

## SCIENTIFIC OPINION

### **Flavouring Group Evaluation 21, Revision 1 (FGE.21Rev1)<sup>1</sup>: Thiazoles, thiophene, thiazoline and thienyl derivatives from chemical group 29 Miscellaneous substances from chemical group 30**

#### **Scientific Opinion of the Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF)**

(Question No EFSA-Q-2009-00481)

**ADOPTED ON 26 MARCH 2009**

#### **PANEL MEMBERS**

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#### **SUMMARY**

The Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (the Panel) was asked to provide scientific advice to the Commission on the implications for human health of chemically defined flavouring substances used in or on foodstuffs in the Member States. In particular, the Panel was asked to evaluate 56 flavouring substances in the Flavouring Group Evaluation 21, Revision 1 (FGE.21Rev1), using the Procedure as referred to in the Commission Regulation (EC) No 1565/2000. These 56 flavouring substances belong to chemical group 29 and 30, Annex I of the Commission Regulation (EC) No 1565/2000.

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In the present FGE.21Rev1, the 56 candidate substances include five- and six-membered sulphur-containing aromatic and non-aromatic heterocycles, which have been arranged into 11 subgroups in order to facilitate comparisons of the data sets between the groups. This division was done on the basis of degree of aromaticity and according to the presence of other heteroatoms (i.e. nitrogen).

Five of the 56 candidate substances possess one chiral centre [FL-no:15.060, 15.077, 15.090, 15.099 and 15.119], two substances [FL-no: 15.054 and 15.129] possess two chiral centres and two substances [FL-no: 15.042 and 15.055] possess three chiral centres. For these nine substances the stereoisomeric composition has not been specified.

Forty-six of the 56 candidate substances are classified in structural class II and 10 candidate substances are classified into structural class III according to the decision tree approach.

Forty-two of 56 the candidate substances have been reported to occur 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 56 flavouring substances in this group have intakes in Europe from 0.0012 to 2.2 microgram/capita/day, which are below the thresholds of concern for structural class II (540 microgram/person/day) and structural class III (90 microgram/person/day).

On the basis of the reported annual production volumes in Europe, the combined estimated daily per capita intakes as flavourings of the candidate substances in subgroups with more than one substance from a structural class range from 0.056 to 3.6 microgram. For none of the subgroups do the total combined intakes exceed the thresholds of concern for compounds belonging to structural class II of 540 microgram/person/day or to structural class III of 90 microgram/person/day.

For the two candidate substances 6-acetyl-2,3-dihydro-1,4-thiazine [FL-no: 15.114] (Register name: 5-acetyl-2,3-dihydro-1,4-thiazine) and 5-acetyl-2,3-dihydro-1,4-thiazine [FL-no: 15.133] from subgroup B-V, which are alpha,beta-unsaturated ketones, i.e. they have a structural alert for genotoxicity, there are no genotoxicity data available and accordingly a concern for genotoxicity could not be ruled out. For the two candidate substances 2-methylthiazolidine [FL-no: 15.090] and 2-propylthiazolidine [FL-no: 15.099] from subgroup B-III, there are indications of a genotoxic potential *in vitro*. Considering the structural similarities between these two thiazolidines in subgroup B-III and the three thiazolines in subgroup B-II (2-methyl-2-thiazoline [FL-no: 15.086], 2,4-dimethyl-3-thiazoline [FL-no: 15.060] and 2-isobutyl-3-thiazoline [FL-no: 15.119]), the Panel

concluded that in the absence of further genotoxicity data the Procedure could not be applied to these seven substances. For the other substances in this FGE the available data considered valid do not give rise to any safety concerns with respect to genotoxicity.

None of the 49 candidate substances evaluated through the procedure can be predicted to be metabolised to innocuous products.

Toxicological data on both candidate and supporting substances were limited and did not include information about chronic or reproductive toxicity. Valid toxicological data which could provide an adequate margin of safety compared to the intakes from use as flavouring substances were only available for the 26 candidate substances from subgroup A-Ic (thiophenes with thiol-containing ring substituents) and subgroup A-II (thiazoles). For the remaining 23 candidate substances belonging to the subgroups of thiophene itself (A-Ia), thiophenes with non-thiol-containing ring substituents (A-Ib), benzothiazoles (A-III), dihydrothiophenes (B-I), dithiazines (B-IV) and thiadiazines (B-VI), the Panel concluded that there were insufficient data available to provide margins of safety from their use as flavouring substances and that additional toxicity data are needed.

It is considered that, on the basis of the default MSDI approach, 26 of the 49 candidate substances evaluated through the Procedure [FL-no: 15.038, 15.039, 15.044, 15.050, 15.051, 15.052, 15.058, 15.061, 15.062, 15.063, 15.067, 15.068, 15.069, 15.071, 15.078, 15.080, 15.082, 15.084, 15.085, 15.087, 15.089, 15.098, 15.108, 15.115, 15.116 and 15.118] are not of safety concern at their estimated levels of intake based on the MSDI approach, whereas for 23 candidate substances additional toxicological data are required [FL-no: 15.037, 15.040, 15.042, 15.043, 15.045, 15.054, 15.055, 15.064, 15.070, 15.072, 15.074, 15.076, 15.077, 15.088, 15.091, 15.092, 15.093, 15.094, 15.096, 15.097, 15.106, 15.107 and 15.129].

The mTAMDI values for the 41 substances evaluated through the Procedure of the 46 candidate substances from structural class II range from 78 to 4000 microgram/person/day. For the eight substances evaluated through the Procedure of the 10 candidate substances from structural class III the mTAMDI values range from 78 to 160 microgram/person/day.

The estimated intakes of 40 of the 41 candidate substances from structural class II are below the thresholds of concern for their structural class. For five of the eight candidate substances belonging to structural class III the estimated intakes are below the thresholds of concern for their structural class. All of these candidate substances are also expected to be metabolised to innocuous products. For the remaining substance in structural class II and the three substances in structural class III more reliable exposure data are required. On the basis of such additional data, these flavouring substances should be reconsidered along the steps of the Procedure. Following this procedure additional toxicological data might become necessary.

In order to determine whether the conclusion for the 49 candidate substances which have been evaluated using the Procedure can be applied to the materials of commerce, it is necessary to consider the available specifications. Adequate specifications including purity and identity for the materials of commerce have been provided for 43 of the 49 flavouring substances evaluated through the Procedure. Information on stereoisomerism is incomplete for five of the substances evaluated through the Procedure [FL-no: 15.042, 15.054, 15.055, 15.077, 15.129] and for one substance evaluated through the Procedure [FL-no: 15.129], an identity test has not been provided. Thus, the final evaluation of the materials of commerce cannot be performed for the five substances [FL-no: 15.042, 15.054, 15.055, 15.077 and 15.129], pending further information.

In conclusion, for the seven substances not evaluated through the Procedure data on genotoxicity are required [FL-no: 15.060, 15.086, 15.090, 15.099, 15.114, 15.119 and 15.133] (Information on stereoisomerism and/or identity test has not been provided for five of these seven substances [FL-no: 15.060, 15.090, 15.099, 15.119 and 15.133]). The final evaluation of the materials of commerce cannot be performed for the five substances [FL-no: 15.042, 15.054, 15.055, 15.077 and 15.129], pending further information on stereoisomerism and/or identity test.

