European Food Safety Authority

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Flavouring Group Evaluation 8 (FGE.08)[1]:

Aliphatic and alicyclic mono-, di-, tri-, and polysulphides with or without additional oxygenated functional groups from chemical group 20

Scientific Opinion of the Panel on Food Additives,

Flavourings, Processing Aids and Materials in Contact with Food (AFC)

(EFSA-Q-2003-151)

Adopted on 08 February 2007

SUMMARY

The Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (the Panel) is asked to advise 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 is asked to evaluate 52 flavouring substances in the Flavouring Group Evaluation 8 (FGE.08), using the Procedure as referred to in the Commission Regulation (EC) No 1565/2000. These 52 flavouring substances belong to chemical groups 20, Annex I of the Commission Regulation (EC) No 1565/2000.

1 For citation purposes: Scientific Opinion of the Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food on a request from Commission on Flavouring Group Evaluation 8 (FGE.08). Aliphatic and alicyclic mono-, di-, tri-, and polysulphides with or without additional oxygenated functional groups from chemical group 20

The present Flavouring Group Evaluation deals with 52 aliphatic and alicyclic mono-and dithiols and mono-, di-, tri-, and polysulphides with or without additional oxygenated functional groups from chemical group 20 of Annex I of Regulation (EC) No 1565/2000

Twelve of the 52 flavouring substances possess one or more chiral centres. In each of these cases, the substance has been presented without any indication that the commercial flavouring substance has dominance of one or the other optical isomer. Five of the 52 substances can exist as geometrical isomers. In each of these cases, no indication has been given that one of the possible isomers has preponderance in the commercial flavouring material.

Thirty-three of the candidate substances belong to structural class I, 15 belong to structural class II and four belong to structural class III.

Forty-one 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 Flavour Industry on the use levels in various foods, it appeared obvious that the MSDI approach in a number of cases would grossly underestimate the intake by regular consumers of products flavoured at the use level reported by the Industry, especially in those cases where the annual production values were reported to be small. In consequence, the Panel had reservations about 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, the 52 flavouring substances in this group have intakes in Europe from 0.0012 to 3.7 microgram/*capita*/day, which are well 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.

Data on genotoxicity of the flavouring substances are limited and the genotoxicity could not be assessed adequately. The data available, however, give rise to some concern of a genotoxic potential of two of the 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 two structurally related flavouring substances, 2-methylbutane-2-thiol [FL-no: 12.172] and 2,4,4-trimethyl-1,3-oxathiane [FL-no: 16.057], until adequate *in vivo* genotoxicity data become available. The genotoxicity data available for the remaining candidate substances do not preclude their evaluation through the Procedure.

For the flavouring substances in this group the metabolic pathways are unlikely to be saturated, given the extremely low levels of exposure to sulphides and thiols from their use as flavouring substances. However, due to the reactivity of the metabolites, the flavouring substances cannot be predicted to be metabolised to innocuous products.

It was noted that where toxicity data were available they were consistent with the conclusions in the present flavouring group evaluation.

The candidate substances was divided into nine subgroups. Except for subgroups II [FL-no: 12.120 and 15.102] and VIII [FL-no: 12.199], adequate repeated-dose toxicity studies are available for supporting substances from the different subgroups, allowing derivation of adequate margins of safety by comparing the NOAEL values with the MSDI. So, for the three substances [FL-no: 12.120, 12.199 and 15.102] additional toxicity data are required.

When the estimated intakes were based on the mTAMDI they ranged from 46 to 240 microgram/person/day for the 33 candidate substances from structural class I. These intakes were below the threshold of concern for structural class I of 1800 microgram/person/day. The estimated intakes for the 15 candidate substances assigned to structural class II, based on the mTAMDI, ranged from 46 to 78 microgram/person/day, which are below the threshold of concern for structural class II of 540 microgram/person/day. The estimated intakes for the four candidate substances assigned to structural class III, based on the mTAMDI, range from 78 to 370 microgram/person/day. For one of the substances [FL-no: 15.081] the mTAMDI is below the threshold of concern of 90 microgram/person/day.

Thus, for three of the 52 flavouring substances considered in this Opinion the intakes, estimated on the basis of the mTAMDI, exceed the relevant threshold for their structural class, to which the flavouring substance has been assigned. Therefore, for these three substances [FL-no: 12.120, 12.136 and 12.159] more reliable exposure data are required. On the basis of such additional data, two of these flavouring substances [FL-no: 12.120 and 12.136] should be reconsidered along the steps of the Procedure. For [FL-no: 12.159] adequate *in vivo* genotoxicity data should be made available. Following this Procedure additional toxicological data might become necessary.

In order to determine whether the conclusion for the 48 flavouring substances evaluated through the Procedure can be applied to the material of commerce, it is necessary to consider the available specifications:

Adequate specifications including complete purity criteria and identity tests for the materials of commerce have been provided for the 48 flavouring substances evaluated through the Procedure, except that information on chirality is missing for 11 of the substances [FL-no: 12.104, 12.106, 12.120, 12.135, 12.177, 12.178, 12.180, 12.214, 15.047, 15.048 and 15.083] and information on geometrical isomerism is missing for five of the substances [FL-no: 12.098, 12.163, 12.164, 15.056 and 15.110].

For the three substances [FL-no: 12.120, 12.199 and 15.102] additional toxicity data are required.

Thus, the final evaluation of the materials of commerce cannot be performed for 18 substances [FL-no: 12.098, 12.104, 12.106, 12.120, 12.135, 12.163, 12.164, 12.177, 12.178, 12.180, 12.199, 12.214, 15.047, 15.048, 15.056, 15.083, 15.102 and 15.110], pending further information. The remaining 30 flavouring substances of the 48 flavouring substances evaluated through the Procedure [FL-no: 12.096, 12.097, 12.099, 12.100, 12.103, 12.111, 12.112, 12.116, 12.117, 12.124, 12.125, 12.127, 12.129, 12.136, 12.151, 12.152, 12.158, 12.165, 12.166, 12.167, 12.181, 12.183, 12.189, 12.191, 12.196, 12.200, 12.221, 15.081, 15.103, 15.111] would present no safety concern at the levels of intake estimated on the basis of the MSDI approach.

Note: Since the adoption of this FGE in November 2007, new data have become available. Accordingly, for six tri- and polysulphides [FL-no: 12.097, 12.100, 12.112, 12.116, 12.164, 12.167] the conclusion might be revised by the Panel, in light of the new information.

KEYWORDS

Flavourings, safety, aliphatic, alicyclic, monosulphides, disulphides, trisulphides, polysulphides.

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BACKGROUND

Regulation (EC) No 2232/96 of the European Parliament and the Council (EC, 1996) 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 others 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 2008/478/EC (EC, 2008). 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, 2000), which is broadly based on the Opinion of the Scientific Committee on Food (SCF, 1999). For the submission of data by the manufacturer, deadlines have been established by Commission Regulation (EC) No 622/2002 (EC, 2002b).

After the completion of the evaluation programme the positive 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, 1996).

TERMS OF REFERENCE

The European Food safety Authority (EFSA) is requested to carry out a risk assessment on flavouring substances prior to their authorisation and inclusion in a positive list according to Commission Regulation (EC) No 1565/2000 (EC, 2000). In addition, the Commission requested EFSA to evaluate newly notified flavouring substances, where possible, before finalising the evaluation programme.

ASSESSMENT

1. Presentation of the substances in the Flavouring Group Evaluation 8

1.1. Description

The present Flavouring Group Evaluation 8 (FGE.08), using the Procedure as referred to in the Commission Regulation (EC) No 1565/2000 (the Procedure – shown in schematic form in Annex I), deals with 52 flavouring substances (candidate substances) from chemical group 20 of Annex I of Commission Regulation (EC) No 1565/2000 (EC, 2000).

The 52 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, and structures are listed in Table 1 and Table 2a.

The 52 candidate substances are straight, branched chain or heterogeneous ring aliphatic hydrocarbons containing one or more sulphur atoms. The sulphur atoms are present as thiols, sulphides or sulphones. Based on their structures, the candidate substances can be subdivided into nine 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, 12.183 and 12.214]
Subgroup II)	Cyclic sulphides: [FL-no: 12.120 and 15.102]
Subgroup III)	Monothiols: [FL-no: 12.104, 12.135, 12.136, 12.172, 12.174, 12.180 and 12.191]
Subgroup IV)	Dithiols: [FL-no: 12.103]
Subgroup V)	Acyclic di-, tri- and polysulphides: [FL-no: 12.097, 12.098, 12.100, 12.111, 12.112, 12.116, 12.151, 12.164 and 12.167]
Subgroup VI)	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 and 16.057]
Subgroup VII)	Thioesters: [FL-no: 12.106, 12.125, 12.165, 12.189, 12.196 and 12.221]
Subgroup VIII)	Thioic acids: [FL-no: 12.199]
Subgroup IX)	Sulphoxides/sulphones and sulphonates: [FL-no: 12.159].

The hydrolysis products of the candidate esters and thioesters are listed in Table 2b. In addition, the following potential hydrolysis products may theoretically be formed from the candidate thioacetals in an acid environment: formaldehyde, 2-methylpropanal, 3-methylbutanal, 3-methyl-3-mercaptobutan-1-ol, ethanthiol and hydrogen sulphide.

The 52 candidate substances are closely related structurally 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.

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 variation of 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.).

Twelve of the 52 flavouring substances possess one or more chiral centres [FL-no: 12.104, 12.106, 12.120, 12.135, 12.177, 12.178, 12.180, 12.214, 15.047, 15.048, 15.083 and 16.057]. In each of these cases, the substance has been presented without sufficient information whether the commercial flavouring substance has dominance of one or the other stereoisomer (see Table 1).

Due to the presence and the position of double bonds, three of the 52 substances [FL-no: 12.098, 12.163 and 12.164] 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. In each of these cases, no indication of the preponderance of either of the possible isomers in the commercial flavouring material has been given (see Table 1).

1.3. Natural Occurrence in Food

Forty-one of the 52 flavouring substances have been reported to occur in 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 (durian, grapefruit juice), cheese, egg, clam, mushroom (shiitake and *Agaricus*), tea (black), beer, wine (red, white), rum, spices, peanuts and sesame seed. Quantitative data on the natural occurrence in food have been reported for 12 of these substances (TNO, 2000).

These reports are:

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

According to TNO the remaining 11 candidate substances have not been reported in any food items (TNO, 2000): Allyl methyl tetrasulphide [FL-no: 12.097], S-2-butyl 3-methylbutanethioate [FL-no: 12.106], 3-mercapto-2-oxopropionic acid [FL-no: 12.136], 3-(methylthio)butyric acid [FL-no: 12.178], S-prenyl thioisobutyrate [FL-no: 12.196], ethanethionic acid [FL-no: 12.199], 1,1-bis(ethylthio)-ethane [FL-no: 12.200], isobutyl-3-(methylthio)butyrate [FL-no: 12.214], S-prenyl thioisopentanoate [FL-no: 12.221], 3-methyl-1,2,4-trithiolane [FL-no: 15.083] and 2,4,4-trimethyl-1,3-oxathiane [FL-no: 16.057].

2. Specifications

Purity criteria for the 52 substances have been provided by the Flavour Industry (EFFA, 2002g).

Judged against the requirements in Annex II of Commission Regulation (EC) No 1565/2000 (EC, 2000), the information is adequate for all substances, except that information on geometrical stereoisomerism and chirality is needed for five and 12 substances, respectively (see Section 1.2 and Table 1).

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, 1999).

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, 1999).

One of the alternatives is the "Theoretical Added Maximum Daily Intake" (TAMDI) approach, which is calculated on the basis of standard portions and upper use levels (SCF, 1995) for flavourable beverages and foods in general, with exceptional levels for particular foods. This method is regarded as a conservative estimate of the actual intake in 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, 2000). 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 Maximised Survey-derived Daily Intake (MSDI) (SCF, 1999) 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, 1995). The intake approach does not consider the possible natural occurrence in food.

Average *per capita* intake (MSDI) is estimated on the assumption that the amount added to food is consumed by 10 % of the EU population [2] (Eurostat, 1998). This is derived for candidate substances from estimates of annual volume of production provided by Industry and incorporates a correction factor of 0.6 to allow for incomplete reporting (60 %) in the Industry surveys (SCF, 1999).

[2] 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 (Eurostat, 1998).

In the present Flavouring Group Evaluation 8 (FGE.08) the total annual volume of production of the 52 candidate substances for use as flavouring substances in Europe has been reported to be approximately 110 kg (EFFA, 2002h). For 68 of the 127 supporting substances the annual volume of production is 740 kg (JECFA, 2000b). The annual volumes of production in Europe for 59 of the supporting substances were not reported.

On the basis of the annual volumes of production reported for the 52 candidate substances, the MSDI values for each of these flavourings have been estimated (see Table 2a).

Eighty percent of the total annual volumes of production for the candidate substances is accounted for by nine of these flavourings: allyl methyl sulphide [FL-no: 12.096], allyl propyl sulphide [FL-no: 12.099], butane-1,4-dithiol [FL-no: 12.103], S-2-butyl 3-methylbutanethioate [FL-no: 12.106], dibutyl disulphide [FL-no: 12.111], 2,8-epithio-p-menthane [FL-no: 12.120], 3-mercapto-2-oxopropionic acid [FL-no: 12.136], 8-(methylthio)-p-menthan-3-one [FL-no: 12.177] and 1,2,4-trithiolane [FL-no: 15.111]. The total estimated daily *per capita* intake of those nine candidate substances from use as flavouring substance is 11 microgram. The daily *per capita* intakes for the remaining 43 substances are for each less than one microgram, and in total less than 2.5 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 the 52 candidate substances, information on food categories and normal and maximum use levels[3,4,4] were submitted by the Flavour Industry (EFFA, 2002g; EFFA, 2007a).

The 52 candidate substances are used in flavoured food products divided into the food categories, outlined in Annex III of the Commission Regulation (EC) No 1565/2000 (EC, 2000), 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.

[3] "Normal use" is defined as the average of reported usages and "maximum use" is defined as the 95th percentile of reported usages (EFFA, 2002i).
[4] 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).
[4] 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).

Food category	Description	Flavourings used
Category 1	Dairy products, excluding products of category 2	All 52
Category 2	Fats and oils, and fat emulsions (type water-in-oil)	All 52
Category 3	Edible ices, including sherbet and sorbet	All 52
Category 4.1	Processed fruits	All 52
Category 4.2	Processed vegetables (incl. mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds	None
Category 5	Confectionery	All 52
Category 6	Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery	All 52 except [FL-no: 12.221]
Category 7	Bakery wares	All 52 except [FL-no: 12.127]
Category 8	Meat and meat products, including poultry and game	All 52
Category 9	Fish and fish products, including molluscs, crustaceans and echinoderms	All 52
Category 10	Eggs and egg products	None
Category 11	Sweeteners, including honey	None
Category 12	Salts, spices, soups, sauces, salads, protein products etc.	All 52
Category 13	Foodstuffs intended for particular nutritional uses	All 52 except [FL-no: 15.081]
Category 14.1	Non-alcoholic ("soft") beverages, excl. dairy products	All 52 except [FL-no: 12.116, 12.200, 15.047, 15.048]
Category 14.2	Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts	All 52
Category 15	Ready-to-eat savouries	All 52 except [FL-no: 12.165, 12.181]
Category 16	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 1 – 15	All 52 except [FL-no: 15.111]

According to the Flavour Industry the normal use levels for the 52 candidate substances are in the range of 0.1 - 2 mg/kg food, and maximum use levels are in the range of 0.2 – 10.5 mg/kg (EFFA, 2002g; EFFA, 2002i, EFFA, 2007a).(Table II.1.2, Annex I).

The mTAMDI values for the 33 candidate substances from structural class I (see Section 5) range from 46 to 240 microgram/person/day. For the 15 candidate substances from structural class II the mTAMDI range from 46 to 78 microgram/person/day. For the four candidate substances from structural class III the mTAMDI range from 78 to 370 microgram/person/day.

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

4. Absorption, Distribution, Metabolism and Elimination

All the candidate substances in the evaluation are sufficiently lipophilic to be efficiently absorbed from the gastrointestinal tract.

