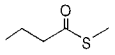
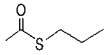
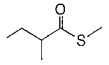
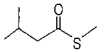
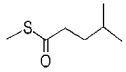
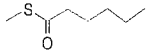
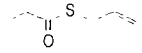
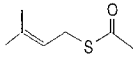
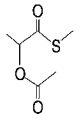
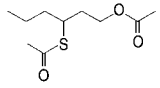
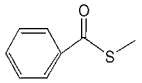
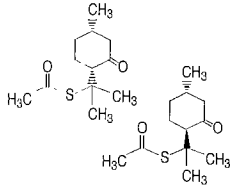
Ethyl thioacetate (*S*-ethyl ethanethioate)	483	625-60-5 	No Europe: 0.02 USA: 0.02	Yes, a NOEL of 6.5 mg/kg of body weight per day was reported in a 90-day study in rats treated at only that dose	NR	No safety concern
Methyl thiobutyrate (*S*-methyl butanethioate)	484	2432-51-1 	No Europe: 5 USA: 5	Yes, a NOEL of 1000 mg/kg of body weight per day was reported in a 90-day study in rats treated at multiple doses	NR	
Propyl thioacetate (*S*-propyl thioacetate)	485	2307-10-0 	No Europe: 0.4 USA: 0.02	Yes, related substance nos 483 and 484	NR	
S-Methyl 2-methylbutanethioate	486	42075-45-6 	No Europe: 0.2 USA: 0.1	Yes, related substance nos 483 and 484	NR	
S-Methyl 3-methylbutanethioate	487	23747-45-7 	No Europe: ND USA: 0.2	Yes, related substance nos 483 and 484	NR	
S-Methyl 4-methylpentanethioate	488	61122-71-2 	No Europe: ND USA: 0.001	Yes, related substance nos 483 and 484	NR	
S-Methyl hexanethioate	489	2432-77-1 	No Europe: ND USA: 0.1	Yes, related substance nos 483 and 484	NR	
Allyl thiopropionate (*S*-2-propenyl propanethioate)	490	41820-22-8 	No Europe: ND USA: 0.1	Yes, related substance nos 483 and 484; related substance no. 587, subgroup ix, which is predicted to be metabolized to allyl disulfide and allyl mercaptan	NR	
Prenyl thioacetate	491	33049-93-3 	No Europe: ND USA: 0.2	Yes, related substance nos 483 and 484; related substance no. 587, subgroup ix, which is predicted to be metabolized to allyl disulfide and allyl mercaptan	NR	

Table 1 (*continued*)

Substance[b]	No.	CAS no. and structure	*Step B3*[c] Does intake exceed the threshold for human intake?	*Step B4* Adequate NOEL for substance or structurally related substance?	*Step B5* Intake >1.5 µg/day?	Conclusion based on current intake
Subgroup xi — continued						
Structural class I (continued)						
Methylthio 2-(acetyloxy)propionate (1-[(methylthio)methyl]ethyl acetate)	492	74586-09-7 	No Europe: ND USA: 9	Yes, related substance nos 483 and 484	NR	No safety concern
Methylthio 2-(propionyloxy) propionate (*S*-methyl 2-(propionyloxy)propanethioate)	493	— 	No Europe: ND USA: 9	Yes, related substance nos 483 and 484	NR	
3-(Acetylmercapto)hexyl acetate	494	136954-25-1 	No Europe: ND USA: 0.4	Yes, related substance nos 483 and 484	NR	

Structural class II						
S-Methyl benzothioate (*S*-methyl thiobenzoate)	504	5925-68-8 	No Europe: ND USA: 0.001	Yes, related substance nos 483 and 484	NR	No safety concern
cis- and *trans*-Menthone-8-thioacetate (*S*-[1-methyl-1-(4-methyl-2-oxocyclohexyl)ethyl] ethanethioate)	506	94293-57-9 	No Europe: ND USA: 0.4	Yes, related substance nos 483 and 484	NR	
Subgroup xii — sulfoxides						
Structural class III						
Methylsulfinylmethane (dimethyl sulfoxide)	507	67-68-5 	No Europe: ND USA: 0.001	Yes, a NOEL of 3000 mg/kg of body weight per day was reported in a study in monkeys treated by gavage at multiple doses for 74–87 weeks; data in support of the NOEL were reported from studies in rats, dogs and humans	NR	No safety concern

CAS: Chemical Abstracts Service; ND: no intake data reported; NR: not required for evaluation because an adequate NOEL for the substance or a related substance was identified at step B4 of the Procedure.

