFA, 2001c)	ND		
12.122	Ethyl 2-(methylthio)acetate		3835 4455-13-4	475 JECFA specification (JECFA, 2000d)	ND		
12.128	2-Ethylhexane-1-thiol		3833 7341-17-5	519 JECFA specification (JECFA, 2003b)	ND		
12.132	Hexane-1-thiol		3842 11487 111-31-9	518 JECFA specification (JECFA, 2000d)	ND		
12.137	3-Mercapto-3-methylbutan-1-ol		3854 34300-94-2	544 JECFA specification (JECFA, 2000d).	ND		
12.138	3-Mercapto-3-methylbutyl formate		3855 50746-10-6	549 JECFA specification (JECFA, 1999c).	ND		
12.143	1-Mercaptopropan-2-one		3856 24653-75-6	557 JECFA specification (JECFA, 2005b)	ND		

Table 3: Supporting Substances Summary

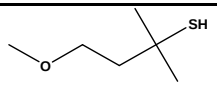
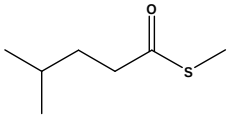
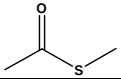
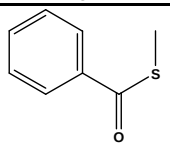
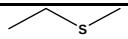
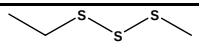
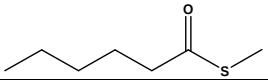
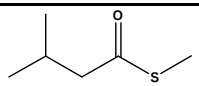
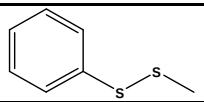
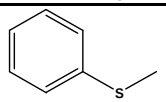
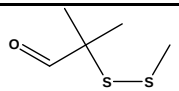
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
12.145	4-Methoxy-2-methylbutane-2-thiol		3785 94087-83-9	548 JECFA specification (JECFA, 2003b).	ND		
12.148	S-Methyl 4-methylpentanethioate		3867 61122-71-2	488 JECFA specification (JECFA, 2003b)	ND		
12.149	S-Methyl acetothioate		3876 1534-08-3	482 JECFA specification (JECFA, 2000d)	ND		
12.150	S-Methyl benzothioate		3857 11505 5925-68-8	504 Tentative JECFA specification (JECFA, 1999c)	ND		
12.154	Methyl ethyl sulfide		3860 11474 624-89-5	453 JECFA specification (JECFA, 1999c)	ND		
12.155	Methyl ethyl trisulfide		3861 31499-71-5	583 JECFA specification (JECFA, 2003b).	ND		
12.156	S-Methyl hexanethioate		3862 11515 20756-86-9	489 JECFA specification (JECFA, 2003b)	ND		
12.157	S-Methyl isopentanethioate		3864 11506 23747-45-7	487 JECFA specification (JECFA, 2000d)	ND		
12.161	Methyl phenyl disulfide		3872 11532 14173-25-2	576 JECFA specification (JECFA, 1999c)	ND		
12.162	Methyl phenyl sulfide		3873 11533 100-68-5	459 JECFA specification (JECFA, 1999c).	0.012		JECFA adopted at step B5 (1.5 microgram/person/day) (JECFA, 2000b).
12.168	2-Methyl-2-(methyldithio)propanal		3866 67952-60-7	580 JECFA specification (JECFA, 2001c).	ND		

Table 3: Supporting Substances Summary

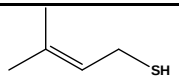
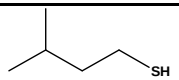
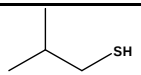
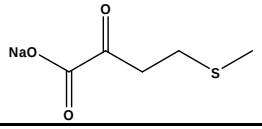
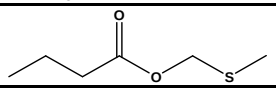
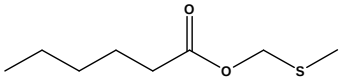
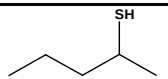
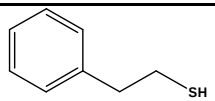
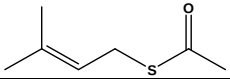
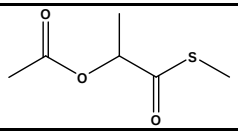
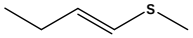
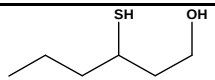
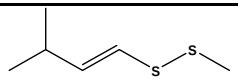
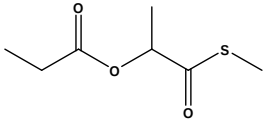
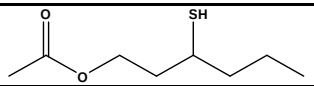
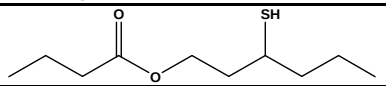
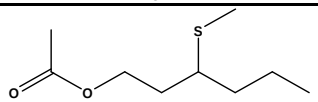
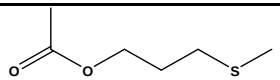
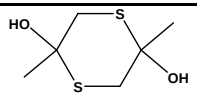
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
12.170	3-Methylbut-2-ene-1-thiol		3896 11511 5287-45-6	522 JECFA specification (JECFA, 1999c).	ND		
12.171	3-Methylbutane-1-thiol		3858 541-31-1	513 JECFA specification (JECFA, 2000d).	ND		
12.173	2-Methylpropane-1-thiol		 11536 513-44-0	512 JECFA specification (JECFA, 2000d).	ND		
12.175	Methylsulfinylmethane		3875 67-68-5	507 JECFA specification (JECFA, 2000d).	ND		
12.176	4-(Methylthio)-2-oxobutyric acid		3881 583-92-6	501 JECFA specification (JECFA, 1999c).	ND		JECFA CASrn 51828-97-8.
12.187	Methylthiomethyl butyrate		3879 74758-93-3	473 JECFA specification (JECFA, 2003b).	ND		
12.188	Methylthiomethyl hexanoate		3880 74758-91-1	479 JECFA specification (JECFA, 2003b).	ND		
12.192	Pentane-2-thiol		3792 2084-19-7	514 JECFA specification (JECFA, 2000d).	1.5		
12.194	2-Phenylethane-1-thiol		3894 11561 4410-99-5	527 JECFA specification (JECFA, 1999c).	ND		
12.195	S-Prenyl thioacetate		3895 33049-93-3	491 JECFA specification (JECFA, 1999c).	ND		
12.197	Propane-2-thiol		3897 11565 75-33-2	510 JECFA specification (JECFA, 2001c).	ND		

Table 3: Supporting Substances Summary

FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
12.203	Methylthio 2-(acetyloxy)propionate		3788 74586-09-7	492 JECFA specification (JECFA, 2002d).	ND		
12.211	But-1-enyl methyl sulphide		3820	457 JECFA specification (JECFA, 2001c).	ND		JECFA evaluated (1-Buten-1-yl) methyl sulfide (CASrn 32951-19-2).
12.217	3-Mercaptohexan-1-ol		3850	545 JECFA specification (JECFA, 1999c).	ND		JECFA evaluated 3-mercaptohexan-1-ol (CASrn 51755-83-0).
12.218	Methyl-3-methyl-1-butenyl disulphide		3865	571 JECFA specification (JECFA, 2003b).	ND		
12.227	Methylthio-2-(propionyloxy)propionate		3790	493 JECFA specification (JECFA, 2002d).	ND		
12.234	3-Mercaptohexyl acetate		3851 136954-20-6	554 JECFA specification (JECFA, 1999c).	ND		
12.235	3-Mercaptohexyl butyrate		3852 136954-21-7	555 JECFA specification (JECFA, 1999c).	ND		
12.236	3-(Methylthio)hexyl acetate		3789 51755-85-2	481 JECFA specification (JECFA, 2000d).	ND		
12.237	3-(Methylthio)propyl acetate		3883 16630-55-0	478 JECFA specification (JECFA, 2001c).	ND		
15.006	2,5-Dihydroxy-2,5-dimethyl-1,4-dithiane		3450 2322 55704-78-4	562 JECFA specification (JECFA, 2001c).	0.15	Category B a)	

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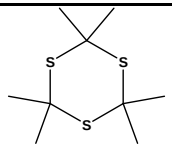
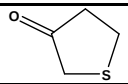
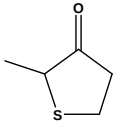
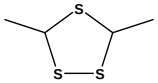
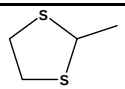
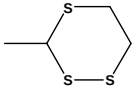
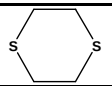
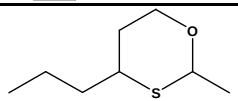
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
15.009	Trithioacetone		3475 2334 828-26-2	543 JECFA specification (JECFA, 2001c).	1.5	Category B a)	
15.012	4,5-Dihydrothiophen-3(2H)-one		3266 2337 1003-04-9	498 JECFA specification (JECFA, 2000d).	0.44	Category B a)	
15.023	4,5-Dihydro-2-methylthiophene-3(2H)-one		3512 11601 13679-85-1	499 JECFA specification (JECFA, 2000d).	12		
15.025	3,5-Dimethyl-1,2,4-trithiolane		3541 11883 23654-92-4	573 JECFA specification (JECFA, 2000d).	0.024		
15.034	2-Methyl-1,3-dithiolane		3705 5616-51-3	534 JECFA specification (JECFA, 1999c).	0.061		
15.036	3-Methyl-1,2,4-trithiane		3718 43040-01-3	574 JECFA specification (JECFA, 2000d).	0.073		
15.066	1,4-Dithiane		3831 505-29-3	456 JECFA specification (JECFA, 2000d).	ND		
16.030	2-Methyl-4-propyl-1,3-oxathiane		3578 11540 67715-80-4	464 JECFA specification (JECFA, 2000d).	1.3		

1) EU MSDI: Amount added to food as flavouring substance in (kg / year) x 10E9 / (0.1 x population in Europe (= 375 x 10E6) x 0.6 x 365) = µg/capita/day.

2) Category 1: Considered safe in use, Category 2: Temporarily considered safe in use, Category 3: Insufficient data to provide assurance of safety in use, Category 4: Not acceptable due to evidence of toxicity.

3) No safety concern at estimated levels of intake.

4) Category A: Flavouring substance, which may be used in foodstuffs, Category B: Flavouring substance which can be used provisionally in foodstuffs.

a) (CoE, 1992).

ND) No intake data reported

ANNEX I: PROCEDURE FOR THE SAFETY EVALUATION

The approach for a safety evaluation of chemically defined flavouring substances as referred to in Commission Regulation (EC) No 1565/2000 (EC, 2000a), named the "Procedure", is shown in schematic form in Figure I.1. The Procedure is based on the Opinion of the Scientific Committee on Food expressed on 2 December 1999 (SCF, 1999a), 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; JECFA, 1996a; JECFA, 1997a; JECFA, 1999b).

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 1800, 540 or 90 microgram/person/day, respectively, are derived from a large database containing data on subchronic and chronic animal studies (JECFA, 1996a).

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[9] (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[10] (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.

[9] "Innocuous metabolic products": Products that are known or readily predicted to be harmless to humans at the estimated intakes of the flavouring agent" (JECFA, 1997a).
[10] "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, 1997a).

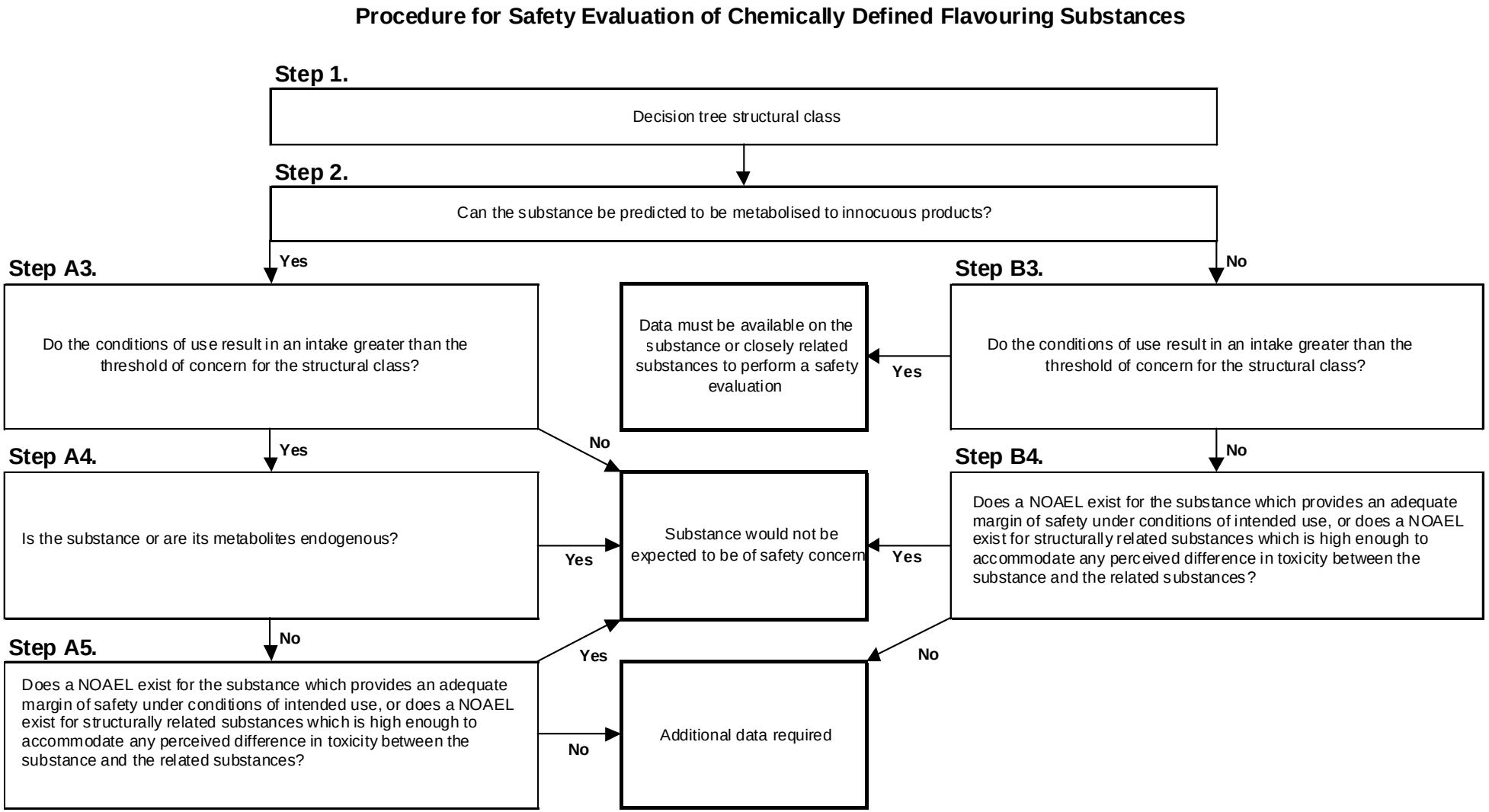


Figure I.1 *Procedure for Safety Evaluation of Chemically Defined Flavouring Substances*

ANNEX II: USE LEVELS / MTAMDI

II.1 Normal and Maximum Use Levels

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

Table II.1.1 Food categories according to Commission Regulation (EC) No 1565/2000 (EC, 2000a)

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

The "normal and maximum use levels" are provided by Industry for the 71 of the 80 candidate substances in the present flavouring group (Table II.1.2).

Table II.1.2. Normal and Maximum use levels (mg/kg) for the candidate substances in FGE.08, Revision 4 (EFFA, 2002g; EFFA, 2002h; EFFA, 2002i; EFFA, 2004ak; EFFA, 2007a; Flavour Industry, 2006q; Flavour Industry, 2006r; Flavour Industry, 2009e; Flavour Industry, 2009o; Flavour Industry, 2010h; Flavour Industry, 2011d).

FL-no	Food Categories Normal use levels (mg/kg) Maximum use levels (mg/kg)																	
	01.0	02.0	03.0	04.1	04.2	05.0	06.0	07.0	08.0	09.0	10.0	11.0	12.0	13.0	14.1	14.2	15.0	16.0
12.093	0,2 1	0,1 0,5	0,2 1	0,2 1	- -	0,2 1	0,1 0,5	0,2 1	0,1 0,2	0,1 0,2	- -	- -	0,1 0,5	0,2 1	0,1 0,5	0,2 1	0,4 2	0,1 0,5
12.094	0,2 1	0,1 0,5	0,2 1	0,2 1	- -	0,2 1	0,1 0,5	0,2 1	0,1 0,2	0,1 0,2	- -	- -	0,1 0,5	0,2 1	0,1 0,5	0,2 1	0,4 2	0,1 0,5
12.096	0,2 1	0,1 0,5	0,2 1	0,2 1	- -	0,2 1	0,1 0,5	0,2 1	0,1 0,2	0,1 0,2	- -	- -	0,1 0,5	0,2 1	0,1 0,3	0,2 1	0,4 2	0,1 0,5
12.097	0,2 1	0,1 0,5	0,2 1	0,2 1	- -	0,2 1	0,1 0,5	0,2 1	0,1 0,2	0,1 0,2	- -	- -	0,1 0,5	0,2 1	0,1 0,3	0,2 1	0,4 2	0,1 0,5
12.098	0,2 1	0,1 0,5	0,2 1	0,2 1	- -	0,2 1	0,1 0,5	0,2 1	0,1 0,2	0,1 0,2	- -	- -	0,1 0,5	0,2 1	0,1 0,3	0,2 1	0,4 2	0,1 0,5
12.099	0,2 1	0,1 0,5	0,2 1	0,2 1	- -	0,2 1	0,1 0,5	0,2 1	0,1 0,2	0,1 0,2	- -	- -	0,1 0,5	0,2 1	0,1 0,5	0,2 1	0,4 2	0,1 0,5
12.100	0,2 1	0,1 0,5	0,2 1	0,2 1	- -	0,2 1	0,1 0,5	0,2 1	0,1 0,2	0,1 0,2	- -	- -	0,1 0,5	0,2 1	0,1 0,3	0,2 1	0,4 2	0,1 0,5
12.103	0,2 1	0,1 0,5	0,2 1	0,2 1	- -	0,2 1	0,1 0,5	0,2 1	0,1 0,2	0,1 0,2	- -	- -	0,1 0,5	0,2 1	0,1 0,3	0,2 1	0,4 2	0,1 0,5
12.104	0,2	0,1	0,2	0,2	-	0,2	0,1	0,2	0,1	0,1	-	-	0,1	0,2	0,1	0,2	0,4	0,1

Table II.1.2. Normal and Maximum use levels (mg/kg) for the candidate substances in FGE.08, Revision 4 (EFFA, 2002g; EFFA, 2002h; EFFA, 2002i; EFFA, 2004ak; EFFA, 2007a; Flavour Industry, 2006q; Flavour Industry, 2006r; Flavour Industry, 2009e; Flavour Industry, 2009o; Flavour Industry, 2010h; Flavour Industry, 2011d).