Depending on the type of sulphur-containing functional groups, the candidate substances can be subdivided into nine 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		
	12.096	Allyl methyl sulfide

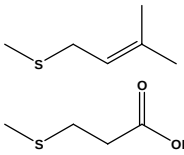
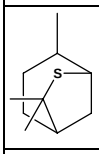
Table 4.1 Subgroups. The supporting substances are listed in brackets.		
	FL-no	**EU Register name**
 and	12.099	Allyl propyl sulfide
	12.117	Dipentyl sulfide
	12.124	Ethyl butyl sulfide
	12.127	Ethyl propyl sulfide
	12.129	3-(Ethylthio)propan-1-ol
	12.152	Methyl butyl sulfide
	12.158	Methyl isoprenyl sulfide
	12.163	Methyl prop-1-enyl sulfide 1)
	12.166	Methyl propyl sulfide
	12.177	8-(Methylthio)-p-menthan-3-one 1)
	12.178	3-(Methylthio)butyric acid 1)
	12.181	1-(Methylthio)pentan-3-one
	12.183	3-(Methylthio)propionic acid
	12.214	Isobutyl-3-(methylthio)butyrate 1)
	(12.001)	3-(Methylthio)propionaldehyde
	(12.002)	Methyl 3-(methylthio)propionate
	(12.006)	Dimethyl sulfide
	(12.007)	Dibutyl sulfide
	(12.040)	2-Methylthioacetaldehyde
	(12.041)	1-(Methylthio)butan-2-one
	(12.042)	2-(Methylthio)phenol
	(12.052)	Di-(3-oxobutyl) sulfide
	(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 sulfide
	(12.078)	4-(Methylthio)butan-1-ol
	(12.084)	Ethyl 4-(methylthio)butyrate
	(12.086)	Methyl 2-(methylthio)butyrate
	(12.088)	Diallyl sulfide
	(12.089)	Ethyl 3-(methylthio)butyrate
	(12.113)	Diethyl sulfide
	(12.118)	2,4-Dithiapentane
	(12.122)	Ethyl 2-(methylthio)acetate
	(12.154)	Methyl ethyl sulfide
	(12.162)	Methyl phenyl sulfide
	(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		
	12.120	2,8-Epithio-p-menthane 1)
	15.102	Tetrahydrothiophene
	(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		

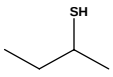
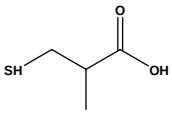
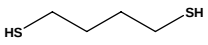
Table 4.1 Subgroups. The supporting substances are listed in brackets.		
	FL-no	**EU Register name**
 and 	12.104	Butane-2-thiol 1)
	12.135	3-Mercapto-2-methylpropionic acid 1)
	12.136	3-Mercapto-2-oxopropionic acid
	12.172	2-Methylbutane-2-thiol
	12.174	2-Methylpropane-2-thiol
	12.180	1-(Methylthio)ethane-1-thiol 1)
	12.191	Pentane-1-thiol
	(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
	(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
	(12.022)	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

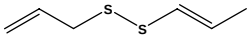
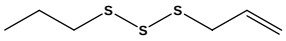
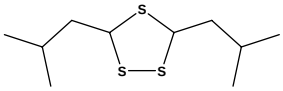
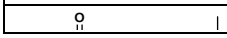
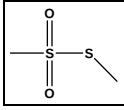
Table 4.1 Subgroups. The supporting substances are listed in brackets.		
	FL-no	**EU Register name**
	(12.073)	Butane-1,3-dithiol
	(12.076)	Propane-1,3-dithiol
	(12.168)	2-Methyl-2-(methyldithio)propanal
V: ACYCLIC DI-, TRI- AND POLYSULPHIDES		
 and 	12.097	Allyl methyl tetrasulfide
	12.098	Allyl prop-1-enyl disulfide 1)
	12.100	Allyl propyl trisulfide
	12.111	Dibutyl disulfide
	12.112	Dibutyl trisulfide
	12.116	Dimethyl tetrasulfide
	12.151	Methyl butyl disulfide
	12.164	Methyl prop-1-enyl trisulfide 1)
	12.167	Methyl propyl tetrasulfide
	(12.008)	Diallyl disulfide
	(12.009)	Diallyl trisulfide
	(12.013)	Dimethyl trisulfide
	(12.014)	Dipropyl disulfide
	(12.019)	Methyl propyl disulfide
	(12.020)	Methyl propyl trisulfide
	(12.023)	Dipropyl trisulfide
	(12.026)	Dimethyl disulfide
	(12.028)	Dicyclohexyl disulfide
	(12.037)	Allyl methyl disulfide
	(12.043)	Diphenyl disulfide
	(12.044)	Prop-1-enyl propyl disulfide
	(12.045)	Methyl allyl trisulfide
	(12.068)	Benzyl methyl disulfide
	(12.074)	Diallyl polysulfides
	(12.075)	Methyl prop-1-enyl disulfide
	(12.081)	Dibenzyl disulfide
	(12.109)	Di-isopropyl disulfide
	(12.121)	Ethyl 2-(methyldithio)propionate
	(12.155)	Methyl ethyl trisulfide
	(12.161)	Methyl phenyl disulfide
	(12.168)	2-Methyl-2-(methyldithio)propanal
	(12.218)	Methyl-3-methyl-1-butenyl disulphide
VI : 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 1)
	15.048	3,5-Di-isopropyl-1,2,4-trithiolane 1)
	15.056	3,6-Dimethyl-1,2,4,5-tetrathiane 1)
	15.081	Lenthionine
	15.083	3-Methyl-1,2,4-trithiolane 1)
	15.103	1,2,4,5-Tetrathiane
	15.110	2,4,6-Trimethyl-1,3,5-trithiane 1)
	15.111	1,2,4-Trithiolane
	16.057	2,4,4-Trimethyl-1,3-oxathiane 1)
	(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
VII: THIOESTERS		
	12.106	S-2-Butyl 3-methylbutanethioate 1)

Table 4.1 Subgroups. The supporting substances are listed in brackets.		
	FL-no	**EU Register name**
	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
	(12.018)	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
	(12.203)	Methylthio 2-(acetyloxy)propionate
	(12.227)	Methylthio-2-(propionyloxy)propionate
VIII: THIOIC ACIDS		
	12.199	Ethanethioic acid
IX: SULPHOXIDES/SULPHONES AND SULPHONATES		
	12.159	Methyl methanethiosulfonate
	(12.175)	Methylsulfinylmethane

1) Stereoisomeric composition not specified

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

Acyclic and cyclic monosulphides (thioethers) primarily undergo S-oxidation, catalysed by cytochrome P450 and flavin-containing monoxygenases, 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.

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.

One of the candidate substances from subgroup I is an ester, isobutyl-3-(methylthio)butyrate [FL-no: 12.214], which is anticipated to be hydrolysed to 2-methylpropanol [FL-no: 02.001] and 3-(methylthio)butyric acid [FL-no: 12.178]. One of the substances from subgroup VIII, ethanethioic acid [FL-no: 12.199] converts to acetic acid [FL-no: 08.002]. See Table 2b.

The only candidate substance of subgroup IX is methyl methanethiosulfonate [FL-no: 12.159], which is anticipated to be hydrolysed to methanesulfonic acid and hydrogensulfide. 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-sulphenic, sulphinic and 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-recognised 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 hydrogensulfide [not a Register substance]. The hydrolysis products are shown in Table 2b.

Subgroup V (Acyclic di-, tri- and polysulphides)

Polysulphides are easily converted to the corresponding disulphides, which are more labile: the disulphide bond is rapidly and extensively reduced to the corresponding thiol through a reversible reaction *in vivo*. Consequently, the above-mentioned metabolic options available to thiols are also available to di-, tri- and polysulphides.

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

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 & Satchell, 1990; Smith & March, 2001). It is thus to be anticipated that these substances may reach the intestinal lumen primarily intact and may be absorbed as such. Otherwise, the flavouring substances in this subgroup are anticipated to be metabolised like the cyclic sulphides in subgroup II.

Subgroup VII (Thioesters) and VIII (Thioic acids)

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 six candidate thioesters are shown in Table 2b.

The candidate 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 low levels of exposure to the flavouring substances in FGE.08.

Due to the reactivity of certain of the anticipated metabolites, the candidate substances cannot 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 two of the candidate substances, 2-methylpropane-2-thiol [FL-no: 12.174] and methyl methanethiosulphonate [FL-no: 12.159], there is 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 two substances, nor to the two structurally related candidates, 2-methylbutane-2-thiol [FL-no: 12.172] and 2,4,4-trimethyl-1,3-oxathiane [FL-no: 16.057].

For the safety evaluation of the remaining 48 candidate substances from chemical group 20 the Procedure as outlined in Annex I was applied, based on the MSDI approach. The stepwise evaluations of the 48 substances are summarised in Table 2a.

Step 1.

The candidate substances were classified following the procedure established by Cramer et al. (1978). For the remaining 48 candidate substances, 31 substances were classified in structural class I. Further 14 substances were classified in structural class II. The final three substances were classified in 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 48 candidate substances proceeds via the B-side of the Procedure scheme (Annex I).

Step B3.

The 31 substances in structural class I have estimated European daily *per capita* intakes ranging from 0.001 to 0.8 microgram, which is below the threshold of concern of 1800 microgram/person/day. The 14 substances in structural class II have estimated European daily *per capita* intakes ranging from 0.0012 to 2.4 microgram, which is below the threshold of concern for class II of 540 microgram/person/day. The three substances in structural class III have estimated European daily *per capita* intakes ranging from 0.012 to 3.7 microgram, which is below the threshold of concern for class III of 90 microgram/person/day. Accordingly, all 48 candidate substances proceed to step B4 of the Procedure.

Step B4.

No adequate studies on any 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.

The 15 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 3.8 microgram for the 15 candidate substances in subgroup I corresponds to 0.063 microgram/kg bw/day at a body weight of 60 kg. Thus a margin of safety of 4×10^6 can be calculated. The 15 candidate substances in subgroup I are accordingly not expected to be of safety concern at the estimated levels of intake.

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 two cyclic sulphides in subgroup II [FL-no: 12.120 and 15.102].

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 five remaining candidate substances in this subgroup to be evaluated through the Procedure. In the four studies, no adverse effects were produced by the highest oral dose tested ranging from 0.06 up to 0.7 mg/kg bw/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 0.78 microgram for the five candidate substances in subgroup III corresponds to 0.013 microgram/kg bw/day at a body weight of 60 kg. Thus, a margin of safety of 4.6×10^3 can be calculated. The five candidate substances in subgroup III, evaluated through the Procedure, are accordingly not expected to be of safety concern at the estimated levels of intake.

The candidate substance 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 highest oral doses tested, that is 0.7 mg/kg bw/day, which can be considered a NOAEL. The estimated daily *per capita* intake of 0.3 microgram for the one candidate substance in subgroup IV corresponds to 0.005 microgram/kg bw/day at a body weight of 60 kg. Thus, a margin of safety

of 1.4 x 10^5 can be calculated. The candidate substance in subgroup IV is accordingly not expected to be of safety concern at the estimated level of intake.

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 nine candidate substances in this subgroup. In the two studies, no adverse effects were produced by the highest 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 0.82 microgram for the nine candidate substances in subgroup V corresponds to 0.014 microgram/kg bw/day at a body weight of 60 kg. Thus, a margin of safety of 1.7 x 10^4 can be calculated. The nine candidate substances in subgroup V are accordingly not expected to be of safety concern at the estimated levels of intake.

Within Subgroup VI, adequate 90-day subchronic studies are available for two supporting substances, 3,5-dimethyl-1,2,4-trithiolane [FL-no: 15.025] and 2-methyl-4-propyl-1,3-oxathiane [FL-no:16.030], which can be considered representative of the remaining nine candidate substances in this subgroup to be evaluated through the Procedure. In the two studies, no adverse effects were produced by the highest oral dose tested: 0.44 and 1.88 mg/kg bw/day. By adopting a conservative approach, the lowest value (0.44 mg/kg bw/day) can be considered a NOAEL. The combined estimated daily *per capita* intake of 2.5 microgram for the 10 candidate substances in subgroup VI corresponds to 0.042 microgram/kg bw/day at a body weight of 60 kg. Thus, a margin of safety of 1 x 10^4 can be calculated. The 9 candidate substances in subgroup VI, evaluated through the Procedure, are accordingly not expected to be of safety concern at the estimated levels of intake.

Within subgroup VII, 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 six candidate substances in this subgroup. In the study, no adverse effects were produced by the highest 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 0.91 microgram for the six candidate substances in subgroup VII corresponds to 0.015 microgram/kg bw/day at a body weight of 60 kg. Thus, a margin of safety of 4.4 x 10^5 can be calculated. The six candidate substances in subgroup VII are accordingly not expected to be of safety concern at the estimated levels of intake.

Within subgroup VIII, no data are available for the candidate substance ethanethioic acid [FL-no: 12.199].

The conclusion from step B4 is that for the candidate substances belonging to Subgroups I, III, IV, V, VI and VII adequate NOAELs exist for structurally related substances providing an adequate margin 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 two candidate substances belonging to subgroup II [FL-no: 12.120 and 15.102] and for the candidate substance of subgroup VIII [FL-no: 12.199] additional toxicity data are required.

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

For all 52 candidate substances in this FGE, the intake estimates based on MSDI vs mTAMDI approach have been presented in Table 6.1.

The estimated intakes for the 31 of the 33 candidate substances in structural class I, which have been evaluated through the Procedure, based on the mTAMDI approach, range from 46 to 240 microgram/person/day. For all 31 substances the mTAMDI values are below the threshold of concern for structural class I substances of 1800 microgram/person/day.

The estimated intakes for the 14 of the 15 candidate substances assigned to structural class II, which have been evaluated through the Procedure, 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 three of the four candidate substances assigned to structural class III, which have been evaluated through the Procedure, based on the mTAMDI approach, range from 78 to 370 microgram/person/day. For one of the substances [FL-no: 15.081] the mTAMDI is below the threshold of concern for structural class III substances of 90 microgram/person/day. For the remaining two substances [FL-no: 12.120 and 12.136] the mTAMDI values are above the threshold of concern for structural class III substances of 90 microgram/person/day. For comparison of the intake estimates based on the MSDI approach and the mTAMDI approach, see Table 6.1.
For two candidate substances [FL-no: 12.120 and 12.136] further information is required. This would include more reliable intake data and then, if required, additional toxicological data.

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
12.183	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
12.199	Ethanethioic acid	0.0012	160	Class I	1800
12.200	1,1-bis(Ethylthio)-ethane	0.0012	46	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

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)
12.172	2-Methylbutane-2-thiol	0.15	78	Class I	1800
12.174	2-Methylpropane-2-thiol	0.0012	78	Class I	1800
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
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
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
15.081	Lenthionine	0.012	78	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 Flavouring Group Evaluation 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, 2002h), the combined estimated daily *per capita* intakes as flavourings of the candidate substances evaluated using the Procedure and assigned to structural class I (31 substances), structural class II (14 substances), and structural class III (three substances) are 3.2, 5.8 and 4.0 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 48 candidate substances, to which the Procedure has been applied, are structurally related to 127 supporting substances evaluated by JEFCA at its 53th JECFA meetings (JECFA, 2000b). Based on reported production volumes, European *per capita* intakes (MSDI) could be estimated for 68 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 59 of the supporting substances.

The total estimated 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 640, 115 and 4.0 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 five candidate substances: butane-2-thiol [FL-no: 12.104], 2-methylbutane-2-thiol [FL-no: 12.172], 2-methylpropane-2-thiol [FL-no: 12.174], pentane-1-thiol [FL-no: 12.191] belonging to subgroup III and tetrahydrothiophene [FL-no: 15.102], included in subgroup II. In addition data are available on 38 supporting substances. The LD_{50} values varied from 100 to more than 2000 mg/kg bw.

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

8.2. Subacute, Subchronic, Chronic and Carcinogenicity Studies

A repeated-dose toxicity study (14 days) is available for one candidate substance 3-(methylthio)propionic acid [FL-no: 12.183] in subgroup I. No NOAEL could be derived from this study (Steele et al., 1979).

Data from repeated-dose toxicity studies were available for 34 supporting substances included in subgroup I (3), II (1), III (10), IV (2), V (7), VI (5), VII (4), IX (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.

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-h period preceding the day of weighing. Urine samples were collected during weeks 2, 6 and 14, and examined for volume, appearance, specific gravity, microscopic constituents, and content of glucose, ketones, bile salts and blood. At sacrifice, blood was taken for haematological examinations. Gross abnormalities 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 & 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 same study design as 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 di-, tri- and polysulphides)

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 same study design as 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 disulfide 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).

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

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 as Cox et al. (1974a). 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., 1979).

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 VII (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).

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 four of the 52 candidate substances: 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 IX). In addition studies are available on 14 supporting substances from subgroups I (1), III (4), IV (1), V (4), VI (1), VII (2) and IX (1).

In vivo data are available for one candidate substance [FL-no: 12.159] (subgroup IX) and for four supporting substances from subgroups I (1), III (1), V (2).

Subgroup I (Acyclic sulphides)

Only data on the supporting substance diallyl sulphide [FL-no: 12.088] are available: diallyl sulphide was negative in a limited bacterial reversion assay using one tester 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 group 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-e).

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 compound 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. 2-methylbutane-2-thiol [FL-no: 12.172].

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] 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 di-, tri- and polysulphides)

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

Overall, the data available do not allow an evaluation of the genotoxicity of the compounds of 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.

Subgroup VII (Thioesters)

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

Subgroup VIII (Thioic acids)

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

Subgroup IX (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., 1997). 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.

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 two tertiary thiols [FL-no: 12.172 and 12.174], 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 VI, 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.

In addition, genotoxicity of the candidate substance MMTS [FL-no: 12.159], included in subgroup IX, could not be assessed from the data available. However, due to the similarity with MMS, 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 four candidate substances [FL-no: 12.159, 12.172, 12.174 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.

The genotoxicity data are summarised in Annex IV, Table IV.4 and 5.

9. Conclusions

The 52 candidate substances can be divided into nine subgroups:

Subgroup I)	Acyclic sulphides, 15 candidate substances [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, 12.183 and 12.214]
Subgroup II)	Cyclic sulphides, two candidate substances [FL-no: 12.120 and 15.102]
Subgroup III)	Monothiols, seven candidate substances [FL-no: 12.104, 12.135, 12.136, 12.172, 12.174, 12.180 and 12.191]
Subgroup IV)	Dithiols, one candidate substance [FL-no: 12.103]

Subgroup V)	cyclic di-, tri- and polysulphides, nine candidate substances: [FL-no: 12.097, 12.098, 12.100, 12.111, 12.112, 12.116, 12.151, 12.164 and 12.167]
Subgroup VI)	Mono-, di-, tri- and polysulphides with thioacetal structure, 10 candidate substances [FL-no: 12.200, 15.047, 15.048, 15.056, 15.081, 15.083, 15.103, 15.110, 15.111 and 16.057]
Subgroup VII)	Thioesters, six candidate substances [FL-no: 12.106, 12.125, 12.165, 12.189, 12.196 and 12.221]
Subgroup VIII)	Thioic acids, one candidate substance [FL-no: 12.199]
Subgroup IX)	Sulphoxides/sulphones and sulphonates, one candidate substance [FL-no: 12.159].