For 23 flavouring substances of the 49 flavouring substances evaluated through the Procedure [FL-no: 15.037, 15.040, 15.042, 15.043, 15.045, 15.054, 15.055, 15.064, 15.070, 15.072, 15.074, 15.076, 15.077, 15.088, 15.091, 15.092, 15.093, 15.094, 15.096, 15.097, 15.106, 15.107 and 15.129] the Panel considered that additional toxicity data are needed. For the remaining 26 flavouring substances of the 49 flavouring substances evaluated through the Procedure the Panel considered there were valid toxicological data providing adequate margins of safety compared to the intakes from use as flavouring substances. Therefore, the following 26 flavouring substances evaluated using the Procedure would present no safety concern at their estimated levels of intake estimated on the basis of the MSDI approach: [FL-no: 15.038, 15.039, 15.044, 15.050, 15.051, 15.052, 15.058, 15.061, 15.062, 15.063, 15.067, 15.068, 15.069, 15.071, 15.078, 15.080, 15.082, 15.084, 15.085, 15.087, 15.089, 15.098, 15.108, 15.115, 15.116 and 15.118].

## KEY WORDS

Flavourings, safety, thiophene, thiophene derivatives, dihydrothiophene, benzothiazole derivatives, thiazole derivatives, thiazoline derivatives, thiazolidine derivatives, dihydrothiazine derivatives, dithiazine derivatives.

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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 2009/163/EC (EC, 2009a). Each flavouring substance is attributed a FLAVIS-number (FL-number) and all substances are divided into 34 chemical groups. Substances within a group should have some metabolic and biological behaviour in common.

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

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

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

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).

## HISTORY OF THE EVALUATION

FGE	Opinion Adopted by EFSA	Link	No of Candidate Substances
FGE.21	8 February 2007	<a href="http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178694698331.htm">http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178694698331.htm</a>	54
FGE.21Rev1	26 March 2009		56

The present revision of FGE.21, FGE.21Rev1, includes the assessment of two additional candidate substances [FL-no: 15.129 and 15.133]. No toxicity and/or metabolism data were provided for these two substances.

For the candidate substance [FL-no: 15.114] the Register name is 5-acetyl-2,3-dihydro-1,4-thiazine. However, since the publication of FGE.21 Industry has informed that the correct name for this flavouring substances is 6-acetyl-2,3-dihydro-1,4-thiazine. Throughout this Revision, the correct name for this substance has been used with the incorrect Register name shown in bracket: “6-acetyl-2,3-dihydro-1,4-thiazine [FL-no: 15.114] (Register name: 5-acetyl-2,3-dihydro-1,4-thiazine)”. One of the additional substances evaluated in the present Revision is 5-acetyl-2,3-dihydro-1,4-thiazine [FL-no: 15.133],

## 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, 2000a).

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## ASSESSMENT

### 1. Presentation of the Substances in the Flavouring Group Evaluation 21 Revision 1

#### 1.1. Description

The present Flavouring Group Evaluation 21, Revision 1 (FGE.21Rev1), using the Procedure as referred to in the Commission Regulation (EC) No 1565/2000 (EC, 2000a) (The Procedure – shown in schematic form in Annex I), deals with 56 flavouring substances (candidate substances) from chemical groups 29 and 30, Annex I of Commission Regulation (EC) No 1565/2000 (EC, 2000a). The candidate substances in FGE.21Rev1 fall into the chemical groups of thiazoles (S- and N-containing), thiazolines (S- and N- containing) and thienyl derivatives (S-containing), and thiophene itself (S-containing) (see Table 4.1 in Section 4).

The 56 candidate substances as well as their chemical Register names, FLAVIS- (FL-no), Chemical Abstract Service- (CAS-), Council of Europe- (CoE-) and Flavor and Extract Manufacturers Association- (FEMA-) numbers, structure and specifications, are listed in Table 1 and Table 2.

The 56 candidate substances are structurally closely related to 29 flavouring substances (supporting substances), including two mixtures (one of two isomeric isobutyl-substituted and one of two isomeric isopropyl-substituted dimethyldihydrodithiazine derivatives), evaluated at the 59<sup>th</sup> JECFA meeting (JECFA, 2002c) in the group of “Sulphur-containing heterocyclic compounds” (see Table 3).

#### 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.).

Five of the 56 candidate substances possess one chiral centre [FL-no: 15.060, 15.077, 15.090, 15.099 and 15.119]. Two flavouring substances possess two chiral centres [FL-no: 15.054 and 15.129] and two possess three chiral centres [FL-no: 15.042 and 15.055]. For these nine substances the stereoisomeric composition has not been specified (see Table 1).

### 1.3. Natural Occurrence in Food

Forty-two of the 56 candidate substances have been reported to occur naturally in one or more of the following food items: shellfish, pork, beef, lamb, chicken, vegetables, peanut, wheaten bread, butter, cheese, tea, coffee, cocoa and various types of alcoholic beverages. Quantitative data on the natural occurrence in foods have been reported for 18 of these substances (TNO, 2000). These reports include:

- 2-Acetyl-4-methylthiazole [FL-no: 15.038]: 0.02 mg/kg in kohlrabi.
- 2-Acetylthiophene [FL-no: 15.040]: Trace amounts in asparagus, up to 1.25 mg/kg in coffee, 0.02 mg/kg in kohlrabi, up to 0.00002 mg/kg in pork (grilled, roasted).
- 2,5-Dimethyl-4-ethylthiazole [FL-no: 15.061]: 0.00001 mg/kg in pork (grilled, roasted).
- 2,4-Dimethylthiazole [FL-no: 15.062]: Up to 0.00004 mg/kg in pork (grilled, roasted).
- 2,5-Dimethylthiophene [FL-no: 15.064]: Up to 0.01 mg/kg in papaya, up to 0.0007 mg/kg in whisky.
- 4-Ethyl-2-methylthiazole [FL-no: 15.067]: Trace amounts in pork (grilled, roasted).
- 5-Ethyl-2-methylthiazole [FL-no: 15.068]: Trace amounts in chicken (roasted).
- 4-Ethyl-5-methylthiazole [FL-no: 15.069]: Up to 0.00001 mg/kg in pork (grilled, roasted).
- 2-Ethyl-5-methylthiophene [FL-no: 15.070]: Trace amounts in pork (grilled, roasted).
- 2-Ethylthiophene [FL-no: 15.072]: Trace amounts in pork (grilled, roasted).
- 2-Hexylthiophene [FL-no: 15.076]: Up to 0.002 mg/kg in guinea hen.
- 2-Methylthiazole [FL-no: 15.089]: Trace amounts in chicken (roasted).
- 2-Methylthiophene [FL-no: 15.091]: Up to 0.0076 mg/kg in beef (grilled, roasted), trace amounts in chicken (roasted), up to 0.1 mg/kg in papaya, 0.0009 mg/kg in shrimps (cooked), up to 0.0073 mg/kg in whisky.
- 3-Methylthiophene [FL-no: 15.092]: Up to 0.01 mg/kg in papaya.
- 2-Pentylthiophene [FL-no: 15.096]: 0.001 mg/kg in chicken (roasted).
- 2-Propionylthiophene [FL-no: 15.097]: 0.8 mg/kg in coffee .
- Thiophene [FL-no: 15.106]: 0.0005 mg/kg in chicken (roasted), up to 0.8 mg/kg in tea, up to 0.0032 mg/kg in whisky.
- Thiophene-2-carbaldehyde [FL-no: 15.107]: 0.01 mg/kg in asparagus (cooked), 0.4 mg/kg in camembert, up to 1.8 mg/kg in coffee, up to 0.04 mg/kg in grape brandy, up to 0.03 mg/kg in malt whisky.