FL-no	Food Categories																	
	Normal use levels (mg/kg) Maximum use levels (mg/kg)																	
	01.0	02.0	03.0	04.1	04.2	05.0	06.0	07.0	08.0	09.0	10.0	11.0	12.0	13.0	14.1	14.2	15.0	16.0
	1	0,5	1	1	-	1	0,5	1	0,2	0,2	-	-	0,5	1	0,3	1	2	0,5
12.106	0,3	0,2	1	0,3	-	0,4	0,2	0,4	0,1	0,1	-	-	0,2	0,4	0,2	0,4	1	0,2
	10	1	5	1,5	-	10	1	10	0,4	0,4	-	-	1	2	5	10	5	1
12.111	0,2	0,1	0,2	0,2	-	0,2	0,1	0,2	0,1	0,1	-	-	0,1	0,2	0,1	0,2	0,4	0,1
	1	0,5	1	1	-	1	0,5	1	0,2	0,2	-	-	0,5	1	0,3	1	2	0,5
12.112	0,2	0,1	0,2	0,2	-	0,2	0,1	0,2	0,1	0,1	-	-	0,1	0,2	0,1	0,2	0,4	0,1
	1	0,5	1	1	-	1	0,5	1	0,2	0,2	-	-	0,5	1	0,3	1	2	0,5
12.116	0,2	0,1	0,2	0,2	-	0,2	0,1	0,2	0,1	0,1	-	-	0,1	0,2	-	0,2	0,4	0,1
	1	0,5	1	1	-	1	0,5	1	0,2	0,2	-	-	0,5	1	-	1	2	0,5
12.117	0,2	0,1	0,2	0,2	-	0,2	0,1	0,2	0,1	0,1	-	-	0,1	0,2	0,1	0,2	0,2	0,1
	1	0,5	1	1	-	1	0,5	1	0,2	0,2	-	-	0,5	1	0,3	1	4	0,5
12.120	2	0,2	0,4	0,3	-	0,4	0,2	0,4	0,1	0,1	-	-	0,2	0,4	0,2	0,4	1	0,2
	4	1	2	1,5	-	2	1	2	0,4	0,4	-	-	1	2	1	2	5	1
12.124	1	0,1	0,2	0,2	-	0,2	0,1	0,2	0,1	0,1	-	-	0,1	0,2	0,1	0,2	0,4	0,1
	2	0,5	1	1	-	1	0,5	1	0,2	0,2	-	-	0,5	1	0,3	1	2	0,5
12.125	0,4	0,2	0,4	0,3	-	0,4	0,2	0,4	0,1	0,1	-	-	0,2	0,4	0,2	0,4	1	0,2
	2	1	2	1,5	-	2	1	2	0,4	0,4	-	-	1	2	1	2	5	1
12.127	0,2	0,1	0,2	0,2	-	0,2	0,1	-	0,1	0,1	-	-	0,1	0,2	0,1	0,2	0,4	0,1
	1	0,5	1	1	-	1	0,5	-	0,2	0,2	-	-	0,5	1	0,3	1	2	0,5
12.129	0,4	0,2	0,4	0,3	-	1	0,2	0,4	0,1	0,1	-	-	0,2	0,4	0,2	1	1	0,2
	2	1	2	1,5	-	5	1	2	0,4	0,4	-	-	1	2	1	5	5	1
12.135	0,2	0,1	0,2	0,2	-	0,2	0,1	0,2	0,1	0,1	-	-	0,1	0,2	0,1	0,2	0,4	0,1
	1	0,5	1	1	-	1	0,5	1	0,2	0,2	-	-	0,5	1	0,3	1	2	0,5
12.136	0,4	0,2	0,4	0,3	-	0,4	0,2	0,4	0,1	0,1	-	-	0,2	0,4	0,2	0,4	1	0,2
	2	1	2	1,5	-	2	1	2	0,4	0,4	-	-	1	2	1	2	5	1
12.151	0,2	0,1	0,2	0,2	-	0,2	0,1	0,2	0,1	0,1	-	-	0,1	0,2	0,1	0,2	0,4	0,1
	1	0,5	1	1	-	1	0,5	1	0,2	0,2	-	-	0,5	1	0,3	1	2	0,5
12.152	0,2	0,1	0,2	0,2	-	0,2	0,1	0,2	0,1	0,1	-	-	0,1	0,2	0,1	0,2	0,4	0,1
	1	0,5	1	1	-	1	0,5	1	0,2	0,2	-	-	0,5	1	0,3	1	2	0,5
12.158	0,2	0,1	0,2	0,2	-	0,2	0,1	0,2	0,1	0,1	-	-	0,1	0,2	0,1	0,2	0,4	0,1
	1	0,5	1	1	-	1	0,5	1	0,2	0,2	-	-	0,5	1	0,3	1	2	0,5
12.159	0,4	0,2	0,4	0,3	-	0,4	0,2	0,4	0,1	0,1	-	-	0,2	0,4	0,2	0,4	1	0,2
	2	1	2	1,5	-	2	1	2	0,4	0,4	-	-	1	2	1	2	5	1
12.163	0,2	0	0,2	0,2	-	0,2	0,1	0,2	0,1	0,1	-	-	0,1	0,2	0,1	0,2	0,4	0,1
	1	10,5	1	1	-	1	0,5	1	0,2	0,2	-	-	0,5	1	0,3	1	2	0,5
12.164	0,2	0	0,2	0,2	-	0,2	0,1	0,2	0,1	0,1	-	-	0,1	0,2	0,1	0,2	0,4	0,1
	1	10,5	1	1	-	1	0,5	1	0,2	0,2	-	-	0,5	1	0,3	1	2	0,5
12.165	0,4	0,2	0,4	0,3	-	0,4	0,2	0,4	0,1	0,1	-	-	0,24	0,4	0,1	0,4	-	0,2
	2	1	2	1,5	-	2	1	2	0,4	0,4	-	-	1	2	0,3	2	-	1
12.166	0,2	0,1	0,2	0,2	-	0,2	0,1	0,2	0,1	0,1	-	-	0,1	0,2	0,1	0,2	0,4	0,1
	1	0,5	1	1	-	1	0,5	1	0,2	0,2	-	-	0,5	1	0,3	1	2	0,5
12.167	0,2	0,1	0,2	0,2	-	0,2	0,1	0,2	0,1	0,1	-	-	0,1	0,2	0,1	0,2	0,4	0,1
	1	0,5	1	1	-	1	0,5	1	0,2	0,2	-	-	0,5	1	0,3	1	2	0,5
12.172	0,2	0,1	0,2	0,2	-	0,2	0,1	0,2	0,1	0,1	-	-	0,1	0,2	0,1	0,2	0,4	0,1
	1	0,5	1	1	-	1	0,5	1	0,2	0,2	-	-	0,5	1	0,3	1	2	0,5
12.174	0,2	0,1	0,2	0,2	-	0,2	0,1	0,2	0,1	0,1	-	-	0,1	0,2	0,1	0,2	0,4	0,1
	1	0,5	1	1	-	1	0,5	1	0,2	0,2	-	-	0,5	1	0,3	1	2	0,5
12.177	0,2	0,1	0,2	0,2	-	0,2	0,1	0,2	0,1	0,1	-	-	0,1	0,2	0,1	0,2	0,4	0,1
	1	0,5	1	1	-	1	0,5	1	0,2	0,2	-	-	0,5	1	0,3	1	2	0,5
12.178	0,4	0,2	0,4	0,3	-	0,4	0,2	0,4	0,1	0,1	-	-	0,2	0,4	0,2	0,4	1	0,2
	5	5	2	1,5	-	2	1	5	0,4	0,4	-	-	1	2	1	2	5	1
12.180	0,2	0,1	0,2	0,2	-	0,2	0,1	0,2	0,1	0,1	-	-	0,1	0,2	0,1	0,2	0,4	0,1
	1	0,5	1	1	-	1	0,5	1	0,2	0,2	-	-	0,5	1	0,3	1	2	0,5
12.181	0,2	0,1	0,2	0,2	-	0,2	0,1	0,2	0,1	0,1	-	-	0,1	0,2	0,1	0,2	-	0,1
	1	0,5	1	1	-	1	0,5	1	0,2	0,2	-	-	0,5	1	0,3	1	-	0,5
12.182	0,4	0,2	0,4	0,3	-	0,4	0,2	0,4	0,1	0,1	-	-	0,2	0,4	0,2	0,4	1	0,2
	2	1	2	1,5	-	2	1	2	0,4	0,4	-	-	1	2	1	2	5	1
12.183	0,4	0,2	0,4	0,3	-	0,4	0,2	0,4	0,1	0,1	-	-	0,2	0,4	0,2	0,4	1	0,2
	2	1	2	1,5	-	2	1	2	0,4	0,4	-	-	1	2	1	2	5	1
12.189	0,4	0,2	0,4	0,3	-	0,4	0,2	0,4	0,1	0,1	-	-	0,2	0,4	0,2	0,4	1	0,2
	2	1	2	1,5	-	2	1	2	0,4	0,4	-	-	1	2	1	2	5	1
12.191	0,2	0,1	0,2	0,2	-	0,2	0,1	0,2	0,1	0,1	-	-	0,1	0,2	0,1	0,2	0,4	0,1
	1	0,5	1	1	-	1	0,5	1	0,2	0,2	-	-	0,5	1	0,3	1	2	0,5
12.196	0,4	0,2	0,4	0,3	-	0,4	0,2	0,4	0,1	0,1	-	-	0,2	0,4	0,2	0,4	1	0,2
	2	1	2	1,5	-	2	1	2	0,4	0,4	-	-	1	2	1	2	5	1
12.199	0,4	0,2	0,4	0,3	-	0,4	0,2	0,4	0,1	0,1	-	-	0,2	0,4	0,2	0,4	1	0,2

Table II.1.2. Normal and Maximum use levels (mg/kg) for the candidate substances in FGE.08, Revision 4 (EFFA, 2002g; EFFA, 2002h; EFFA, 2002i; EFFA, 2004ak; EFFA, 2007a; Flavour Industry, 2006q; Flavour Industry, 2006r; Flavour Industry, 2009e; Flavour Industry, 2009o; Flavour Industry, 2010h; Flavour Industry, 2011d).

FL-no	Food Categories																	
	Normal use levels (mg/kg) Maximum use levels (mg/kg)																	
	01.0	02.0	03.0	04.1	04.2	05.0	06.0	07.0	08.0	09.0	10.0	11.0	12.0	13.0	14.1	14.2	15.0	16.0
	2	1	2	1,5	-	2	1	2	0,4	0,4	-	-	1	2	1	2	5	1
12.200	0,2	0,1	0,2	0,2	-	0,2	0,1	0,2	0,1	0,1	-	-	0,1	0,2	-	0,2	0,4	0,1
	1	0,5	1	1	-	1	0,5	1	0,2	0,2	-	-	0,5	1	-	1	2	0,5
12.205	0,4	0,2	0,4	0,3	-	0,4	0,2	0,4	0,1	0,1	-	-	0,2	0,4	0,2	0,4	1	0,2
	2	1	2	1,5	-	2	1	2	0,4	0,4	-	-	1	2	1	2	5	1
12.214	0,4	0,2	0,4	0,3	-	0,4	0,2	0,4	0,1	0,1	-	-	0,2	0,4	0,2	0,4	1	0,2
	2	1	2	1,5	-	2	1	2	0,4	0,4	-	-	1	2	1	2	5	1
12.221	0,4	0,2	0,4	0,3	-	0,2	-	0,4	0,1	0,1	-	-	0,2	0,4	0,2	0,2	1	0,2
	2	1	2	1,5	-	1	-	2	0,4	0,4	-	-	1	2	1	1	5	1
12.250	0,05	0,05	0,5	0,05	0,05	5	-	0,5	0,05	0,05	-	-	0,05	-	5	5	0,05	0,05
	0,25	0,25	2,5	0,25	0,25	25	-	2,5	0,25	0,25	-	-	0,25	-	25	25	0,25	0,25
12.277	5	10	1	0,5	-	1	5	5	5	1	-	-	0,1	-	0,2	0,5	-	0,5
	10	20	5	1	-	5	10	10	20	2	-	-	0,2	-	0,5	1	-	0,1
12.282	0,2	0,2	1	0,2	0,2	20	4	2	0,2	0,2	-	-	0,2	-	20	20	0,2	0,2
	2,5	2,5	25	2,5	2,5	250	50	25	2,5	2,5	-	-	2,5	-	250	250	2,5	2,5
12.298	0,1	0,1	-	0,05	0,05	-	-	0,2	0,05	-	-	-	0,05	-	-	-	-	-
	1	1	-	0,5	0,5	-	-	2	0,5	-	-	-	0,5	-	-	-	-	-
12.299	2	-	1	2	2	9	1	3	1	-	-	-	3	-	1	2	1	-
	15	-	5	20	20	35	5	15	5	-	-	-	115	-	5	10	10	-
12.300	0,04	0,02	-	0,01	0,01	-	0,01	0,03	0,04	-	-	-	0,1	-	0	0	0,01	-
	0,18	0,08	-	0,05	0,05	-	0,05	0,13	0,16	-	-	-	0,1	-	0	0	0,05	-
12.301	-	-	-	0,5	-	-	-	1	0,6	-	-	-	1,5	-	0	0	0,5	-
	-	-	-	1	-	-	-	2	2	-	-	-	3	-	0	0	1	-
12.302	-	-	-	0,2	0,2	-	-	0,4	0,4	-	-	-	0,4	-	0	0	0,4	-
	-	-	-	0,5	0,5	-	-	1	1	-	-	-	1	-	0	0	0,5	-
12.303	-	-	-	0,02	0,02	-	-	0,02	0,02	-	-	-	0,02	-	0	0	0,02	-
	-	-	-	0,1	0,1	-	-	0,2	0,2	-	-	-	0,2	-	0	0	0,2	-
12.304	0,2	-	0,1	0,2	0,2	0,3	0,1	0,1	-	-	-	-	0,4	-	0,2	0,1	-	0,1
	1	-	0,5	1	1	1,5	0,5	0,5	-	-	-	-	2	-	1	0,5	-	0,5
12.305	-	0,06	-	0,23	0,23	0,06	-	0,14	0,06	-	-	-	0,23	-	0,06	0	-	0,1
	-	0,5	-	2	2	0,5	-	1,1	0,5	-	-	-	2	-	0,5	0	-	1
12.306	0,05	0,05	-	0,1	0,1	-	-	0,1	0,1	0,05	0,05	-	0,15	-	0	0	0,05	-
	0,1	0,2	-	0,2	0,2	-	-	0,3	0,2	0,2	0,1	-	0,3	-	0	0	0,1	-
15.047	0,2	0,1	0,2	0,2	-	0,2	0,1	0,2	0,1	0,1	-	-	0,1	0,2	-	0,2	0,4	0,1
	1	0,5	1	1	-	1	0,5	1	0,2	0,2	-	-	0,5	1	-	1	2	0,5
15.048	0,2	0,1	0,2	0,02	-	0,2	0,1	0,2	0,1	0,1	-	-	0,1	0,2	-	0,2	0,4	0,1
	1	0,5	1	1	-	1	0,5	1	0,2	0,2	-	-	0,5	1	-	1	2	0,5
15.056	0,2	0,1	0,2	0,2	-	0,2	0,1	0,2	0,1	0,1	-	-	0,1	0,2	0,1	0,2	0,4	0,1
	1	0,5	1	1	-	1	0,5	1	0,2	0,2	-	-	0,5	1	0,3	1	2	0,5
15.081	0,2	0,1	0,2	0,2	-	0,2	0,1	0,2	0,1	0,1	-	-	0,1	-	0,1	0,2	0,4	0,1
	1	0,5	1	1	-	1	0,5	1	0,2	0,2	-	-	0,5	-	0,3	1	2	0,5
15.083	0,2	0,1	0,2	0,2	-	0,2	0,1	0,2	0,1	0,1	-	-	0,1	0,2	0,1	0,2	0,4	0,1
	1	0,5	1	1	-	1	0,5	1	0,2	0,2	-	-	0,5	1	0,3	1	2	0,5
15.102	0,2	0,1	0,2	0,2	-	0,2	0,1	0,2	0,1	0,1	-	-	0,1	0,2	0,1	0,2	0,4	0,1
	1	0,5	1	1	-	1	0,5	1	0,2	0,2	-	-	0,5	1	0,3	1	2	0,5
15.103	0,2	0,1	0,2	0,2	-	0,2	0,1	0,2	0,1	0,1	-	-	0,1	0,2	0,1	0,2	0,4	0,1
	1	0,5	1	1	-	1	0,5	1	0,2	0,2	-	-	0,5	1	0,3	1	2	0,5
15.110	0,2	0,1	0,2	0,2	-	0,2	0,1	0,2	0,1	0,1	-	-	0,1	0,2	0,1	0,2	0,4	0,1
	1	0,5	1	1	-	1	0,5	1	0,2	0,2	-	-	0,5	1	0,3	1	2	0,5
15.111	0,2	0,1	0,2	0,2	-	0,2	0,1	0,2	0,1	0,1	-	-	0,1	0,2	0,1	0,2	0,4	-
	1	0,5	1	1	-	1	0,5	1	0,2	0,2	-	-	0,5	1	0,3	1	2	-
15.134	-	-	-	-	-	-	-	0,02	0,02	-	-	-	0,02	-	-	-	25	-
	-	-	-	-	-	-	-	7,5	2	-	-	-	1	-	-	-	50	-
16.057	0,2	0,1	0,2	0,2	-	0,2	0,1	0,2	0,1	0,1	-	-	0,1	0,2	0,1	0,2	0,4	0,1
	1	0,5	1	1	-	1	0,5	1	0,2	0,2	-	-	0,5	1	0,3	1	2	0,5
16.114	-	-	0,5	-	-	1	-	-	-	-	-	-	-	-	0,5	0,5	1	0,5
	-	-	3	-	-	5	-	-	-	-	-	-	-	-	3	3	5	3
16.122	-	-	0,1	0,5	0,5	0,7	0,1	0,4	0,1	-	-	-	0,2	-	0,4	0,2	0,2	0,2
	-	-	1	3	3	4	1	1	1	-	-	-	1	-	1	1	1	1

II.2 mTAMDI Calculations

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

the amount of flavourable foods and beverages listed in Table II.2.1. These consumption estimates are then multiplied by the reported use levels in the different food categories and summed up.

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

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

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

- Beverages (SCF, 1995) correspond to food category 14.1 (EC, 2000a)
- Foods (SCF, 1995) correspond to the food categories 1, 2, 3, 4.1, 4.2, 6, 7, 8, 9, 10, 13, and/or 16 (EC, 2000a)
- Exception a (SCF, 1995) corresponds to food category 5 and 11 (EC, 2000a)
- Exception b (SCF, 1995) corresponds to food category 15 (EC, 2000a)
- Exception c (SCF, 1995) corresponds to food category 14.2 (EC, 2000a)
- Exception d (SCF, 1995) corresponds to food category 12 (EC, 2000a)
- Exception e (SCF, 1995) corresponds to others, e.g. chewing gum.

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

	Food categories according to Commission Regulation (EC) No1565/2000	Distribution of the seven SCF food categories		
Key	Food category	Food	Beverages	Exceptions
01.0	Dairy products, excluding products of category 02.0	Food		
02.0	Fats and oils, and fat emulsions (type water-in-oil)	Food		
03.0	Edible ices, including sherbet and sorbet	Food		
04.1	Processed fruit	Food		
04.2	Processed vegetables (incl. mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds	Food		
05.0	Confectionery			Exception a
06.0	Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery	Food		
07.0	Bakery wares	Food		
08.0	Meat and meat products, including poultry and game	Food		
09.0	Fish and fish products, including molluscs, crustaceans and echinoderms	Food		
10.0	Eggs and egg products	Food		
11.0	Sweeteners, including honey			Exception a
12.0	Salts, spices, soups, sauces, salads, protein products, etc.			Exception d
13.0	Foodstuffs intended for particular nutritional uses	Food		

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

	Food categories according to Commission Regulation (EC) No1565/2000	Distribution of the seven SCF food categories	
14.1	Non-alcoholic ("soft") beverages, excl. dairy products	Beverages	
14.2	Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts		Exception c
15.0	Ready-to-eat savouries		Exception b
16.0	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 01.0 - 15.0	Food	

The mTAMDI values (see Table II.2.3) are presented for each of the 71 flavouring substances in the present flavouring group, for which Industry has provided use and use levels (EFFA, 2002g; EFFA, 2002h; EFFA, 2002i; EFFA, 2004ak; EFFA, 2007a; Flavour Industry, 2006q; Flavour Industry, 2006r; Flavour Industry, 2009e; Flavour Industry, 2009o; Flavour Industry, 2010h; Flavour Industry, 2011d). The mTAMDI values are only given for the highest reported normal use levels.