Twelve of the 52 flavouring substances possess one or more chiral centres [FL-no: 12.104, 12.106, 12.120, 12.135, 12.177, 12.178, 12.180, 12.214, 15.047, 15.048, 15.083 and 16.057]. In each of these cases, the substance has been presented without any indication that the commercial flavouring substance has dominance of one or the other stereoisomer. Three of the 52 substances can exist as geometrical isomers [FL-no: 12.098, 12.163 and 12.164]. In each of these cases, no indication has been given that one of the possible isomers has preponderance in the commercial flavouring material.

Thirty-three of the candidate substances belong to structural class I, 15 belong to structural class II and four belong to structural class III.

Forty-one 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, the 52 flavouring substances in this group have intakes in Europe ranging from 0.0012 to 3.7 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.

On the basis of the reported annual production volumes in Europe (MSDI approach), the combined intake of the 31 candidate substances belonging to class I and evaluated through the Procedure, the 15 candidate substances belonging to class II and evaluated through the Procedure, and the three candidate substances belonging to class III and evaluated through the Procedure, would result in total intakes of approximately 3.2, 5.8 and 4.0, respectively, which do not exceed the thresholds of concern. Based on reported production volumes, European *per capita* intakes (MSDI) could be estimated for 68 of the 127 supporting substances. The total combined intakes of the candidate and supporting substances (for which there are European intake data) are approximately 637, 115 and 4 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 two structurally related candidate substances, 2-methylbutane-2-thiol [FL-no: 12.172] and 2,4,4-trimethyl-1,3-oxathiane [FL-no: 16.057] until adequate *in vivo* genotoxicity data become available. 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.

Except for subgroups II [FL-no: 12.120 and 15.102] and VIII [FL-no: 12.199], adequate repeated-dose toxicity studies are available for supporting substances from the different subgroups, allowing derivation of adequate margins of safety by comparing the NOAEL values with the MSDI. So, for the three substances [FL-no: 12.120, 12.199 and 15.102] additional toxicity data are required.

It is considered that on the basis of the default MSDI approach 45 of the 48 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 data are required for the two candidate substances in subgroup II [FL-no: 12.120 and 15.102] and for the candidate substance in subgroup VIII [FL-no: 12.199].

When the estimated intakes were based on the mTAMDI they ranged from 46 to 240 microgram/person/day for the 31 candidate substances from structural class I evaluated through the Procedure. These intakes were below the threshold of concern for structural I of 1800 microgram/person/day. The estimated intakes for the 14 candidate substances assigned to structural class II and evaluated through the Procedure, based on the mTAMDI, ranged from 46 to 78 microgram/person/day, which are below the threshold of concern for structural class II of 540 microgram/person/day. The estimated intakes for the three candidate substances assigned to structural class III and evaluated through the Procedure, based on the mTAMDI, are in the range of 78 to 370 microgram/person/day. For one of the substances [FL-no: 15.081] the mTAMDI is below the threshold of concern of 90 microgram/person/day.

Thus, for two of the 48 flavouring substances evaluated through the Procedure the intakes, estimated on the basis of the mTAMDI, exceed the relevant threshold for their structural class, to which the flavouring substance has been assigned. Therefore, for these two substances [FL-no: 12.120 and 12.136] more reliable 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 48 candidate substances evaluated through the Procedure can be applied to the material of commerce, it is necessary to consider the available specifications:

Adequate specifications including complete purity criteria and identity tests for the materials of commerce have been provided for the 48 flavouring substances evaluated through the Procedure, except that information on chirality is missing for 11 of the substances [FL-no: 12.104, 12.106, 12.120, 12.135, 12.177, 12.178, 12.180, 12.214, 15.047, 15.048 and 15.083] and information on geometrical isomerism is missing for five of the substances [FL-no: 12.098, 12.163, 12.164, 15.056 and 15.110].

For the three substances [FL-no: 12.120, 12.199 and 15.102] additional toxicity data are required.

Thus, the final evaluation of the materials of commerce cannot be performed for 18 substances [FL-no: 12.098, 12.104, 12.106, 12.120, 12.135, 12.163, 12.164, 12.177, 12.178, 12.180, 12.199, 12.214, 15.047, 15.048, 15.056, 15.083, 15.102 and 15.110], pending further information. The remaining 30 flavouring substances of the 48 flavouring substances evaluated through the Procedure [FL-no: 12.096, 12.097, 12.099, 12.100, 12.103, 12.111, 12.112, 12.116, 12.117, 12.124,

12.125, 12.127, 12.129, 12.136, 12.151, 12.152, 12.158, 12.165, 12.166, 12.167, 12.181, 12.183, 12.189, 12.191, 12.196, 12.200, 12.221, 15.081, 15.103, 15.111] would present no safety concern at the levels of intake estimated on the basis of the MSDI approach.

Note: Since the adoption of this FGE in November 2007, new data have become available. Accordingly, for six tri- and polysulphides [FL-no: 12.097, 12.100, 12.112, 12.116, 12.164, 12.167] the conclusion might be revised by the Panel, in light of the new information.

TABLE 1: SPECIFICATION SUMMARY OF THE SUBSTANCES IN THE FLAVOURING GROUP EVALUATION 8

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 8

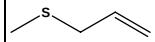
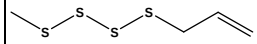
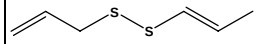
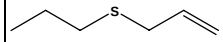
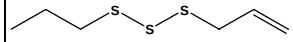
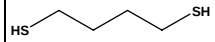
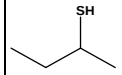
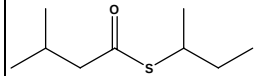
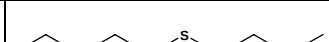
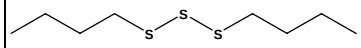
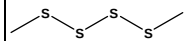
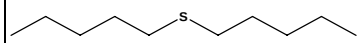
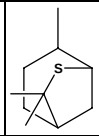
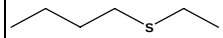
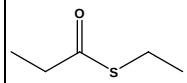
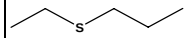
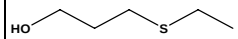
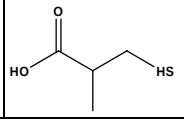
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys. form Mol. formula Mol. weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec. gravity 5)	Specification comments
12.096	Allyl methyl sulfide		11429 10152-76-8	Liquid C_4H_8S 88.17	Practically insoluble or insoluble 1 ml in 1 ml	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 1 ml in 1 ml	267 23 MS 95 %	n.a. n.a.	
12.098	Allyl prop-1-enyl disulfide 6)		11433 33368-82-0	Liquid $C_6H_{10}S_2$ 146.28	Practically insoluble or insoluble 1 ml in 1 ml	205 NMR 95 %	1.541-1.547 1.004-1.010	(Z) or (E) isomer not specified by Register CASrn.
12.099	Allyl propyl sulfide		11434 27817-67-0	Liquid $C_6H_{12}S$ 148.29	Practically insoluble or insoluble 1 ml in 1 ml	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 1 ml in 1 ml	253 MS 95 %	1.584-1.590 1.050-1.056	
12.103	Butane-1,4-dithiol		1191-08-8	Liquid $C_4H_{10}S_2$ 122.24	Slightly soluble 1 ml in 1 ml	73 (13 hPa) MS 95 %	1.524-1.530 1.041-1.047	
12.104	Butane-2-thiol 6)		513-53-1	Liquid $C_4H_{10}S$ 90.18	Slightly soluble 1 ml in 1 ml	85 MS 95 %	1.431-1.437 0.826-0.832	CASrn in Register refers to the racemate
12.106	S-2-Butyl 3-methylbutanethioate 6)		2432-91-9	Liquid $C_9H_{18}OS$ 174.30	Practically insoluble or insoluble 1 ml in 1 ml	181 MS 98 %	1.452-1.459 0.898-0.906	(R) or (S) isomer not specified by Register CASrn
12.111	Dibutyl disulfide		629-45-8	Liquid $C_8H_{18}S_2$ 178.35	Practically insoluble or insoluble 1 ml in 1 ml	101 (13 hPa) MS 95 %	1.488-1.494 0.934-0.940	

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 8

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
12.112	Dibutyl trisulfide		5943-31-7	Liquid $C_8H_{18}S_3$ 210.41	Practically insoluble or insoluble 1 ml in 1 ml	139 (16 hPa) NMR 95 %	1.525-1.531 1.015-1.021	
12.116	Dimethyl tetrasulfide		11459 5756-24-1	Liquid $C_2H_6S_4$ 158.31	Practically insoluble or insoluble 1 ml in 1 ml	60 (1.3 hPa) MS 95 %	1.658-1.664 1.303-1.309	
12.117	Dipentyl sulfide		872-10-6	Liquid $C_{10}H_{22}S$ 174.34	Practically insoluble or insoluble 1 ml in 1 ml	108 (20 hPa) MS 95 %	1.450-1.456 0.836-0.842	
12.120	2,8-Epithio-p-menthane 6)		68398-18-5	Liquid $C_{10}H_{18}S$ 170.31	Practically insoluble or insoluble 1 ml in 1 ml	114 (31 hPa) MS 95 %	1.511-1.517 0.999-1.005	(R) or (S) enantiomer not specified by CASrn in Register.
12.124	Ethyl butyl sulfide		638-46-0	Liquid $C_6H_{14}S$ 118.24	Practically insoluble or insoluble 1 ml in 1 ml	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 1 ml in 1 ml	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 1 ml in 1 ml	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 1 ml in 1 ml	99 (13 hPa) NMR 95 %	1.480-1.486 0.989-0.995	
12.135	3-Mercapto-2-methylpropionic acid 6)		26473-47-2	Solid $C_4H_8O_2S$ 120.17	Soluble 1 ml in 1 ml	113 (13 hPa) 43 NMR 95 %	n.a. n.a.	(R) or (S) enantiomer not specified by CASrn in Register.

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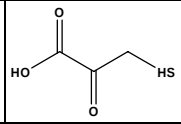
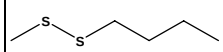
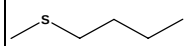
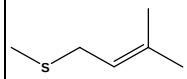
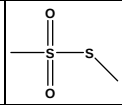
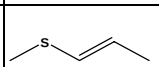
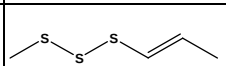
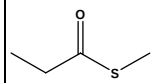
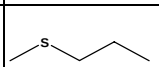
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys. form Mol. formula Mol. weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec. gravity 5)	Specification comments
12.136	3-Mercapto-2-oxopropionic acid		2464-23-5	Solid $C_3H_4O_3S$ 120.12	Soluble 1 ml in 1 ml	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 1 ml in 1 ml	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 1 ml in 1 ml	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 1 ml in 1 ml	145 NMR 95 %	1.478-1.484 0.862-0.868	
12.159	Methyl methanethiosulfonate		11520 2949-92-0	Liquid $C_2H_6O_2S_2$ 126.19	Slightly soluble 1 ml in 1 ml	104 (13 hPa) MS 95 %	1.507-1.513 1.315-1.321	
12.163	Methyl prop-1-enyl sulfide 6)		11538 10152-77-9	Liquid C_4H_8S 88.17	Practically insoluble or insoluble 1 ml in 1 ml	103 NMR 95 %	1.487-1.493 0.867-0.873	(Z) or (E) isomer not specified by CASrn in Register.
12.164	Methyl prop-1-enyl trisulfide 6)		11539 33368-80-8	Liquid $C_4H_8S_3$ 152.17	Practically insoluble or insoluble 1 ml in 1 ml	223 NMR 95 %	1.586-1.592 1.112-1.118	(Z) or (E) isomer not specified by CASrn in Register.
12.165	S-Methyl propanethioate		5925-75-7	Liquid C_4H_8OS 104.17	Practically insoluble or insoluble 1 ml in 1 ml	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 1 ml in 1 ml	96 MS 95 %	1.438-1.444 0.834-0.840	

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 8

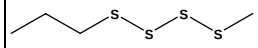
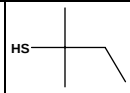
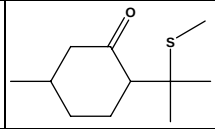
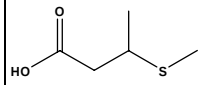
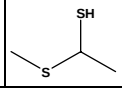
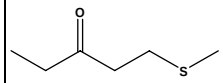
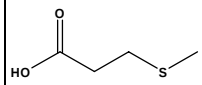
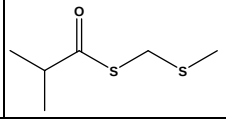
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys. form Mol. formula Mol. weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec. gravity 5)	Specification comments
12.167	Methyl propyl tetrasulfide		87148-08-1	Liquid $C_4H_{10}S_4$ 186.18	Practically insoluble or insoluble 1 ml in 1 ml	259 NMR 95 %	1.622-1.628 1.197-1.203	
12.172	2-Methylbutane-2-thiol		1679-09-0	Liquid $C_5H_{12}S$ 104.21	Practically insoluble or insoluble 1 ml in 1 ml	99 MS 95 %	1.432-1.438 0.809-0.815	
12.174	2-Methylpropane-2-thiol		11537 75-66-1	Liquid $C_4H_{10}S$ 90.18	Slightly soluble 1 ml in 1 ml	64 MS 95 %	1.417-1.423 0.797-0.803	
12.177	8-(Methylthio)-p-menthan-3-one 6)		32637-94-8	Liquid $C_{11}H_{20}OS$ 200.34	Practically insoluble or insoluble 1 ml in 1 ml	72 (0.1 hPa) NMR 95 %	1.495-1.501 0.951-0.957	CASrn in Register refers to (Z) isomer.
12.178	3-(Methylthio)butyric acid 6)		16630-65-2	Liquid $C_5H_{10}O_2S$ 134.19	Soluble 1 ml in 1 ml	127 (13 hPa) MS 95 %	1.479-1.486 1.102-1.108	(R) or (S) enantiomer not specified by CASrn in Register.
12.180	1-(Methylthio)ethane-1-thiol 6)		31331-53-0	Liquid $C_3H_8S_2$ 108.22	Slightly soluble 1 ml in 1 ml	58 (35 hPa) NMR 95 %	1.522-1.528 0.879-0.885	(R) or (S) enantiomer not specified by CASrn in Register.
12.181	1-(Methylthio)pentan-3-one		66735-69-1	Liquid $C_6H_{12}OS$ 132.22	Practically insoluble or insoluble 1 ml in 1 ml	88 (16 hPa) MS 95 %	1.467-1.473 0.987-0.993	
12.183	3-(Methylthio)propionic acid		646-01-5	Liquid $C_4H_8O_2S$ 120.17	Soluble 1 ml in 1 ml	125 (16 hPa) MS 95 %	1.485-1.491 1.155-1.161	
12.189	S-(Methylthiomethyl) 2-methylpropanethioate		77974-85-7	Liquid $C_6H_{12}OS_2$ 164.03	Practically insoluble or insoluble 1 ml in 1 ml	273 NMR 95 %	1.452-1.456 1.031-1.037	

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 8

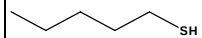
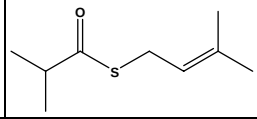
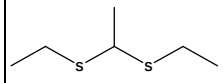
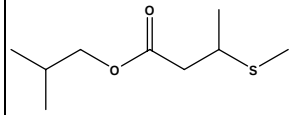
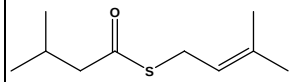
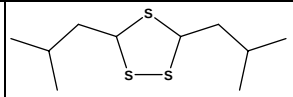
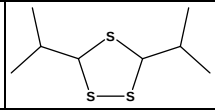
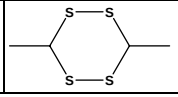
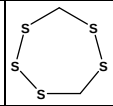
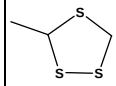
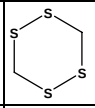
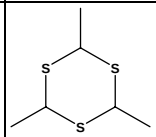
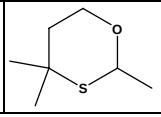
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys. form Mol. formula Mol. weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec. gravity 5)	Specification comments
12.191	Pentane-1-thiol		110-66-7	Liquid $C_5H_{12}S$ 104.21	Slightly soluble 1 ml in 1 ml	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 1 ml in 1 ml	100 (20 hPa) NMR 95 %	1.483-1.489 1.109-1.115	
12.199	Ethanethioic acid		507-09-5	Liquid C_2H_4OS 76.11	Slightly soluble 1 ml in 1 ml	88 MS 95 %	1.459-1.465 1.066-1.072	
12.200	1,1-bis(Ethylthio)-ethane		14252-42-7	Liquid $C_6H_{14}S_2$ 150.30	Practically insoluble or insoluble 1 ml in 1 ml	80 (13 hPa) MS 95 %	1.499-1.505 0.967-0.973	
12.214	Isobutyl-3-(methylthio)butyrate 6)		127931-21-9	Liquid $C_9H_{18}O_2S$ 190.30	Practically insoluble or insoluble 1 ml in 1 ml	224 NMR 95 %	1.458-1.464 0.875-0.881	(R) or (S) enantiomer not specified by CASrn in Register.
12.221	S-Prenyl thioisopentanoate		75631-91-3	Liquid $C_{10}H_{18}OS$ 186.28	Practically insoluble or insoluble 1 ml in 1 ml	248 MS 95 %	1.475-1.481 1.003-1.009	
15.047	3,5-Di-isobutyl-1,2,4-trithiolane 6)		92900-67-9	Solid $C_{10}H_{20}S_3$ 236.40	Practically insoluble or insoluble 1 ml in 1 ml	295 156 NMR 95 %	n.a. n.a.	CASrn in Register refers to the racemate.
15.048	3,5-Di-isopropyl-1,2,4-trithiolane 6)		54934-99-5	Solid $C_8H_{16}S_3$ 208.39	Practically insoluble or insoluble 1 ml in 1 ml	263 133 MS 95 %	n.a. n.a.	CASrn in Register refers to the racemate.
15.056	3,6-Dimethyl-1,2,4,5-tetrathiane 6)		67411-27-2	Solid $C_4H_8S_4$ 184.35	Practically insoluble or insoluble 1 ml in 1 ml	264 198 MS 95 %	n.a. n.a.	CASrn in Register does not specify stereoisomeric composition.