[a] None of the substances in this group are predicted to be metabolized to innocuous products. They were placed in subgroups i–xii on the basis of the position of the sulfur atom.

[b] The substance names are given as they appear in the specifications monograph (FAO Food and Nutrition Paper, No. 52, Add. 7, 1999). In cases where substances were evaluated under their trivial name, the systematic name is given in parentheses.

[c] The thresholds for human intake of classes I, II and III are 1800, 540 and 90 μg per day, respectively. All intake values are expressed in μg per day.

for methyl 3-(methylthio)propionate (no. 472) in Europe and for bis(methylthio)methane (no. 533) in the USA and 0.0003–0.2 μg/kg of body weight for 3-(methylthio)propionaldehyde (no. 466), ethyl 3-(methylthio)propionate (no. 476), methyl mercaptan (methanethiol; no. 508) and allyl disulfide (no. 572) in Europe and benzenethiol (no. 525) and 3-methyl-1,2,4-trithiane (no. 574) in the USA. The intake of each substance in the group in μg per day per person in Europe and the USA is reported in Table 1.

Simple aliphatic and aromatic sulfides and thiols have been detected in a variety of foods and beverages, including onion, garlic, cabbage, tea, coffee and beer. Of the 137 substances in this group, 106 have been reported to occur naturally in foods. Quantitative data on the natural occurrence of 19 substances in the group demonstrate that they are consumed predominantly in traditional foods, with the exception of methyl 3-(methylthio)propionate (no. 472) and ethyl 3-(methylthio)propionate (no. 476).

4.1.2 *Absorption, metabolism and elimination*

The group of 137 flavouring agents considered at this meeting was divided into 12 subgroups on the basis of the position of the sulfur atom, to facilitate assessment of their metabolism and toxicity. The subgroups are:

- Subgroup i — simple sulfides (thioethers), in which the sulfur is located between two unoxidized alkyl or aryl side-chains (nos 452–455, 457–460 and 533).
- Subgroup ii — acyclic sulfides with oxidized side-chains, in which an alcohol, aldehyde, ketone, ester, carboxylic acid or phenol group is present (nos 461–463, 465–481, 495–497, 500–503 and 505).
- Subgroup iii — cyclic sulfides (nos 456, 464, 498, 499, 534, 543, 550 and 562).
- Subgroup iv — simple thiols with unoxidized aliphatic or aromatic side-chains (nos 508–531).
- Subgroup v — thiols with oxidized side-chains, in which an alcohol, aldehyde, ketone, ester or carboxylic acid group is present (nos 544–549, 551–561 and 563).
- Subgroup vi — dithiols (nos 532 and 535–542).
- Subgroup vii — simple disulfides (nos 564–572 and 575–579).
- Subgroup viii — disulfides with oxidized side-chains (nos 580 and 581).
- Subgroup ix — trisulfides and polysulfides (nos 582–588).
- Subgroup x — heterocyclic disulfides (nos 573 and 574).
- Subgroup xi — thioesters (nos 482–494, 504, 506a and 506b).
- Subgroup xii — sulfoxides (no. 507).

All of the sulfur substances considered are of low relative molecular mass and are sufficiently lipophilic to be absorbed from the intestine. These flavouring agents would be metabolized via many different pathways. As metabolism would usually result in increased polarity and a greater likelihood of excretion, these substances would not be expected to accumulate in the body. Many substances, such as thiols and disulfides, would be able to form disulfide bonds with endogenous thiols. Disulfides formed with cysteine could be excreted in the urine as the xenobiotic cysteine disulfide, whereas formation of disulfides with endogenous macromolecules would delay elimination and could result in effects such as enzyme inhibition.