Fourteen of the substances have not been reported to occur naturally in any food items according to TNO (TNO, 2000). These are: 2-butyl-4-methyl(4H)pyrrolidino-[1,2d]-1,3,5-dithiazine [FL-no: 15.042], 2-butylthiazole [FL-no: 15.044], 2,4-dimethyl(4H)pyrrolidino[1,2e]-1,3,5-dithiazine [FL-no: 15.055], 5-ethylthiophene-2-carbaldehyde [FL-no: 15.074], 4-hydroxy-2,5-dimethylthiophen-3(2H)-one [FL-no: 15.077], 3-mercaptothiophene [FL-no: 15.082], 2-methyl-3-mercaptothiophene [FL-no: 15.087], 2-thiophenemethanethiol [FL-no: 15.108], 6-acetyl-2,3-dihydro-1,4-thiazine [FL-no: 15.114] (Register name: 5-acetyl-2,3-dihydro-1,4-thiazine), 2-isobutyl-4-methylthiazole [FL-no: 15.115], 2-acetyl-4-ethylthiazole [FL-no: 15.116], 2-isobutyl-3-thiazoline [FL-no: 15.119], tetrahydro-2,4,6-trimethyl-1,3,5(2H)-thiadiazine [FL-no: 15.129] and 5-acetyl-2,3-dihydro-1,4-thiazine [FL-no: 15.133].

## 2. Specifications

Purity criteria for the 56 candidate substances have been provided by the flavour industry (EFFA, 2004i). Judged against the requirements in Annex II of Commission Regulation (EC) No 1565/2000 (EC, 2000a), the information is adequate for all 56 substances, except that information on stereoisomerism is needed for nine candidate substances [FL-no: 15.042, 15.054, 15.055, 15.060, 15.077, 15.090, 15.099, 15.119 and 15.129] (see Section 1.2 and Table 1). For two substances [FL-no: 15.129 and 15.133] an identity test is missing.

## 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, 2000a). However, it is considered as a suitable tool to screen and prioritise the flavouring substances according to the need for refined intake data (EFSA, 2004a).

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

The 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 population<sup>2</sup> (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).

The total annual volume of production of the 56 candidate substances from use as flavouring substances in Europe has been reported to be approximately 64 kg (EFFA, 2004f). 2-Acetylthiophene [FL-no: 15.040] accounts for 18 kg. For 18 of the 29 supporting substances the annual volume of production is approximately 3700 kg in Europe (JECFA, 2003a). Approximately 2500 kg is accounted for by thiamine hydrochloride [FL-no: 16.027] and approximately 1200 kg is accounted for by 5-(2-hydroxyethyl)-4-methylthiazole [FL-no: 15.014]. There was no reported production in Europe for eight of the supporting substances in the Register [FL-no: 15.002, 15.005, 15.008, 15.027, 15.029, 15.030, 15.109 and 15.113].

On the basis of the annual volumes of production reported for the 56 candidate substances, the daily per capita intakes for each of these flavourings have been estimated (Table 2).

The estimated daily per capita intake of 2-acetylthiophene [FL-no: 15.040] from use as a flavouring substance is 2.2 microgram, of 4-butylthiazole [FL-no: 15.118] 1.3 microgram, of each of 2,4-dimethylthiazole [FL-no: 15.062], tetrahydro-2,4,6-trimethyl-1,3,5(2H)-thiadiazine [FL-no: 15.129] and 5-acetyl-2,3-dihydro-1,4-thiazine [FL-no: 15.133] 0.61 microgram. For the remaining 51 substances the estimated daily per capita intakes are in the range of 0.0012 to 0.24 microgram (Table 2).

### 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.

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<sup>2</sup> EU figure 375 millions (Eurostat, 1998). 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.

For the present evaluation of the 56 candidate substances, information on food categories and normal and maximum use levels<sup>3,4,5</sup> were submitted by the Flavour Industry (EFFA, 2004g; EFFA, 2004i; EFFA, 2007a; Flavour Industry, 2004-5). The 56 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, 2000a), as shown in Table 3.1. For the present calculation of the 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.

Food category	Description	Flavourings used
Category 01.0	Dairy products, excluding products of category 2	All 56
Category 02.0	Fats and oils, and fat emulsions (type water-in-oil)	All 56
Category 03.0	Edible ices, including sherbet and sorbet	All 56 except [FL-no: 15.094]
Category 04.1	Processed fruits	All 56
Category 04.2	Processed vegetables (including mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds	[FL-no: 15.062, 15.129 & 15.133]
Category 05.0	Confectionery	All 56 except [FL-no: 15.062]
Category 06.0	Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery	All 56
Category 07.0	Bakery wares	All 56
Category 08.0	Meat and meat products, including poultry and game	All 56
Category 09.0	Fish and fish products, including molluscs, crustaceans and echinoderms	All 56 except [FL-no: 15.039]
Category 10.0	Eggs and egg products	None
Category 11.0	Sweeteners, including honey	None
Category 12.0	Salts, spices, soups, sauces, salads, protein products, etc.	All 56 except [FL-no: 15.089]
Category 13.0	Foodstuffs intended for particular nutritional uses	All 56 except [FL-no: 15.129 & 15.133]
Category 14.1	Non-alcoholic ("soft") beverages, excluding dairy products	All 56
Category 14.2	Alcoholic beverages, including alcohol-free and low-alcoholic counterparts	[FL-no: 15.089, 15.129 & 15.133]
Category 15.0	Ready-to-eat savouries	All 56 except [FL-no: 15.068]
Category 16.0	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 1 – 15	All 56

According to the Flavour Industry the normal use levels for the 56 candidate substances are in the range of 0.1-10 mg/kg food, and the maximum use levels are in the range of 0.2-250 mg/kg (EFFA, 2004g; EFFA, 2004i; EFFA, 2007a; Flavour Industry, 2004-5).

The mTAMDI values for the 46 candidate substances from structural class II (see Section 6) range from 78 to 4000 microgram/person/day. For the 10 candidate substances from structural class III (see Section 6) the mTAMDI range from 78 to 4000 microgram/person/day.

<sup>3</sup> "Normal use" is defined as the average of reported usages and "maximum use" is defined as the 95th percentile of reported usages (EFFA, 2002i).

<sup>4</sup> 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).

<sup>5</sup> 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).

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

The 56 candidate substances are structurally related to 29 supporting substances evaluated by the JECFA in “Sulphur-containing heterocyclic compounds” (JECFA, 2003a). The substances are divided into 11 subgroups based on the nature of the ring (aromatic (clustered in subgroups A-Ia,b,c to A-III) vs. non-aromatic (clustered in subgroups B-I to B-VI)) depending on type and number of ring heteroatoms (sulphur or sulphur with nitrogen), and the degree of saturation in the non-aromatic rings. The assignment of the individual substances to the different subgroups is presented in Table 4.1. The structures of the substances and a description of the characteristic features of the 11 subgroups are also given in Table III.1 and the accompanying text in Annex III.