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 8

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
15.081	Lenthionine		11619 292-46-6	Solid $C_2H_4S_5$ 188.35	Practically insoluble or insoluble 1 ml in 1 ml	287 61 MS 95 %	n.a. n.a.	
15.083	3-Methyl-1,2,4-trithiolane 6)		51647-38-2	Solid $C_3H_6S_3$ 138.28	Practically insoluble or insoluble 1 ml in 1 ml	198 111 MS 95 %	n.a. n.a.	(R) or (S) enantiomer not specified by CASrn in Register.
15.102	Tetrahydrothiophene		110-01-0	Liquid C_4H_8S 88.17	Slightly soluble 1 ml in 1 ml	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 1 ml in 1 ml	239 126 MS 95 %	n.a. n.a.	
15.110	2,4,6-Trimethyl-1,3,5-trithiane 6)		2765-04-0	Solid $C_6H_{12}S_3$ 180.34	Practically insoluble or insoluble 1 ml in 1 ml	246 125 MS 95 %	n.a. n.a.	CASrn in Register does not specify stereoisomeric composition.
15.111	1,2,4-Trithiolane		289-16-7	Solid $C_2H_4S_3$ 124.23	Practically insoluble or insoluble 1 ml in 1 ml	102 (13 hPa) 104 MS 95 %	n.a. n.a.	
16.057	2,4,4-Trimethyl-1,3-oxathiane 6)		72472-02-7	Solid $C_7H_{14}OS$ 146.25	Practically insoluble or insoluble 1 ml in 1 ml	70 (25 hPa) 32 NMR 95 %	n.a. n.a.	(R) or (S) enantiomer not specified by CASrn in Register.

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.

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)

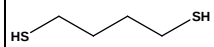
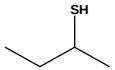
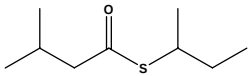
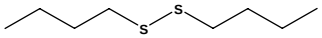
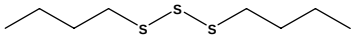
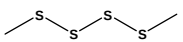
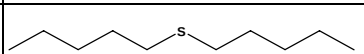
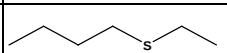
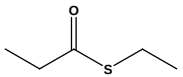
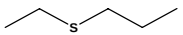
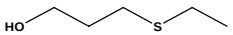
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
12.103	Butane-1,4-dithiol		0.3	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
12.104	Butane-2-thiol		0.18	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	7)	
12.106	S-2-Butyl 3-methylbutanethioate		0.8	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	7)	
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: Adequate NOAEL exists	4)	6)	
12.116	Dimethyl tetrasulfide		0.016	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
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)	

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

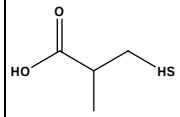
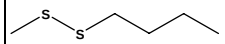
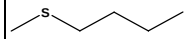
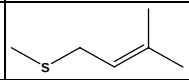
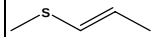
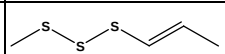
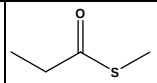
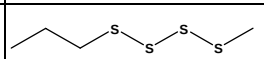
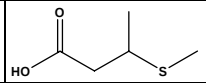
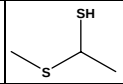
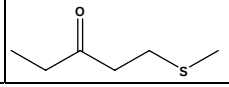
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
12.135	3-Mercapto-2-methylpropionic acid		0.12	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	7)	
12.151	Methyl butyl disulfide		0.0061	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
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)	7)	
12.164	Methyl prop-1-enyl trisulfide		0.0061	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	7)	
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: Adequate NOAEL exists	4)	6)	
12.178	3-(Methylthio)butyric acid		0.12	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	7)	
12.180	1-(Methylthio)ethane-1-thiol		0.12	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	7)	
12.181	1-(Methylthio)pentan-3-one		0.12	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)

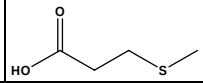
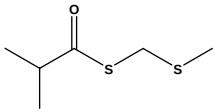
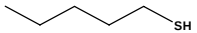
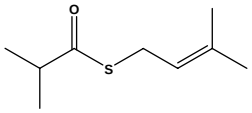
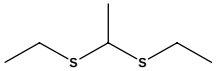
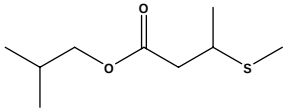
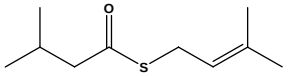
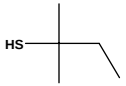
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
12.183	3-(Methylthio)propionic acid		0.21	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
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		
12.200	1,1-bis(Ethylthio)-ethane		0.0012	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)	7)	
12.221	S-Prenyl thioisopentanoate		0.012	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
12.172	2-Methylbutane-2-thiol		0.15	Class I No evaluation			a)
12.174	2-Methylpropane-2-thiol		0.0012	Class I No evaluation			a)

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

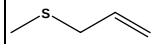
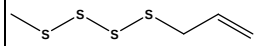
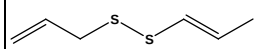
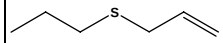
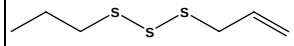
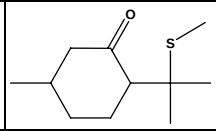
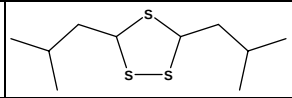
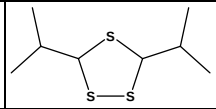
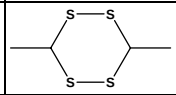
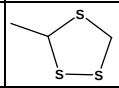
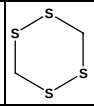
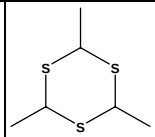
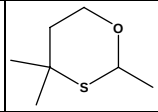
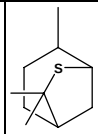
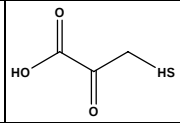
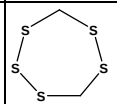
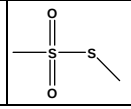
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
12.096	Allyl methyl sulfide		0.99	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
12.097	Allyl methyl tetrasulfide		0.012	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
12.098	Allyl prop-1-enyl disulfide		0.17	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	7)	
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: Adequate NOAEL exists	4)	6)	
12.177	8-(Methylthio)-p-menthan-3-one		0.37	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	7)	
15.047	3,5-Di-isobutyl-1,2,4-trithiolane		0.024	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	7)	
15.048	3,5-Di-isopropyl-1,2,4-trithiolane		0.0061	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	7)	
15.056	3,6-Dimethyl-1,2,4,5-tetrathiane		0.0024	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	7)	
15.083	3-Methyl-1,2,4-trithiolane		0.0024	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	7)	
15.102	Tetrahydrothiophene		0.024	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		

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
15.103	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)	7)	
15.111	1,2,4-Trithiolane		2.4	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
16.057	2,4,4-Trimethyl-1,3-oxathiane		0.0012	Class II No evaluation			a)
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)	
15.081	Lenthionine		0.012	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
12.159	Methyl methanethiosulfonate		0.061	Class III No evaluation			a)

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, Class II = 540, 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) Evaluation deferred pending in vivo genotoxicity data

TABLE 2B: EVALUATION STATUS OF HYDROLYSIS PRODUCTS OF CANDIDATE THIOESTERS AND ESTERS

Table 2b: Evaluation Status of Hydrolysis Products of Candidate Thioesters and Esters

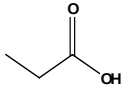
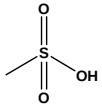
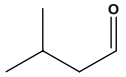
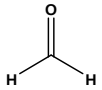
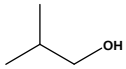
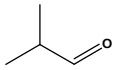
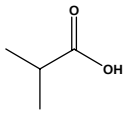
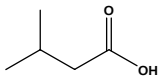
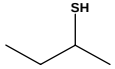
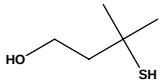
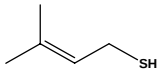
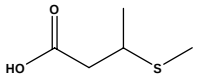
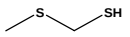
FL-no	EU Register name JECFA no	Structural formula	SCF status 1) JECFA status 2) CoE status 3) EFSA status	Structural class 4) Procedure path (JECFA) 5)	Comments
	Proprionic acid		Not evaluated as flavouring substance		Not in EU-Register.
	Methanesulfonic acid		Not evaluated as flavouring substance		Not in EU-Register.
	Hydrogensulfide	H_2S	Not evaluated as flavouring substance		Not in EU-Register.
	3-Methylbutanaldehyde		Not evaluated as flavouring substance		Not in EU-Register.
	Formaldehyde		Not evaluated as flavouring substance		Not in EU-Register.
02.001	2-Methylpropan-1-ol 251		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake below threshold	
05.001	Acetaldehyde 80		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake below threshold	
05.004	2-Methylpropanal 252		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake below threshold	
08.002	Acetic acid 81		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake below threshold	

Table 2b: Evaluation Status of Hydrolysis Products of Candidate Thioesters and Esters

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
08.006	2-Methylpropionic acid 253		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake below threshold	
08.008	3-Methylbutyric acid 259		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake below threshold	
12.003	Methanethiol 508		No safety concern e) Category B c)	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	
12.017	Ethanethiol 1659		Category B c)	Class I No evaluation	
12.104	Butane-2-thiol			Class I B3: Intake below threshold, B4: Adequate NOAEL exists	
12.137	3-Mercapto-3-methylbutan-1-ol 544		No safety concern e)	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	
12.170	3-Methylbut-2-ene-1-thiol 522		No safety concern e)	Class I B3: Intake below threshold, B4: Adequate NOAEL exists	
12.178	3-(Methylthio)butyric acid			Class I B3: Intake below threshold, B4: Adequate NOAEL exists	
12.242	Methylthiomethylmercaptan 1675				

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, Class II = 540, 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) (JECFA, 1999b).

c) (CoE, 1992).

d) (JECFA, 2001a).

e) (JECFA, 2000b).

TABLE 3: SUPPORTING SUBSTANCES SUMMARY

Table 3: Supporting Substances Summary

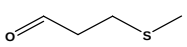
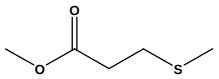
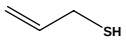
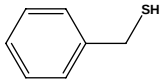
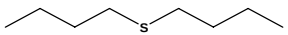
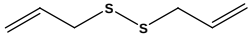
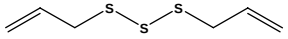
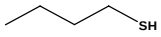
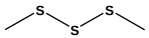
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (μg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
12.001	3-(Methylthio)propionaldehyde		2747 125 3268-49-3	466 JECFA specification (JECFA, 2000d)	28	No safety concern a) Category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

Table 3: Supporting Substances Summary

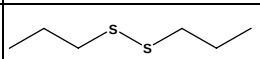
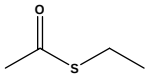
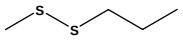
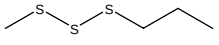
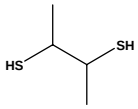
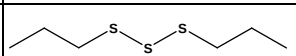
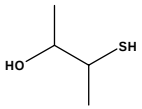
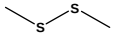
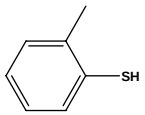
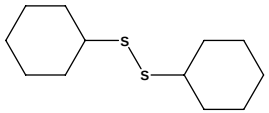
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
12.014	Dipropyl disulfide		3228 540 629-19-6	566 JECFA specification (JECFA, 2002d)	3.4	No safety concern a) Category B b)	
12.018	S-Ethyl acetothioate		3282 11665 625-60-5	483 JECFA specification (JECFA, 2002d)	0.012	No safety concern a) Deleted b)	
12.019	Methyl propyl disulfide		3201 585 2179-60-4	565 JECFA specification (JECFA, 2000d)	3.9	No safety concern a) Category B b)	
12.020	Methyl propyl trisulfide		3308 586 17619-36-2	584 JECFA specification (JECFA, 2000d)	0.21	No safety concern a) Category A b)	
12.022	Butane-2,3-dithiol		3477 725 4532-64-3	539 JECFA specification (JECFA, 1999c)	0.049	No safety concern a) Category A b)	
12.023	Dipropyl trisulfide		3276 726 6028-61-1	585 JECFA specification (JECFA, 2000d)	7.3	No safety concern a) Category A b)	
12.024	3-Mercaptobutan-2-ol		3502 760 37887-04-0	546 JECFA specification (JECFA, 2000d)	4.0	No safety concern a) Category A b)	
12.026	Dimethyl disulfide		3536 2175 624-92-0	564 JECFA specification (JECFA, 2002d)	6.9	No safety concern a) Category B b)	
12.027	2-Methylbenzene-1-thiol		3240 2272 137-06-4	528 JECFA specification (JECFA, 2000d)	17	No safety concern a) Category A b)	
12.028	Dicyclohexyl disulfide		3448 2320 2550-40-5	575 JECFA specification (JECFA, 2000d)	0.012	No safety concern a) Category A b)	

Table 3: Supporting Substances Summary

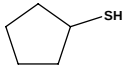
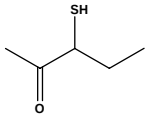
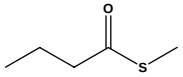
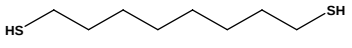
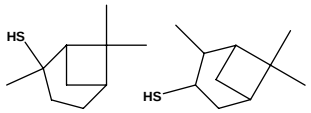
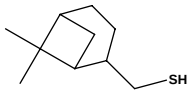
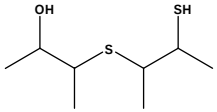
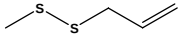
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
12.029	Cyclopentanethiol		3262 2321 1679-07-8	516 JECFA specification (JECFA, 2000d)	ND	No safety concern a) Category B b)	
12.031	3-Mercaptopentan-2-one		3300 2327 67633-97-0	560 JECFA specification (JECFA, 2000d)	ND	No safety concern a) Category A b)	
12.032	S-Methyl butanethioate		3310 2328 2432-51-1	484 JECFA specification (JECFA, 2000d)	2.9	No safety concern a) Category A b)	
12.034	Octane-1,8-dithiol		3514 2331 1191-62-4	541 JECFA specification (JECFA, 1999c)	2.1	No safety concern a) Category A b)	
12.035	2-,3- and 10-Mercaptopinane	 **2-Mercaptopinane** **3-Mercaptopinane**  **10-Mercaptopinane**	3503 2332	520 JECFA specification (JECFA, 2000d)	0.037	No safety concern a) Category A b)	
12.036	3-[(2-Mercapto-1-methylpropyl)thio]butan-2-ol		3509 2353 54957-02-7	547 JECFA specification (JECFA, 1999c)	ND	No safety concern a) Category A b)	
12.037	Allyl methyl disulfide		3127 11866 2179-58-0	568 JECFA specification (JECFA, 2003b)	0.0012	No safety concern a)	

Table 3: Supporting Substances Summary

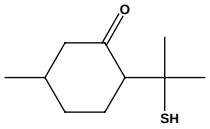
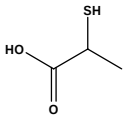
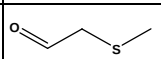
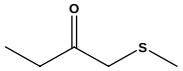
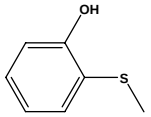
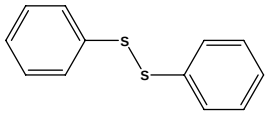
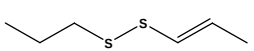
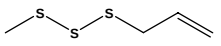
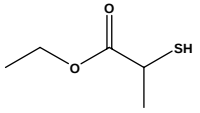
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (μg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
12.038	8-Mercapto-p-menthan-3-one		3177 11789 38462-22-5	561 JECFA specification (JECFA, 2000d)	10	No safety concern a)	
12.039	2-Mercaptopropionic acid		3180 11790 79-42-5	551 JECFA specification (JECFA, 2002d)	2.1	No safety concern a)	
12.040	2-Methylthioacetaldehyde		3206 11686 23328-62-3	465 JECFA specification (JECFA, 2003b)	ND	No safety concern a)	
12.041	1-(Methylthio)butan-2-one		3207 11543 13678-58-5	496 JECFA specification (JECFA, 1999c)	0.0037	No safety concern a)	
12.042	2-(Methylthio)phenol		3210 11553 1073-29-6	503 JECFA specification (JECFA, 2000d)	0.61	No safety concern a)	
12.043	Diphenyl disulfide		3225 11757 882-33-7	578 JECFA specification (JECFA, 1999c)	ND	No safety concern a)	
12.044	Prop-1-enyl propyl disulfide		3227 11699 5905-46-4	570 JECFA specification (JECFA, 2005b)	ND	No safety concern a)	
12.045	Methyl allyl trisulfide		3253 11867 34135-85-8	586 JECFA specification (JECFA, 2003b)	ND	No safety concern a)	
12.046	Ethyl 2-mercaptopropionate		3279 11469 19788-49-9	552 JECFA specification (JECFA, 2000d)	0.39	No safety concern a)	