TableII.2.3 Estimated intakes based on the mTAMDI approach

FL-no	EU Register name	mTAMDI (µg/person/day)	Structural class	Threshold of concern (µg/person/day)
12.103	Butane-1,4-dithiol	78	Class I	1800
12.104	Butane-2-thiol	78	Class I	1800
12.106	S-2-Butyl 3-methylbutanethioate	240	Class I	1800
12.111	Dibutyl disulfide	78	Class I	1800
12.112	Dibutyl trisulfide	78	Class I	1800
12.116	Dimethyl tetrasulfide	46	Class I	1800
12.117	Dipentyl sulfide	74	Class I	1800
12.124	Ethyl butyl sulfide	190	Class I	1800
12.125	Ethyl propanethioate	160	Class I	1800
12.127	Ethyl propyl sulfide	78	Class I	1800
12.129	3-(Ethylthio)propan-1-ol	190	Class I	1800
12.135	3-Mercapto-2-methylpropionic acid	78	Class I	1800
12.151	Methyl butyl disulfide	78	Class I	1800
12.152	Methyl butyl sulfide	78	Class I	1800
12.158	Methyl isoprenyl sulfide	78	Class I	1800
12.163	Methyl prop-1-enyl sulfide	78	Class I	1800
12.164	Methyl prop-1-enyl trisulfide	78	Class I	1800
12.165	S-Methyl propanethioate	110	Class I	1800
12.166	Methyl propyl sulfide	78	Class I	1800
12.167	Methyl propyl tetrasulfide	78	Class I	1800
12.178	3-(Methylthio)butyric acid	160	Class I	1800
12.180	1-(Methylthio)ethane-1-thiol	78	Class I	1800
12.181	1-(Methylthio)pentan-3-one	70	Class I	1800
12.182	2-(Methylthio)propionic acid	160	Class I	1800
12.183	3-(Methylthio)propionic acid	160	Class I	1800
12.189	S-(Methylthiomethyl) 2-methylpropanethioate	160	Class I	1800
12.191	Pentane-1-thiol	78	Class I	1800
12.196	S-Prenyl thioisobutyrate	160	Class I	1800
12.199	Ethanethioic acid	160	Class I	1800
12.200	1,1-bis(Ethylthio)-ethane	46	Class I	1800
12.205	Mercaptoacetaldehyde	160	Class I	1800
12.214	Isobutyl-3-(methylthio)butyrate	160	Class I	1800
12.221	S-Prenyl thioisopentanoate	150	Class I	1800
12.250	3-Mercaptohexanal	1900	Class I	1800
12.266	Methyl-2-mercaptopropionate		Class I	1800
12.277	3-(Methylthio)propyl butyrate	1400	Class I	1800
12.278	3-Acetyl-mercaptohexyl acetate		Class I	1800
12.282	(S)-Methyl octanethioate	8000	Class I	1800
12.298	Di-(1-propenyl)-sulfid (mixture)	28	Class I	1800
12.299	3-(Methylthio)propyl hexanoate	1100	Class I	1800
12.303	3-Pentanethiol	3.5	Class I	1800
12.306	3-(Methylthio)-decanal	17	Class I	1800
12.304	Ethyl-2-mercapto-2-methyl propanoate	110	Class I	1800

TableII.2.3 Estimated intakes based on the mTAMDI approach

FL-no	EU Register name	mTAMDI (µg/person/day)	Structural class	Threshold of concern (µg/person/day)
12.172	2-Methylbutane-2-thiol	78	Class I	1800
12.174	2-Methylpropane-2-thiol	78	Class I	1800
12.268	3-Mercaptooctanal		Class I	1800
12.269	3-Mercaptodecanal		Class I	1800
12.271	Methanedithiol diacetate		Class I	1800
12.093	Diallyl hexasulfide	78	Class II	540
12.094	Diallyl heptasulfide	78	Class II	540
12.096	Allyl methyl sulfide	78	Class II	540
12.097	Allyl methyl tetrasulfide	78	Class II	540
12.098	Allyl prop-1-enyl disulfide	78	Class II	540
12.099	Allyl propyl sulfide	78	Class II	540
12.100	Allyl propyl trisulfide	78	Class II	540
12.177	8-(Methylthio)-p-menthan-3-one	78	Class II	540
12.302	2-Butanol, 4-mercapto-3-methyl	69	Class II	540
12.305	2-Mercapto-4-heptanol	56	Class II	540
15.047	3,5-Di-isobutyl-1,2,4-trithiolane	46	Class II	540
15.048	3,5-Di-isopropyl-1,2,4-trithiolane	46	Class II	540
15.056	3,6-Dimethyl-1,2,4,5-tetrathiane	78	Class II	540
15.083	3-Methyl-1,2,4-trithiolane	78	Class II	540
15.102	Tetrahydrothiophene	78	Class II	540
15.103	1,2,4,5-Tetrathiane	78	Class II	540
15.110	2,4,6-Trimethyl-1,3,5-trithiane	78	Class II	540
15.111	1,2,4-Trithiolane	78	Class II	540
15.125	4-Tetrahydrothiopyranone		Class II	540
12.295	3,5-Dimethyl-1,2-dithiolane-4-one		Class II	540
16.057	2,4,4-Trimethyl-1,3-oxathiane	78	Class II	540
12.120	2,8-Epithio-p-menthane	370	Class III	90
12.136	3-Mercapto-2-oxopropionic acid	160	Class III	90
12.300	1,1-Propanedithiol	7.5	Class III	90
12.301	Methyl-2-oxo-propyl disulfide	170	Class III	90
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)		Class III	90
15.081	Lenthionine	78	Class III	90
15.134	2,5-Dihydroxy-1,4-dithiane	500	Class III	90
16.062	trans-2-Methyl-4-propyl-1,3-oxathiane		Class III	90
16.114	2-Pentyl-4-propyl-1,3-oxathiane	290	Class III	90
16.122	4-Methyl, 2-propyl, 1-3-oxathiane	230	Class III	90
12.159	Methyl methanethiosulfonate	160	Class III	90

ANNEX III: METABOLISM

III.1. Introduction

The group comprises 80 straight, branched chain or heterogeneous ring aliphatic hydrocarbons containing one or more sulphur atoms. Depending on the type of sulphur-containing functional group(s), the candidate substances can be subdivided into 11 subgroups (see Table III.1).

The candidate substances are structurally closely related to 127 supporting flavouring substances evaluated at the 53rd meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in the groups "Simple aliphatic and aromatic sulphides and thiols" (JECFA, 2000b; JECFA, 2000c);. These supporting substances have been allocated to 11 subgroups in the same way as has been indicated for the candidate substances in Table III.1.

Table III.1 Subgroups. The supporting substances are listed in brackets

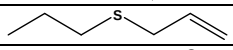
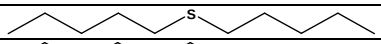
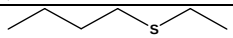
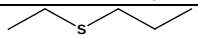
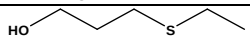
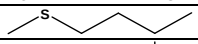
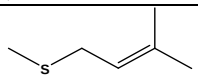
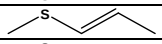
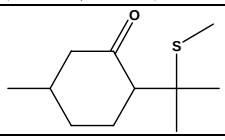
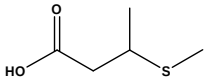
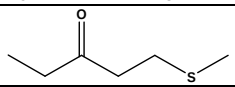
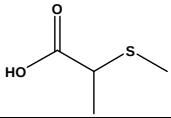
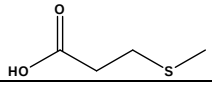
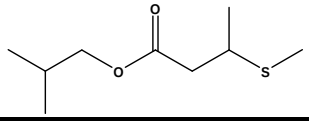
FL-no	EU Register name	Structural formula	Structural Class
I: ACYCLIC SULPHIDES			
12.096	Allyl methyl sulphide		II
12.099	Allyl propyl sulphide		II
12.117	Dipentyl sulphide		I
12.124	Ethyl butyl sulphide		I
12.127	Ethyl propyl sulphide		I
12.129	3-(Ethylthio)propan-1-ol		I
12.152	Methyl butyl sulphide		I
12.158	Methyl isoprenyl sulphide		I
12.163	Methyl prop-1-enyl sulfide		I
12.166	Methyl propyl sulphide		I
12.177	8-(Methylthio)-p-menthan-3-one		II
12.178	3-(Methylthio)butyric acid		I
12.181	1-(Methylthio)pentan-3-one		I
12.182	2-(Methylthio)propionic acid		I
12.183	3-(Methylthio)propionic acid		I
12.214	Isobutyl-3-(methylthio)butyrate		I

Table III.1 Subgroups. The supporting substances are listed in brackets

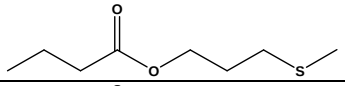
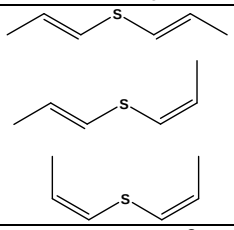
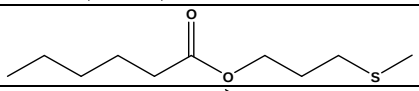
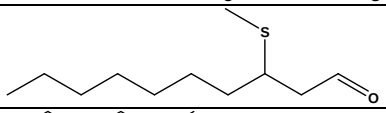
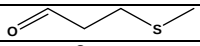
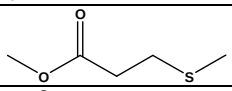
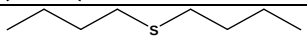
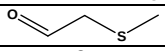
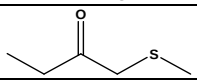
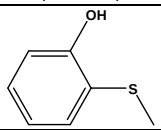
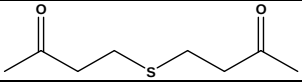
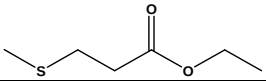
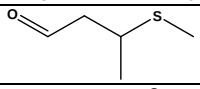
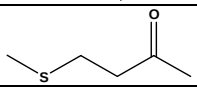
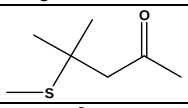
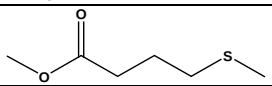
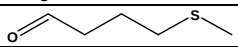
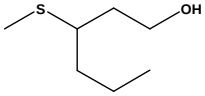
FL-no	EU Register name	Structural formula	Structural Class
12.277	3-(Methylthio)propyl butyrate		I
12.298	Di-(1-propenyl)-sulfid (mixture)		I
12.299	3-(Methylthio)propyl hexanoate		I
12.306	3-(methylthio)-decanal		I
(12.001)	3-(Methylthio)propionaldehyde		I
(12.002)	Methyl 3-(methylthio)propionate		I
(12.006)	Dimethyl sulphide		I
(12.007)	Dibutyl sulphide		I
(12.040)	2-Methylthioacetaldehyde		I
(12.041)	1-(Methylthio)butan-2-one		I
(12.042)	2-(Methylthio)phenol		II
(12.052)	Di-(3-oxobutyl) sulphide		I
(12.053)	Ethyl 3-(methylthio)propionate		I
(12.056)	3-(Methylthio)butanal		I
(12.057)	4-(Methylthio)butan-2-one		I
(12.058)	4-(Methylthio)-4-methylpentan-2-one		I
(12.060)	Methyl 4-(methylthio)butyrate		I
(12.061)	4-(Methylthio)butanal		I
(12.062)	3-(Methylthio)propan-1-ol		I
(12.063)	3-(Methylthio)hexan-1-ol		I

Table III.1 Subgroups. The supporting substances are listed in brackets

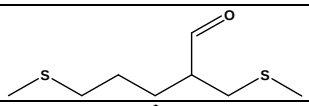
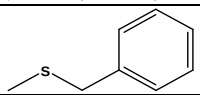
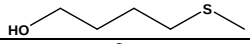
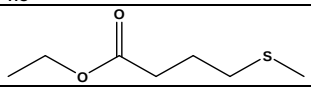
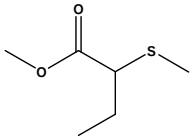
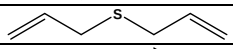
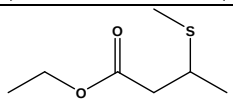
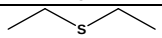
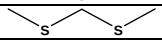
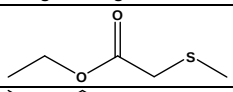
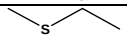
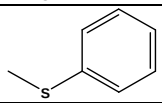
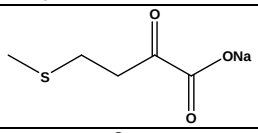
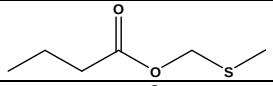
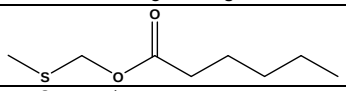
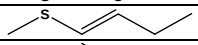
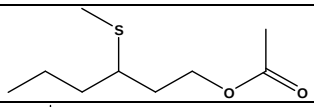
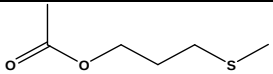
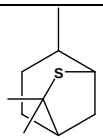
FL-no	EU Register name	Structural formula	Structural Class
(12.065)	2,8-Dithianon-4-en-4-carboxaldehyde		I
(12.077)	Benzyl methyl sulphide		II
(12.078)	4-(Methylthio)butan-1-ol		I
(12.084)	Ethyl 4-(methylthio)butyrate		I
(12.086)	Methyl 2-(methylthio)butyrate		II
(12.088)	Diallyl sulphide		II
(12.089)	Ethyl 3-(methylthio)butyrate		I
(12.113)	Diethyl sulphide		I
(12.118)	2,4-Dithiapentane		I
(12.122)	Ethyl 2-(methylthio)acetate		I
(12.154)	Methyl ethyl sulphide		I
(12.162)	Methyl phenyl sulphide		II
(12.176)	4-(Methylthio)-2-oxobutyric acid		III
(12.187)	Methylthiomethyl butyrate		I
(12.188)	Methylthiomethyl hexanoate		I
(12.211)	But-1-enyl methyl sulphide		I
(12.236)	3-(Methylthio)hexyl acetate		I
(12.237)	3-(Methylthio)propyl acetate		I
II: CYCLIC SULPHIDES			
12.120	2,8-Epithio-p-menthane		III
15.102	Tetrahydrothiophene		II

Table III.1 Subgroups. The supporting substances are listed in brackets

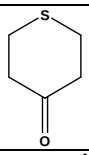
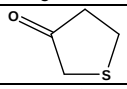
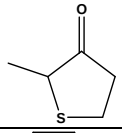
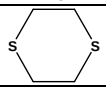
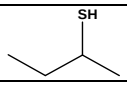
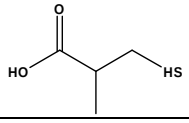
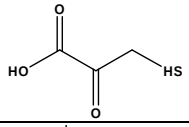
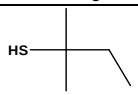
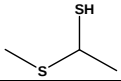
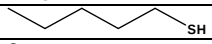
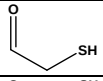
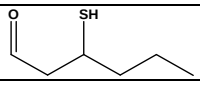
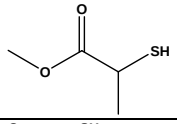
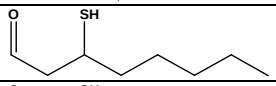
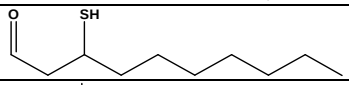
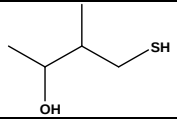
FL-no	EU Register name	Structural formula	Structural Class
15.125	4-Tetrahydrothiopyranone		II
(15.012)	4,5-Dihydrothiophen-3(2H)-one		II
(15.023)	4,5-Dihydro-2-methylthiophene-3(2H)-one		II
(15.066)	1,4-Dithiane		II
III: MONOTHIOLS			
12.104	Butane-2-thiol		I
12.135	3-Mercapto-2-methylpropionic acid		I
12.136	3-Mercapto-2-oxopropionic acid		III
12.172	2-Methylbutane-2-thiol		I
12.174	2-Methylpropane-2-thiol		I
12.180	1-(Methylthio)ethane-1-thiol		I
12.191	Pentane-1-thiol		I
12.205	Mercaptoacetaldehyde		I
12.250	3-Mercaptohexanal		I
12.266	Methyl-2-mercaptopropionate		I
12.268	3-Mercaptooctanal		I
12.269	3-Mercaptodecanal		I
12.302	2-Butanol, 4-mercapto-3-methyl		II

Table III.1 Subgroups. The supporting substances are listed in brackets

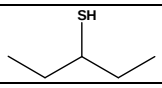
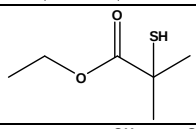
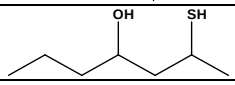
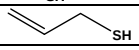
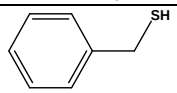
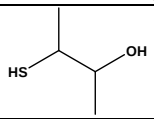
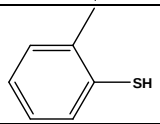
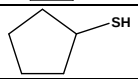
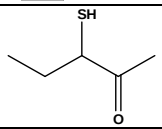
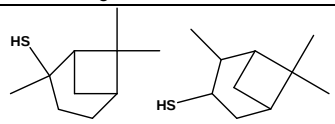
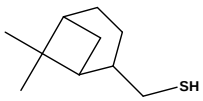
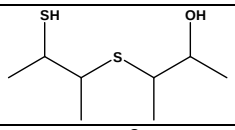
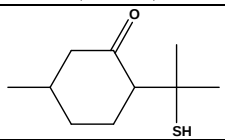
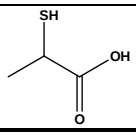
FL-no	EU Register name	Structural formula	Structural Class
12.303	3-Pentanethiol		I
12.304	Ethyl-2-mercapto-2-methyl propanoate		I
12.305	2-Mercapto-4-heptanol		II
(12.003)	Methanethiol		I
(12.004)	Allylthiol		II
(12.005)	Phenylmethanethiol		II
(12.010)	Butane-1-thiol		I
(12.024)	3-Mercaptobutan-2-ol		I
(12.027)	2-Methylbenzene-1-thiol		II
(12.029)	Cyclopentanethiol		II
(12.031)	3-Mercaptopentan-2-one		I
(12.035)	2-,3- and 10-Mercaptopinane	 **2-Mercaptopinane** **3-Mercaptopinane**  **10-Mercaptopinane**	II
(12.036)	3-[(2-Mercapto-1-methylpropyl)thio]butan-2-ol		I
(12.038)	8-Mercapto-p-menthan-3-one		II
(12.039)	2-Mercaptopropionic acid		I

Table III.1 Subgroups. The supporting substances are listed in brackets

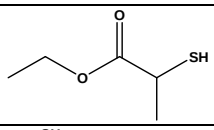
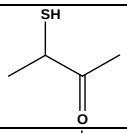
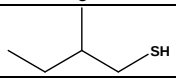
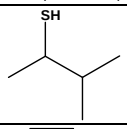
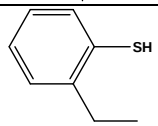
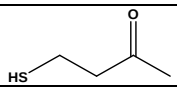
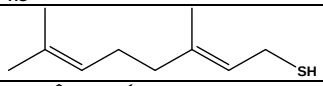
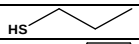
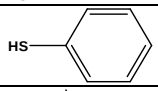
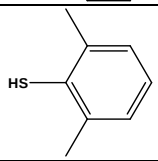
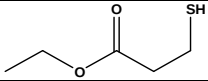
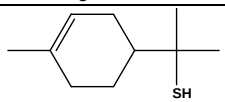
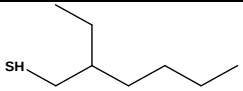
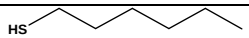
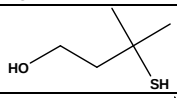
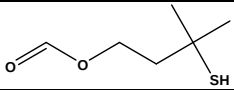
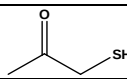
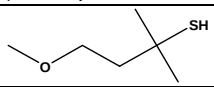
FL-no	EU Register name	Structural formula	Structural Class
(12.046)	Ethyl 2-mercaptopropionate		I
(12.047)	3-Mercaptobutan-2-one		I
(12.048)	2-Methylbutane-1-thiol		I
(12.049)	3-Methylbutane-2-thiol		I
(12.054)	2-(Ethylthio)phenol		III
(12.055)	4-Mercaptobutan-2-one		I
(12.064)	Thiogeraniol		I
(12.071)	1-Propane-1-thiol		I
(12.080)	Thiophenol		II
(12.082)	2,6-(Dimethyl)thiophenol		II
(12.083)	Ethyl 3-mercaptopropionate		I
(12.085)	p-Menth-1-ene-8-thiol		II
(12.128)	2-Ethylhexane-1-thiol		I
(12.132)	Hexane-1-thiol		I
(12.137)	3-Mercapto-3-methylbutan-1-ol		I
(12.138)	3-Mercapto-3-methylbutyl formate		I
(12.143)	1-Mercaptopropan-2-one		I
(12.145)	4-Methoxy-2-methylbutane-2-thiol		I