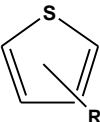
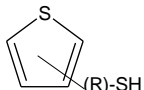

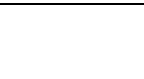
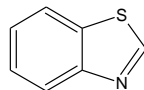


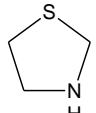
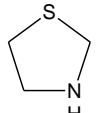


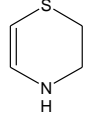
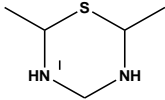
Table 4.1 Division of candidate substances into structural subgroups		
Subgroup and common ring structure	Register name	FL-no
<b>A: Aromatic subgroups:</b>		
<b>A-Ia: Thiophene</b> 	Thiophene	15.106
<b>A-Ib: Thiophenes: (with non-thiol-containing ring substituents)</b> 	2-Methylthiophene	15.091
	3-Methylthiophene	15.092
	2-Ethylthiophene	15.072
	2-Butylthiophene	15.045
	2-Pentylthiophene (Register name: <i>sec</i> -Pentylthiophene)	15.096
	2-Hexylthiophene	15.076
	2-Octylthiophene	15.093
	2,5-Dimethylthiophene	15.064
	2-Ethyl-5-methylthiophene	15.070
	2-Butyl-5-ethylthiophene	15.043
	2-Acetylthiophene	15.040
	2-Propionylthiophene	15.097
	2-Pentanoylthiophene	15.094
	2-Acetyl-3-methylthiophene	15.037
Thiophene-2-carbaldehyde	15.107	
5-Ethylthiophene-2-carbaldehyde	15.074	
<b>A-Ic: Thiophenes: (with thiol-containing ring substituents)</b> 	3-Mercaptothiophene	15.082
	2-Thiophenemethanethiol	15.108
<b>A-II: Thiazoles</b> 	2-Methylthiazole	15.089
	2-Ethylthiazole	15.071
	2-Propylthiazole	15.098
	2-Butylthiazole	15.044
	4-Butylthiazole	15.118
	2,4-Dimethylthiazole	15.062
	2,5-Dimethylthiazole	15.063
	5-Ethyl-2-methylthiazole	15.068
	4-Ethyl-2-methylthiazole	15.067
	2-Methyl-3-mercaptothiophene	15.087

Table 4.1 Division of candidate substances into structural subgroups		
Subgroup and common ring structure	Register name	FL-no
	4-Ethyl-5-methylthiazole	15.069
	2,5-Diethylthiazole	15.052
	5-Methyl-2-pentylthiazole	15.084
	4,5-Dimethyl-2-ethylthiazole	15.058
	2,5-Dimethyl-4-ethylthiazole	15.061
	2,5-Diethyl-4-methylthiazole	15.050
	2,5-Diethyl-4-propylthiazole	15.051
	2-Isobutyl-4-methylthiazole	15.115
	2-Isobutyl-4,5-dimethylthiazole	15.078
	2-Isopropyl-4,5-dimethylthiazole	15.080
	2-Acetyl-4-methylthiazole	15.038
	2-Acetyl-5-methylthiazole	15.039
	2-Acetyl-4-ethylthiazole	15.116
	4-Methyl-2-propionylthiazole	15.085
<b>A-III: Benzothiazoles</b> 	2-Methyl-4,5-benzothiazole	15.088
<b>B: Non-aromatic subgroups:</b>		
<b>B-I : Dihydrothiophenes</b> 	4-Hydroxy-2,5-dimethylthiophen-3(2H)-one	15.077
<b>B-II: Thiazolines</b> 	2-Methyl-2-thiazoline	15.086
	2,4-Dimethyl-3-thiazoline	15.060
<b>B-III: Thiazolidines</b> 	2-Isobutyl-3-thiazoline	15.119
	2-Methylthiazolidine	15.090
<b>B-III: Thiazolidines</b> 	2-Propylthiazolidine	15.099
	<b>B-IV: Dithiazines</b> 	Dihydro-2,4,6-triethyl-1,3,5(4H)-dithiazine
<b>B-IV: Dithiazines</b> 	2,4-Dimethyl(4H)pyrrolidino[1,2c]-1,3,5-dithiazine	15.055
	2-Butyl-4-methyl(4H)pyrrolidino[1,2d]-1,3,5-dithiazine	15.042
<b>B-V: Dihydrothiazines</b> 	6-Acetyl-2,3-dihydro-1,4-thiazine (Register name: 5-acetyl-2,3-dihydro-1,4-thiazine)	15.114
	5-Acetyl-2,3-dihydro-1,4-thiazine	15.133

Subgroup and common ring structure	Register name	FL-no
<b>B-VI: Thiadiazine</b> 	Tetrahydro-2,4,6-trimethyl-1,3,5(2H)-thiadiazine	15.129

From the few data available on absorption, distribution and elimination of the aromatic candidate substances in this FGE, it is anticipated that they may be absorbed and eliminated after biotransformation. Some volatile substances may also be eliminated unchanged via exhalation. For the non-aromatic substances there are no data available for the candidate or for the supporting substances.

For the evaluation of the metabolism of the candidate substances, only limited data were available. These were confined to a few studies on thiophene, and some thiophene-, thiazole- and benzothiazole-derivatives (i.e. only directly relevant for the evaluation of subgroups-A (A-Ia, -Ib, -Ic, -II, -III)), the aromatic candidate substances. Other information was found in several review papers. However, virtually no data were found on the metabolism of substances from the B-subgroups (B-I, -II, -III, -IV, -V, -VI) possessing non-aromatic ring structures.

#### *Metabolism of substances in subgroup A-I*

Thiophene and the ring-substituted thiophenes (A-Ia, b and c) may undergo S-oxidation to give sulphoxides. These primary metabolites may react spontaneously with glutathione (GSH), and it is likely that they also exhibit reactivity towards protein thiols.

Only very limited information was submitted to show whether side chain oxidation of the ring-substituted thiophenes could also occur. Based on some studies with a substituted thiazole (chlormethiazole) such side chain reactions may be anticipated. For the substances in subgroup A-Ib, metabolites of the side chain oxidation pathways may be expected to be conjugated, e.g. with glucuronic acid. Similar reactions (e.g. omega or omega-1 oxidations) for ring-substituent chains have been discussed for alkylated pyrazines (EFSA, 2005d). The two thiophene carbaldehyde candidate substances [FL-no: 15.107 and 15.074] may be expected to be oxidised to the corresponding carboxylic acids. Where applicable, conjugation with amino acids (e.g. glycine) or glucuronic acid may also be expected to occur (see FGE.13 (EFSA, 2005c); FGE.14 (EFSA, 2005e); FGE.17 (EFSA, 2005d)). The acyl-substituted thiophenes [FL-no: 15.037, 15.040, 15.094 and 15.097] may be subject to keto-reduction, possibly followed by conjugation, similar to acetyl derivatives of furans (EFSA, 2005c) and of pyrazines (EFSA, 2005d).

The mercapto-group in the ring-substituent of the substances in subgroup A-Ic may undergo S-methylation to produce the corresponding methyl thioether, with further oxidation to the corresponding sulphoxide and sulphone. They may also react with glutathione or other endogenous thiol substances to form mixed disulphides, which may undergo reduction to thiols or oxidative desulphuration. Alternatively, they may undergo enzymatic oxygenation resulting in the formation of the corresponding sulphinic or sulphonic acids. These metabolites are all expected to be excreted

in the urine. A more detailed discussion on the metabolism of sulphur compounds can be found in FGE.13 (EFSA, 2005c).