Table 3: Supporting Substances Summary

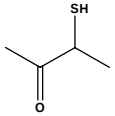
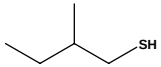
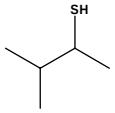
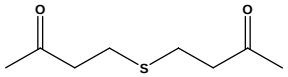
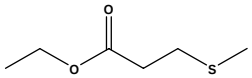
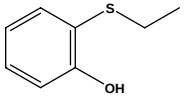
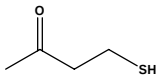
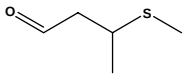
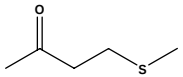
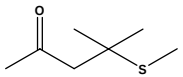
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
12.047	3-Mercaptobutan-2-one		3298 11497 40789-98-8	558 JECFA specification (JECFA, 2000d)	3.2	No safety concern a)	
12.048	2-Methylbutane-1-thiol		3303 11509 1878-18-8	515 JECFA specification (JECFA, 1999c)	0.3	No safety concern a)	
12.049	3-Methylbutane-2-thiol		3304 11510 2084-18-6	517 JECFA specification (JECFA, 1999c)	0.012	No safety concern a)	
12.052	Di-(3-oxobutyl) sulfide		3335 11441 40790-04-3	502 JECFA specification (JECFA, 2003b)	ND	No safety concern a)	
12.053	Ethyl 3-(methylthio)propionate		3343 11476 13327-56-5	476 JECFA specification (JECFA, 2002d)	24	No safety concern a)	
12.054	2-(Ethylthio)phenol		3345 11666 4500-58-7	529 JECFA specification (JECFA, 2000d)	0.00012	No safety concern a)	
12.055	4-Mercaptobutan-2-one		3357 11498 34619-12-0	559 JECFA specification (JECFA, 2003b)	ND	No safety concern a)	
12.056	3-(Methylthio)butanal		3374 11687 16630-52-7	467 JECFA specification (JECFA, 2000d)	0.085	No safety concern a)	
12.057	4-(Methylthio)butan-2-one		3375 11688 34047-39-7	497 JECFA specification (JECFA, 2000d)	0.012	No safety concern a)	
12.058	4-(Methylthio)-4-methylpentan-2-one		3376 11551 23550-40-5	500 JECFA specification (JECFA, 2000d)	0.024	No safety concern a)	

Table 3: Supporting Substances Summary

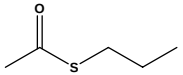
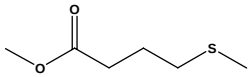
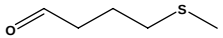
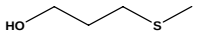
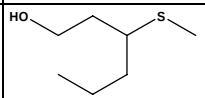
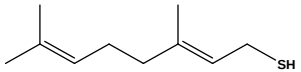
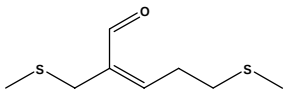
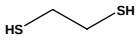
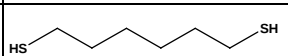
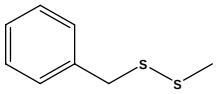
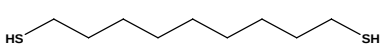
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
12.059	Propyl thioacetate		3385 11576 2307-10-0	485 JECFA specification (JECFA, 1999c)	0.27	No safety concern a)	
12.060	Methyl 4-(methylthio)butyrate		3412 11526 53053-51-3	474 JECFA specification (JECFA, 1999c)	0.061	No safety concern a)	
12.061	4-(Methylthio)butanal		3414 11542 42919-64-2	468 JECFA specification (JECFA, 2003b)	ND	No safety concern a)	
12.062	3-(Methylthio)propan-1-ol		3415 11554 505-10-2	461 JECFA specification (JECFA, 2001c)	2.8	No safety concern a)	
12.063	3-(Methylthio)hexan-1-ol		3438 11548 51755-66-9	463 JECFA specification (JECFA, 1999c)	3.2	No safety concern a)	
12.064	Thiogeraniol		3472 11583 39067-80-6	524 JECFA specification (JECFA, 2000d)	1.1	No safety concern a)	
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) a)	JECFA adopted at step B5 (1.5 microgram/person/day) (JECFA, 2000b).
12.066	Ethane-1,2-dithiol		3484 11467 540-63-6	532 JECFA specification (JECFA, 1999c)	0.0012	No safety concern a)	
12.067	Hexane-1,6-dithiol		3495 11486 1191-43-1	540 JECFA specification (JECFA, 2002d)	1.6	No safety concern a)	
12.068	Benzyl methyl disulfide		3504 11508 699-10-5	577 JECFA specification (JECFA, 1999c)	0.012	No safety concern a)	
12.069	Nonane-1,9-dithiol		3513 11558 3489-28-9	542 JECFA specification (JECFA, 2002d)	0.0012	No safety concern a)	

Table 3: Supporting Substances Summary

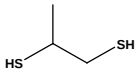
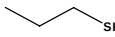
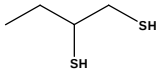
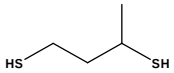
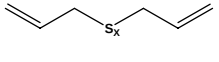
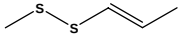
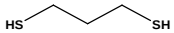
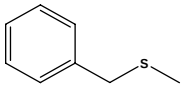
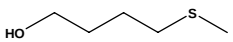
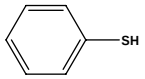
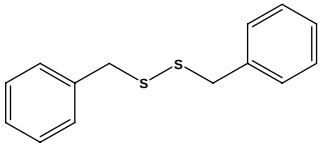
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
12.070	Propane-1,2-dithiol		3520 11564 814-67-5	536 JECFA specification (JECFA, 2000d)	ND	No safety concern a)	
12.071	1-Propane-1-thiol		3521 11816 107-03-9	509 JECFA specification (JECFA, 2000d)	2.2	No safety concern a)	
12.072	Butane-1,2-dithiol		3528 11909 16128-68-0	537 JECFA specification (JECFA, 1999c)	ND	No safety concern a)	
12.073	Butane-1,3-dithiol		3529 11910 24330-52-7	538 JECFA specification (JECFA, 1999c)	ND	No safety concern a)	
12.074	Diallyl polysulfides	 X=2,3,4 or 5	3533 11912 72869-75-1	588 JECFA specification (JECFA, 2000d)	1.2	No safety concern a)	
12.075	Methyl prop-1-enyl disulfide		3576 11712 5905-47-5	569 JECFA specification (JECFA, 2003b)	ND	No safety concern a)	
12.076	Propane-1,3-dithiol		3588 11929 109-80-8	535 JECFA specification (JECFA, 1999c)	0.85	No safety concern a)	
12.077	Benzyl methyl sulfide		3597 766-92-7	460 JECFA specification (JECFA, 1999c)	0.13	JECFA adopted at step B5 (1.5 microgram/person/day) a)	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	No safety concern a)	
12.080	Thiophenol		3616 11585 108-98-5	525 JECFA specification (JECFA, 1999c)	0.73	No safety concern a)	
12.081	Dibenzyl disulfide		3617 150-60-7	579 JECFA specification (JECFA, 2000d)	0.012	No safety concern a)	

Table 3: Supporting Substances Summary

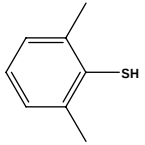
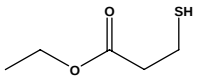
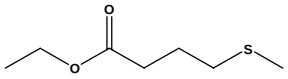
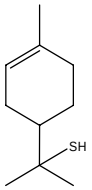
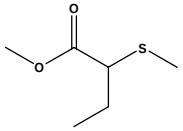
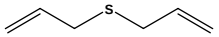
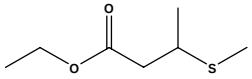
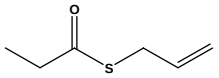
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
12.082	2,6-(Dimethyl)thiophenol		3666 118-72-9	530 JECFA specification (JECFA, 1999c)	1.3	No safety concern a)	
12.083	Ethyl 3-mercaptopropionate		3677 5466-06-8	553 JECFA specification (JECFA, 2002d)	0.073	No safety concern a)	
12.084	Ethyl 4-(methylthio)butyrate		3681 22014-48-8	477 JECFA specification (JECFA, 1999c)	ND	No safety concern a)	
12.085	p-Menth-1-ene-8-thiol		3700 71159-90-5	523 JECFA specification (JECFA, 2000d)	0.34	No safety concern a)	
12.086	Methyl 2-(methylthio)butyrate		3708 51534-66-8	486 JECFA specification (JECFA, 2000d)	0.097	No safety concern a)	JECFA evaluated S-methyl 2-methylbutanethioate (CASrn 42075-45-6).
12.088	Diallyl sulfide		2042 11846 592-88-1	458 JECFA specification (JECFA, 2000d)	ND	JECFA adopted at step B5 (1.5 microgram/person/day) a)	JECFA adopted at step B5 (1.5 microgram/person/day) (JECFA, 2000b).
12.089	Ethyl 3-(methylthio)butyrate		3836 11475	480 JECFA specification (JECFA, 2003b)	ND	No safety concern a)	
12.101	Allyl thiopropionate		3329 11436 41820-22-8	490 JECFA specification (JECFA, 2002d)	ND	No safety concern a)	

Table 3: Supporting Substances Summary

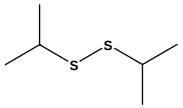
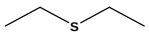
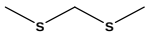
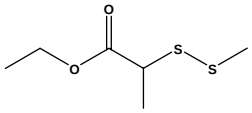
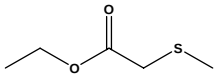
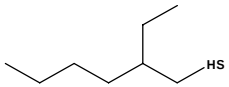
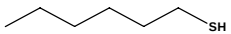
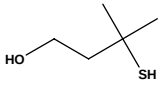
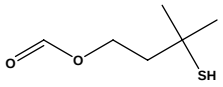
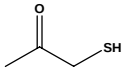
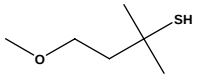
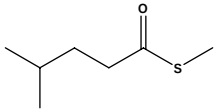
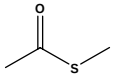
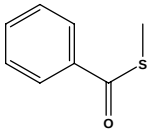
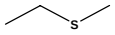
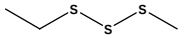
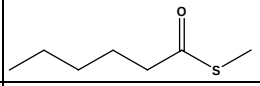
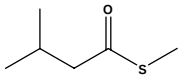
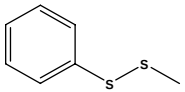
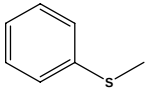
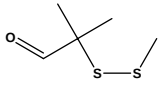
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
12.109	Di-isopropyl disulfide		3827 11455 4253-89-8	567 JECFA specification (JECFA, 1999c)	ND	No safety concern a)	
12.113	Diethyl sulfide		3825 11450 352-93-2	454 JECFA specification (JECFA, 1999c)	ND	No safety concern a)	
12.118	2,4-Dithiapentane		3878 1618-26-4	533 JECFA specification (JECFA, 2000d)	ND	No safety concern a)	
12.121	Ethyl 2-(methyldithio)propionate		3834 11471 23747-43-5	581 JECFA specification (JECFA, 2001c)	ND	No safety concern a)	
12.122	Ethyl 2-(methylthio)acetate		3835 4455-13-4	475 JECFA specification (JECFA, 2000d)	ND	No safety concern a)	
12.128	2-Ethylhexane-1-thiol		3833 7341-17-5	519 JECFA specification (JECFA, 2003b)	ND	No safety concern a)	
12.132	Hexane-1-thiol		3842 11487 111-31-9	518 JECFA specification (JECFA, 2000d)	ND	No safety concern a)	
12.137	3-Mercapto-3-methylbutan-1-ol		3854 34300-94-2	544 JECFA specification (JECFA, 2000d)	ND	No safety concern a)	
12.138	3-Mercapto-3-methylbutyl formate		3855 50746-10-6	549 JECFA specification (JECFA, 1999c)	ND	No safety concern a)	
12.143	1-Mercaptopropan-2-one		3856 24653-75-6	557 JECFA specification (JECFA, 2005b)	ND	No safety concern a)	
12.145	4-Methoxy-2-methylbutane-2-thiol		3785 94087-83-9	548 JECFA specification (JECFA, 2003b)	ND	No safety concern 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
12.148	S-Methyl 4-methylpentanethioate		3867 61122-71-2	488 JECFA specification (JECFA, 2003b)	ND	No safety concern a)	
12.149	S-Methyl acetothioate		3876 1534-08-3	482 JECFA specification (JECFA, 2000d)	ND	No safety concern a)	
12.150	S-Methyl benzothioate		3857 11505 5925-68-8	504 Tentative JECFA specification (JECFA, 1999c)	ND	No safety concern a)	
12.154	Methyl ethyl sulfide		3860 11474 624-89-5	453 JECFA specification (JECFA, 1999c)	ND	No safety concern a)	
12.155	Methyl ethyl trisulfide		3861 31499-71-5	583 JECFA specification (JECFA, 2003b)	ND	No safety concern a)	
12.156	S-Methyl hexanethioate		3862 11515 20756-86-9	489 JECFA specification (JECFA, 2003b)	ND	No safety concern a)	
12.157	S-Methyl isopentanethioate		3864 11506 23747-45-7	487 JECFA specification (JECFA, 2000d)	ND	No safety concern a)	
12.161	Methyl phenyl disulfide		3872 11532 14173-25-2	576 JECFA specification (JECFA, 1999c)	ND	No safety concern a)	
12.162	Methyl phenyl sulfide		3873 11533 100-68-5	459 JECFA specification (JECFA, 1999c)	ND	JECFA adopted at step B5 (1.5 microgram/person/day) a)	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	No safety concern a)	

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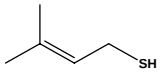
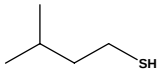
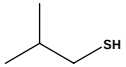
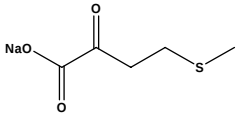
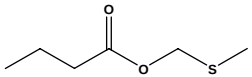
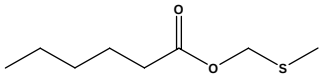
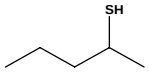
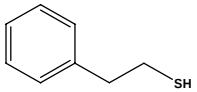
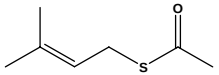
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
12.170	3-Methylbut-2-ene-1-thiol		3896 11511 5287-45-6	522 JECFA specification (JECFA, 1999c)	ND	No safety concern a)	
12.171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

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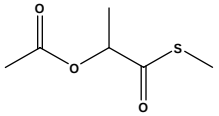
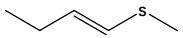
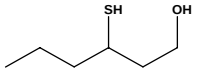
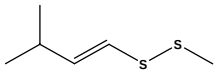
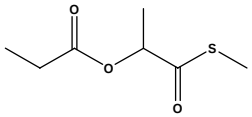
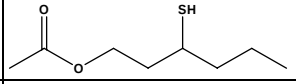
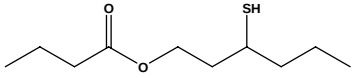
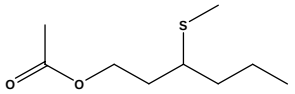
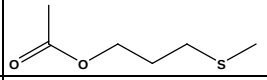
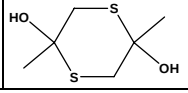
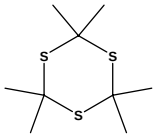
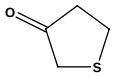
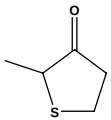
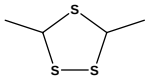
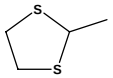
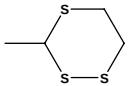
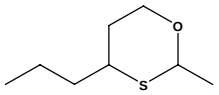
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
12.203	Methylthio 2-(acetyloxy)propionate		3788 74586-09-7	492 JECFA specification (JECFA, 2002d)	ND	No safety concern a)	
12.211	But-1-enyl methyl sulphide		3820	457 JECFA specification (JECFA, 2001c)	ND	No safety concern a)	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	No safety concern a)	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	No safety concern a)	
12.227	Methylthio-2-(propionyloxy)propionate		3790	493 JECFA specification (JECFA, 2002d)	ND	No safety concern a)	
12.234	3-Mercaptohexyl acetate		3851 136954-20-6	554 JECFA specification (JECFA, 1999c)	ND	No safety concern a)	
12.235	3-Mercaptohexyl butyrate		3852 136954-21-7	555 JECFA specification (JECFA, 1999c)	ND	No safety concern a)	
12.236	3-(Methylthio)hexyl acetate		3789 51755-85-2	481 JECFA specification (JECFA, 2000d)	ND	No safety concern a)	
12.237	3-(Methylthio)propyl acetate		3883 16630-55-0	478 JECFA specification (JECFA, 2001c)	ND	No safety concern a)	
15.006	2,5-Dihydroxy-2,5-dimethyl-1,4-dithiane		3450 2322 55704-78-4	562 JECFA specification (JECFA, 2001c)	0.15	No safety concern a) Category B b)	

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
15.009	Trithioacetone		3475 2334 828-26-2	543 JECFA specification (JECFA, 2001c)	1.5	No safety concern a) Category B b)	
15.012	4,5-Dihydrothiophen-3(2H)-one		3266 2337 1003-04-9	498 JECFA specification (JECFA, 2000d)	0.44	No safety concern a) Category B b)	
15.023	4,5-Dihydro-2-methylthiophene-3(2H)-one		3512 11601 13679-85-1	499 JECFA specification (JECFA, 2000d)	12	No safety concern a)	
15.025	3,5-Dimethyl-1,2,4-trithiolane		3541 11883 23654-92-4	573 JECFA specification (JECFA, 2000d)	0.024	No safety concern a)	
15.034	2-Methyl-1,3-dithiolane		3705 5616-51-3	534 JECFA specification (JECFA, 1999c)	0.061	No safety concern a)	
15.036	3-Methyl-1,2,4-trithiane		3718 43040-01-3	574 JECFA specification (JECFA, 2000d)	0.073	No safety concern a)	
15.066	1,4-Dithiane		3831 505-29-3	456 JECFA specification (JECFA, 2000d)	ND	No safety concern a)	
16.030	2-Methyl-4-propyl-1,3-oxathiane		3578 11540 67715-80-4	464 JECFA specification (JECFA, 2000d)	1.3	No safety concern a)	

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) (JECFA, 2000b).

b) (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, 2000), 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, 1999), which is derived from the evaluation procedure developed by the Joint FAO/WHO Expert Committee on Food Additives at its 44th, 46th and 49th meetings (JECFA, 1995; 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[5] (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[6] (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.