Table III.1 Subgroups. The supporting substances are listed in brackets

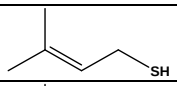
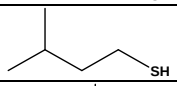
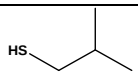
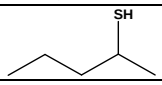
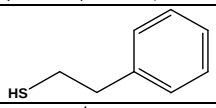
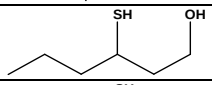
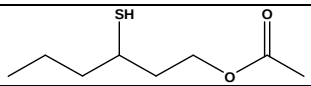
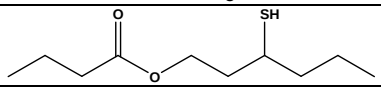
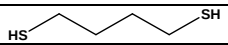
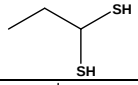
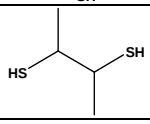
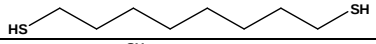
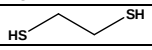
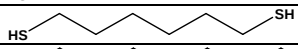
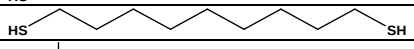
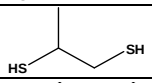
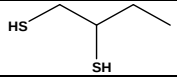
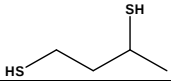
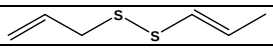
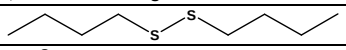
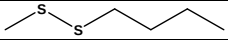
FL-no	EU Register name	Structural formula	Structural Class
(12.170)	3-Methylbut-2-ene-1-thiol		I
(12.171)	3-Methylbutane-1-thiol		I
(12.173)	2-Methylpropane-1-thiol		I
(12.192)	Pentane-2-thiol		I
(12.194)	2-Phenylethane-1-thiol		II
(12.197)	Propane-2-thiol		I
(12.217)	3-Mercaptohexan-1-ol		I
(12.234)	3-Mercaptohexyl acetate		I
(12.235)	3-Mercaptohexyl butyrate		I
IV: DITHIOLS			
12.103	Butane-1,4-dithiol		I
12.300	1,1-Propanedithiol		III
(12.022)	Butane-2,3-dithiol		I
(12.034)	Octane-1,8-dithiol		I
(12.066)	Ethane-1,2-dithiol		I
(12.067)	Hexane-1,6-dithiol		I
(12.069)	Nonane-1,9-dithiol		I
(12.070)	Propane-1,2-dithiol		I
(12.072)	Butane-1,2-dithiol		I
(12.073)	Butane-1,3-dithiol		I
(12.076)	Propane-1,3-dithiol		I
V: ACYCLIC AND CYCLIC DISULPHIDES			
12.098	Allyl prop-1-enyl disulfide 1)		II
12.111	Dibutyl disulfide		I
12.151	Methyl butyl disulfide		I

Table III.1 Subgroups. The supporting substances are listed in brackets

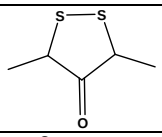
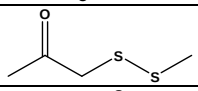
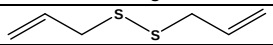
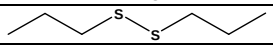
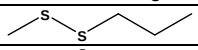
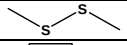
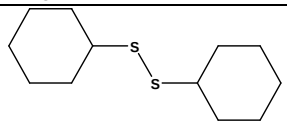
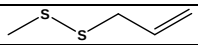
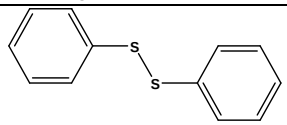
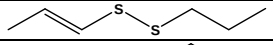
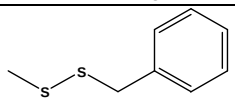
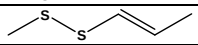
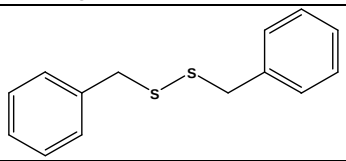
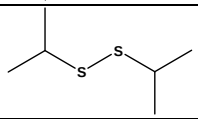
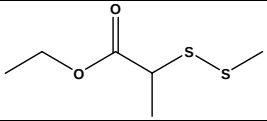
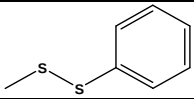
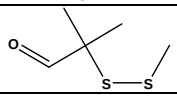
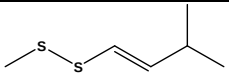
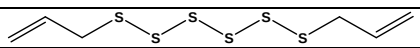
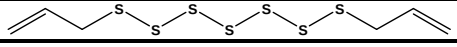
FL-no	EU Register name	Structural formula	Structural Class
12.295	3,5-Dimethyl-1,2-dithiolane-4-one 1)		II
12.301	Methyl-2-oxo-propyl disulfide		III
(12.008)	Diallyl disulfide		II
(12.014)	Dipropyl disulfide		I
(12.019)	Methyl propyl disulfide		I
(12.026)	Dimethyl disulfide		I
(12.028)	Dicyclohexyl disulfide		II
(12.037)	Allyl methyl disulfide		II
(12.043)	Diphenyl disulfide		III
(12.044)	Prop-1-enyl propyl disulfide		I
(12.068)	Benzyl methyl disulfide		II
(12.075)	Methyl prop-1-enyl disulfide		I
(12.081)	Dibenzyl disulfide		II
(12.109)	Di-isopropyl disulfide		I
(12.121)	Ethyl 2-(methyldithio)propionate		I
(12.161)	Methyl phenyl disulfide		II
(12.168)	2-Methyl-2-(methyldithio)propanal		I
(12.218)	Methyl-3-methyl-1-butenyl disulphide		I
VI: ACYCLIC POLYSULPHIDES			
12.093	Diallyl hexasulfide		II
12.094	Diallyl heptasulfide		II

Table III.1 Subgroups. The supporting substances are listed in brackets

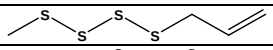
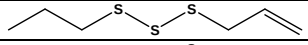
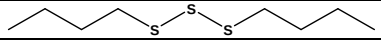
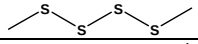
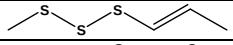
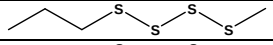
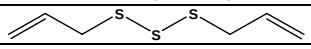
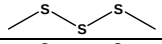
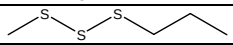
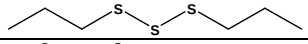
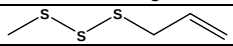
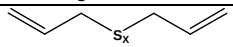
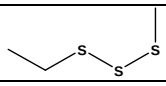
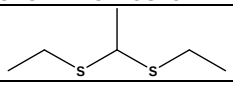
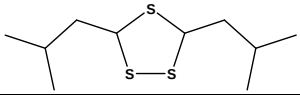
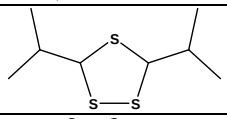
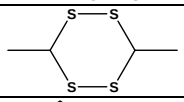
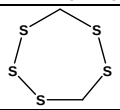
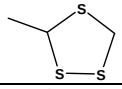
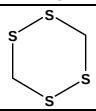
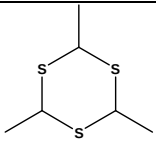
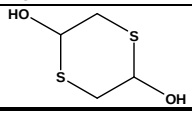
FL-no	EU Register name	Structural formula	Structural Class
12.097	Allyl methyl tetrasulfide		II
12.100	Allyl propyl trisulfide		II
12.112	Dibutyl trisulfide		I
12.116	Dimethyl tetrasulfide		I
12.164	Methyl prop-1-enyl trisulfide		I
12.167	Methyl propyl tetrasulfide		I
(12.009)	Diallyl trisulfide		II
(12.013)	Dimethyl trisulfide		I
(12.020)	Methyl propyl trisulfide		I
(12.023)	Dipropyl trisulfide		I
(12.045)	Methyl allyl trisulfide		II
(12.074)	Diallyl polysulfides	 X=2,3,4 or 5	II
(12.155)	Methyl ethyl trisulfide		I
VII: MONO-, DI- , TRI- AND POLYSULPHIDES WITH THIOACETAL STRUCTURE			
12.200	1,1-bis(Ethylthio)-ethane		I
15.047	3,5-Di-isobutyl-1,2,4-trithiolane		II
15.048	3,5-Di-isopropyl-1,2,4-trithiolane		II
15.056	3,6-Dimethyl-1,2,4,5-tetrathiane		II
15.081	Lenthionine		III
15.083	3-Methyl-1,2,4-trithiolane		II
15.103	1,2,4,5-Tetrathiane		II
15.110	2,4,6-Trimethyl-1,3,5-trithiane		II
15.111	1,2,4-Trithiolane		II
15.134	2,5-Dihydroxy-1,4-dithiane		III

Table III.1 Subgroups. The supporting substances are listed in brackets

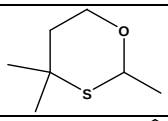
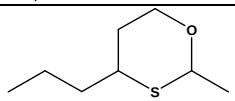
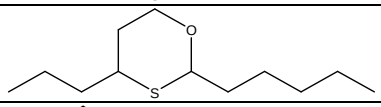
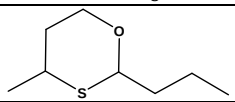
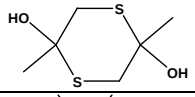
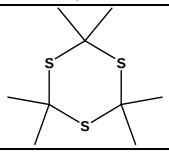
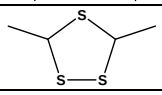
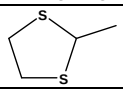
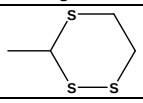
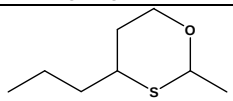
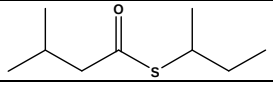
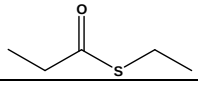
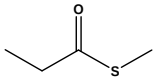
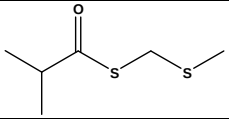
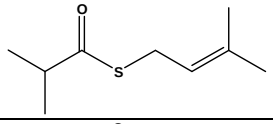
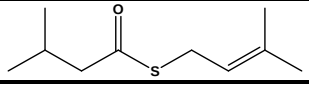
FL-no	EU Register name	Structural formula	Structural Class
16.057	2,4,4-Trimethyl-1,3-oxathiane		II
16.062	trans-2-Methyl-4-propyl-1,3-oxathiane		III
16.114	2-Pentyl-4-propyl-1,3-oxathiane		III
16.122	4-Methyl, 2-propyl, 1-3-oxathiane		III
(15.006)	2,5-Dihydroxy-2,5-dimethyl-1,4-dithiane		I
(15.009)	Trithioacetone		II
(15.025)	3,5-Dimethyl-1,2,4-trithiolane		II
(15.034)	2-Methyl-1,3-dithiolane		II
(15.036)	3-Methyl-1,2,4-trithiane		II
(16.030)	2-Methyl-4-propyl-1,3-oxathiane		II
VIII: THIOESTERS			
12.106	S-2-Butyl 3-methylbutanethioate		I
12.125	Ethyl propanethioate		I
12.165	S-Methyl propanethioate		I
12.189	S-(Methylthiomethyl) 2-methylpropanethioate		I
12.196	S-Prenyl thioisobutyrate		I
12.221	S-Prenyl thioisopentanoate		I

Table III.1 Subgroups. The supporting substances are listed in brackets

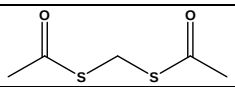
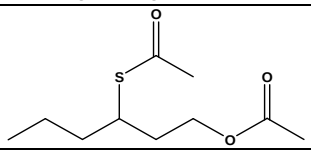
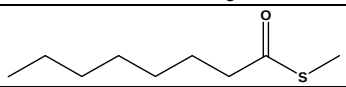
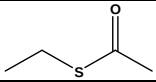
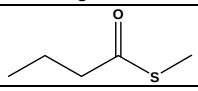
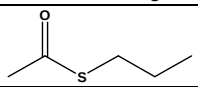
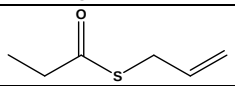
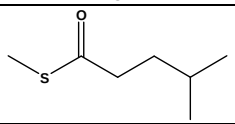
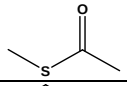
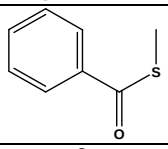
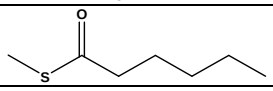
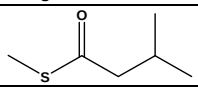
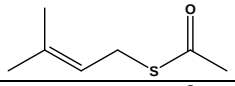
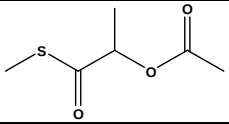
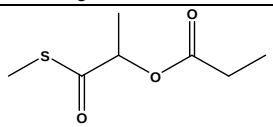
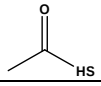
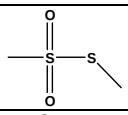
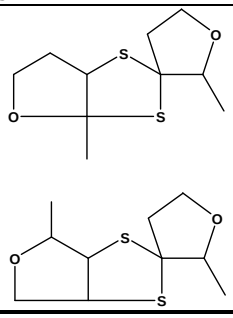
FL-no	EU Register name	Structural formula	Structural Class
12.271	Methanedithiol diacetate		I
12.278	3-Acetyl-mercaptohexyl acetate		I
12.282	(S)-Methyl octanethioate		I
(12.018)	S-Ethyl acetothioate		I
(12.032)	S-Methyl butanethioate		I
(12.059)	Propyl thioacetate		I
(12.101)	Allyl thiopropionate		I
(12.148)	S-Methyl 4-methylpentanethioate		I
(12.149)	S-Methyl acetothioate		I
(12.150)	S-Methyl benzothioate		II
(12.156)	S-Methyl hexanethioate		I
(12.157)	S-Methyl isopentanethioate		I
(12.195)	S-Prenyl thioacetate		I
(12.203)	Methylthio 2-(acetyloxy)propionate		I
(12.227)	Methylthio-2-(propionyloxy)propionate		I
IX: THIOIC ACID			
12.199	Ethanethioic acid		I
X: SULPHOXIDES/SULPHONES AND SULPHONATES			

Table III.1 Subgroups. The supporting substances are listed in brackets

FL-no	EU Register name	Structural formula	Structural Class
12.159	Methyl methanethiosulfonate		III
(12.175)	Methylsulfinylmethane		III
XI: CYCLIC THIOKETAL WITH FUSED OXOLANE RINGS			
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)		III

The general metabolic reactions that the candidate substances may be expected to undergo, and which are discussed below, are one or several of the following:

- S-oxidation
- reductions
- carbon-sulphur bond formation and/or fission
- oxidative desulphuration
- oxidative dealkylation
- S-methylation
- conjugation with glutathione and/or glucuronic acid
- hydrolysis

Very few data are available on candidate substances. However, based on data on structurally related compounds, both the supporting substances included in the present evaluation and others not used as flavouring substances, the following conclusion can be drawn.

There are not sufficient data available to determine to what degree the candidate substances may be absorbed from the gastro-intestinal tract. Lipophilicity and water solubility of these substances indicate a varying degree of absorption efficiency. For the purpose of this evaluation it is assumed that all substances will be absorbed.

Data on absorption of supporting substances are equally insufficient. However, available data on solubility and lipophilicity of both candidate and supporting substances outline that the supporting substances used for deriving NOAELs for the different subgroups of this evaluation have equal lipophilicity and equal or less water solubility than the corresponding candidate substances. This indicates that the candidate substances

may be absorbed to the same degree as the corresponding supporting substances, and that the use of NOAELs from these supporting substances does not underestimate the toxicity of the candidate substances in this respect.

III.2. Sulphides, Sulphoxides/Sulphones and Sulphonates

The following description is pertinent to subgroups I, II, IX and X.

All the sulphides (or thioethers) among the candidate substances are sufficiently lipophilic to be efficiently absorbed from the gastrointestinal (GI) tract. Oral doses of the drugs sulphinpyrazone and sulindac are completely absorbed and their metabolites excreted in the bile of humans (Renwick et al., 1982; Renwick et al., 1986; Strong et al., 1984b), while dimethyl sulphoxide and dimethyl sulphone are excreted in the urine as metabolites of methyl sulphide administered subcutaneously to rabbits (Williams et al., 1966).

Once alkyl and aromatic sulphides enter systemic circulation, they are rapidly oxidised to sulphoxides, and, depending on the structure of the sulphide, may be further oxidised to the sulphone (Figure III.1). The products of S-oxidation reactions may react spontaneously with glutathione, and it is likely that they also exhibit reactivity towards nucleophilic sites in cellular macromolecules. The S-reaction is favoured by the presence of a lone reactive pair of electrons on divalent sulphur in monosulphides (Damani, 1987), as shown by the excretion in the urine of dimethyl sulphoxide and dimethyl sulphone after methyl sulphide subcutaneous administration to rabbits (Williams et al., 1966).

Although S-oxidation generally yields mixtures of sulphone and sulphoxide metabolites, the relative amounts of excretion products are dependent upon the polarity of the sulphide. In rats, polar aliphatic sulphides give rise to higher proportion of the sulphoxide metabolites (Damani, 1987). This is probably due to the water-solubility of the sulphoxides, which presumably limits their partitioning into the catalytic sites on the microsomal monooxygenase systems (P450 and FMO), involved in the S-oxidation reaction (Damani, 1987).

The first oxidation from sulphide to sulphoxide is reversible, whereas the sulphone group is stable and is not reduced back to the sulphoxide; this latter irreversibility seems to be related to the substrate specificity of the reductase (Renwick, 1989). The reduction of sulphoxide is mediated by the GI tract microflora as well as by hepatic and extra hepatic mammalian reductase. In many cases the reversible nature of the sulphide-sulphoxide reaction depends on the dynamic metabolising system provided by intestinal flora (1010 bacteria/g of gut content). Anaerobic organisms populate the upper intestines and stomach of mice and rats. Their distribution is concentrated in the lower intestines in rabbits and humans, possibly due to lower gastric pH. In all species, reduction predominates in the lower gut, mainly the cecum and colon. Therefore, if gut flora is involved in the metabolism of monosulphide- and thiol-containing flavouring substances, the sulphur derivatives must either be incompletely absorbed or reach the lower gut as biliary metabolites (Renwick and George, 1989), then entering the enterohepatic circulation.

In vitro under anaerobic conditions, the sulphoxide anti-inflammatory drug sulphinpyrazone is reduced approximately six times faster in cultures with cecum contents than with liver cell homogenates from either rats (Renwick et al., 1982) or rabbits (Strong et al., 1984a). Oral doses of the sulphoxide drugs sulindac and sulphinpyrazone, which are completely absorbed and excreted in the bile of humans, are bioactivated by reduction to the corresponding monosulphides (Renwick et al., 1982; Renwick et al., 1986; Strong et al., 1984b). The gut microflora is considered the major site of reduction of sulphinpyrazone to its sulphide in man (Renwick et al., 1982; Renwick et al., 1986; Strong et al., 1984b), whereas the reduction of sulindac to its sulphide takes place mainly in the liver, although gut microflora is partially involved (Renwick, 1989).