Potential toxicity can be deduced by comparison with structural analogues on the basis of metabolic similarities. In the absence of information on the toxicity of structural analogues, however, it is not possible to conclude a priori that the substances are metabolized to innocuous products.

Subgroup i — simple sulfides (thioethers)

Once alkyl and aromatic thioethers, commonly called "sulfides", enter the systemic circulation, they are rapidly oxidized to sulfoxides and, depending on the structure of the sulfide, may be further oxidized to sulfones. Sulfoxides and sulfones are the major urinary metabolites of simple sulfides. Aliphatic sulfides (nos 452–455, 457, 458 and 533) and sulfides containing an aromatic ring (nos 459 and 460) yield mixtures of sulfoxide and sulfone metabolites. Enzymes of the cytochrome P450 superfamily and flavin-containing monooxygenases catalyse the oxidation of sulfides to sulfoxides. Oxidation of sulfoxides to the corresponding sulfones occurs both in tissues and in aerobic microorganisms and is an irreversible metabolic reaction in mammals. Sulfoxides may also be converted back to the corresponding sulfides by aldehyde oxidase, by thioredoxin and thioredoxin reductase, and by the anaerobic microflora in the lower bowel.

The methyl aromatic sulfides (nos 459 and 460) are predicted to be major metabolites of the corresponding aromatic thiols (nos 525 and 526, subgroup iv) and would be oxidized to sulfoxides and sulfones, which would be excreted.

Subgroup ii — acyclic sulfides with oxidized side-chains

The presence of other functional groups, such as alcohols (nos 461–463), aldehydes (nos 465–471 and 505), esters (nos 472–481), acids (no. 501), β-ketones (nos 495–497, 500 and 502) and phenols (no. 503), provides centres of greater polarity and additional sites for the biotransformation of sulfides. The presence of these polar groups

would also result in increased renal excretion. The biotransformation of oxygenated, carbon-containing, functional groups is well characterized and has been described for groups of flavouring agents previously evaluated by the Committee. Concurrent metabolism of various substrates at both sulfur and oxygenated functional groups has been reported, and sulfoxide formation usually predominates as the major metabolic pathway of detoxification. Experiments in vitro suggest that hydrolysis of carboxyl esters occurs in the presence of thioether (sulfide) groups. In consequence, sulfides with oxidized side-chains would be expected to be eliminated more rapidly than simple sulfides.

Subgroup iii — cyclic sulfides

Oxidation of unsubstituted and methyl-substituted cyclic sulfides by the cytochrome P450 superfamily produces the corresponding sulfoxides. The mono-sulfoxides are predicted to be the main urinary metabolites of simple cyclic sulfides (nos 456, 534 and 543). The metabolism of cyclic sulfides containing oxidized carbon atoms (nos 464, 498, 499, 550 and 562) has not been studied but would be predicted to involve extensive *S*-oxidation and possibly oxidation or conjugation of alcohol groups. The polarity of the hydroxy thioethers (nos 550 and 562) may allow their elimination unchanged.

Subgroup iv — simple thiols

The simple thiol flavouring agents considered were alkyl and alicyclic thiols (nos 508–524, 526 and 527) and aromatic thiols (thiophenols; nos 525 and 528–531). These substances can be metabolized via several pathways. Simple aliphatic and aromatic thiols undergo *S*-methylation in mammals to produce the corresponding methyl thioether or sulfide. *S*-Methylation is catalysed by thiopurine *S*-methyltransferase in the cytosol and thiol *S*-methyltransferase in microsomes; both reactions require *S*-adenosyl-L-methionine as a methyl group donor. Thiopurine *S*-methyltransferase is present in human liver, kidney and erythrocytes, and its preferred substrates include aromatic and heterocyclic thiols. *S*-Methylation of aliphatic thiols is catalysed by microsomal thiol *S*-methyltransferase, and the resulting methyl thioether (sulfide) metabolite undergoes *S*-oxidation to give the corresponding methyl sulfoxide and methyl sulfone analogues, which are excreted in the urine.