[5] *"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).*

[6] *"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).*

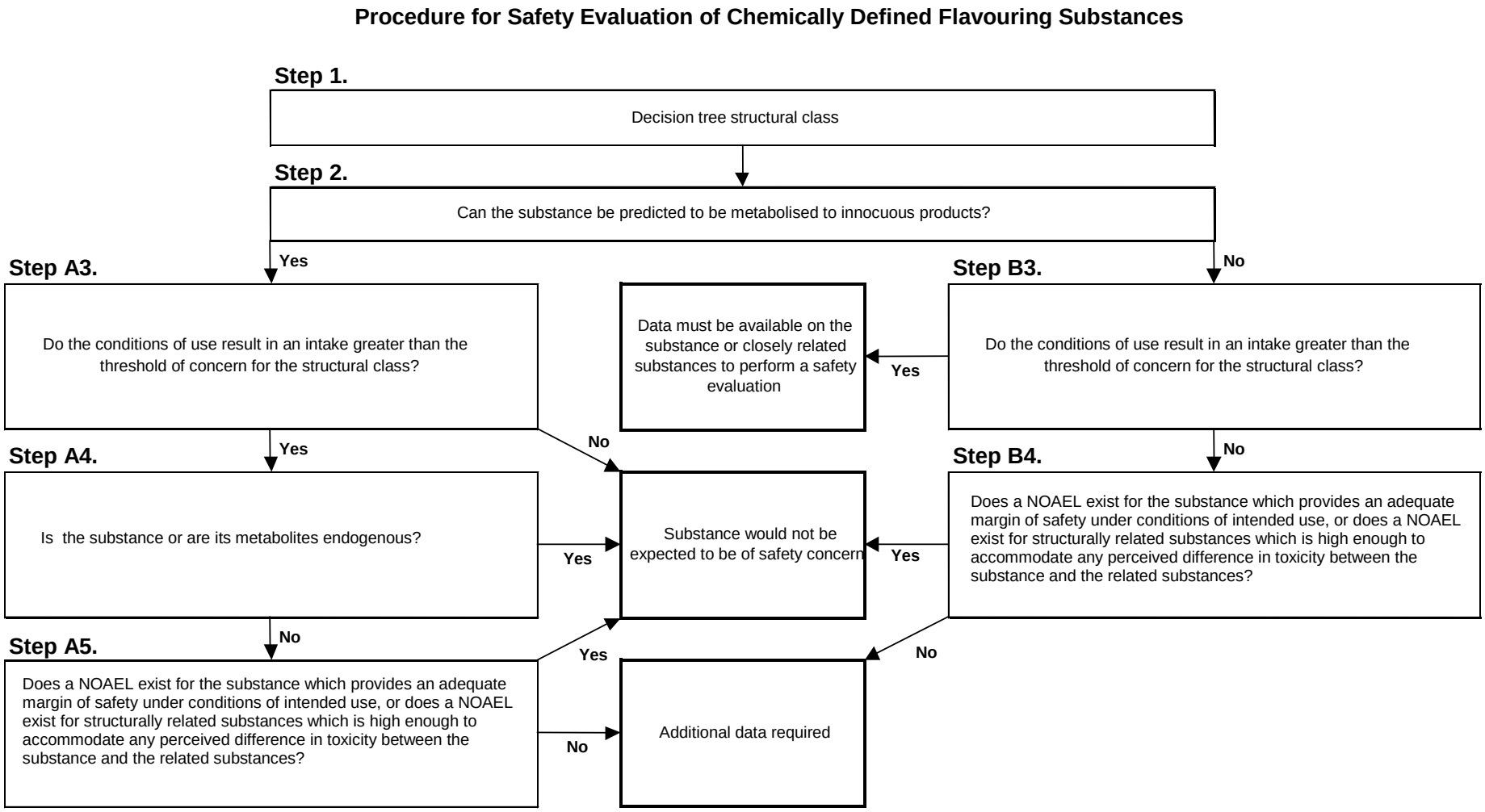


Figure I.1 *Procedure for Safety Evaluation of Chemically Defined Flavouring Substances*

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, 2000). 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, 2000)

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

The "normal and maximum use levels" are provided by Industry for all 52 candidate substances in the present flavouring group (Table II.1.2).

Table II.1.2 Normal and Maximum use levels (mg/kg) for candidate substances in FGE.08 (EFFA, 2002g, EFFA, 2007a)

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
12.096	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 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
12.106	0,3 10	0,2 1	1 5	0,3 1,5	- -	0,4 10	0,2 1	0,4 10	0,1 0,4	0,1 0,4	- -	- -	0,2 1	0,4 2	0,2 5	0,4 10	1 5	0,2 1
12.111	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
12.112	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
12.116	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	- -	0,2 1	0,4 2	0,1 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

Table II.1.2 Normal and Maximum use levels (mg/kg) for candidate substances in FGE.08 (EFFA, 2002g, EFFA, 2007a)

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 1	0,5 1	1 1	1 1	- -	1 1	0,5 1	1 1	0,2 0,2	0,2 0,2	- -	- -	0,5 1	1 1	0,3 1	1 1	4 4	0,5 1
12.120	2 4	0,2 1	0,4 2	0,3 1,5	- -	0,4 2	0,2 1	0,4 2	0,1 0,4	0,1 0,4	- -	- -	0,2 1	0,4 2	0,2 1	0,4 2	1 5	0,2 1
12.124	1 2	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
12.125	0,4 2	0,2 1	0,4 2	0,3 1,5	- -	0,4 2	0,2 1	0,4 2	0,1 0,4	0,1 0,4	- -	- -	0,2 1	0,4 2	0,2 1	0,4 2	1 5	0,2 1
12.127	0,2 1	0,1 0,5	0,2 1	0,2 1	- -	0,2 1	0,1 0,5	- -	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
12.129	0,4 2	0,2 1	0,4 2	0,3 1,5	- -	1 5	0,2 1	0,4 2	0,1 0,4	0,1 0,4	- -	- -	0,2 1	0,4 2	0,2 1	1 5	1 5	0,2 1
12.135	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
12.136	0,4 2	0,2 1	0,4 2	0,3 1,5	- -	0,4 2	0,2 1	0,4 2	0,1 0,4	0,1 0,4	- -	- -	0,2 1	0,4 2	0,2 1	0,4 2	1 5	0,2 1
12.151	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
12.152	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
12.158	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
12.159	0,4 2	0,2 1	0,4 2	0,3 1,5	- -	0,4 2	0,2 1	0,4 2	0,1 0,4	0,1 0,4	- -	- -	0,2 1	0,4 2	0,2 1	0,4 2	1 5	0,2 1
12.163	0,2 1	0 10,5	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
12.164	0,2 1	0 10,5	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
12.165	0,4 2	0,2 1	0,4 2	0,3 1,5	- -	0,4 2	0,2 1	0,4 2	0,1 0,4	0,1 0,4	- -	- -	0,24 1	0,4 2	0,1 0,3	0,4 2	- -	0,2 1
12.166	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
12.167	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
12.172	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
12.174	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
12.177	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
12.178	0,4 5	0,2 5	0,4 2	0,3 1,5	- -	0,4 2	0,2 1	0,4 5	0,1 0,4	0,1 0,4	- -	- -	0,2 1	0,4 2	0,2 1	0,4 2	1 5	0,2 1
12.180	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
12.181	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	- -	0,1 0,5
12.183	0,4 2	0,2 1	0,4 2	0,3 1,5	- -	0,4 2	0,2 1	0,4 2	0,1 0,4	0,1 0,4	- -	- -	0,2 1	0,4 2	0,2 1	0,4 2	1 5	0,2 1
12.189	0,4 2	0,2 1	0,4 2	0,3 1,5	- -	0,4 2	0,2 1	0,4 2	0,1 0,4	0,1 0,4	- -	- -	0,2 1	0,4 2	0,2 1	0,4 2	1 5	0,2 1
12.191	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
12.196	0,4 2	0,2 1	0,4 2	0,3 1,5	- -	0,4 2	0,2 1	0,4 2	0,1 0,4	0,1 0,4	- -	- -	0,2 1	0,4 2	0,2 1	0,4 2	1 5	0,2 1
12.199	0,4 2	0,2 1	0,4 2	0,3 1,5	- -	0,4 2	0,2 1	0,4 2	0,1 0,4	0,1 0,4	- -	- -	0,2 1	0,4 2	0,2 1	0,4 2	1 5	0,2 1
12.200	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	- -	0,2 1	0,4 2	0,1 0,5
12.214	0,4 2	0,2 1	0,4 2	0,3 1,5	- -	0,4 2	0,2 1	0,4 2	0,1 0,4	0,1 0,4	- -	- -	0,2 1	0,4 2	0,2 1	0,4 2	1 5	0,2 1
12.221	0,4 2	0,2 1	0,4 2	0,3 1,5	- -	0,2 1	- -	0,4 2	0,1 0,4	0,1 0,4	- -	- -	0,2 1	0,4 2	0,2 1	0,2 1	1 5	0,2 1
15.047	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	- -	0,2 1	0,4 2	0,1 0,5
15.048	0,2 1	0,1 0,5	0,2 1	0,02 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	- -	0,2 1	0,4 2	0,1 0,5
15.056	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
15.081	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	- -	0,1 0,3	0,2 1	0,4 2	0,1 0,5
15.083	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
15.102	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

Table II.1.2 Normal and Maximum use levels (mg/kg) for candidate substances in FGE.08 (EFFA, 2002g, EFFA, 2007a)

FL-no	Food Categories Normal use levels (mg/kg) Maximum use levels (mg/kg)																	
	01.0	02.0	03.0	04.1	04.2	05.0	06.0	07.0	08.0	09.0	10.0	11.0	12.0	13.0	14.1	14.2	15.0	16.0
15.103	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
15.110	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
15.111	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	- -
16.057	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

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, 2000) 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, 2000)
- 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, 2000)
- Exception a (SCF, 1995) corresponds to food category 5 and 11 (EC, 2000)
- Exception b (SCF, 1995) corresponds to food category 15 (EC, 2000)
- Exception c (SCF, 1995) corresponds to food category 14.2 (EC, 2000)
- Exception d (SCF, 1995) corresponds to food category 12 (EC, 2000)
- 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, 2000) into the seven SCF food categories used for TAMDI calculation (SCF, 1995)

	Food categories according to Commission Regulation 1565/2000	Distribution of the seven SCF food categories		
Key	Food category	Food	Beverages	Exceptions
01	Dairy products, excluding products of category 02.0	Food		

02	Fats and oils, and fat emulsions (type water-in-oil)	Food		
03	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	Confectionery			Exception a
06	Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery	Food		
07	Bakery wares	Food		
08	Meat and meat products, including poultry and game	Food		
09	Fish and fish products, including molluscs, crustaceans and echinoderms	Food		
10	Eggs and egg products	Food		
11	Sweeteners, including honey			Exception a
12	Salts, spices, soups, sauces, salads, protein products, etc.			Exception d
13	Foodstuffs intended for particular nutritional uses	Food		
14.1	Non-alcoholic ("soft") beverages, excl. dairy products		Beverages	
14.2	Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts			Exception c
15	Ready-to-eat savouries			Exception b
16	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 52 flavouring substances in the present flavouring group, for which Industry has provided use and use levels (EFFA, 2002g; EFFA, 2004ak; Flavour Industry, 2006q; Flavour Industry, 2006r). The mTAMDI values are only given for highest reported normal use levels (see Table II.2.3).

Table II.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
12.183	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
12.214	Isobutyl-3-(methylthio)butyrate	160	Class I	1800
12.221	S-Prenyl thioisopentanoate	150	Class I	1800
12.172	2-Methylbutane-2-thiol	78	Class I	1800
12.174	2-Methylpropane-2-thiol	78	Class I	1800

Table II.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)
12.096	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
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
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
15.081	Lenthionine	78	Class III	90
12.159	Methyl methanethiosulfonate	160	Class III	90

ANNEX III: METABOLISM

III.1. Absorption, Distribution, Metabolism and Elimination

Introduction

The group comprises 52 straight, branched chain or heterogeneous ring aliphatic hydrocarbons containing one or more sulphur atoms. Depending on the type of sulphur-containing functional group(s) in the molecule, the candidate substances can be subdivided into 12 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 sulfides and thiols" (JECFA, 2000c; JECFA, 2000b). These supporting substances have been allocated to eight 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

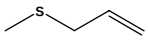
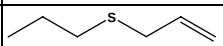
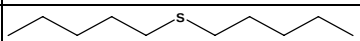
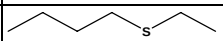
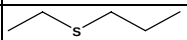
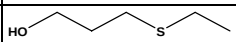
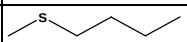
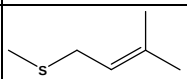
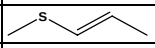
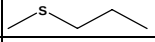
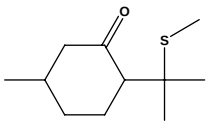
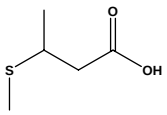
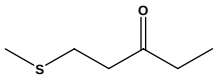
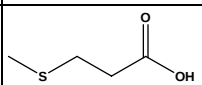
FL-no	EU Register name	Structural formula	Structural Class
I: ACYCLIC SULPHIDES			
12.096	Allyl methyl sulfide		II
12.099	Allyl propyl sulfide		II
12.117	Dipentyl sulfide		I
12.124	Ethyl butyl sulfide		I
12.127	Ethyl propyl sulfide		I
12.129	3-(Ethylthio)propan-1-ol		I
12.152	Methyl butyl sulfide		I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

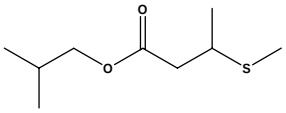
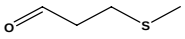
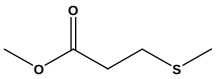
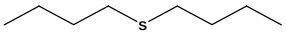
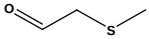
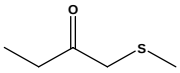
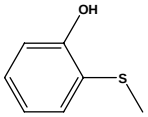
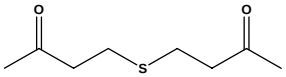
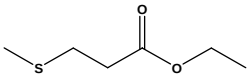
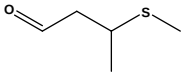
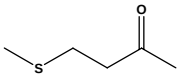
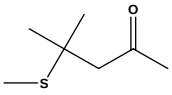
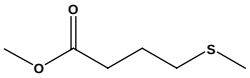
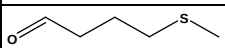
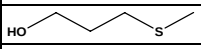
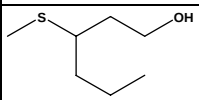
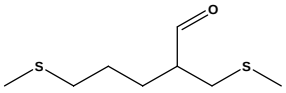
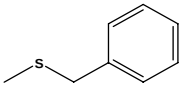
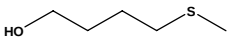
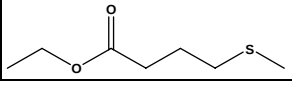
Table III.1 Subgroups. The supporting substances are listed in brackets			
FL-no	**EU Register name**	**Structural formula**	**Structural Class**
12.214	Isobutyl-3-(methylthio)butyrate 1)		I
(12.001)	3-(Methylthio)propionaldehyde		I
(12.002)	Methyl 3-(methylthio)propionate		I
(12.006)	Dimethyl sulfide		I
(12.007)	Dibutyl sulfide		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) sulfide		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
(12.065)	2,8-Dithianon-4-en-4-carboxaldehyde		I
(12.077)	Benzyl methyl sulfide		II
(12.078)	4-(Methylthio)butan-1-ol		I
(12.084)	Ethyl 4-(methylthio)butyrate		I

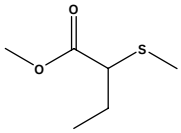
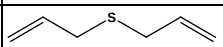
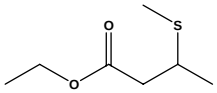
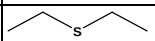
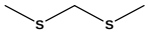
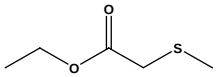
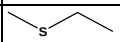
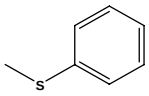
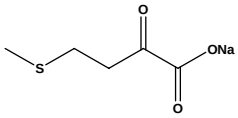
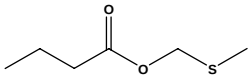
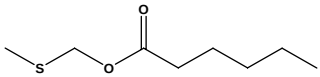
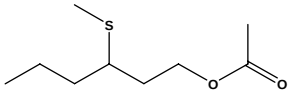
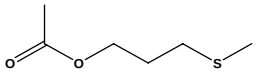
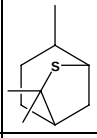
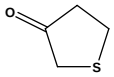
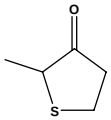
Table III.1 Subgroups. The supporting substances are listed in brackets			
FL-no	**EU Register name**	**Structural formula**	**Structural Class**
(12.086)	Methyl 2-(methylthio)butyrate		II
(12.088)	Diallyl sulfide		II
(12.089)	Ethyl 3-(methylthio)butyrate		I
(12.113)	Diethyl sulfide		I
(12.118)	2,4-Dithiapentane		I
(12.122)	Ethyl 2-(methylthio)acetate		I
(12.154)	Methyl ethyl sulfide		I
(12.162)	Methyl phenyl sulfide		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 1)		III
15.102	Tetrahydrothiophene		II
(15.012)	4,5-Dihydrothiophen-3(2H)-one		II
(15.023)	4,5-Dihydro-2-methylthiophene-3(2H)-one		II