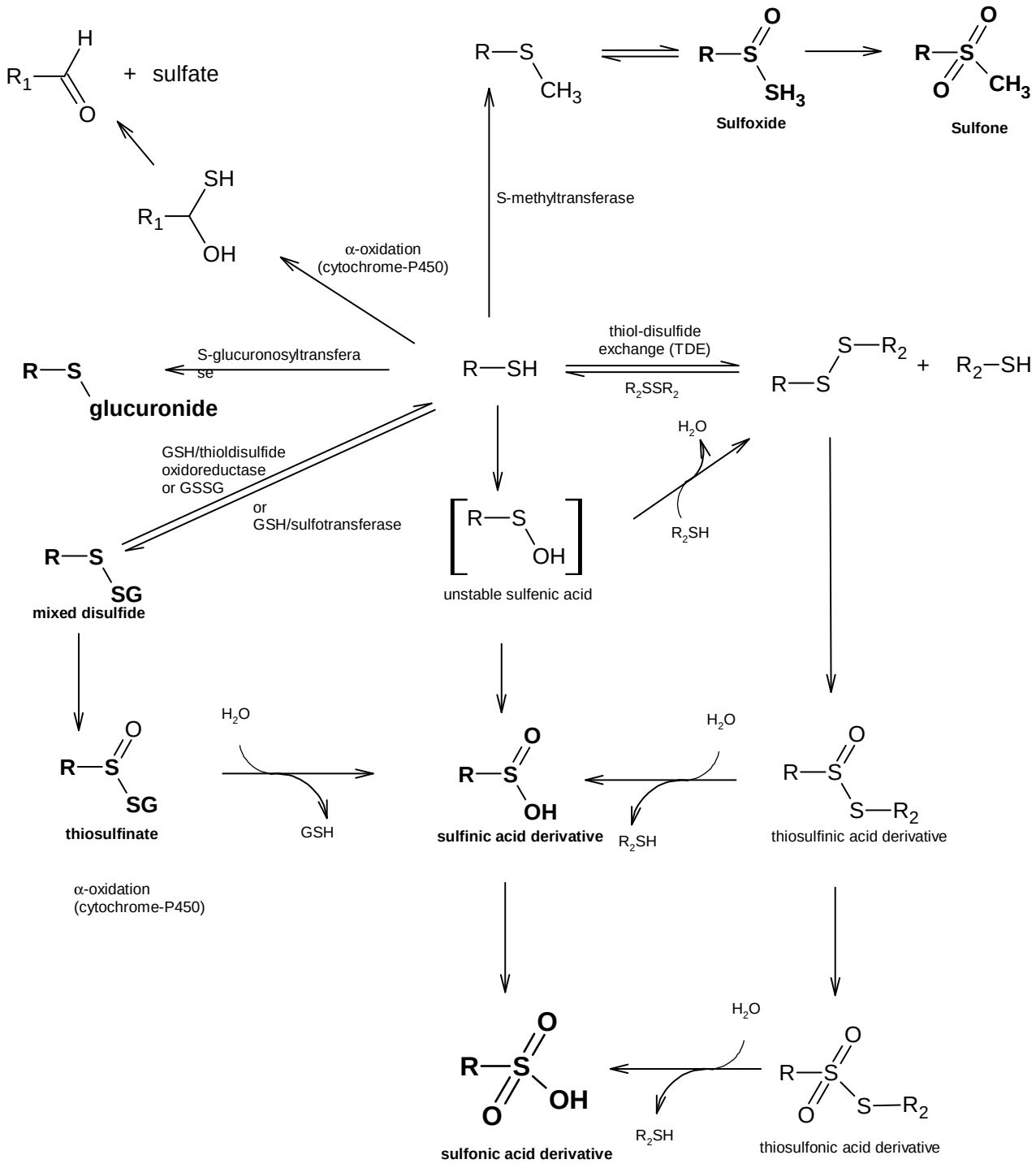


Figure III.1: Biotransformation of disulphides, thiols, and related sulphur substances (Excretion products in bold)

The metabolism of dipropyl sulphide (as supporting for compounds in subgroups I), dipropyl sulphoxide, and dipropyl sulphone has been studied extensively in rats (Nickson and Mitchell, 1994; Nickson et al., 1995). Dipropyl sulphide is metabolised mainly to the corresponding sulphoxide. Other excreted metabolites include small amounts of the sulphone and trace amounts of inorganic sulphate. Individual studies on the sulphoxide and sulphone indicate that these metabolites are relatively stable under physiologic conditions.

Ten male Wistar rats were given a single oral dose of 513 mg/kg bw [^{35}S]-dipropyl sulphide in corn oil by gavage. The majority of radioactivity (92.8 %) recovered over the following three days was in the urine (66 %), with lesser amounts in exhaled air (17.7 %), faeces (4.6 %) and carcass (1.5 %). Plasma profiles showed a slow continuous absorption with peak plasma levels occurring at 12 - 15 hours. The sulphoxide was the only species detected in the plasma. In the urine, about 25 % of the radioactivity was accounted for on day 1 and 39 % on day 2. This delayed urinary excretion was related to enterohepatic cycling of the major metabolite dipropyl sulphoxide. Approximately 25 % of the radioactivity passed through the bile duct over 48 hours, with only 5 % being excreted in the faeces. The only biliary metabolites detected were the sulphoxide (80 %) and sulphone (20 %). Urinary metabolites collected during the first 24 hours included the sulphoxide (92.5 %), sulphone (5 %) and sulphate (3 %). On days 2 and 3, the sulphoxide accounted for more than 98 % of daily urinary metabolites (Nickson and Mitchell, 1994).

In a parallel study, eight rats were each given 580 mg [^{35}S]-dipropyl sulphoxide/kg bw. Essentially the entire administered radioactivity was recovered over the following three days in the urine (80 %), exhaled air (1.4 %), faeces (5.0 %) and carcass (13.0 %). Peak plasma levels occurred slightly later (15 - 20 hours) for the sulphoxide compared to that for the sulphide (12 - 15 hours). In the urine, about 28 % of the radioactivity was accounted for on day 1 and 47 % on day 2. The delayed urinary excretion paralleled that for the sulphide and supports the conclusion that enterohepatic cycling of sulphoxide delays the urinary excretion. In the bile, radioactivity was excreted as the sulphoxide (70 %) and sulphone (30 %) (Nickson and Mitchell, 1994). The profile of urinary metabolites was the same after administration of the sulphoxide or the sulphide. The principal quantitative difference was that more sulphone (18 % on day 1) was excreted after sulphoxide administration.

In rats, dipropyl sulphone is physiologically stable and is excreted unchanged in the urine (Nickson et al., 1995). The pattern of absorption, distribution and excretion was similar to that of sulphide and sulphoxide.

Urine was the major route of excretion (83 %), again with a greater percentage of radioactivity excreted on day two (47 %) than on day one (28 %). As with the sulphide and sulphoxide, biliary excretion played a key role with 33 % of the dose passing through the bile within 48 hours. The metabolism of the administered sulphone appeared quite limited. More than 98 % was excreted unchanged in the urine along with trace amounts of inorganic sulphate. No reduction of the sulphone group or oxidation of the hydrocarbon chain was observed.

Based on the results of these three studies, it can be concluded that dipropyl sulphide is metabolised in the rat via S-oxidation to dipropyl sulphoxide and, to a small extent, dipropyl sulphone. The sulphoxide and sulphone are physiologically stable, and for the most part excreted unchanged.

The fate of sulphoxides in humans is similar to that in rats. Dimethyl sulphoxide (DMSO) is the primary metabolite of methyl sulphide (as supporting for compounds in subgroups I). When an oral dose of 1 g/kg bw DMSO was given to six subjects, peak serum concentrations (1 - 3 mg/ml) were observed approximately four hours after administration (Hucker et al., 1967). Peak dimethyl sulphone concentrations (1 - 5 mg/ml) were measured at 72 - 96 hours. Approximately 51 % of the dose was excreted in the urine unchanged over the first 120 hours. Up to 22 % of the dose was excreted as dimethyl sulphone beginning 20 hours after dosing. Repeated daily oral administration of 0.5 g/kg bw/day DMSO for 14 days to one adult human showed similar peak serum levels (2 mg/ml) of DMSO achieved by day 8 of the study. Urinary excretion of DMSO was linear throughout the dosing period. After day 14, the DMSO concentration decreased to non-detectable levels.

In Rhesus monkeys the absorption, metabolism and excretion of DMSO are similar, although more rapid, to that for humans. Three monkeys were given a daily oral dose of 3 mg DMSO/kg bw for 14 days (Layman and Jacob, 1985). DMSO was rapidly absorbed, reached peak serum concentration after about four hours, and was cleared from the blood within 72 hours after termination of treatment. Dimethyl sulphone was

detected in the blood two hours after treatment and reached a steady state concentration after four days. It was cleared from the blood 120 hours after treatment ended. Urinary excretion of DMSO and dimethyl sulphone accounted for approximately 60 % and 16 %, respectively, of the total ingested dose. Neither DMSO nor dimethyl sulphone were detected in the faeces (Layman and Jacob, 1985).

Aliphatic, heterocyclic and aryl sulphides participate in the same oxidation pathway. Ring sulphoxidation have been reported in some sulphur heterocyclic drugs (Damani, 1987). When the supporting substance methyl phenyl sulphide [FL-no: 12.162] was administered orally to rats, methyl phenyl sulphone and hydroxylated sulphones (i.e., hydroxy methyl phenyl sulphone and conjugates of hydroxy methyl phenyl sulphone) were detected in the urine (McBain and Menn, 1969). Similarly, 4-chlorophenyl methyl sulphide was reported to be oxidised by FMO and P450 to the sulphoxide and sulphone derivatives in vitro (Nnane and Damani, 1995). The aromatic sulphoxide, diphenyl sulphoxide, perfused with intact guinea-pig liver is oxidised exclusively to the corresponding sulphone under normoxic conditions (Yoshihara and Tatsumi, 1990).

The oxidation to sulphoxides is mainly catalysed by two enzyme systems, P450 and FMO (Renwick, 1989). Any organosulphur compound may be a substrate for both the enzyme systems, although with different affinity, essentially dependent on the electromolecular environment in which the sulphur is located; the more nucleophilic divalent sulphur are primarily oxidised by FMO and to a lesser extent by P450. This is the case for simple aliphatic (e.g. the supporting substance diethyl sulphide [FL-no: 12.113]), alicyclic (e.g. thiolane) and aromatic (e.g., ethyl p-tolyl sulphide) sulphides (Hoodi and Damani, 1984; Damani, 1987). Moreover, another important determinant is the tissue-specific distribution of the two different enzymatic systems, especially in extrahepatic tissues, as well as the differential presence of single isoforms, with different catalytic activities.

Both P450- and FMO-catalysed oxidations may be accompanied by stereoselectivity.

A series of 2-aryl-1,3-dithiolanes incubated with rabbit lung microsomes, pulmonary FMO fractions or pulmonary P450 fractions were oxidised primarily to the trans sulphoxide isomer; the enantioselectivity produced by FMO was higher when compared to P450 (Cashman et al., 1990; Cashman and Williams, 1990). Different isoenzymes of FMO, c-DNA expressed in *E.coli* have been used to investigate further the stereochemistry of sulphoxidation in humans (Rettie et al., 1994). When methyl p-tolyl sulphide was incubated with human foetal liver and human kidney microsomes from which P450 had been inhibited, the resulting sulphoxide contained an enantiomeric excess (> 86 %) of the (R)-isomer. Decreasing stereo selectivity was observed with increasing size of the alkyl group (i.e. ethyl, propyl or isopropyl) (Sadeque et al., 1992) and increasing pH (i.e. 8.5 to 10) (Rettie et al., 1990). Stereoselectivity was also dependent on the isoform involved in the reaction; oxidation of the propyl and butyl p-tolyl sulphide with the dominant human liver FMO isozyme, FMO3, showed a preference for the (R)-enantiomer (73 - 88 %), whereas oxidation of the methyl or ethyl derivative by human FMO5 showed greater than 90 % preference for the formation of the (S)-stereoisomer of the sulphoxide (Sadeque et al., 1995).

Oxidation of unsubstituted and methyl-substituted cyclic sulphides by a rabbit liver phenobarbital-type P450 yielded corresponding sulphoxides, but corresponding sulphones were not detected (Takata et al., 1983). In a subsequent experiment to the Takata et al. (1983) study using rabbit liver phenobarbital-type P450, pig liver microsomal FMO was used to elucidate mechanisms involved in the oxygenation of simple aryl or alkyl sulphides. The experiment demonstrated that oxygenation of sulphide with pig liver microsomal FMO involves the nucleophilic attack of the divalent sulphur on the reactive oxygen atom at the enzyme active site, i.e. electrophilic oxygenation of sulphide; whereas the oxygenation with the rabbit liver phenobarbital-type P450 is initiated by a single electron transfer from the sulphide to the enzyme active species (Oae et al., 1985). P450 can also catalyse the dealkylation of sulphides, but only when S is bonded to an electronegative substituent (e.g. an acyl-group) (Oae et al., 1985).

Oxygenated functional groups provide additional sites for the biotransformation of sulphides. Therefore, when a substance contains both a sulphide and an oxygenated functional group (i.e. alcohol, aldehyde, acid or ketone function), C-oxidation and/or conjugation may compete with S-oxidation. However, even in the presence of oxygenated functional groups, sulphoxide formation is usually the major metabolic pathway.

Examples of concurrent metabolism via both sulphur and oxygenated functional groups have been reported for various substrates (Black et al., 1993; Feng and Solsten, 1991; Gachon et al., 1988; Karim El Fatih et al., 1988). In all of them, the predominance of S-oxidation pathway has been reported. As an example, when 40 mg/kg bw of [^{13}C4, ^{35}S]-thiodiglycol ($(HOCH_2CH_2)_2S$) was administered intraperitoneally to male Porton rats, the major urinary metabolites were the corresponding sulphoxide (90 %) and carboxylic acid, S-(2-hydroxyethylthio)acetic acid (10 %). The corresponding sulphone and combined C- and S-oxidation product, S-(2-hydroxyethylsulphinyl) acetic acid, were only minor metabolites (Black et al., 1993).

Analogously, the corresponding sulphoxide is the principal urinary metabolite of the mucolytic drug S-carboxymethyl-L-cysteine (S-containing amino acid) (Damani, 1987); in the case of the histamine antagonist cimetidine (S-containing amidine) in humans, the unchanged compound and the sulphoxide were identified in faecal samples, whereas the urinary metabolites were the glucuronide, the sulphoxide and a very low amount of the 5-hydroxymethyl-cimetidine (Mitchell et al., 1982).

In summary, sulphides undergo FMO and P450 catalysed oxidation to yield chiral sulphoxides. Subsequent oxidation of the sulphoxide to the sulphone is an irreversible reaction that is mainly catalysed by P450. The relative amounts of sulphoxide and sulphone excreted are dependent upon the stability and hydrophilicity of the sulphoxide (Damani, 1987). However, the sulphoxide is generally the predominant urinary metabolite of simple sulphides, such as methyl sulphide (Williams et al., 1966).

Based on the numerous examples of successive oxidation of sulphides to sulphoxides and sulphones by FMO and P450 enzymes in a variety of test systems (Cashman and Williams, 1990; Cashman et al., 1990; Cashman et al., 1995a; Cashman et al., 1995b; Elfarra et al., 1995) and (Nnane and Damani, 1995; Rettie et al., 1990; Sadeque et al., 1992; Sadeque et al., 1995; Yoshihara and Tatsumi, 1990), it is concluded that the oxidation pathway is the major route of biotransformation of (mono)sulphides in humans (Ziegler, 1980; Nickson and Mitchell, 1994). The same applies to sulphides containing an oxygenated functional group (i.e., alcohol, aldehyde, acid or ketone function); indeed, although C-oxidation and/or conjugation may compete with S-oxidation, sulphoxide formation is usually the major metabolic pathway.

Three of the candidate substances from subgroup I are esters, isobutyl-3-(methylthio)butyrate [FL-no: 12.214], 3-(methylthio)propyl butyrate [FL-no: 12.277] and 3-(methylthio)propyl hexanoate [FL-no: 12.299], which are anticipated to be hydrolysed, respectively, to 2-methylpropanol [FL-no: 02.001] and 3-(methylthio)butyric acid [FL-no: 12.178], to 3-(methylthio)propan-1-ol [FL-no: 12.062] and butyric acid [FL-no: 08.005] and to 3-(methylthio)propan-1-ol [FL-no: 12.062] and hexanoic acid [FL-no: 08.009]. The candidate substance methyl methanethiosulphonate [FL-no: 12.159] from subgroup X is anticipated to be hydrolysed to methanol and methanethiosulphonic acid. The substance from subgroup IX, ethanethioic acid [FL-no: 12.199], converts to acetic acid [FL-no: 08.002]. See Table 2b.

Studies for Candidate Substances

<u>3-(Methylthio)propionic acid [FL-no: 12.183] (Subgroup I)</u>

The metabolism of [methyl-^{14}C]- and 3-methyl [^{35}S]thiopropionate (the salt of 3-(methylthio)propionic acid) was studied in a rat liver homogenate system. In addition to carbon dioxide and sulphate, methanethiol and hydrogen sulphide are intermediary or excreted metabolites of the salt of the candidate substance 3-(methylthio)propionic acid (Steele and Benevenga, 1979). The developmental changes for rats in the metabolism of the salt of 3-(methylthio)propionic acid were measured for animals from 1 to 400 days of age.

The metabolic capacity of liver homogenates to produce methanethiol and hydrogen sulphide from 3-methyl [^{35}S]thiopropionate increased six-fold during the first week of life, remained at that level through weaning, and gradually decreased to essentially the value observed in the one-day old rat by 400 days of age.

This pattern is not altered when the data are expressed in relation to tissue O_2 consumption, implying that the greater ability of young rats to produce methanethiol and hydrogen sulphide from 3-methyl[^{35}S]thiopropionate is not simply a reflection of greater metabolic rate (Finkelstein and Benevenga, 1984).

Methyl propyl sulphide [FL-no: 12.166] (Subgroup I)

Information may be derived from a study on the biotransformation of methyl, ethyl, isopropyl and propyl thiols, studied in rabbit liver microsomes. The results demonstrate that the thiols are primarily converted to the sulphoxides; then rabbit liver catalyses the S-methylation of shortchain alkane to yield the corresponding methyl sulphides. The coenzyme in this process, as with most other methyltransferases, is S-adenosyl-L-methionine. The resulting methyl sulphides, including the candidate substance methyl propyl sulphide [FL-no: 12.166] are further transformed by formation of the corresponding sulphoxide and sulphone. The methylation of short-chain alkane thiols to methylthioethers acts as a detoxication mechanism for the reactive sulphhydryl group (Holloway et al., 1979).

Allyl methyl sulphide [FL-no: 12.096] (Subgroup I)

Expiration of human subjects was trapped and analysed by GC-MS for volatile sulphur derivatives after subjects chewed and ate 1000 mg of grated raw or grated heat-treated garlic for 30 seconds. Allyl methyl sulphide, allyl mercaptan and methyl mercaptan were determined to be the important volatile low-molecular weight sulphur compounds expired. Analytical concentrations for the candidate substance allyl methyl sulphide [FL-no: 12.096] for raw garlic and heated garlic at the first measurement time point (0 minutes) were about 0.03 ppm and 0.05 ppm, respectively, and after 30 minutes had decreased to approximately 0.01 and < 0.05 (Tamaki and Sonoki, 1999). It was determined that the major volatile metabolite detected in breath and plasma from human subjects which had consumed dehydrated granular garlic and an enteric-coated garlic preparation is allyl methyl sulphide (Rosen et al., 2000; Rosen et al., 2001). Its formation is very likely due to the action of allicin, released by garlic preparations, which decomposes in the stomach or in the intestine to release allyl sulphides, disulphides and other volatile sulphur compounds.

Primary rat hepatocytes prepared by collagenase perfusion were incubated with diallyl disulphide or diallyl sulphide and the metabolites were identified. Allyl mercaptan and allyl methyl sulphide are the metabolites of diallyl disulphide. The highest amount of allyl methyl sulphide (0.93 ± 0.08 µg/ml at 90 minutes) is much less than that of allyl mercaptan (46.2 ± 6.6 µg/ml at 60 minutes) (Sheen et al., 1999).