#### *Metabolism of substances in subgroup A-II*

Thiazoles may be subject to ring S- and N-oxidation. From the various studies with thiazole derivatives (A-II), it can be seen that their metabolites may react spontaneously with glutathione, and it is likely that they also exhibit reactivity towards biomacromolecules. In addition, for some thiazole derivatives, ring C-oxidation may be accompanied by heterocyclic ring cleavage which can result in the formation of alpha-diketone and thioamide intermediates. For the latter a relationship with nephro- and hepatotoxicity has been established, especially after GSH depletion, but this is a high-dose phenomenon. Due to the compound specificity of these reactions, the extent of formation cannot be generalised; however, these thioamide intermediates and the S-oxides seem to be quantitatively minor metabolites.

Similarly to the situation with the ring-substituted thiophenes, only very limited information was available which could demonstrate whether side chain oxidation of the ring-substituted thiazoles could occur. Based on some studies with a substituted thiazole (chlormethiazole) such side chain reactions may be anticipated. Metabolites of the side chain oxidation pathways may be expected to be conjugated, e.g. with glucuronic acid. Similar reactions (e.g. omega or omega-1 oxidations) for ring-substituent chains have been discussed for alkylated pyrazines (EFSA, 2005d). The acyl-substituted thiazoles [FL-no: 15.038, 15.039, 15.116 and 15.085] may be subject to keto-reduction, possibly followed by conjugation, similarly to acetyl derivatives of furanes (EFSA, 2005c) and pyrazines (EFSA, 2005d).

#### *Metabolism of substances in subgroup A-III*

In addition to ring cleavage as mentioned for the thiazoles in subgroup A-II, oxidation of the fused benzene ring has also been reported for some structural analogues of the candidate substance, 2-methyl-4,5-benzothiazole, in subgroup A-III [FL-no: 15.088]. No further information was available on the metabolism of this substance or on structurally related substances.

#### *Conclusions for the substances in the subgroups A-I to A-III*

From the very limited data available on the aromatic subgroups (A) it is not anticipated that the thiophenes (A-I) and thiazoles (A-II) utilise similar metabolic pathways. These sulphur-containing heterocyclic and heteroaromatic derivatives can be expected to participate in metabolic pathways principally involving side-chain C-oxidation, oxidation of the ring-S and (for the thiazoles) ring-N to yield sulphoxide or sulphones and N-oxides, respectively. Furthermore, from the data available it is anticipated that the unsubstituted thiophene (A-Ia) and benzothiazole (A-III) are metabolised differently from their ring-substituted derivatives.

In the light of the above considerations and as a consequence of the expected reactivity of the thiol groups and the possible reactions of the ring side-chains it cannot be anticipated that the substances in the aromatic subgroups (A) are metabolised to innocuous substances. The Panel was aware of the potential bio-activation of candidate substances with heteroaromatic rings to yield intermediates via ring-scission or S/N-oxidation which could be reactive to proteins or DNA. However, taking into account available data on structurally related thiazoles and considering the available genotoxicity data on thiazoles in the present FGE.21Rev1 (negative Ames tests), the Panel concluded that there are not sufficient indications to preclude the application of the Procedure to the thiophenes, thiazoles and benzothiazole (A-I to A-III).

### *Metabolism of the substances in subgroups B-I to B-VI*

No specific information was available on the metabolism of the dihydrothiophene-, thiazoline-, thiazolidine-, dithiazine-, dihydrothiazine-, thiadiazine-derivatives or related substances for any of these non-aromatic substances in the B-subgroups. It may be speculated that the substances in these groups are metabolised primarily by oxidation of the ring-S, or, if applicable, via N-oxidation. In addition, metabolism of the ring substituents is likely to occur.

The substances exhibiting thioacetal structures could be subject to acid hydrolysis in the stomach, similar to oxygen-containing acetals. However, thioacetals are more resistant than oxygen acetals. It is thus to be anticipated that these substances may reach the intestinal lumen intact and also may be absorbed as such.

### *Conclusions for the substances in the subgroups B-I to B-VI*

Due to the lack of metabolism data it cannot be concluded that the candidate substances in the B-subgroups will be metabolised to innocuous products.

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

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

For the two candidate substance 6-acetyl-2,3-dihydro-1,4-thiazine [FL-no: 15.114] (Register name: 5-acetyl-2,3-dihydro-1,4-thiazine) and 5-acetyl-2,3-dihydro-1,4-thiazine [FL-no: 15.133] from subgroup B-V, which are alpha,beta-unsaturated ketones, i.e. they have a structural alert for genotoxicity (EFSA, 2008b), there are no genotoxicity data available and accordingly a concern for genotoxicity could not be ruled out.

For the two candidate substances 2-methylthiazolidine [FL-no: 15.090] and 2-propylthiazolidine [FL-no: 15.099] from subgroup B-III, there are indications of a genotoxic potential *in vitro*. Considering the structural similarities between these two thiazolidines in subgroup B-III and the three thiazolines in subgroup B-II (2-methyl-2-thiazoline [FL-no: 15.086], 2,4-dimethyl-3-thiazoline [FL-no: 15.060] and 2-isobutyl-3-thiazoline [FL-no: 15.119]), the Panel concluded that in the absence of further genotoxicity data the Procedure could not be applied to these five substances from subgroup B-II and B-III.

Thus, the Panel concluded that in the absence of further genotoxicity data the Procedure could not be applied to the above mentioned seven substances.

For the safety evaluation of the remaining 49 candidate substances from chemical groups 29 and 30 the Procedure as outlined in Annex I was applied, based on the MSDI approach. The evaluations of the 49 substances are summarised in Table 2.



### Step 1:

According to the decision tree approach, presented by Cramer et al. (Cramer et al., 1978) 41 candidate substances, which are evaluated through the Procedure, are classified into structural class II and eight into structural class III, see Table 2.

### Step 2:

None of the 49 candidate substances can be predicted to be metabolised to innocuous products. Therefore, the evaluation of all 49 candidate substances proceeds via the B-side of the Procedure.

### Step B3:

The 41 candidate substances in structural class II have estimated European daily per capita intakes (MSDI) ranging from 0.0012 microgram to 2.2 microgram (Tables 2 and 6.1). These intakes are below the threshold of concern of 540 microgram/person/day for structural class II substances.

Similarly, the estimated daily per capita intakes of all eight candidate substances in structural class III, ranging from 0.001 microgram to 0.12 microgram (Tables 2 and 6.1), are below the threshold of concern for structural class III substances of 90 microgram/person/day.

Accordingly, the evaluation of all 49 candidate substances proceeds to step B4.

### Step B4:

#### **Subgroup A-Ia: Thiophene**

No valid toxicity study, from which a no-observed-adverse-effect-level (NOAEL) could be established, was available neither for the candidate substance nor for any relevant supporting substance. Accordingly, further data are required for thiophene [FL-no: 15.106].

#### **Subgroup A-Ib: Thiophenes with non-thiol-containing ring substituents**

No valid toxicity study from which a NOAEL could be established was available for the candidate or for any relevant supporting substance. Therefore, the Panel concluded that additional toxicity data are needed for the 16 substituted thiophenes in subgroup A-Ib.

#### **Subgroup A-Ic: Thiophenes with thiol-containing ring substituents**

A NOAEL of 0.29 mg/kg bw/day was reported for the supporting substance 2-thienyl disulfide [FL-no: 15.008] in a single-dose level 90-day study in rats. The combined estimated daily per capita intake of 0.14 microgram for the three candidate substances in subgroup A-Ic corresponds to 0.0023 microgram/kg bw/day at a body weight of 60 kg. Thus, a margin of safety of  $1.2 \times 10^5$  can be calculated.