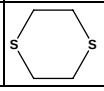
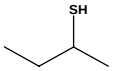
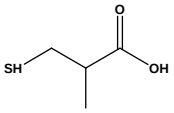
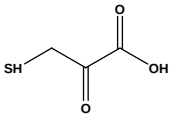
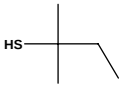
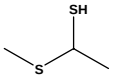
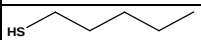
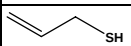
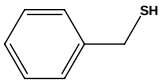
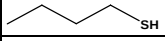
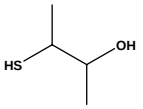
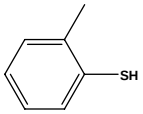
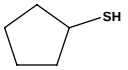
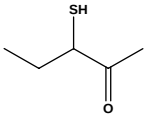
Table III.1 Subgroups. The supporting substances are listed in brackets			
FL-no	**EU Register name**	**Structural formula**	**Structural Class**
(15.066)	1,4-Dithiane		II
III: MONOTHIOLS			
12.104	Butane-2-thiol 1)		I
12.135	3-Mercapto-2-methylpropionic acid 1)		I
12.136	3-Mercapto-2-oxopropionic acid		III
12.172	2-Methylbutane-2-thiol		I
12.174	2-Methylpropane-2-thiol		I
12.180	1-(Methylthio)ethane-1-thiol 1)		I
12.191	Pentane-1-thiol		I
(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

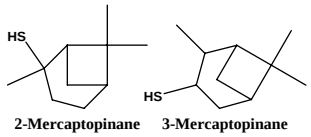
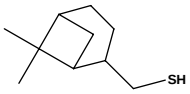
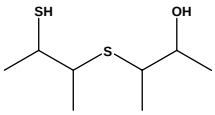
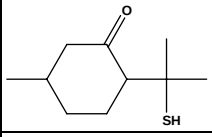
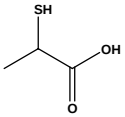
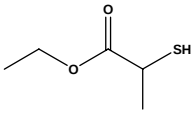
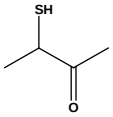
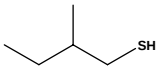
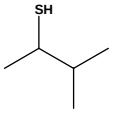
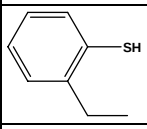
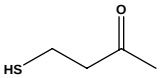
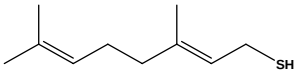
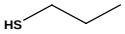
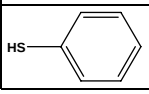
Table III.1 Subgroups. The supporting substances are listed in brackets			
FL-no	**EU Register name**	**Structural formula**	**Structural Class**
(12.035)	2-,3- and 10-Mercaptopinane	 **2-Mercaptopinane** **3-Mercaptopinane**  **10-Mercaptopinane**	II
(12.036)	3-[(2-Mercapto-1-methylpropyl)thio]butan-2-ol		I
(12.038)	8-Mercapto-p-menthan-3-one		II
(12.039)	2-Mercaptopropionic acid		I
(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

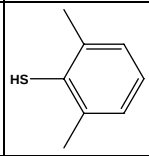
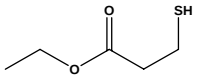
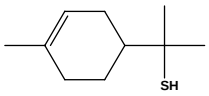
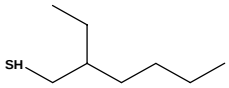
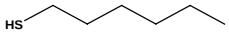
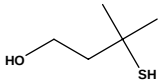
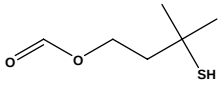
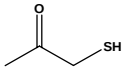
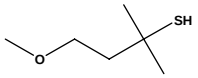
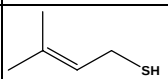
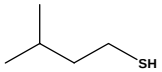
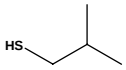
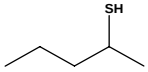
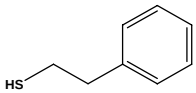
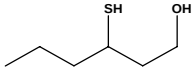
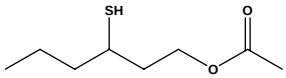
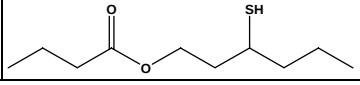
Table III.1 Subgroups. The supporting substances are listed in brackets			
FL-no	**EU Register name**	**Structural formula**	**Structural Class**
(12.082)	2,6-(Dimethyl)thiophenol		II
(12.083)	Ethyl 3-mercaptopropionate		I
(12.085)	p-Menth-1-ene-8-thiol		II
(12.128)	2-Ethylhexane-1-thiol		I
(12.132)	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
(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			

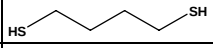
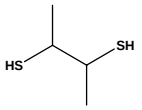
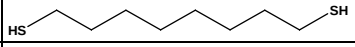
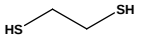
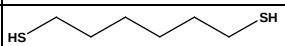
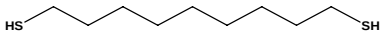
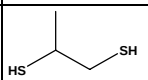
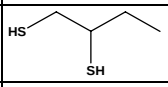
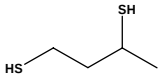
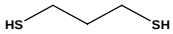
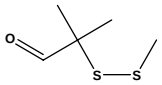
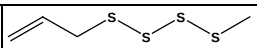
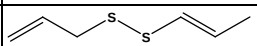
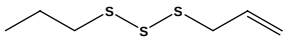
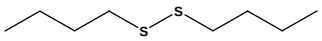
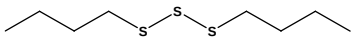
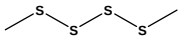
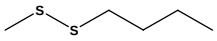
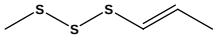
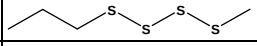
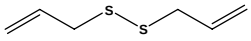
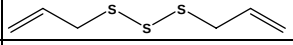
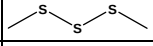
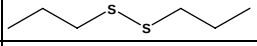
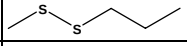
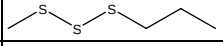
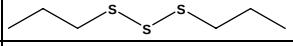
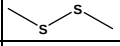
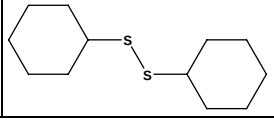
Table III.1 Subgroups. The supporting substances are listed in brackets			
FL-no	**EU Register name**	**Structural formula**	**Structural Class**
12.103	Butane-1,4-dithiol		I
(12.022)	Butane-2,3-dithiol		I
(12.034)	Octane-1,8-dithiol		I
(12.066)	Ethane-1,2-dithiol		I
(12.067)	Hexane-1,6-dithiol		I
(12.069)	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
(12.168)	2-Methyl-2-(methyldithio)propanal		I
V: ACYCLIC DI-, TRI- AND POLYSULPHIDES			
12.097	Allyl methyl tetrasulfide		II
12.098	Allyl prop-1-enyl disulfide 1)		II
12.100	Allyl propyl trisulfide		II
12.111	Dibutyl disulfide		I
12.112	Dibutyl trisulfide		I
12.116	Dimethyl tetrasulfide		I
12.151	Methyl butyl disulfide		I
12.164	Methyl prop-1-enyl trisulfide 1)		I
12.167	Methyl propyl tetrasulfide		I
(12.008)	Diallyl disulfide		II
(12.009)	Diallyl trisulfide		II
(12.013)	Dimethyl trisulfide		I
(12.014)	Dipropyl disulfide		I
(12.019)	Methyl propyl disulfide		I
(12.020)	Methyl propyl trisulfide		I
(12.023)	Dipropyl trisulfide		I
(12.026)	Dimethyl disulfide		I
(12.028)	Dicyclohexyl disulfide		II

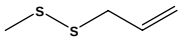
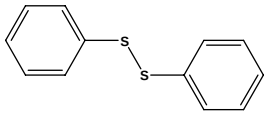
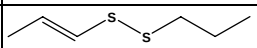
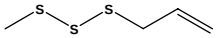
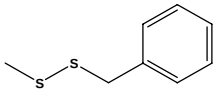
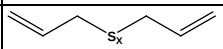
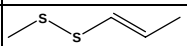
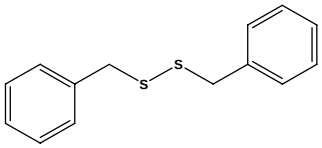
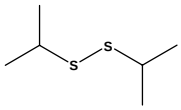
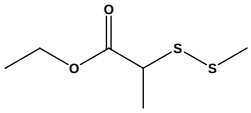
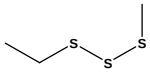
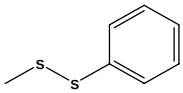
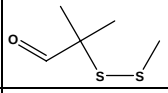
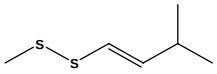
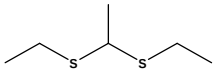
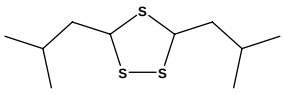
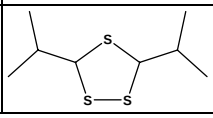
Table III.1 Subgroups. The supporting substances are listed in brackets			
FL-no	**EU Register name**	**Structural formula**	**Structural Class**
(12.037)	Allyl methyl disulfide		II
(12.043)	Diphenyl disulfide		III
(12.044)	Prop-1-enyl propyl disulfide		I
(12.045)	Methyl allyl trisulfide		II
(12.068)	Benzyl methyl disulfide		II
(12.074)	Diallyl polysulfides	 X=2,3,4 or 5	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
(12.155)	Methyl ethyl trisulfide		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: MONO-, DI- AND POLYSULPHIDES WITH THIOACETAL STRUCTURE			
12.200	1,1-bis(Ethylthio)-ethane		I
15.047	3,5-Di-isobutyl-1,2,4-trithiolane 1)		II
15.048	3,5-Di-isopropyl-1,2,4-trithiolane 1)		II

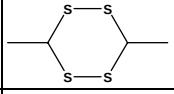
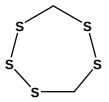
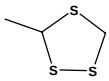
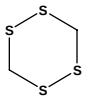
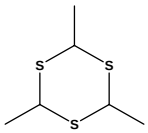
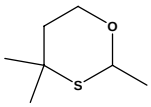
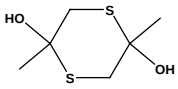
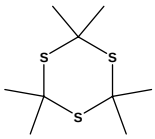
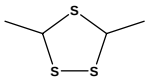
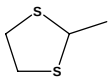
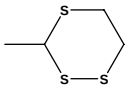
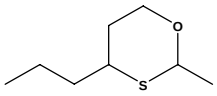
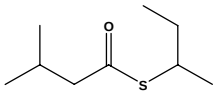
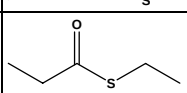
Table III.1 Subgroups. The supporting substances are listed in brackets			
FL-no	**EU Register name**	**Structural formula**	**Structural Class**
15.056	3,6-Dimethyl-1,2,4,5-tetrathiane 1)		II
15.081	Lenthionine		III
15.083	3-Methyl-1,2,4-trithiolane 1)		II
15.103	1,2,4,5-Tetrathiane		II
15.110	2,4,6-Trimethyl-1,3,5-trithiane 1)		II
15.111	1,2,4-Trithiolane		II
16.057	2,4,4-Trimethyl-1,3-oxathiane 1)		II
(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
VII: THIOESTERS			
12.106	S-2-Butyl 3-methylbutanethioate 1)		I
12.125	Ethyl propanethioate		I

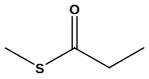
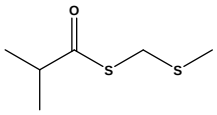
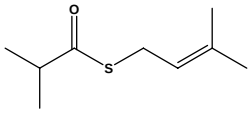
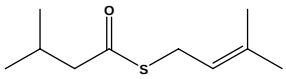
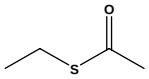
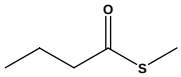
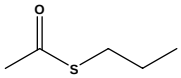
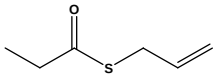
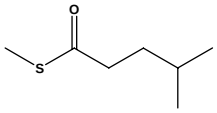
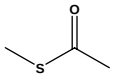
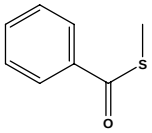
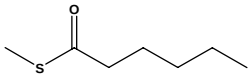
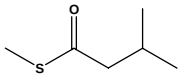
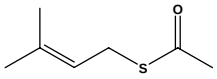
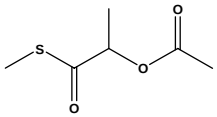
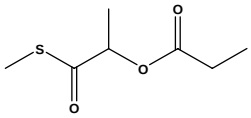
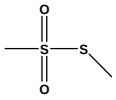
Table III.1 Subgroups. The supporting substances are listed in brackets			
FL-no	**EU Register name**	**Structural formula**	**Structural Class**
12.165	S-Methyl propanethioate		I
12.189	S-(Methylthiomethyl) 2-methylpropanethioate		I
12.196	S-Prenyl thioisobutyrate		I
12.221	S-Prenyl thioisopentanoate		I
(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
VIII: THIOIC ACID			

Table III.1 Subgroups. The supporting substances are listed in brackets			
FL-no	**EU Register name**	**Structural formula**	**Structural Class**
12.199	Ethanethioic acid		I
IX: SULPHOXIDES/SULPHONES AND SULPHONATES			
12.159	Methyl methanethiosulfonate		III
(12.175)	Methylsulfinylmethane		III

1) Stereoisomeric composition not specified

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.

III.2. Sulphides

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

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; Strong et al., 1984b; Renwick et al., 1986), 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).

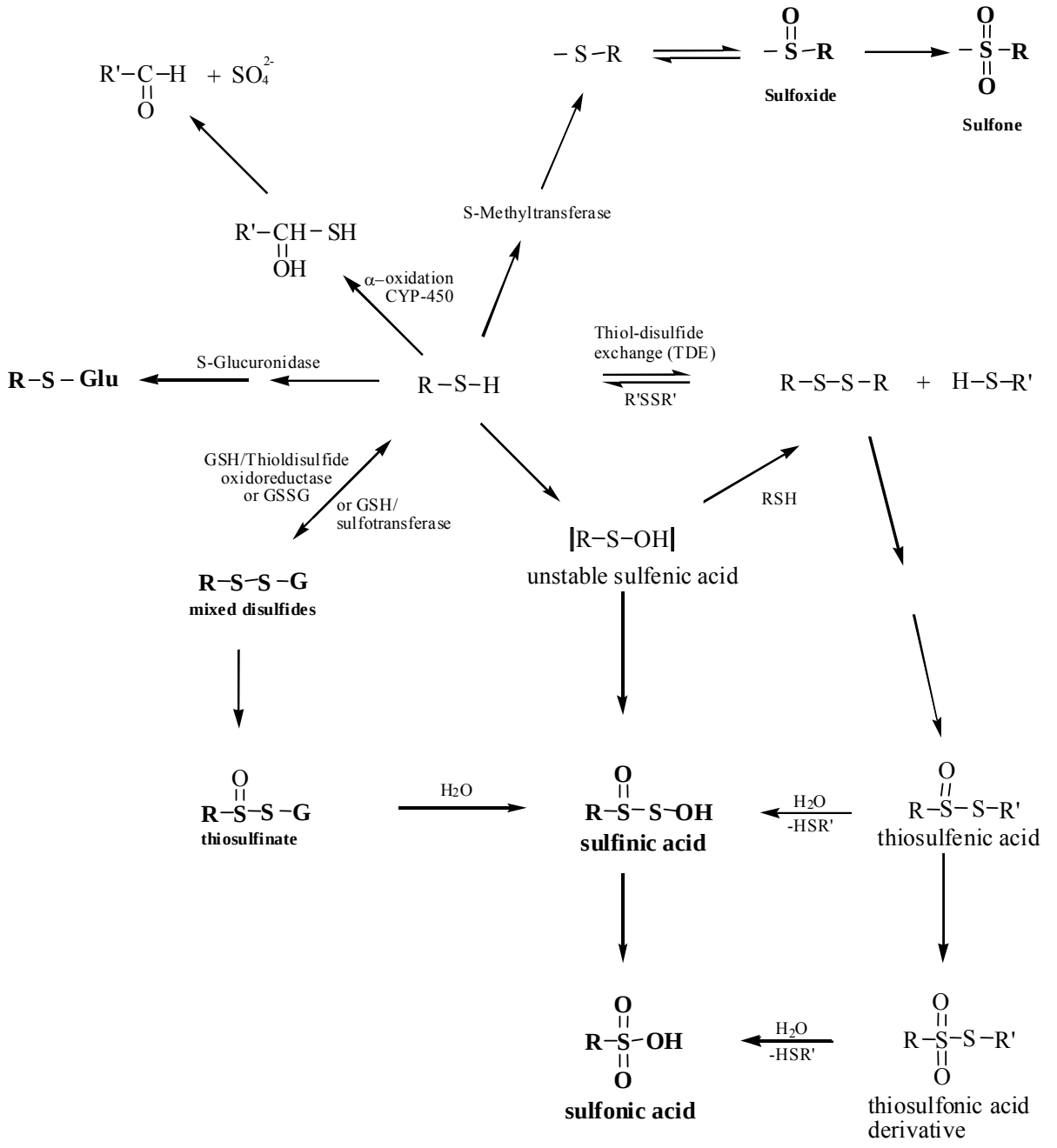


Figure III.1: *Biotransformation of disulphides, thiols, and related sulphur substances (Excretion products in bold)*

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 extrahepatic mammalian reductase. In many cases the reversible nature of the sulphide-sulphoxide reaction depends on the dynamic metabolising system provided by intestinal flora (10^{10} 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 & 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; Strong et al., 1984b; Renwick et al., 1986). The gut microflora is considered the major site of reduction of sulphinpyrazone to its sulphide in man (Renwick et al., 1982; Strong et al., 1984b; Renwick et al., 1986), whereas the reduction of sulindac to its sulphide takes place mainly in the liver, although gut microflora is partially involved (Renwick, 1989).