Tetrahydrothiophene [FL-no: 15.102] (Subgroup II)

In a study on the metabolism of 1,4-dibromobutane, six rats were injected intraperitoneally with 20.3 mg of the test substance in arachis oil. Urine samples were collected during the 24-hour period prior to dosing, and at 24 and 48 hours after dosing. Tetrahydrothiophene [FL-no: 15.102] and the corresponding hydroxylated sulphone, 3-hydroxysulpholane, were the only stable sulphur-containing metabolites identified and they were quantified for the 0 - 24, 24 - 48 and 0 - 48 time intervals using GLC with FID detection. At 48 hours, tetrahydrothiophene and 3-hydroxysulpholane in excreted urine were determined to be 5.8 ± 1.1 and 57 ± 15 % of the dose of the parent compound, respectively. The authors concluded that 1,4-dibromobutane is extensively metabolised via GSH conjugation, resulting in the efficient detoxification of the parent compound. The initial conjugation to GSH in the biotransformation leads to the formation of a relatively stable cyclic sulphonium ion, *N*-acetyl-S-(beta-alanyl) tetrahydrothiophenium salt. This sulphonium salt is excreted to a minor extent as such; however, the major fraction decomposes *in vivo* to tetrahydrothiophene,

which is further metabolised to yield 3-hydroxysulpholane, and both metabolites are excreted in the urine (Onkenhout et al., 1986).

III.3. Thiols

The following discussion is pertinent to subgroups III and IV.

Thiols are highly reactive *in vivo*, mainly because most thiols exist in the ionised form at physiologic pH. Metabolic options for thiols include oxidation to form unstable sulphenic acids (RSOH), which may be oxidised to the corresponding sulphinic (RSO_2H) and sulphonic acids (RSO_3H); methylation to yield methyl sulphides, which then form sulphoxides and sulphones; reaction with physiologic thiols (either present in small molecules such as cysteine and glutathione or in biomacromolecules) to form mixed disulphides, or conjugation with glucuronic acid; and/or oxidation of the alpha carbon, resulting in desulphuration and formation of an aldehyde intermediate (McBain and Menn, 1969; Dutton and Illing, 1972; Maiorino et al., 1989; Richardson et al., 1991).

In the candidate substance 1,1-propanedithiol [FL-no: 12.300] the two SH groups are attached to the same carbon atom. This would not affect the stability or reactivity of these SH groups and therefore this candidate substance is anticipated to undergo the same metabolic reactions as monothiols or dithiols in which the SH groups are connected to different carbon atoms (cf. the candidates in subgroup IV) (Magnusson, 1962).

Oxidation to Sulphonic Acid

Enzymatic oxygenation of thiols results in the reactive sulphenic acid, sulphinic acid and sulphonic acid (see Figure III.1). The sulphenic acid almost instantaneously react with thiols to produce disulphides. The resulting disulphides can either be reduced to yield thiols or be further oxidised to yield sulphonic acid derivatives via thiosulphinic and thiosulphinic acid intermediates. Alternatively, S-oxigenation of disulphide may be followed by hydrolytic cleavage of the S-S bond. Among thiols, the sulphenic acid preferentially reacts with GSH, yielding mixed disulphide, the reduction of which by GSH would generate the foreign thiols, as follows:

$$RSOH + GSH \longrightarrow RSSG \xrightarrow{GSH} RSH + GSSG$$

This oxidation/reduction cycle may be the main cause of GSH tissue depletion and/or alteration of the cellular oxidative status (Ziegler, 1980).

Dermal administration of pyridine-2-thiol-N-oxide gave rise to the corresponding sulphonic acid as the major metabolite in rats, with the disulphide present in much smaller amounts (Min et al., 1970).

Methylation

Simple aliphatic and aromatic thiols undergo S-methylation in mammals to produce the corresponding methyl sulphides, which may be successively oxidised to the corresponding sulphoxides and sulphones. Principally two enzymes, both of which require S-adenosyl-L-methionine as a methyl group donor, catalyse the methylation reaction.

In microsomes, S-methylation is catalysed by thiol methyltransferase (TMT), which exhibits a substrate preference for ‘non-physiological’ aliphatic thiols. Compounds such as 2-mercaptoethanol, methylmercaptan and 2-mercaptopropionic acid are substrates for TMT (Bremer and Greenberg, 1961), but the endogenous aliphatic thiols, homocysteine and glutathione are not. TMT is an adenosine-L-methionine-dependent

membrane-bound enzyme. In human red blood cells membranes TMT exhibits high and low affinity activities, which show distinct pH dependence.

In the cytoplasm of all mammalian tissues, S-methylation is catalysed by thiopurine methyltransferase (TPMT). This enzyme has similar levels of activity in human liver, kidney and erythrocytes (Szumlanski et al., 1988). Preferential substrates for this enzyme are thiopurines and thiopyrimidines, but other aromatic and heterocyclic thiols are also metabolised, although apparent K_m values of thiophenols are at least two orders of magnitude less than those for thiopurines (Ames et al., 1986; Woodson and Weinshilboum, 1983; Woodson et al., 1983).

TPMT activity in human tissue is regulated by a common genetic polymorphism (Woodson et al., 1982). Results of family studies indicate that the polymorphism is due to a single genetic locus with two alleles, $TPMT^H$ for high activity and $TPMT^L$ for low activity, with 94 % and 6 % gene frequencies, respectively. This fact results in a trimodal frequency distribution of TPMT activities in the general population. Of 298 subjects, 89 % showed high TPMT activity (homozygous for the high activity allele), 11.1 % being heterozygous showed intermediate activity and 0.3 % ($TPMT^L$-$TPMT^L$) showed no activity (Woodson et al., 1982).

The impact of inherited differences in "methylator status" on the metabolism of thiols at extremely low levels of exposure via the diet is not currently known. However, microsomal TMT and cytoplasmic TPMT activities are regulated independently in human tissue (Keith et al., 1983). Therefore, S-methylation of thiols may occur even in individuals showing no TPMT activity, although with different rates. Furthermore, alternative metabolic pathways such as S-oxidation and conjugation reaction are active, suggesting that thiol-containing flavouring substances would be metabolised even in the absence of TPMT activity.

Examples of S-methylation cover a broad spectrum of aliphatic and aromatic substrates. Ethyl methyl sulphide was detected in the urine of guinea pigs and mice following an oral dose of diethyl disulphide. Presumably, diethyl disulphide was reductively cleaved to form ethanethiol, which was subsequently methylated to form ethyl methyl sulphide. Minor urinary metabolites of ethyl methyl sulphide were the sulphoxide and sulphone (Snow, 1957).

The urine of rats orally dosed with 6 mg/kg [^{35}S]-phenyl mercaptan contained metabolites derived from S-methylation of the administered parent mercaptane. Phenyl methyl sulphide metabolites included phenyl methyl sulphone, and o- and p-hydroxylated phenyl methyl sulphone (McBain and Menn, 1969). The alkyl thiol, captopril, undergoes S-methylation in the presence of S-adenosyl-L-methionine when incubated with microsomal fractions prepared from human liver, renal cortex, renal medulla or intestinal mucosa (Pacifici et al., 1991a).

The urine of rats given a 10 mg/kg oral dose of S-benzyl-N-malonyl-L-cysteine contained the sulphoxide and sulphone derivative of benzyl methyl sulphide. Presumably, benzyl methyl sulphide forms via methylation of the intermediary metabolite benzyl mercaptan (Richardson et al., 1991).

Reaction with Glutathione

Thiols react with glutathione to form mixed disulphides. Both membrane-bound and cytosolic thioltransferases have been reported to catalyse the formation of mixed disulphides. Mixed disulphides can undergo reduction and oxidative desulphuration or oxidation to sulphonic acid via the intermediates, thiosulphinate and sulphinic acid (Figure III.1).

The mixed disulphides formed from glutathione and thiols are not substrates for the potentially intoxicating enzyme, cysteine conjugate beta-lyase. The beta-lyase is vitamin B6-dependent and catalyses the reduction

of cysteine conjugates of selected halogenated substrates, yielding unstable thiols that induce renal toxicity (Shaw and Blagbrough, 1989; Tateishi et al., 1978).

Oxidation and Desulphuration

Low molecular weight thiols undergo oxidative desulphuration *in vivo* to yield CO_2 and SO_4^{2-}.

When ^{14}C-labeled methanethiol (supporting substance [FL-no: 12.003]) was administered to rats by intraperitoneal injection, 40 % of the label was expired as CO_2 and 6.4 % was expired as unchanged methanethiol within six hours. Only 2.3 % of methanethiol was excreted in the urine (Canellakis and Tarver, 1953). In a separate experiment using 35S-labeled methanethiol, 31 % of the label was excreted in the urine as sulphate ion. The labelled carbon also was detected in the beta-carbon of serine and the methyl groups of methionine, choline and creatine (Canellakis and Tarver, 1953). Formaldehyde has been shown *in vitro* to be an intermediate in the oxidation of methanethiol (Mazel et al., 1964). Although the carbon atom from thiols may be utilised in the biosynthesis of amino acids, the sulphur atom is not utilised significantly in the synthesis of sulphur-containing amino acids (Mazel et al., 1964). Methanethiol has been reported to be a metabolite in normal humans (Williams, 1959a).

Hydrolysis

One of the candidate substances in subgroup III, 1-(methylthio)ethane-1-thiol [FL-no: 12.180] is a thioacetal, which can be hydrolysed to acetaldehyde [FL-no: 05.001], methyl mercaptan [FL-no: 12.003] and hydrogensulphide [not a Register substance]. Two candidate substances from subgroup III, methyl 2-mercaptopropionate [FL-no: 12.266] and ethyl-2-mercapto-2-methyl propanoate [FL-no: 12.304] are carboxylic acid esters, which are anticipated to be hydrolysed, respectively, to methanol and 2-mercaptopropionic acid [FL-no: 12.039] and to ethanol and to 2-mercapto-2-methyl propionic acid. The hydrolysis products are shown in Table 2b.

Studies for Candidate Substances

<u>Butane-1,4-dithiol [FL-no: 12.103] (Subgroup IV)</u>

Microsomal thiol S-methyltransferase activity in rat salivary glands was found to be specific to aliphatic thiols compared to S-containing amino acids and simple aliphatic diols. Relative activity of 4 mM butane-1,4-dithiol [FL-no: 12.103] is 95.6 % (relative to dithiothreitol 100 %), whereas relative activity for 4 mM L-cysteine or 2,3-butanediol are only 3.0 and 0.7 %, respectively. The authors suggest that microsomal thiol S-methyltransferase activity in rat salivary glands detoxicates extracellular thiols and/or intracellular hydrogen sulphide to protect normal secretory functions (Yashiro and Takatsu, 2001).

<u>3-Mercapto-2-oxopropionic acid [FL-no: 12.136] (Subgroup III)</u>

The transamination pathway (3-mercaptopyruvate pathway) of L-cysteine metabolism in rats was studied, in part, to determine the metabolic fate of the intermediate product, the salt of 3-mercapto-2-oxopropionic acid [FL-no: 12.136]. It was determined that it is metabolised by reduction and trans-sulphuration to yield 3-mercaptolactatecysteine mixed disulphide [S-(2-hydroxy-2-carboxyethylthio) cysteine, HCETC] and inorganic sulphate, respectively. The reduction of the salt of 3-mercapto-2-oxopropionic acid is catalysed by lactate dehydrogenase as indicated by the use of anti-lactate dehydrogenase antiserum. Formation of HCETC is favoured at low 3-mercaptopyruvate sulphurtransferase activity (Ubuka et al., 1992).

III.4. Disulphides, trisulphides and Polysulphides

The following discussion is pertinent to subgroups V and VI.

Disulphides

Disulphides may be reduced to two thiol molecules. Consequently, metabolic options available for thiols (see section III.3) may also be applicable to disulphides. The disulphide bond may in certain circumstances also be reduced to the corresponding dithiol in a reversible reaction *in vivo*.

A proposed metabolic pathway for the candidate cyclic disulphide 3,5-dimethyl-1,2-dithiolane-4-one [FL-no: 12.295] would be, ringopening and disulphide reduction to form a dithiol, and then further metabolism following the scheme suggested for thiols in Section III.3. Analoguous to this, lipoic acid is a five-membered cyclic disulphide that undergoes rapid redox cycling between ring disulphide and open dithiol.

Thiol-disulphide exchange (TDE) reactions occur *in vivo* and result from nucleophilic substitution by sulphur. These reactions require the presence of a thiolate ion, proximity and appropriate orientation of the disulphide, and enzymes capable of catalysing these reactions (Myers et al., 1977a). TDE reactions control cellular concentrations of endogenous thiols (i.e. GSH) and disulphides (i.e. GSSG). The GSH/GSSH ratio decreases when cells undergo oxidative stress. Cells combat this decrease by rapidly switching glucose equivalents away from glycolysis and into the production of NADPH-reducing equivalents via the pentose phosphate pathway (Brigelius, 1985; Sies et al., 1987). The NADPH-reducing equivalents are used to convert GSSG back to GSH. Therefore, disturbance of the redox balance of thiol components and/or over expression of TDE could initiate acute cytotoxicity (Cotgreave et al., 1989).

Examples of *in vivo* reduction of naturally occurring disulphides include the metabolism of asparagusic acid (the disulphide of 1,3-dithio-2-propanecarboxylic acid) in asparagus. Five volunteers ingested 500 g of asparagus and the urinary metabolites detected after ingestion were methanethiol, dimethyl sulphide, dimethyl sulphoxide, dimethyl sulphone, dimethyl disulphide and bis(methylthio)methane. Presumably, asparagusic acid is reduced to the dithiol, which may then be methylated, followed by oxidation of adjacent carbons, liberating methanethiol. Subsequent oxidation, methylation and dimerisation of methanethiol would produce the other detected metabolites (Waring et al., 1987).

Incubation of dimethyl or diethyl disulphides with mouse lung and liver tissues *in vitro* resulted in the rapid generation of thiols (Oginsky et al., 1956).

Sulphate and ethyl methyl sulphide were detected in the urine of guinea pigs and mice following an oral dose of diethyl disulphide. The diethyl disulphide was reductively cleaved to form ethanethiol, which was subsequently methylated to form ethyl methyl sulphide (Snow, 1957). An unidentified metabolite was presumed to be the sulphoxide or sulphone of ethyl methyl sulphide or the glucuronic acid conjugate of ethanethiol.

Disulphides are also oxidised to thiosulphinic acid derivatives (Figure III.1). Thiosulphinic acid derivatives may be hydrolysed to the corresponding sulphinic and sulphonic acids or oxidised to yield thiosulphonic acid derivatives (Ziegler, 1982; Ziegler, 1985). Thiosulphonates (thiosulphonic acid derivatives) are unstable and are readily hydrolysed to the corresponding sulphonic acid (see Figure III.1) (Ziegler, 1984).

Tri-, tetra- and polysulphides

Tri-, tetra- and polysulphides may react with endogenous thiols such as reduced glutathione (GSH) or cysteine forming a thiol and a hydropersulphide or perthiol (RSH + R'SSH or R'SSSH or R'S_xH, respectively) (Münchberg et al., 2007). Compared to thiols, perthiols may be strong reducing agents,

reacting rapidly with oxidants to form reactive products. According to several authors the biological activity of sulphides increase in the order mono-< di- < tri-< tetrasulphide.

In a study by Munday et al (2003) the ability of di-, tri- and tetrasulphides to cause oxidative damage to erythrocytes *in vitro* was investigated. In this experiment sulphides (dipropyl sulphide, diallyl sulphide, dipropyl disulphide, diallyl disulphide, dipropyl trisulphide, diallyl trisulphide and diallyl tetrasulphide) were added to suspensions of rat erythrocytes. Percentage of methemoglobin was assayed after incubation of erythrocytes with 1 mM of respective sulphide for 2 hours. In control, erythrocytes and erythrocytes incubated with monosulphides, small amounts of haemoglobin were oxidised to methemoglobin. (~0.5 - 0.6 %), more with disulphides (~3 - 12 %), and the most with tri- and tetrasulphides (~38 - 46 %). Sulphhemoglobin was not detected in erythrocytes incubated with mono- and disulphides, nor in control erythrocytes, but was detected (~1 - 5 %) in erythrocytes incubated with tri- and tetrasulphides. Heinz bodies were observed in a proportion of the cells incubated with tri- and tetrasulphides. Formation of hydrogen peroxide was measured and erythrocytic levels of glutathione determined in erythrocytes incubated with 50 µM of respective sulphide for 1 hour. No hydrogen peroxide was detected in control cells or cells incubated with monosulphides or dipropyl disulphide. In erythrocytes incubated with diallyl disulphide, trisulphides or tetrasulphide hydrogen peroxide was detected in increasing amounts (~21 - 96 % inhibition of erythrocytic catalase). The diallyl sulphides being more active than the dipropyl sulphides in this regard. Decrease in erythrocytic GSH-levels was not noticed in control cells or cells incubated with monosulphides. The greatest decrease was found in cells incubated with tetrasulphides (~91 - 92 %) followed by trisulphides (~73 - 74 %) and disulphides (~17 - 32 %).

The ability of di-, tri- and tetrasulphides to cause hemolytic anemia *in vivo* in rats was also studied (Munday et al, 2003). Groups of six female rats were dosed with the test sulphides (same as in the in vitro experiments described above) in soybean oil by gavage for 5 days. All compounds were given at 500 µmoles/kg bw/day (57 and 59 mg/kg for the monosulphides, 73 and 75 mg/kg for the disulphides, 89 and 91 mg/kg for the trisulphides and 105 and 107 mg/kg for the tetrasulphides). Rats were killed on the 6th day of the experiment. All rats were in good health during the experimental period. Rats dosed with diallyl disulphide and the tri- and tetrasulphides were anemic at the end of the 6 days experiment. The anemia was associated with pronounced formation of Heinz bodies. Splenic enlargement was seen in animals receiving tri- and tetrasulphides, and the histopathology was consistent with haemolytic anemia with compensatory erythropoiesis. The ability of the sulphides to increase the activity of the enzymes quinone reductase (QR) and glutathione-S-transferase (GST) was measured in liver, kidney, spleen, lungs, heart, digestive tract and urinary bladder from the experimental animals. While dially tri- and tetrasulphides increased QR-activities in all the tissues studied, the propyl derivatives did not have significant effects in these tissues. Allyl sulphides had smaller and less widespread effects on GST activities, and no effects were seen with the propyl derivatives.

The authors drew the conclusion that the activity of the sulphides increased in the order di- < tri- < tetrasulphide. In the paper it is discussed that trisulphides are readily cleaved by GSH to form an equimolar mixture of thiol and perthiol, while tetrasulphides are symmetrically cleaved forming two molecules of perthiol. Redox cycling and production of "active oxygen" may be expected with tri-, tetra- and polysulphides. In this context the chain of reduction is proposed to start with GSH, which reduces the polysulphide and continues via the perthiol and haemoglobin to O_2 which is reduced to H_2O_2.

RSSSR + 2 GSH → RSSH + RSH + GSSG

RSSSSR + 2 GSH → 2 RSSH + GSSG (Munday et al., 2003)

Experiments with a synthetic persulphide, benzyl hydrodisulphide (benzyl-SSH) gave evidence that persulphides may produce reactive oxygen species (O_2^{*-}, H_2O_2 and HO^*) under physiologically relevant

conditions. This was proposed to be the mechanism behind the cytotoxicity of some naturally occurring products (Chatterji et al., 2005).

The ability of allyl sulphides (diallyl monosulphide, diallyl disulphide and diallyl trisulphide) to induce apoptosis and supress cell proliferation was investigated in human colon cancer cells. Whereas the growth of cells was significantly depressed by diallyl trisulphide, neither diallyl monosulphide nor diallyldisulphide showed such an effect. Apoptosis of cells was proposed to be associated with oxidative modification of β-tubulin (Hosono et al., 2005).

III.5. Sulphides with Thioacetal and Thioketal Structure

The following discussion is pertinent to subgroup VII.

The thioacetals could be subject to acid-hydrolysis in the stomach, similar to oxygen-containing acetals forming aldehydes and thiols. The potential hydrolysis products of the 14 candidate substances of subgroup VII are shown in Table 2b. However, thioacetals are more resistant to hydrolysis than oxygen-acetals (Satchell and Satchell, 1990; Smith and March, 2001). It is thus to be anticipated that these substances may reach the intestinal lumen intact and may also be absorbed as such.

The following text concerns subgroup XI.