Thiols may react with glutathione and other endogenous thiol substances to form mixed disulfides. Both microsomal and cytosolic thioltransferases have been reported to catalyse the formation of mixed disulfides. The resulting mixed disulfides can undergo

reduction back to thiols, oxidative desulfuration or oxidation to the corresponding sulfonic acid via the intermediate thiosulfinate and sulfinic acid. The principal form in the circulation would probably be a mixed disulfide formed with albumin.

S-Glucuronidation of aromatic thiols has been reported, and this may be a pathway for the metabolism of aromatic thiols (thiophenols; nos 525 and 528–531) and simple aromatic disulfides (nos 576 and 578; subgroup vii) after their reduction (see below). Glucuronyl transferases behave similarly towards hydroxyl and sulfhydryl functional groups, and the two activities have the same subcellular location and optimal pH.

Thiols may be oxidized to form sulfenic acids (RSOH), which are unstable and readily undergo further oxidation to sulfinic (RSO_2H) and sulfonic (RSO_3H) acids or combine with nucleophiles. The sulfonic acid group is highly polar and renders molecules very soluble in water. In general, sulfonic acids are not extensively metabolized.

Alkyl thiols of low relative molecular mass undergo oxidative desulfuration in vivo to yield carbon dioxide and sulfate. This reaction has been shown to occur, for example, with methyl mercaptan (no. 508). Whereas the carbon atoms from thiols may be used in the biosynthesis of amino acids, the sulfur atoms are not used significantly in the synthesis of sulfur-containing amino acids.

Subgroup v — thiols with oxidized side-chains

Although alkyl thiols with oxidized side-chains (nos 544–549, 551–561 and 563) comprise a significant proportion of the flavouring agents evaluated, their metabolic fate has not been studied. Their metabolism is predicted to involve a combination of the pathways described above for simple thiols and further oxidation or conjugation of the oxidized side-chain. The compound that is in structural class III, sodium 3-mercapto-oxopropionate (sodium 3-mercaptopyruvate; no. 563), would be expected to be eliminated very rapidly after metabolism at both the thiol and keto-acid groups.

Subgroup vi — dithiols

The metabolism of the simple aliphatic dithiols (nos 532 and 535–542) is predicted to involve the pathways described above for simple thiols. Urinary metabolites could result from *S*-methylation, *S*-oxidation of one sulfur atom to yield a polar sulfonate or the formation of mixed disulfides of low relative molecular mass such as cysteine, an endogenous thiol. The longer, linear dithiols (nos 540–542) could form intramolecular disulfide bonds, with interconversion between the dithiol and cyclic disulfide forms.

Subgroup vii — simple disulfides

The reduction of xenobiotic disulfides is believed to be extensive, and the reaction may be catalysed enzymatically by thioltransferases and chemically by exchange with glutathione, thioredoxin, cysteine and other endogenous thiols. Reduction of the non-cyclic disulfides considered in the group (nos 564–572 and 575–579) would result in the formation of thiols of low relative molecular mass, which would then be metabolized by the various pathways described above for simple thiols.

Subgroup viii — disulfides with oxidized side-chains

As discussed above for acyclic sulfides with oxidized side-chains and cyclic sulfides (subgroups ii and iii), the presence of additional sites of carbon oxidation would result in greater polarity and further oxidation or conjugation of the flavouring agents evaluated (nos 580 and 581). By analogy to thiols with oxidized side-chains (subgroup v), the oxidized side-chains in this group are susceptible to reductive cleavage, which would be expected to be the initial metabolic reaction. The polarity of the side-chains would primarily affect elimination of the thiol fragments.