On the basis of the application of the Procedure, 3-mercaptothiophene [FL-no: 15.082], 2-methyl-3-mercaptothiophene [FL-no: 15.087] and 2-thiophenemethanethiol [FL-no: 15.108] are not expected to be of safety concern at their estimated levels of intake.

#### **Subgroup A-II: Thiazoles**

A NOAEL of 25 mg/kg bw/day was reported for the supporting substance 5-acetyl-2,4-dimethylthiazole [FL-no: 15.011] in a single-dose level 90-day study in rats. The combined estimated daily per capita intake of 2.3 microgram for the 23 candidate substances in subgroup A-II

corresponds to 0.038 microgram/kg bw/day at a body weight of 60 kg. Thus, a margin of safety of  $6.6 \times 10^5$  can be calculated.

On the basis of the application of the Procedure, the 23 candidate substances in subgroup A-II are not expected to be of safety concern at their estimated levels of intake.

### **Subgroup A-III: Benzothiazoles**

There were no valid toxicological data available on the candidate substance 2-methyl-4,5-benzothiazole [FL-no: 15.088] nor for any sufficiently structurally related substances. The Panel considered that data on the supporting substance benzothiazole [FL-no: 15.016] could not be used in the Procedure for 2-methyl-4,5-benzothiazole, since the unsubstituted benzothiazole is anticipated to be metabolised differently from the substituted benzothiazole in subgroup A-III. Therefore, the Panel concluded that additional toxicity data are needed for 2-methyl-4,5-benzothiazole [FL-no: 15.088].

### **Subgroup B-I: Dihydrothiophenes**

No toxicological data were available on 4-hydroxy-2,5-dimethylthiophen-3(2H)-one [FL-no: 15.077] or for structurally related supporting substances. Therefore, the Panel concluded that additional toxicity data are needed for this substance.

### **Subgroup B-II: Thiazolines**

The candidate substances were not evaluated through the Procedure.

### **Subgroup B-III: Thiazolidines**

The candidate substances were not evaluated through the Procedure.

### **Subgroup B-IV: Dithiazines**

There were no toxicological data available on the candidate substances in this subgroup and the Panel concluded that the data available on supporting substances could not be used for deriving a NOAEL to support the candidate substances in this subgroup B-IV. Accordingly, additional data are needed for 2-butyl-4-methyl(4H)pyrrolidino[1,2d]-1,3,5-dithiazine [FL-no: 15.042], dihydro-2,4,6-triethyl-1,3,5(4H)-dithiazine [FL-no: 15.054] and 2,4-dimethyl(4H)pyrrolidino[1,2e]-1,3,5-dithiazine [FL-no: 15.055] in subgroup B-IV.

### **Subgroup B-V: Dihydrothiazines**

6-acetyl-2,3-dihydro-1,4-thiazine [FL-no: 15.114] (Register name: 5-acetyl-2,3-dihydro-1,4-thiazine) or on 5-acetyl-2,3-dihydro-1,4-thiazine [FL-no: 15.133] not evaluated through the Procedure.

### **Subgroup B-VI: Thiadiazines**

No toxicological data were available on tetrahydro-2,4,6-trimethyl-1,3,5(2H)-thiadiazine [FL-no: 15.129] or on structurally related supporting substances. Therefore, the Panel concluded that additional toxicity data are needed for this substance from subgroup B-VI.

### *Summary of application of the Procedure*

In total, 26 of the candidate substances are not of safety concern at their estimated levels of intake based on the MSDI approach whereas for 23 candidate substances additional data are required (see Table 2).

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

The estimated intakes, based on the mTAMDI, for the 41 candidate substances assigned to structural class II and evaluated using the Procedure range from 78 to 4000 microgram/person/day. For 40 candidate substances the mTAMDI values are below the threshold of concern of 540 microgram/person/day for structural class II. For one substance [FL-no: 15.129] the mTAMDI value is 4000 microgram/person/day, which is above the threshold of concern of 540 microgram/person/day for structural class II. For comparison of the intake estimates based on the MSDI approach and the mTAMDI approach, see Table 6.1.

The estimated intakes, based on the mTAMDI, for the eight candidate substances assigned to structural class III and evaluated using the Procedure range from 78 to 160 microgram/person/day. For three of these candidate substances (2-butyl-4-methyl(4H)pyrrolidino[1,2d]-1,3,5-dithiazine [FL-no: 15.042], 2,4-dimethyl(4H) pyrrolidino[1,2e]-1,3,5-dithiazine [FL-no: 15.055], 2-methyl-4,5-benzothiazole [FL-no: 15.088] the mTAMDI values are above the threshold of concern for structural class III substances of 90 microgram/person/day. For comparison of the MSDI and mTAMDI values, see Table 6.1.

Further information is therefore required on these four substances. This would include more reliable intake data as well as the additional data already identified as a consequence of application of the Procedure.

FL-no	EU Register name	MSDI ( $\mu\text{g/capita/day}$ )	mTAMDI ( $\mu\text{g/person/day}$ )	Structural class	Threshold of concern ( $\mu\text{g/person/day}$ )
15.037	2-Acetyl-3-methylthiophene	0.18	78	Class II	540
15.038	2-Acetyl-4-methylthiazole	0.0049	160	Class II	540
15.039	2-Acetyl-5-methylthiazole	0.0024	160	Class II	540
15.040	2-Acetylthiophene	2.2	78	Class II	540
15.043	2-Butyl-5-ethylthiophene	0.0012	78	Class II	540
15.044	2-Butylthiazole	0.011	160	Class II	540
15.045	2-Butylthiophene	0.012	78	Class II	540
15.050	2,5-Diethyl-4-methylthiazole	0.012	160	Class II	540
15.051	2,5-Diethyl-4-propylthiazole	0.0012	160	Class II	540
15.052	2,5-Diethylthiazole	0.015	160	Class II	540
15.054	Dihydro-2,4,6-triethyl-1,3,5(4H)-dithiazine	0.0012	160	Class II	540
15.058	4,5-Dimethyl-2-ethylthiazole	0.015	130	Class II	540
15.061	2,5-Dimethyl-4-ethylthiazole	0.011	160	Class II	540
15.062	2,4-Dimethylthiazole	0.61	140	Class II	540
15.063	2,5-Dimethylthiazole	0.0061	160	Class II	540
15.064	2,5-Dimethylthiophene	0.23	78	Class II	540
15.067	4-Ethyl-2-methylthiazole	0.0037	160	Class II	540
15.068	5-Ethyl-2-methylthiazole	0.0061	220	Class II	540
15.069	4-Ethyl-5-methylthiazole	0.012	160	Class II	540
15.070	2-Ethyl-5-methylthiophene	0.061	78	Class II	540
15.071	2-Ethylthiazole	0.028	160	Class II	540
15.072	2-Ethylthiophene	0.0012	78	Class II	540
15.074	5-Ethylthiophene-2-carbaldehyde	0.0012	78	Class II	540
15.076	2-Hexylthiophene	0.12	78	Class II	540
15.078	2-Isobutyl-4,5-dimethylthiazole	0.12	160	Class II	540