Cyclic oxygen-acetals may be very resistant to hydrolysis, the same is expected for cyclic thioketals (Deslongchamps et al., 2000). It is thus anticipated that the candidate substance [FL-no: 15.007] may be absorbed as such. Acetals may be hydrolysed by enzymatic hydrolysis, however the process may be slow and incomplete (Edsbacker et al., 1987; Hitchcock and Nelson, 1943; Levine et al., 1940; Thurston et al., 1968).

There is no information on metabolism of the candidate substance. In general, methylsubstituted cyclic thioethers and acetals are expected to undergo S-oxidation to the corresponding sulphoxide. In a study of the metabolism of 7-(1,3-dithiolan-2-ylmethyl)-1,3-dimethylxanthine by rat liver microsomes, enzymatic oxidation occurred at the sulphur atom, which was the major nucleophilic center of the molecule. The presence of sulphur atoms suppressed the metabolic activity at the acetal carbon, and sulphoxidation was the preferred metabolic pathway. The oxidation occurred at the sulphur which was most accessible, and no further oxidation to disulphide or sulphone was detected during incubation. This was explained by the polarity of the sulphoxides, which made them poor substrates for microsomal enzymes (Grosa et al., 1991).

The fate of 2-aryl-1,3-dithiolanes was studied in rabbit lung enzyme preparations. The sulphuroxide was the only detectable product formed during the incubation time. Studies on the biochemical mechanism suggested that the reaction preferentially was catalysed by flavin-containing monooxygenase, even though cytochromes P-450 also may contribute to sulphur oxidation. The monooxygenase only catalysed formation of the trans-isomer of the sulphoxide, at the *pro*-R-sulphur atom (Cashman and Williams, 1990).

Takata et al (1983) studied the enzymatic oxygenation of sulphides with cytochrome P450 from rabbit liver. Various dialkyl-, aryl-, alkyl-, and diaryl-sulphides were readily oxygenated to the corresponding sulphoxides, but no sulphones were detected. The yield of sulphoxide was markedly affected by the structure of the sulphide, i.e. by substituents on the sulphur. It was concluded that the enzymatic oxygenation of the cyclic sulphide predominantly took place at the opposite side of the alkyl substituent at *alpha*-position, forming mainly the trans-sulphoxide (Takata et al., 1983).

The candidate substance, a cyclic thioketal with fused oxolane rings, is expected to be resistant to hydrolysis, and to be mainly absorbed as such. The sulphur atoms of the molecule are expected to be the

main target for metabolic activity. The proposed pathway of metabolism is sulphoxidation to yield the corresponding sulphoxide.

III.6. Thioesters and thioc acid

The following discussion is pertinent to subgroup VIII and IX.

S-Thioesters are rapidly hydrolysed by lipases and esterases forming primarily the corresponding carboxylic acids and thiols (Kurooka et al., 1976). The rate of hydrolysis of thioesters increases as the C-chain length of the carboxylic acid fragment increases (Greenzaid and Jencks, 1971) and decreases as oxygenation of the carbon chain in the thiol moiety increases (Kurooka et al., 1976).

The hydrolysis products of the candidate thioesters and ethanethioc acid [FL-no: 12.199] are shown in Table 2b.

Thioesters with a polar anionic group, such as carboxylic acid one or more carbon atoms away from the sulphur, are inhibitors of rather than substrates for FMO (Taylor and Ziegler, 1987) and probably would be eliminated without S-oxidation.

III.7. Sulphoxides/Sulphones and Sulphonates

The only candidate substance of subgroup X is methyl methanethiosulphonate [FL-no: 12.159], which is anticipated to be hydrolysed to methanesulphonic acid and methanethiol and/or methanethiosulphonic acid and methanol (See Table 2b).

III.8. Conclusions

The candidate substances and supporting substances are expected to participate in common routes of absorption, distribution and metabolism, and exhibit similar toxicological properties. Saturation of these metabolic pathways is unlikely, given the extremely low levels of exposure to sulphides and thiols from their use as flavouring substances.

Organosulphur compounds and their oxygenated derivatives are readily metabolised to excretable metabolites. Monosulphides primarily undergo S-oxidation to sulphoxides and sulphones, whereas thiols and polysulphides may follow a combination of pathways including S-oxidation, reduction, oxidative desulphuration, alkylation, and conjugation with glutathione and/or glucuronic acid. The oxidation of thiols leads to reactive sulphenic (R-S-OH) acid, which is readily further oxidised to sulphinic ($R\text{-}SO_2H$) acid. Once formed, sulphenic acid can react with excess thiol (preferentially GSH), yielding the corresponding disulphide, which can be either reduced back to thiols or be oxidised to thio-sulphinic, sulphinic and sulphonic ($R\text{-}SO_3H$) acid. In the likely event that thiols and disulphides form mixed disulphides, reacting with endogenous thiols present in cellular macromolecules, an adverse effect could be produced.

Tri-, tetra- and polysulphides may react with endogenous thiols such as reduced glutathione (GSH) or cysteine forming a thiol and a hydropersulphide or perthiol (RSH + R'SSH or R'SSSH or R'SxH, respectively). Compared to thiols, perthiols may be strong reducing agents, reacting rapidly with oxidants and to form reactive products. *In vitro* and *in vivo* studies indicate that the biological activity of sulphides increase in the order mono- < di- < tri- < tetrasulphides.

The presence of additional oxygen-containing functional group in the molecule seems not to significantly affect the rate of the above described pathways of organosulphur compound biotransformations, although very low amounts of metabolites can be produced via the well recognised metabolic pathways of alcohols, aldehydes, acids and ketones.

Due to the reactivity of the electrophilic metabolites, (e.g. by either ring scission or S-oxidation) towards cellular nucleophilic sites, the 80 candidate substances are not predicted to be metabolised to innocuous products.

ANNEX IV: TOXICITY

Oral acute toxicity data are available for four candidate substances of the present Flavouring Group Evaluation from chemical group 20 and 30, and for 35 supporting substances evaluated by the JECFA at the 53rd meeting. The supporting substances are listed in brackets.

Table IV.1: ACUTE TOXICITY

Chemical Name [FL-no]	Species	Sex	Route	LD_{50} (mg/kg bw)	Reference	Comments
Subgroup I – Acyclic Sulphides						
(Dimethyl sulphide [12.006])	Mouse	NR	Gavage	3700	(Koptyaev, 1967b)	
	Rat	NR	Gavage	3300	(Koptyaev, 1967b)	
(Dibutyl sulphide [12.007])	Rat	NR	Oral	2220	(Moreno, 1975g)	
(3-(Methylthio)propionaldehyde [12.001])	Rat	M, F	Oral	M: 1000 F: 1680	(Ballantyne and Myers, 2000)	
(Ethyl 3-(methylthio)propionate [12.053])	Rat	M, F	Gavage	>5000	(Panasevich et al., 1980)	
(2-(Methylthio)phenol [12.042])	Rat	M, F	Gavage	M: 1740 F: 2400	(Butterworth and Mason, 1981)	
	Mouse	M, F	Gavage	M: 1560 F: 1750	(Butterworth and Mason, 1981)	
(Methyl 2-(methylthio)butyrate [12.086])	Rat	M, F	Gavage	2108	(Piccirillo and Lunchicki, 1982)	
Subgroup II – Cyclic Sulphides						
Tetrahydrothiophene [15.102]	Rat	M, F 5/sex/group	Gavage	M: 2000 F. 1750	(Auletta and Daly, 1985)	
	Rat	NR	Oral	1200 (100% survival rate) 3000 (100% fatality rate)	(Dow Chemical Company, 1992a)	
Subgroup III – Monothiols						
(1-Propane-1-thiol [12.071])	Rat	NR	Gavage	134	(Elf Atochem, 1981b)	Referred to as 3-mercapto-1-propanol in reference.
	Rat	NR	Gavage	1790	(Fairchild and Stokinger, 1958)	
2-Methylpropane-2-thiol [12.174]	Rat	NR	Gavage	4729	(Fairchild and Stokinger, 1958)	
	Rat	M, F	Gavage	8400	(Phillips Petroleum Company, 1990a)	
(Butane-1-thiol [12.010])	Rat	NR	Gavage	1500	(Fairchild and Stokinger, 1958)	
(2-Methylpropane-1-thiol [12.173])	Rat	NR	Gavage	7168	(Fairchild and Stokinger, 1958)	
Butane-2-thiol [12.104]	Rat	NR	Gavage	5176	(Elf Atochem, 1981a)	
2-Methylbutane-2-thiol [12.172]	Rat	M 6/group	Gavage	>5000	(Elf Atochem, 1977)	
(Pentane-2-thiol [12.192])	Rat	M, F	Gavage	>5000	(Collinson, 1989a)	
(3-Methylbutane-2-thiol [12.049])	Rat	M, F	Gavage	540	(Harper and Ginn, 1964)	
(Cyclopentanethiol [12.029])	Mouse	M, F 5/group	Oral	2680	(Oser, 1970c)	Use of both sexes not clear from reference.
(*p*-Menth-1-ene-8-thiol [12.085])	Rat	M, F	Oral	>6000	(Mondino and Peano, 1982)	
(Thiophenol [12.080])	Rat	NR	Gavage	46	(Fairchild and Stokinger, 1958)	

Table IV.1: ACUTE TOXICITY

Chemical Name [FL-no]	Species	Sex	Route	LD_{50} (mg/kg bw)	Reference	Comments
(3-Mercaptopentan-2-one [12.031])	Mouse	M, F	Gavage	M: 540 F: 455	(Shellenberger, 1971b)	
(2,6-(Dimethyl)thiophenol [12.082])	Rat	M, F 5/sex/group	Oral	3150	(Mondino and Peano, 1979a)	
Subgroup IV – Dithiols						
(Ethane-1,2-dithiol [12.066])	Rat	M, F	Oral	144	(Phillips Petroleum Company, 1990b)	
	Mouse	M, F	Oral	342	(Moran et al., 1980)	
	Mouse	M, F	Gavage	342	(Fogleman and DeProspo, 1974)	
	Mouse	NR	Oral	120	(Pharmacology Research, Inc., 1963)	
	Rat	M, F	Oral	218	(Phillips Petroleum Company, 1990b)	
(Propane-1,3-dithiol [12.076])	Rat	NR	Oral	100-200	(Eastman Kodak Co., 1955b)	
	Mouse	NR	Oral	1070[2]	(Schafer and Bowles, 1985)	
(Propane-1,2-dithiol [12.070])	Mouse	M, F	Oral	153	(Bailey, 1976a)	
	Mouse	M, F	Gavage	170	(Fogleman and Suppers, 1974c)	
(Octane-1,8-dithiol [12.034])	Mouse	M, F 5/sex/group	Oral	882 (940 M, 1300 F)	(Bailey, 1976b)	
	Mouse	M, F	Oral	1262	(Moran et al., 1980)	
Subgroup V – Acyclic and cyclic disulphides						
(Dimethyl disulphide [12.026])	Rat	M, F	Oral	190	(Shapiro et al., 1985)	
(Dipropyl disulphide [12.014])	Rat	M, F	Oral	2000	(Elf Atochem, 1992)	Reference is dipropyl disulphide.
	Rat	M	Gavage	6000[3]	(Rohm & Haas Co., 1980)	
(Diallyl disulphide [12.008])	Rat	M	Oral	260	(Moreno, 1980h)	Paper reports compound as allyl sulphide.
	Rat	NR	Oral	<5000[4]	(Platte Chemical Co., 1995)	
(Benzyl methyl disulphide [12.068])	Mouse	M, F	Oral	1080	(Bailey, 1976c)	
Subgroup VI –Acyclic polysulphides						
(Diallyl trisulphide [12.009])	Mouse	M, F	Oral	100-400	(Moran et al., 1980)	Not definitive test.
(Dipropyl trisulphide [12.023])	Mouse	M, F	Oral	800-1600	(Moran et al., 1980)	Not definitive test.
Subgroup VII – Mono-, di-, tri- and polysulphides with thioacetal structure						
(3-Methyl-1,2,4-trithiane [15.036])	Rat	M, F	Oral	440	(Mondino and Peano, 1979b)	
(3,5-Dimethyl-1,2,4-trithiolane [15.025])	Rat	Not specified	Oral	115	(BIBRA, 1976)	
(2,5-Dihydroxy-2,5-dimethyl-1,4-dithiane [15.006])	Mouse	F 5/group	Gavage	360	(Fogleman and DeProspo, 1973a)	
(2-Methyl-4-propyl-1,3-oxathiane [16.030])	Rat	NR	Gavage	6000[1]	(BIBRA, 1976)	
(2-Methyl-1,3-dithiolane [15.034])	Rat	M, F	Gavage	1610 (1.61 g/kg)	(Griffiths et al., 1979a)	
(Trithioacetone [15.009])	Mouse	M, F	Gavage	M: 2600 F: 2000	(Fenwick and Hanley, 1985)	
Subgroup VIII – Thioesters						
(Methylthio 2-(acetyloxy)propionate [12.203])	Rat	M, F	Gavage	1050	(Watanabe and Kinosaki, 1989a)	
(Methylthio-2-(propionyloxy) propionate [12.227])	Rat	M, F	Gavage	1330	(Watanabe and Kinosaki, 1989b)	

Table IV.1: ACUTE TOXICITY

Chemical Name [FL-no]	Species	Sex	Route	LD_{50} (mg/kg bw)	Reference	Comments
Subgroup X – Sulphoxides/Sulphones and Sulphonates						
(Methylsulphinylmethane [12.175])	Rat	M, F	Gavage	20000	(Brown et al., 1963)	
	Mouse	M, F	Gavage	20000	(Brown et al., 1963)	
	Mouse	M, F	Oral	21400	(Willson et al., 1965)	
	Rat	M, F	Oral	28300	(Willson et al., 1965)	
	Mouse	M, F	Oral	16500	(Sommer and Tauberger, 1964)	
	Rat	M, F	Oral	19700	(Sommer and Tauberger, 1964)	
	Mouse	NR	Oral	3100	(Fishman et al., 1969)	
	Rat	NR	Oral	14500	(Fishman et al., 1969)	

NR = Not Reported.

M = Male; F = Female.

1 Estimated value.

2 Reported as ALD (Approximate Lethal Dose).

3 Value does not represent a true LD50 value. Test conducted with a mixture of seven components. Mixture contained 1.9% of diisopropyl disulphide.

4 Value does not represent an LD50 value. Value reported is an LD100 value.

Subacute / Subchronic / Chronic / Carcinogenic toxicity data are available for three candidate substances of the present flavouring group evaluation from chemical group 20 and 30, and for 33 supporting substances evaluated by the JECFA at the 53rd meeting. The supporting substances are listed in brackets.

Table IV.2: Subacute / Subchronic / Chronic / Carcinogenicity Studies

Chemical Name [FL-no]	Species; Sex No/Group	Route	Dose levels (mg/kg/day)	Duration	NOAEL (mg/kg/day)	Reference	Comments
Subgroup I – Acyclic Sulphides							
(Dimethyl sulphide [12.006])	Rat; M, F 15/sex/group	Oral (gavage in corn oil)	0 (control group), 2.5, 25, 250	14 Weeks	No adverse effect measured at the highest tested dose (250)[1]	(Butterworth et al., 1975b)	Study published on a peer reviewed journal. Acceptable quality.
	Rat (sex unspecified) 5/group	Oral (gavage)	0 (control group), 0.0015, 0.015, 0.6, 15	225 days	0.6	(Koptyaev, 1967b)	Insufficiently reported study. Validity cannot be evaluated – no histopathology data.
	Rabbit (sex unspecified) 18 (reported as total number)	Oral (gavage)	0 (control group), 0.0015, 0.015, 0.6, 15	225 days	0.6	(Koptyaev, 1967b)	Insufficiently reported study. Validity cannot be evaluated – no histopathology data.
	Rabbit; M, F 10/group	Oral (in drinking water)	0 (control group), 2000	13 Weeks	No adverse effect measured at the highest tested dose (2000)[1]	(Wood et al., 1971)	Limited relevance (The only end-point followed was lenticular changes).
(2,8-Dithianon-4-ene-4-carboxaldehyde [12.065])	Rat; M, F 5/sex/group	Oral (gavage in corn oil)	0 (control group), 0.33, 3.3	2 Weeks	No adverse effect measured at the highest tested dose (3.3)[1]	(deGroot et al., 1974)	Unpublished report; limited quality due to scant data reporting.
3-(Methylthio)propionic acid [12.183]	Rat; M 5/group	Diet	0 (control group), 2.57% (corresponding to 2570 mg)	2 Weeks	Not determined: effects observed at the only tested dose	(Steele et al., 1979)	Study published on a peer reviewed journal Acceptable quality.
Subgroup II – Cyclic Sulphides							
(4,5-Dihydro-3(2H)-thiophenone [15.012])	Rat; M, F 15/sex/group	Diet	0 (control group), 9.16 (nominal dose; actual dose = 10)	90 Days	No adverse effect measured at the only tested dose (10)[1]	(Morgareidge and Oser, 1970a)	Unpublished /uncompleted report: histopathology results not available.
2,8-Epithio-p-menthane [12.120]	Rat; M, F 10/sex/group	Oral (gavage)	0 (control group), 10	28 Days	No adverse effect measured at the only tested dose (10)[1]	(Finlay, 2004)	Unpublished report: acceptable quality.
Subgroup III –Monothiols							
(2-Mercapto-3-butanol [12.024])	Rat; M, F 15/sex/group	Diet	0 (control group), 0.752 (nominal dose; actual dose = 0.705)	90 Days	No adverse effect measured at the only tested dose (0.705)[1]	(Cox et al., 1974a)	Unpublished report: acceptable quality.
(*o*-Toluenethiol [12.027])	Rat; M, F 20-32	Diet	0 (control group), 0.52	90 Days	No adverse effect measured at the only tested dose (0.52)[1]	(Posternak et al., 1969)	Poorly reported study (only a summary available).
(Cyclopentanethiol [12.029])	Rat; M, F 15/sex/group	Diet	0 (control group), 0.49 (nominal dose; actual dose =0.56)	90 Days	No adverse effect measured at the only tested dose (0.56)[1]	(Morgareidge and Oser, 1970b)	Unpublished report: acceptable quality.
(3-Mercapto-2-pentanone [12.031])	Mouse; M, F 15/sex/group	Diet	0 (control group), 1.7 (nominal dose; actual dose = 1.89)	90 Days	No adverse effect measured at the only tested dose (1.89)[1]	(Morgareidge, 1971b)	Unpublished /incomplete report: histopathology results not available.
(2,3- and 10-mercaptopinane [12.035])	Rat; M, F 17/sex/group	Diet	0 (control group), 0.06	90 Days	No adverse effect measured at the only	(Oser, 1966)	Unpublished report: acceptable quality.