Subgroup ix — trisulfides and polysulfides

The trisulfide of glutathione is labile and readily converted to the disulfide, the sulfur being released as hydrogen sulfide. The trisulfides and polysulfide in this subgroup (nos 582–587 and no. 588) are predicted to be converted rapidly to the corresponding disulfides and reduced to thiols, which would then be metabolized via the pathways described above for simple thiols. The potential toxicity of trisulfides and polysulfides is probably related to their metabolic lability and to the nature of the resultant thiol (e.g. allyl thiol).

Subgroup x — heterocyclic disulfides

The heterocyclic disulfides (nos 573 and 574) are five- and six-carbon rings which also contain a cyclic thioether bond. A related substance, lipoic acid, which is endogenous, undergoes rapid redox cycling between the ring disulfide and open dithiol forms. On the basis of the known metabolism of lipoic acid, the principal metabolic pathways of the substances in this group are predicted to be reduction of the disulfide with opening of the ring to produce a dithiol, and *S*-oxidation of the cyclic thioether.

Subgroup xi — thioesters

Thioester groups (—S—CO—) are present in a number of the flavouring agents in this subgroup (nos 482–494, 504 and 506). The hydrolysis of esters has been considered previously by the Committee, but not that of thioesters. Thioesters are hydrolysed by lipase and

esterases, and the rate of hydrolysis increases with increasing length of the carbon chain of the carboxylic acid fragment and decreases with increasing oxygenation of the carbon chain in the thiol moiety.

The thioesters in this subgroup are predicted to be hydrolysed to the corresponding thioic acid and alcohol, or the corresponding carboxylic acid and thiol (the metabolic fates of which are outlined above). Data on dithioic acids and esters indicate that the esters of monothioic acids would be poor substrates for oxidation, but the monothioic acid released by hydrolysis would be oxidized to the corresponding dioxo acid. Other possibilities for elimination in vivo include urinary excretion of thiocarboxylic acid. The substances evaluated (with the exceptions of prenyl thioacetate (no. 491) and allyl thiopropionate (*S*-2-propenyl propanethioate; no. 490)) are simple linear alkyl compounds, branched-chain alkyl compounds or their side-chain hydroxyester analogues, so that their toxicity can reasonably be compared.

Subgroup xii — sulfoxides

The sulfoxides are predicted to be metabolized via the same pathways as thioethers (subgroup i). The only sulfoxide flavouring agent evaluated was methylsulfinylmethane (dimethyl sulfoxide; no. 507), since data were available on both its metabolism and its toxicity in experimental animals and humans. Methylsulfinylmethane is readily absorbed and excreted in urine as the parent sulfoxide and dimethyl sulfone.

4.1.3 *Application of the Procedure for the Safety Evaluation of Flavouring Agents*

Step 1

In applying the Procedure for the Safety Evaluation of Flavouring Agents to the above-mentioned aliphatic and aromatic sulfides and thiols, the Committee assigned 97 of the 137 substances (nos 452–455, 457, 461–463, 465–497, 500, 502, 508–515, 517–519, 522, 524, 532, 533, 535–542, 544–560, 562, 564–567, 569–571 and 580–585) to structural class I. These substances are simple aliphatic thiols and sulfides, which may or may not contain an additional oxygenated functional group, and have the lowest toxic potential. The Committee assigned 34 of the 137 substances to structural class II because they are aromatic sulfides or thiols (nos 459, 460, 503, 504, 525–528, 530, 531, 576, 577 and 579), alicyclic substances (nos 506, 516, 520, 523, 561 and 575), heterocyclic substances (nos 456, 464, 498, 499, 534, 543, 573 and 574) or allyl mercaptan (2-propene-1-thiol) or sulfides (nos 458, 521, 568, 572 and 586–588), which are common components of food. The Committee assigned aromatic thiols or sulfides that are not common components

of food (nos 505, 529 and 578) to structural class III. The remaining three substances were also assigned to structural class III by virtue of the fact that they are aliphatic thiols or sulfides containing more than three functional groups (nos 501 and 563) or do not contain divalent sulfur (no. 507).