<b>Table 6.1 Estimated intakes based on the MSDI approach and the mTAMDI approach</b>					
FL-no	EU Register name	MSDI ( $\mu\text{g/capita/day}$ )	mTAMDI ( $\mu\text{g/person/day}$ )	Structural class	Threshold of concern ( $\mu\text{g/person/day}$ )
15.080	2-Isopropyl-4,5-dimethylthiazole	0.012	160	Class II	540
15.084	5-Methyl-2-pentylthiazole	0.0037	160	Class II	540
15.085	4-Methyl-2-propionylthiazole	0.0037	160	Class II	540
15.089	2-Methylthiazole	0.018	150	Class II	540
15.091	2-Methylthiophene	0.019	78	Class II	540
15.092	3-Methylthiophene	0.12	78	Class II	540
15.093	2-Octylthiophene	0.012	160	Class II	540
15.094	2-Pentanylthiophene	0.0012	160	Class II	540
15.096	sec-Pentylthiophene	0.24	160	Class II	540
15.097	2-Propionylthiophene	0.12	160	Class II	540
15.098	2-Propylthiazole	0.085	160	Class II	540
15.107	Thiophene-2-carbaldehyde	0.21	78	Class II	540
15.115	2-Isobutyl-4-methylthiazole	0.011	160	Class II	540
15.116	2-Acetyl-4-ethylthiazole	0.024	160	Class II	540
15.118	4-Butylthiazole	1.3	160	Class II	540
15.060	2,4-Dimethyl-3-thiazoline	0.012	160	Class II	540
15.086	2-Methyl-2-thiazoline	0.24	160	Class II	540
15.090	2-Methylthiazolidine	0.024	160	Class II	540
15.099	2-Propylthiazolidine	0.012	160	Class II	540
15.119	2-Isobutyl-3-thiazoline	0.011	160	Class II	540
15.129	Tetrahydro-2,4,6-trimethyl-1,3,5(2H)-thiadiazine	0.61	4000	Class II	540
15.042	2-Butyl-4-methyl(4H)pyrrolidino[1,2d]-1,3,5-dithiazine	0.0012	160	Class III	90
15.055	2,4-Dimethyl(4H)pyrrolidino[1,2e]-1,3,5-dithiazine	0.055	160	Class III	90
15.077	4-Hydroxy-2,5-dimethylthiophen-3(2H)-one	0.12	78	Class III	90
15.082	3-Mercaptothiophene	0.011	78	Class III	90
15.087	2-Methyl-3-mercaptothiophene	0.12	78	Class III	90
15.088	2-Methyl-4,5-benzothiazole	0.0085	160	Class III	90
15.106	Thiophene	0.12	78	Class III	90
15.108	2-Thiophenemethanethiol	0.0073	78	Class III	90
15.114	5-Acetyl-2,3-dihydro-1,4-thiazine	0.012	160	Class III	90
15.133	5-Acetyl-2,3-dihydro-1,4-thiazine	0.61	4000	Class III	90

## 7. Considerations of Combined Intakes from Use as Flavouring Substances

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

The total estimated combined daily per capita intake is estimated by summing the MSDIs for individual substances.

Seven of the candidate substances [FL-no: 15.060, 15.086, 15.090, 15.099, 15.114, 15.119 and 15.133] have not been evaluated through the Procedure (see Section 5) and are therefore not considered together with the other 49 candidate substances evaluated in FGE.21Rev1 in the combined intake.

On the basis of the reported annual production volumes in Europe (EFFA, 2004f), the combined estimated daily per capita intakes as flavourings of the candidate substances in subgroups with more than one substance from a structural class (sc), are: subgroup A-Ib (16 substances from sc II) 3.6 microgram; subgroup A-Ic (three substances from sc II) 0.14 microgram; subgroup A-II (23 substances from sc II) 2.3 microgram; and subgroup B-IV (two substances from sc III) 0.056 microgram.

The 49 candidate substances are structurally related to 29 supporting substances evaluated by the JECFA at its 59<sup>th</sup> JECFA meeting (JECFA, 2002c). The total combined daily per capita intakes (in Europe) of candidate and supporting substances in each of the four subgroups for which supporting substances were available are: subgroup A-Ib (18 substances from sc II) 22 microgram; subgroup A-Ic (four substances from sc III) 0.15 microgram; subgroup A-II (36 substances from sc II) 500 microgram and subgroup A-III (two substances from sc III) 1.2 microgram.

For none of the subgroups do the total combined intakes exceed the thresholds of concern for compounds belonging to structural class II of 540 microgram/person/day or to structural class III of 90 microgram/person/day.

## 8. Toxicity

### 8.1. Acute Toxicity

Data are available for two of the 56 candidate substances, thiophene [FL-no: 15.106] and thiophene-2-carbaldehyde [FL-no: 15.107] and for 17 of the 29 supporting substances. The Oral LD<sub>50</sub> values in rats or mice ranged from 180 to 3700 mg/kg body weight (bw).

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

### 8.2. Subacute, Subchronic, Chronic and Carcinogenicity Studies

Subacute toxicity data were available for two of the candidate substances, thiophene [FL-no: 15.106] and thiophene-2-carbaldehyde [FL-no: 15.107], for 11 supporting substances, 9 in the Register [FL-no: 15.002, 15.004, 15.005, 15.008, 15.011, 15.016, 15.020, 15.024 and 15.029] and for two mixtures of structurally related substances: one mixture of 2-isobutyl-4,6-dimethyldihydro-1,3,5-dithiazine and 4-isobutyl-2,6-dimethyldihydro-1,3,5-dithiazine, and one mixture of 2-isopropyl-4,6-dimethyldihydro-1,3,5-dithiazine and 4-isopropyl-2,6-dimethyldihydro-1,3,5-dithiazine.

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

#### **Subgroup A-Ia: Thiophene**

Based on the available data on metabolism, the toxicity of the candidate substance thiophene [FL-no: 15.106] is considered separately from the substituted thiophenes in subgroup A-Ib. One short-term toxicity test is available on thiophene, administered to groups of 5 male rats at dose levels of 0 - 1000 mg/kg bw/day by gavage for up to 19 days (O'Donoghue, 1979). Evidence of central

nervous system toxicity was seen in higher dose groups, accompanied by haematological and clinical chemistry changes and increased relative liver weights. Histologically, hepatic necrosis was present in the 500 and 1000 mg/kg bw groups and one rat in the 1000 mg/kg bw groups showed thymic cortical atrophy, splenic lymphoid depletion and hypocellular bone marrow. The reported No Observed Adverse Effect Level (NOAEL) was 100 mg/kg bw/day. The Panel considered that this study was inadequate for deriving an appropriate NOAEL to be used in the Procedure because of the short duration and the limited study details given.

#### **Subgroup A-Ib: Thiophenes with non-thiol-containing ring substituents**

The candidate substance thiophene-2-carbaldehyde [FL-no: 15.107] was tested in a subacute toxicity test, in which groups of 5 rats (sex not specified) were exposed to 0, 95 and 840 mg/kg bw/day for 11 days (Sharp, 1979). No adverse signs or changes in behaviour were noted in treated animals. The reported NOAEL was 95 mg/kg bw/day. Data are also available from short-term tests on two supporting substances in this subgroup A-Ib, 5-methyl-2-thiophenecarbaldehyde [FL-no: 15.004] and 3-acetyl-2,5-dimethylthiophene [FL-no: 15.024], administered to male and female rats for 14 days at 10 mg/kg bw/day (Gill & Van Miller, 1987b). Both substances produced minor body weight changes in both sexes and organ weight changes in males only. The Panel considered that all three studies were inadequate for deriving an appropriate NOAEL to be used in the Procedure because of the short durations and the limited study details given.