Table IV.2: Subacute / Subchronic / Chronic / Carcinogenicity Studies

Chemical Name [FL-no]	Species; Sex No/Group	Route	Dose levels (mg/kg/day)	Duration	NOAEL (mg/kg/day)	Reference	Comments
					tested dose (0.06)[1]		
(2,6-Dimethylthiophenol [12.082])	Rat; M, F 16/sex/group	Oral (gavage in corn oil)	0 (control group), 0.43	13 Weeks	No adverse effect measured at the only tested dose (0.43)[1]	(Peano et al., 1981)	Good quality unpublished report.
(3-Mercapto-3-methylbutyl formate [12.138])	Rat; M, F 5/sex/group	Diet	0 (control group), 10	2 Weeks	No adverse effect measured at the only tested dose (10)[1]	(Wnorowski, 1996e)	Good quality GLP study.
(Prenylthiol [12.170])	Rat; M, F 5/sex/group	Diet	0 (control group), 12.8 (M & F)	2 Weeks	No adverse effect measured at the only tested dose (12.8)[1]	(Wnorowski, 1997a)	Good quality GLP study.
(3-Mercaptohexanol [12.217])	Rat; M, F 5/sex/group	Diet	0 (control group), 11.80(M) and 10.73 (F)	2 Weeks	No adverse effect measured at the only tested dose (11.8)[1]	(Wnorowski, 1996d)	Good quality GLP study.
(3-Mercaptohexyl acetate [12.234])	Rat; M, F 5/sex/group	Diet	0 (control group), 11.66	2 Weeks	No adverse effect measured at the only tested dose (11.66)[1]	(Wnorowski, 1996a)	Good quality GLP study.
(3-Mercaptohexyl butyrate [12.235])	Rat; M, F 5/sex/group	Diet	0 (control group), 11.87 (M) and 11.99 (F)	2 Weeks	No adverse effect measured at the only tested dose (11.9)[1]	(Wnorowski, 1996b)	Good quality GLP study.
Subgroup IV –Dithiols							
(2,3-Butanedithiol [12.022])	Rat; M, F 15/sex/group	Oral	0 (control group), 0.752 (nominal dose; actual dose = 0.703)	90 Days	No adverse effect measured at the only tested dose (0.703)[1]	(Cox et al., 1974c)	Unpublished report: acceptable quality.
(1,8-Octanedithiol [12.034])	Rat; M, F 15/sex/group	Oral	0 (control group), 0.752 (nominal dose; actual dose = 0.705)	90 Days	No adverse effect measured at the only tested dose (0.705)[1].	(Cox et al., 1974d)	Unpublished report: acceptable quality.
Subgroup V – Acyclic and cyclic disulphides							
(Diallyl disulphide [12.008])	Rat; F 12 (control group) 6 (treatment group)	Oral (gavage in peanuts oil)	0 (control group), 36.5, 146, 732	6 Days	146 (hemolytic anemia at the higher dose)	(Munday and Manns, 1994)	Study published on a peer reviewed journal. Acceptable quality.
(Dipropyl disulphide [12.014])	Rat; F 12 (control group) 6 (treatment group)	Oral (gavage in peanuts oil)	0 (control group), 37.6, 150.4, 752	6 Days	150.4 (hemolytic anemia at the higher dose)	(Munday and Manns, 1994)	Study published on a peer reviewed journal. Acceptable quality.
	Rat; M 10-16	Diet	7.29	90 Days	No adverse effect measured at the only tested dose (7.29)[1]	(Posternak et al., 1969)	Poorly reported study (only a summary is available).
(Dicyclohexyl disulphide [12.028])	Rat; M, F 15/sex/group	Diet	0 (control group), 0.752 (nominal dose; actual dose = 0.232)	90 Days	No adverse effect measured at the only tested dose (0.23)[1]	(Cox et al., 1974e)	Unpublished report: acceptable quality.
(Diphenyl disulphide [12.043])	Rat; F 6	Oral (gavage in peanuts oil)	0 (control group), 218	6 Days	< 218	(Munday et al., 1990)	Limited validity: the study was only looking at some haematological parameters.
(Benzyl methyl disulphide [12.068])	Rat; M, F 15/sex/group	Diet	0 (control group), 1.13 (nominal dose; actual dose = 1.15)	90 Days	No adverse effect measured at the only tested dose (1.15)[1]	(Gallo et al., 1976a)	Unpublished report: acceptable quality.
Subgroup VI – Acyclic polysulphides							

Table IV.2: Subacute / Subchronic / Chronic / Carcinogenicity Studies

Chemical Name [FL-no]	Species; Sex No/Group	Route	Dose levels (mg/kg/day)	Duration	NOAEL (mg/kg/day)	Reference	Comments
(Diallyl trisulphide [12.009])	Rat; M, F 15/sex/group	Diet	0 (control group), 4.16 (nominal dose; actual dose = 4.6)	90 Days	No adverse effect measured at the only tested dose (4.6)[1]	(Morgareidge and Oser, 1970d)	Unpublished /incomplete report: histopathology results not available.
(Dipropyl trisulphide [12.023])	Rat; M, F 15/sex/group	Diet	0 (control group), 4.16 (nominal dose; actual dose = 4.8)	90 Days	No adverse effect measured at the only tested dose (4.8)[1]	(Morgareidge and Oser, 1970c)	Unpublished /incomplete report: results of histopathology not available.
Subgroup VII – Mono-, di-, tri- and polysulphides with thioacetal structure							
(2,5-dihydroxy-2,5-dimethyl-1,4-dithiane [15.006])	Rat; M, F 15/sex/group	Diet	0 (control group), 3.14	90 Days	No adverse effect measured at the only tested dose (3.14)[1]	(Cox et al., 1973a)	Unpublished report; acceptable quality.
(Trithioacetone [15.009])	Rat; M, F 15/sex/group	Diet	0 (control group), 0.2338 (nominal dose; actual dose = 0.2)	90 Days	No adverse effect measured at the only tested dose (0.2)[1]	(Cox et al., 1973b)	Unpublished report; acceptable quality.
(3,5-Dimethyl-1,2,4-trithiolane [15.025])	Rat; M, F 15/sex/group	Oral (gavage in corn oil)	0 (control group), 1.88	90 Days	No adverse effect measured at the only tested dose (1.88)[1]	(BIBRA, 1976)	Unpublished report; acceptable quality.
(2-Methyl-1,3-dithiolane [15.034])	Rat; M, F 16/sex/group	Oral (gavage in water/ propylglcol)	0 (control group), 7	91 Days	No adverse effect measured at the only tested dose (7.0)[1]	(Griffiths et al., 1979a)	Unpublished report; acceptable quality.
(3-Methyl-1,2,4-trithiane [15.036])	Rat; M, F 16/sex/group	Oral (gavage in corn oil)	0 (control group), 0.3	13 Weeks	No adverse effect measured at the only tested dose (0.3)[1]	(Mondino, 1981a)	Good quality unpublished report.
(2-Methyl-4-propyl-1,3-oxathiane [16.030])	Rat; M, F 15/sex/group	Oral (gavage in corn oil)	0 (control group), 0.44	13 Weeks	No adverse effect measured at the only tested dose (0.44)[1]	(BIBRA, 1976)	Unpublished report; acceptable quality. Note that this is the 'same' substance name as [16.062], however [16.030] is 80 - 90 % cis-isomer and [16.062] is trans-isomer. In the study no mention is made of possible isomers or racemate.
Subgroup VIII – Thioesters							
(Ethyl thioacetate [12.018])	Rat; M, F 12/sex/group	Diet	0 (control group), 6.48 (nominal dose; actual dose =6.63 (M) and 6.7 (F)	90 Days	No adverse effect measured at the only tested dose (6.7)[1]	(Shellenberger, 1970b)	Unpublished report: acceptable quality.
(Prenyl thioacetate [12.195])	Rat; M, F 7/sex/group	Oral (gavage in corn oil)	0 (control group), 10	2 Weeks	No adverse effect measured at the only tested dose (10)[1]	(Wnorowski, 1997b)	Good quality GLP study.
(Methylthio-2-(acetyloxy)propionate [12.203])	Rat; M, F 5/sex/group	Diet	0 (control group), 500	2 Weeks	Not determined: some effects on food consumption and relative kidney weight at 500	(Hermansky and Weaver, 1990)	Unpublished /incomplete report: results are reported as a summary - validity of conclusions could not be evaluated.
(Methylthio-2-(propionyloxy) propionate [12.227])	Rat; M, F 5/sex/group	Diet	0 (control group), 500	2 Weeks	Not determined: some effects on food consumption and relative kidney weight at 500	(Hermansky and Weaver, 1990)	Unpublished /incomplete report: results are reported as a summary - validity of conclusions could not be evaluated.
Subgroup X – Sulphoxides/sulphones and sulphonates							
(Methylsulphinylmethane [12.175])	Rat; M, F 50/sex/group	Oral (gavage in 50% aqueous solution)	Control group (receiving 9 ml distilled water), 1, 3, 9 ml[2,3]	18 Months	1 ml/kg (corresponding to 1100 mg/kg)	(Noel et al., 1975)	Study published on a peer reviewed journal. No histopathology reported.

Table IV.2: Subacute / Subchronic / Chronic / Carcinogenicity Studies

Chemical Name [FL-no]	Species; Sex No/Group	Route	Dose levels (mg/kg/day)	Duration	NOAEL (mg/kg/day)	Reference	Comments
	Dog; M, F 5/sex/group	Oral (gavage in 50% aqueous solution)	Control group (receiving 1 ml distilled water), 1, 3, 9 ml[2,4]	2 Years	Not determined: effects observed at the lowest tested dose	(Noel et al., 1975)	Study published on a peer reviewed journal. No histopathology was reported (with exception of the eye).
	Monkey; M, F 3-4	Oral (gavage in DMSO)	control group, 1, 3, 9 ml/kg[2]	74 - 87 Weeks	2970	(Vogin et al., 1970)	Study published on a peer-reviewed journal. DMSO-induced effects confounded the obtained results, limiting their quality.
Subgroup XI – Cyclic thioketal with oxolane rings							
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) [15.007]	Rats, M 10	Diet	0, 25, 250 mg/kg bw per day, The 3rd dosed group was initially exposed to 250 mg/kg bw per day, increased to 500 mg/kg bw per day after week 1 and to 1000 mg/kg bw per day at week 6.	90 days	25 mg/kg bw	(Wheldon et al., 1970)	Unpublished report. Study with limitations but of sufficient quality.

M = Male; F = Female.

1 This study was performed with either a single dose level or multiple dose levels that produced no adverse effect.

2 Reported as total volume dosed.

3 10/sex/group sacrificed at 52 weeks.

4 After 18 weeks only half of each original group continued to be treated; the rest was observed for signs of recovery.

Developmental and reproductive toxicity data are not available for any candidate substances of the present flavouring group evaluation from chemical group 20 and 30, but for two supporting substance evaluated by the JECFA at the 53rd meeting. Supporting substance listed in brackets.

TABLE IV.3: DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

Chemical Name [FL-no]	Study type Duration	Species/Sex No/group	Route	Dose levels	NOAEL (mg/kg/day) including information on possible maternal toxicity	Reference	Comments
Subgroup III – Monothiols							
(Butane-1-thiol [12.010])	Gestation days 6-16	Mice; F 25	Inhalation	0, 10, 68, 152 ppm total body, 6hr/day	Maternal: 10 ppm Foetal: 10 ppm	(Thomas et al., 1987)	Limited relevance due to the route of exposure.
	Gestation days 6-16	Rat; F 25	Inhalation	0, 10, 68, 152 ppm total body, 6hr/day	Maternal: 152 ppm Foetal: 152 ppm	(Thomas et al., 1987)	Limited relevance due to the route of exposure.
(Thiophenol [12.080])	Gestation days 6-19	Rabbit; F 15-26	Oral	10, 30, 40 mg/kg/d	Maternal: 10 Foetal: 40	(George et al., 1995)	Limited relevance: abstract only, the quality could not be checked.
	Gestation days 6-15	Rat; F 25	Oral	20, 35, 50 mg/kg/d	Maternal: < 20 Foetal: 10 ppm	(George et al., 1995)	Limited relevance: abstract only, the quality could not be checked.
	>48 weeks	Rat; F, M 40	Gavage	0, 9, 19, 35 mg/kg	Maternal: Not determined[1] Reproduction: 9	(NTP, 1996b)	Good quality study.

1 Liver and kidney weights accompanied by histological changes at the lowest dose tested.

In vitro mutagenicity/genotoxicity data are available for five candidate substances of the present flavouring group evaluation from chemical group 20 and 30, and for 14 supporting substances evaluated by the JECFA at the 53rd meeting. Supporting substances are listed in brackets.

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test system	Test Object	Concentration	Result	Reference	Comments
Subgroup I – Acyclic Sulphides						
(Diallyl sulphide [12.088])	Ames test	*S. typhimurium* TA100	0.004 – 0.44 µg/ml	Negative (±S9)	(Eder et al., 1982a)	Review. No details on method and results reported. Only TA100 used.
	Sister chromatid exchange	Chinese hamster ovary cells	200 - 600 µg/ml	Positive[1]	(Musk et al., 1997)	Limited quality of study. Insufficiently reported.
	Chromosomal aberrations	Chinese hamster ovary cells	200 - 600 µg/ml	Positive[1]	(Musk et al., 1997)	Limited quality of study. Insufficiently reported.
Di-(1-propenyl)-sulphid (mixture) [12.298]	*Ames test*	*S. typhimurium* TA98, TA100, TA102, TA1535, TA1537	1 - 100 µg/plate	Negative[1]	(Stien, 2005c)	Un-published GLP study. Study considered valid.
Subgroup II – Cyclic Sulphides						
Tetrahydrothiophene [15.102]	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537	50 - 5000 µg/plate	Negative(±S9)	(Pennwalt Corporation, 1987a-d)	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-d)	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-d)	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, 1987e)	Validity of this study cannot be fully evaluated (only abstract provided).
	Unscheduled DNA synthesis	Human epithelial cells	2.5 - 5120 µg/ml	Negative (±S9)	(Pennwalt Corporation, 1987a-d)	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 - 12024 µg/plate)	Positive (-S9) Negative (+S9)	(Lee et al., 1994a)	Only two strains were tested, otherwise acceptable study.
	Sister chromatid exchange	Chinese hamster ovary cells	2000 µM (240 µg/ml)	Negative(±S9)	(Lee et al., 1994a)	Insufficient quality.
Subgroup III – Monothiols						
2-Methylpropane-2-thiol [12.174]	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	10000 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	1000 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	1350 µg/ml	Negative (+S9)[2]	(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 – 1400 µg/ml)	Negative (±S9)	(Eder et al., 1980)	Acceptable quality.

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test system	Test Object	Concentration	Result	Reference	Comments
(Benzyl mercaptan [12.005])	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	3.6 mg/plate (3600 µg/plate)	Negative (±S9)	(Wild et al., 1983)	Review. Methods and results insufficiently documented.
(2-Mercaptopropionic acid [12.039])	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	3.6 mg/plate (3600 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 5000 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 and cyclic disulphides						
(Diallyl disulphide [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 disulphide [12.026])	Ames test	*S. typhimurium* TA98, TA100, TA102	0.000011 - 1.1 mmol/plate (1.04 - 104000 µg/plate)	Negative (±S9)	(Aeschbacher et al., 1989)	Limited quality (only 3 strains used).
(Phenyl disulphide [12.043])	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	3.6 mg/plate (3600 µg/plate)	Negative (±S9)	(Wild et al., 1983)	Review. Methods and results insufficiently documented.
(Benzyl disulphide [12.081])	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	3.6 mg/plate (3600 µg/plate)	Negative (±S9)	(Wild et al., 1983)	Review. Methods and results insufficiently documented.
Dibutyl disulphide [12.111]	Forward mutational assay	Mouse lymphoma cells	NR	Negative (-S9)	(Dooley et al., 1987)	Validity cannot be fully evaluated (only abstract provided).
Subgroup VIII – Thioesters						
(Methylthio 2-(acetyloxy)propionate [12.203])	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537, *E. Coli* WP2uvrA	0.156 - 5.0 mg/plate (156-5000 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 - 5000 µg/plate)	Negative (±S9)	(Watanabe and Morimoto, 1989b)	Acceptable quality.
Subgroup X – Sulphoxides/sulphones and sulphonates						
Methyl methanethiosulphonate [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[6].
	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[6].

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name [FL-no]	Test system	Test Object	Concentration	Result	Reference	Comments
	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[6].
	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[6].
	Ames test	*S. typhimurium* TA98, TA100, TA2637	NR	Negative[3]	(Dorange et al., 1983)	Test is not appropriate for antimicrobial agents[6].
	Ames test	*S. typhimurium* TA98, TA100, TA2637	0.6 - 200 µg/plate	Negative[4]	(Dorange et al., 1983)	Test is not appropriate for antimicrobial agents[6].
	Yeast assay	*S. cerevisiae* Strain D7	1 - 300 µg/ml	Negative (±S9)	(Dorange et al., 1983)	Test is not appropriate for antimicrobial agents[6].
	Yeast assay	*S. cerevisiae* Haploid strain N123	1 - 100 µg/ml	Negative (±S9)	(Dorange et al., 1983)	Test is not appropriate for antimicrobial agents[6].
(Methylsulphinyl methane [12.175]) (synonym: dimethylsulphoxid, DMSO)	Ames test	*S. typhimurium* TA97, TA98, TA100	100000 - 300000 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 - 10000 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 (100000 - 400000 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 (100000 - 400000 µg/plate)	Positive (-S9) [5]	(Hakura et al., 1993)	Good quality study. Positive at high doses with reduced bacterial survival. Doses routinely used in Ames test were negative.

NR: Not reported.

1 With and without metabolic activation at clearly cytotoxic concentrations.

2 A statistically significant increase in the number of SCEs per chromosome was seen at 1350 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.

3 With 100 µl/plate fecalase.

4 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.

5 Positive results obtained at doses where lethal toxicity was observed. Negative results obtained at doses routinely used in Ames test.

6 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.

In vivo mutagenicity/genotoxicity data are available for one candidate substance of the present flavouring group evaluation from chemical group 20 and 30, and for four supporting substances evaluated by the JECFA at the 53rd meeting. Supporting substances are listed in brackets.

Table IV.5: GENOTOXICITY (*in vivo*)

Chemical Name [FL-no]	Test System	Test Object	Route	Dose	Result	Reference	Comments
Subgroup I – Acyclic Sulphides							
(Diallyl sulphide [12.088])	*In vivo* mouse micronucleus test	Mouse	gavage	0.33 - 0.67 mM/kg (38 - 77 mg/kg)[1]	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 (1061 µg/ml)	Negative	(Wild et al., 1983)	Limited quality (insufficiently documented). The article compiles results obtained with 76 substances in 3 test systems.
Subgroup V – Acyclic and cyclic disulphides							
(Allyl disulphide [12.008])	*In vivo* mouse micronucleus test	Mouse	gavage	0.33 - 0.67 mM/kg (48 - 98 mg/kg) [1]	Negative	(Marks et al., 1992)	Insufficient quality. Mixture of three substances was tested.
Subgroup VI – Acyclic polysulphides							
(Diallyl trisulphide [12.009])	*In vivo* mouse micronucleus test	Mouse	gavage	0.33 - 0.67 mM/kg (59 - 120 mg/kg)[1]	Negative	(Marks et al., 1992)	Insufficient quality. Mixture of three substances was tested.
Subgroup X – Sulphoxides/sulphones and sulphonates							
Methyl methane-thiosulphonate [12.159]	*In vivo* genetic mutation	*Nicotiana tabacum* seeds	-	2 - 4 mg/ml (2000 - 4000 µg/ml)	Negative	(Dorange et al., 1983)	Obscure test system[2]. 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[2]. This assay cannot be regarded as standard test.

[1] *Study used a mixture of allyl sulphide, allyl disulphide and ally trisulphide in the respective ratio, 68:20:12.*

[2] *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*.

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ABBREVIATIONS

ADI	Acceptable Daily Intake
BW	Body weight
CAS	Chemical Abstract Service
CEF	Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids Chemical Abstract Service
CHO	Chinese hamster ovary (cells)
CoE	Council of Europe
DMSO	Dimethyl Sulphoxide
DNA	Deoxyribonucleic acid
EC	European Commission
EFFA	European Flavour and Fragrance Association
EFSA	The European Food Safety Authority
EU	European Union
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)
FMO	Flavin-containing monooxygenases
GC	Gas Chromatography
GI	Gastrointestinal
GSH	Glutathione
GST	Glutathione-S-Transferase
HPRT	Hypoxanthine-guanine phosphoribosyltransferase
ID	Identity
IOFI	International Organization of the Flavour Industry
IR	Infrared spectroscopy
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
LD_{50}	Lethal Dose, 50 %; Median lethal dose
MS	Mass spectrometry
MSDI	Maximised Survey-derived Daily Intake
mTAMDI	Modified Theoretical Added Maximum Daily Intake
MMS	Methyl methanesulphonate
MMTS	Methyl methanethiosulphonate
NAD	Nicotinamide Adenine Dinucleotide
NADP	Nicotinamide Adenine Dinucleotide Phosphate

NADPH	Nicotinamide Adenine Dinucleotide Phosphate – reduced form
No	Number
NOAEL	No Observed Adverse Effect Level
NOEL	No Observed Effect Level
NTP	National Toxicology Program
QR	Quinone Reductase
SCE	Sister Chromatid Exchange
SCF	Scientific Committee on Food
SMART	Somatic Mutation and Recombination Test
TAMDI	Theoretical Added Maximum Daily Intake
TDE	Thiol-Disulphide Exchange
TMT	Thiol Methyl Transferase
TPMT	Thio Purine Methyl Transferase
UDS	Unscheduled DNA Synthesis
WHO	World Health Organisation