Step 2

None of the 137 substances in this group can be predicted to be metabolized to innocuous products. The evaluation of these substances therefore proceeded via the right-hand side of the decision tree (Fig. 1).

Step B3

The estimated daily per capita intakes of the substances in this group for which data were available were below the thresholds for the structural classes to which they were assigned (1800μg for class I, 540μg for class II and 90μg for class III).

Step B4

The Committee considered the results of toxicity studies of at least 90 days' duration in rodents or monkeys for 27 substances in this group of flavouring agents (nos 452, 464, 483, 484, 498, 505, 507, 516, 520, 528, 530, 531, 534, 539, 541, 543, 546, 547, 560, 562, 566, 573–575, 577, 585 and 587).

The Committee noted that the single or multiple doses of the flavouring agents tested in a number of such studies had no effect in rats and that the NOELs were consequently derived from the results of studies that did not show toxic effects. The results of long-term studies in rats, dogs and monkeys were considered for one substance, methylsulfinylmethane (no. 507). To facilitate comparisons of the toxicity of structurally related substances, the flavouring agents were considered in 12 subgroups, as defined above (see Tables 1 and 2). Toxicity was compared within and across subgroups, with no restriction on the basis of structural class assignment.

Subgroup i — simple sulfides (thioethers). This subgroup comprises nine simple thioethers. The NOEL for methyl sulfide (no. 452) in a 14-week study in rats treated with multiple doses by gavage was 250mg/kg of body weight per day. This NOEL provided an adequate basis for evaluating the toxicity of five structurally and metabolically related substances (nos 453–455, 457 and 533). However, the Committee considered it inappropriate for the evaluation of allyl sulfide (no. 458) and two aromatic sulfides, methyl phenyl sulfide (no. 459) and benzyl methyl sulfide (no. 460). The evaluation of these three substances therefore proceeded to step B5.

Table 2
Comparison of the toxicity and intake data used in the safety evaluation of 137 aliphatic and aromatic sulfides and thiols, by subgroup[a]

Subgroup	Adequate NOEL for substance[b]	Adequate NOEL for structurally related substance[b]	No adequate NOEL for substance or related substance, but intake <1.5 µg/day[c]
(i) Simple sulfides (thioethers)	no. 452	nos 453–455, 457, 533	nos 458–460
(ii) Acyclic sulfides with oxidized side-chains	no. 505	nos 461–463, 465–469, 472–481, 495–497, 500–503	nos 470, 471
(iii) Cyclic sulfides	nos 464, 498, 534, 543, 562	nos 456, 499, 550	—
(iv) Thiols	nos 516, 520, 528, 530, 531	nos 508–515, 517–519, 521–527, 529	—
(v) Thiols with oxidized side-chains	nos 546, 547, 560	nos 544, 545, 548, 549, 551–559, 561, 563	—
(vi) Dithiols	nos 539, 541	nos 532, 535–538, 540, 542	—
(vii) Simple disulfides	nos 566, 575, 577	nos 564, 565, 567–572, 576, 578, 579	—
(viii) Disulfides with oxidized side-chains	—	nos 580, 581	—
(ix) Trisulfides and polysulfides	nos 585, 587	nos 582–584, 586, 588	—
(x) Heterocyclic disulfides	no. 573	no. 574	—
(xi) Thioesters	nos 483, 484	nos 482, 485–494, 504, 506	—
(xii) Sulfoxides	no. 507	—	—

[a] See Table 1 for further details of the evaluations.
[b] See Fig. 1, step B4 and pages 60–65 for further information.
[c] See Fig. 1, step B5 and page 65 for further information.

Subgroup ii — acyclic sulfides with oxidized side-chains. This subgroup comprises 28 acyclic thioethers with oxidized side-chains. The NOEL for 2-(methylthiomethyl)-3-phenylpropenal (2-[(methylthio)methyl]-3-phenyl-2-propenal; no. 505) in a 90-day study in rats treated with a single dose was 1.4 mg/kg of body weight per day.