The metabolism of dipropyl sulphide (as supporting for compounds in subgroups I), dipropyl sulphoxide, and dipropyl sulphone has been studied extensively in rats (Nickson & 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 >98 % of daily urinary metabolites (Nickson & Mitchell, 1994).

In a parallel study, eight rats were each given 580 mg/kg bw [^{35}S]-dipropyl sulphoxide. 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 & 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 gm DMSO/kg bw for 14 days (Layman & 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 & 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 & Menn, 1969). Similarly, 4-chlorophenyl sulphide was reported to be oxidised by FMO and P450 to the sulphoxide and sulphone derivatives *in vitro* (Nnane & Damani, 1995). The aromatic sulphoxide, diphenyl sulphoxide, perfused with intact guinea-pig liver is oxidised exclusively to the corresponding sulphone under normoxic conditions (Yoshihara & 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 diethyl sulphide [FL-no: 12.113]), alicyclic (e.g. thiolane, as supporting of compounds in subgroup III) and aromatic (e.g. ethyl p-tolyl sulphide) sulphides (Hoodi & 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 (as supporting of compounds in subgroup VI) incubated with rabbit lung microsomes, pulmonary FMO fractions or pulmonary P450 fractions were oxidized primarily to the trans sulphoxide isomer; the enantioselectivity produced by FMO was higher when compared to P450 (Cashman et al., 1990; Cashman & 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 stereoselectivity 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 (Gachon et al., 1988; Karim El Fatih et al., 1988; Feng & Solsten, 1991; Black et al., 1993). In all of them, the predominance of S-oxidation pathway has been reported. As an example, when 40 mg/kg bw of [$^{13}C_4$, ^{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 & Williams, 1990; Cashman et al., 1990; Yoshihara & Tatsumi, 1990; Cashman et al., 1995a; Cashman et al., 1995b; Elfarra et al., 1995) and (Rettie et al., 1990; Sadeque et al., 1992; Nnane & Damani, 1995; Sadeque et al., 1995), it is concluded that the oxidation pathway is the major route of biotransformation of (mono)sulphides in humans (Ziegler, 1980; Nickson & 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.

One of the candidate substances from subgroup I is an ester, isobutyl-3-(methylthio)butyrate [FL-no: 12.214], which is anticipated to be hydrolysed to 2-methylpropanol [FL-no: 02.001] and 3-(methylthio)butyric acid [FL-no: 12.178]. One of the substances from subgroup VIII, ethanethioic acid [FL-no: 12.199] converts to acetic acid [FL-no: 08.002]. See Table 2b.

Studies for Candidate Substances

3-(Methylthio)propionic acid [FL-no: 12.183] (Subgroup I)

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 & 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 & 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 methyltransferase, is S-adenosyl-L-methionine. The resulting methyl sulphides, including the candidate substance methyl propyl sulfide [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 & 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 & Menn, 1969; Dutton & Illing, 1972; Maiorino et al., 1988; Richardson et al., 1991).

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 reacts 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 thiosulphenic and sulphinic 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 & 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 cell 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 Km values of thiophenols are at least two orders of magnitude less than those for thiopurines (Woodson & Weinshilboum, 1983; Woodson et al., 1983; Ames et al., 1986).

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 % (TPMTL-TPMTL) 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 & 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 (Tateishi et al., 1978; Shaw & Blagbrough, 1989).

Oxidation and Desulphuration

Low molecular weight thiols undergo oxidative desulphuration *in vivo* to yield CO2 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 & Tarver, 1953). In a separate experiment using ^{35}S-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 & 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, 1959).

Hydrolysis

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], methyl mercaptan [FL-no: 12.003] and hydrogensulfide [not a Register substance]. The hydrolysis products are shown in Table 2b.

Studies for Candidate Substances

Butane-1,4-dithiol [FL-no: 12.103] (Subgroup III)

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 & Takatsu, 2001).

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

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 Subgroup V.

The disulphide bond is rapidly and extensively reduced to the corresponding thiol in a reversible reaction *in vivo* (Figure III.1). Consequently, metabolic options available to thiols (see section III.3) are also available to di-, tri- and polysulphides.

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 thiosulphenic acid derivatives (Figure III.1). Thiosulphenic 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).

While specific studies on the biotransformation of dimethyl trisulphide (or other trisulphides) were not located, their simple structure suggests that they would undergo biotransformation by the pathways described above for disulphides.

III.5. Sulphides and Thiols with Additional Oxygenated Functional Groups

The following discussion is pertinent to Subgroup VI.

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 10 candidate substances are shown in Table 2b. However, thioacetals are more resistant to hydrolysis than oxygen-acetals (Satchell & Satchell, 1990; Smith & March, 2001). It is thus to be anticipated that these substances may reach the intestinal lumen intact and may also be absorbed as such.

III.6. Thioesters and thioc acid

The following discussion is pertinent to Subgroups VII.

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 & Jencks, 1971) and decreases as oxygenation of the carbon chain in the thiol moiety increases (Kurooka et al., 1976).

The hydrolysis products of the six candidate thioesters are shown in Table 2b.

Thioethers 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 & Ziegler, 1987) and probably would be eliminated without S-oxidation.

III.7. Sulphoxides/sulphones and sulphonated

The only candidate substance of subgroup IX is methyl methanethiosulfonate [FL-no: 12.159], which is anticipated to be hydrolysed to methanesulfonic acid and hydrogensulfide. 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-sulphenic, 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.

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 52 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 for 38 supporting substances evaluated by JECFA at the 53rd meeting. The supporting substances are listed in brackets.

TABLE IV.1: ACUTE TOXICITY

Table IV.1: ACUTE TOXICITY

Chemical Name [FL-no]	Species	Sex	Route	LD_{50} (mg/kg bw)	Reference	Comments
Subgroup I – Acyclic Sulphides						
(Dimethyl sulfide [12.006])	Mouse	NR	Gavage	3700	(Koptyaev, 1967b)	
	Rat	NR	Gavage	3300	(Koptyaev, 1967b)	
(Dibutyl sulfide [12.007])	Rat	NR	Oral	2220	(Moreno, 1975g)	
(3-(Methylthio)propionaldehyde [12.001])	Rat	M, F	Oral	M: 1000 F: 1680	(Ballantyne & 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 & Mason, 1981)	
	Mouse	M, F	Gavage	M: 1560 F: 1750	(Butterworth & Mason, 1981)	
(Methyl 2-(methylthio)butyrate [12.086])	Rat	M, F	Gavage	2108	(Piccirillo & Lunchicki, 1982)	
Subgroup II – Cyclic Sulphides						
Tetrahydrothiophene [15.102]	Rat	M, F 5/sex/group	Gavage	M: 2000 F. 1750	(Auletta & 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 & Stokinger, 1958)	
2-Methylpropane-2-thiol [12.174]	Rat	NR	Gavage	4729	(Fairchild & Stokinger, 1958)	
	Rat	M, F	Gavage	8400	(Phillips Petroleum Company, 1990a)	
(Butane-1-thiol [12.010])	Rat	NR	Gavage	1500	(Fairchild & Stokinger, 1958)	
(2-Methylpropane-1-thiol [12.173])	Rat	NR	Gavage	7168	(Fairchild & Stokinger, 1958)	
Butane-2-thiol [12.104]	Rat	NR	Gavage	5176	(Elf Atochem, 1981a)	
2-Methylbutane-2-thiol [12.172]	Rat	M	Gavage	>5000	(Elf Atochem, 1977)	

Table IV.1: ACUTE TOXICITY

Chemical Name [FL-no]	Species	Sex	Route	LD_{50} (mg/kg bw)	Reference	Comments
		6/group				
(Pentane-2-thiol [12.192])	Rat	M, F	Gavage	>5000	(Collinson, 1989a)	
(3-Methylbutane-2-thiol [12.049])	Rat	M, F	Gavage	540	(Harper & 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 & Peano, 1982)	
(Thiophenol [12.080])	Rat	NR	Gavage	46	(Fairchild & Stokinger, 1958)	
(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 & 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 & 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 & Bowles, 1985)	
(Propane-1,2-dithiol [12.070])	Mouse	M, F	Oral	153	(Bailey, 1976a)	
	Mouse	M, F	Gavage	170	(Fogleman & 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 Di-, Tri- and Polysulphides						
(Dimethyl disulfide [12.026])	Rat	M, F	Oral	190	(Shapiro et al., 1985)	
(Dipropyl disulfide [12.014])	Rat	M, F	Oral	2000	(Elf Atochem, 1992)	Reference is dipropyl disulfide
	Rat	M	Gavage	6000[3]	(Rohm & Haas Co., 1980)	
(Diallyl disulfide [12.008])	Rat	M	Oral	260	(Moreno, 1980h)	Paper reports compound as allyl sulfide
	Rat	NR	Oral	<5000[4]	(Platte Chemical Co., 1995)	
(Benzyl methyl disulfide [12.068])	Mouse	M, F	Oral	1080	(Bailey, 1976c)	
(Dipropyl trisulfide [12.023])	Mouse	M, F	Oral	800-1600	(Moran et al., 1980)	Not definitive test
(Diallyl trisulfide [12.009])	Mouse	M, F	Oral	100-400	(Moran et al., 1980)	Not definitive test
Subgroup VI –Cyclic Mono-, Di-, Tri- and Polysulphides with thioacetal structure						

Table IV.1: ACUTE TOXICITY

Chemical Name [FL-no]	Species	Sex	Route	LD_{50} (mg/kg bw)	Reference	Comments
(3,5-Dimethyl-1,2,4-trithiolane [15.025])	Rat	Not specified	Oral	115	(BIBRA, 1976)	
(3-Methyl-1,2,4-trithiane [15.036])	Rat	M, F	Oral	440	(Mondino & Peano, 1979b)	
(2,5-Dihydroxy-2,5-dimethyl-1,4-dithiane [15.006])	Mouse	F 5/group	Gavage	360	(Fogleman & 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., 1979)	
(Trithioacetone [15.009])	Mouse	M, F	Gavage	M: 2600 F: 2000	(Fenwick & Hanley, 1985)	
Subgroup VII – Thioesters						
(Methylthio 2-(acetyloxy)propionate [12.203])	Rat	M, F	Gavage	1050	(Watanabe & Kinosaki, 1989a)	
(Methylthio-2-(propionyloxy) propionate [12.227])	Rat	M, F	Gavage	1330	(Watanabe & Kinosaki, 1989b)	
Subgroup IX – Sulphoxides/Sulphones and Sulphonates						
(Methylsulfinylmethane [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 & Tauberger, 1964)	
	Rat	M, F	Oral	19700	(Sommer & 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 one candidate substance of the present flavouring group evaluation from chemical group 20 and for 38 supporting substances evaluated by JECFA at the 53rd meeting. The supporting substances are listed in brackets.

TABLE IV.2: SUBACUTE / SUBCHRONIC / CHRONIC / CARCINOGENICITY STUDIES

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							
(Methyl sulfide [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 & Oser, 1970a)	Unpublished /uncompleted report: histopathology results not available.
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 & 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 /uncomplete report: histopathology results not available.
(2,3- and 10-mercaptopinane [12.035])	Rat; M, F	Diet	0 (control group), 0.06	90 Days	No adverse effect	(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
	17/sex/group				measured at the only 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 Di-, Tri- and Polysulphides							
(Diallyl disulfide [12.008])	Rat; F 12 (control group) 6 (treatment group)	Oral (gavage in peanut oil)	0 (control group), 36.5, 146, 732	6 Days	146 (hemolytic anemia at the higher dose)	(Munday & Manns, 1994)	Study published on a peer reviewed journal. Acceptable quality.
(Diallyl trisulfide [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 & Oser, 1970d)	Unpublished /uncomplete report: histopathology results not available.
(Dipropyl disulfide [12.014])	Rat; F 12 (control group) 6 (treatment group)	Oral (gavage in peanut oil)	0 (control group), 37.6, 150.4, 752	6 Days	150.4 (hemolytic anemia at the higher dose)	(Munday & 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)
(Dipropyl trisulfide [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 & Oser, 1970c)	Unpublished /uncomplete report: results of histopathology not available.
(Dicyclohexyl disulfide [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
(Phenyl disulfide [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

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
(Benzyl methyl disulfide [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 –Cyclic Mono-, Di-, Tri- and Polysulphides with thioacetal structure							
(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/ propylglycol)	0 (control group), 7	91 Days	No adverse effect measured at the only tested dose (7.0)[1]	(Griffiths et al., 1979)	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
Subgroup VII – 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 & Weaver, 1990)	Unpublished /uncomplete 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 & Weaver, 1990)	Unpublished /uncomplete report: results are reported as a summary - validity of conclusions could not be evaluated.
Subgroup IX – Sulphoxides/Sulphones and Sulphonates							
(Methylsulfinylmethane [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 .
	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.

NR: Not reported

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 but for two supporting substance evaluated by JECFA at the 53rd meeting. Supporting substance listed in brackets.

TABLE IV.3: DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

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 6-19	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: 20	(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 four candidate substances of the present flavouring group evaluation from chemical group 20 and for 14 supporting substances evaluated by JECFA at the 53rd meeting. Supporting substances are listed in brackets.

TABLE IV.4: GENOTOXICITY (*IN VITRO*)

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

Chemical Name [FL-no]	Test system	Test Object	Concentration	Result	Reference	Comments
Subgroup I – Acyclic Sulphides						
(Allyl sulfide [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.
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.
(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						

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

Chemical Name [FL-no]	Test system	Test Object	Concentration	Result	Reference	Comments
(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 Di-, Tri-, and Polysulphides						
(Allyl disulfide [12.008])	Modified Ames test	S. *typhimurium* TA98, TA100, TA1535, TA1537, TA1538	0.0015 – 0.15 µg/ml	Negative (±S9)	(Eder et al., 1980)	Acceptable quality.
	Sister chromatid exchange	Chinese hamster ovary cells	2 - 25 µg/ml	Negative (-S9)	(Musk et al., 1997)	Limited quality. Insufficiently reported.
	Chromosomal aberrations	Chinese hamster ovary cells	2 - 25 µg/ml	Positive (-S9)	(Musk et al., 1997)	Limited quality. Insufficiently reported.
(Dimethyl disulfide [12.026])	Ames test	S. *typhimurium* TA98, TA100, TA102	0.000011 – 1.1 mmol/plate (1.04 - 104000 µg/plate)	Negative (±S9)	(Aeschbacher et al., 1989)	Limited quality (only 3 strains used).
(Phenyl disulfide [12.043])	Ames test	S. *typhimurium* TA98, TA100, TA1535, TA1537, TA1538	3.6 mg/plate (3600 µg/plate)	Negative (±S9)	(Wild et al., 1983)	Review. Methods and results insufficiently documented.
(Benzyl disulfide [12.081])	Ames test	S. *typhimurium* TA98, TA100, TA1535, TA1537, TA1538	3.6 mg/plate (3600 µg/plate)	Negative (±S9)	(Wild et al., 1983)	Review. Methods and results insufficiently documented.
Dibutyl disulfide [12.111]	Forward mutational assay	Mouse lymphoma cells	NR	Negative (-S9)	(Dooley et al., 1987)	Validity cannot be fully evaluated (only abstract provided)
Subgroup VII – 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 & 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 & Morimoto, 1989b)	Acceptable quality.
Subgroup IX – Sulphoxides/Sulphones and Sulphonates						
Methyl methane-thiosulfonate [12.159]	Ames test	S. *typhimurium* TA98, TA100, TA1535, TA1537, TA1538, TA2637	0.6 – 60 µg/plate	Negative (-S9)	(Dorange et al., 1983)	Test is not appropriate for antimicrobial agents[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]
	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]
(Methylsulfinyl methane [12.175]) (synonym: dimethylsulfoxid, 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)

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

Chemical Name [FL-no]	Test system	Test Object	Concentration	Result	Reference	Comments
	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] *Thiosulfonates in general, and methyl methane thiosulfonate 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 for four supporting substances evaluated by JECFA at the 53rd meeting. Supporting substances are listed in brackets.

TABLE IV.5: GENOTOXICITY (*IN VIVO*)

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

Chemical Name [FL-no]	Test System	Test Object	Route	Dose	Result	Reference	Comments
Subgroup I – Acyclic Sulphides							
(Allyl sulfide [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 Di-, Tri-, and Polysulphides							
(Allyl disulfide [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.
(Diallyl trisulfide [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 IX – Sulphoxides/Sulphones and Sulphonates							
Methyl methane-thiosulfonate [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 sulfide, allyl disulfide and ally trisulfide 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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