#### **Subgroup A-Ic: Thiophenes with thiol-containing ring substituents**

No toxicity data were available for the three candidate substances in this subgroup [FL-no: 15.082, 15.087 and 15.108]. The supporting substance 2-thienyl disulfide (2,2'-dithiodithiophene) [FL-no: 15.008] was tested in a 90-day dietary feeding study in male and female rats at a single dose level of 0.29 mg/kg bw/day (Morgareidge & Oser, 1970g). Body weight changes, food consumption, haematological and clinical chemistry parameters were assessed, urinalysis was undertaken and a comprehensive range of tissues were examined histopathologically. No changes were detected in any of the parameters assessed, and accordingly a NOAEL of 0.29 mg/kg bw/day could be established.

#### **Subgroup A-II: Thiazoles**

There were no subacute, subchronic or chronic toxicity studies on any of the 23 candidate thiazoles in subgroup A-II.

A 90-day dietary feeding study was carried out on the supporting substance 5-acetyl-2,4-dimethylthiazole [FL-no: 15.011] in male (23) and female (23) rats, at a single dose level of approximately 25 mg/kg bw/day (Shellenberger, 1971d). Body weight, food consumption, haematological and clinical chemistry parameters were assessed, urinalysis was undertaken and a comprehensive range of tissues were examined histopathologically. No changes were detected in any of the parameters assessed and accordingly a NOAEL of 25 mg/kg bw/day could be established.

In a 90-day study on the supporting substance 2-acetylthiazole [FL-no: 15.020], male rats (10/group) were fed diets containing 0, 100, 1000 or 10,000 mg/kg test substance, reported to be equivalent to an average daily intake of 0, 5, 50 and 500 mg/kg bw/day (Wheldon et al., 1970). The dose level in the highest dose group was increased to 20,000 mg/kg at week 6. Test substance-related changes at 10,000-20,000 mg/kg included reduced body weight gain, increased relative liver, adrenal and thyroid weights and minimal fatty changes in the liver. The authors reported a

NOAEL of 50 mg/kg bw/day. However, due to limitations in the study design and reporting, the Panel concluded that this NOAEL could not be used in the Procedure.

Two further 90-day studies were carried out at single dose levels with the supporting substances 2-methyl-5-methoxythiazole [FL-no: 15.002] and 2,4-dimethyl-5-vinylthiazole [FL-no: 15.005] (Posternak et al., 1975). Due to limitations in reporting of experimental details and results, the Panel concluded that these studies could not be used to derive a NOAEL to be used in the Procedure.

Oral intakes of up to 500 mg thiamine/day (8 mg/kg bw/day in a 60 kg individual) have been reported to have no adverse effects in humans (SCF, 2001).

### **Subgroup A-III: Benzothiazoles**

There were no toxicological studies available on the candidate substance 2-methyl-4,5-benzothiazole [FL-no: 15.088] in subgroup A-III. The Panel concluded that data on the supporting substance benzothiazole [FL-no: 15.016] could not be used in the Procedure for 2-methyl-4,5-benzothiazole, since the unsubstituted benzothiazole is anticipated to be metabolised differently from the substituted benzothiazole in subgroup A-III (see Section 4 and Annex III).

### **Subgroup B-I: Dihydrothiophenes**

There were no toxicological data available on the candidate substance 4-hydroxy-2,5-dimethylthiophen-3(2H)-one) [FL-no: 15.077] nor were there any data on supporting substances for subgroup B-I.

### **Subgroup B-II: Thiazolines**

There were no toxicological data available for the three candidate substances in this subgroup. The supporting substance 2-(sec-butyl)-4,5-dimethyl-3-thiazoline [FL-no: 15.029] was tested in a 90-day dietary feeding study in male and female rats (15/sex) at one dose level of approximately 1 mg/kg bw/day (Babish & Re, 1978). Body weight changes, food consumption, limited haematological and clinical chemistry parameters were assessed, urinalysis was undertaken and a comprehensive range of tissues were examined histopathologically. No changes were detected in any of the parameters assessed and accordingly a NOAEL of 1 mg/kg bw/day could be established.

### **Subgroup B-III: Thiazolidines**

There were no toxicological data available on the candidate substances 2-methyl thiazolidine and 2-propyl thiazolidine [FL-no: 15.090 and 15.099] nor were there any data on structurally related substances for subgroup B-III.

### **Subgroup B-IV: Dithiazines**

There were no toxicological data available on the three candidate substances in subgroup B-IV [FL-no: 15.042, 15.054, 15.055]. Short-term toxicity tests are available on isobutyl-2,6-dimethyldihydro-1,3,5-dithiazine (mixture of the 2- and 4-isobutyl isomers) and isopropyl-4,6-dimethyldihydro-1,3,5-dithiazine (mixture of the 2- and 4-isopropyl isomers) (Rush, 1989a; Rush, 1989b). The mixtures were incorporated in the diet of Sprague-Dawley rats (5/sex/group) intake of approximately 10 mg/kg bw/day over a period of 14 days. No treatment-related effects were seen. The Panel considered that these studies were inadequate for deriving a NOAEL to be used in the Procedure, because of the short duration of the studies.

### **Subgroup B-V: Dihydrothiazines**

There were no toxicological data available on the candidate substances 6-acetyl-2,3-dihydro-1,4-thiazine [FL-no: 15.114] (Register name: 5-acetyl-2,3-dihydro-1,4-thiazine) or 5-acetyl-2,3-dihydro-1,4-thiazine [FL-no: 15.133] nor were there any data on supporting substances for subgroup B-V.

### **Subgroup B-VI: Thiadiazines**

No toxicological data were available on tetrahydro-2,4,6-trimethyl-1,3,5(2H)-thiadiazine [FL-no: 15.129] or on structurally related supporting substances.

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

### **8.3. Developmental / Reproductive Toxicity Studies**

No developmental toxicity or reproductive toxicity studies were available for any of the 56 candidate substances or for any of the 29 supporting substances evaluated by the JECFA.

### **8.4. Genotoxicity Studies**

Genotoxicity data were provided for 12 of the 56 candidate substances. These 12 substances belong to subgroup A-Ia: thiophene [FL-no: 15.106]; subgroup A-Ib: 2-methylthiophene [FL-no: 15.091], 3-methylthiophene [FL-no: 15.092], 2,5-dimethylthiophene [FL-no: 15.064], 2-acetylthiophene [FL-no: 15.040], 2-acetyl-3-methylthiophene [FL-no: 15.037], thiophene-2-carbaldehyde [FL-no: 15.107], 5-ethylthiophene-2-carbaldehyde [FL-no: 15.074]; subgroup A-II: 2,4-dimethylthiazole [FL-no: 15.062]; subgroup A-III: 2-methyl-4,5-benzothiazole [FL-no: 15.088]; subgroup B-III: 2-methylthiazolidine [FL-no: 15.090] and 2-propylthiazolidine [FL-no: 15.099]. There were also mutagenicity data on four supporting substances and on four other structurally related substances. All available information on genotoxicity of the 12 candidate and the four supporting substances and of four other structurally related substances is based upon *in vitro* studies only.

#### **Subgroup A-I:**

Thiophene [FL-no: 15.106], 2-methylthiophene [FL-no: 15.091], 3-methyl-thiophene [FL-no: 15.092] and 2,5-dimethylthiophene [FL-no: 15.064] were reported to be negative in microbial mutagenicity assays. 2-Acetylthiophene [FL-no: 15.040] was negative in microbial tests, using *S. typhimurium* strains TA98 and TA100, with and without metabolic activation and in the SOS chromotest with metabolic activation. 2-Acetylthiophene was reported to be positive without metabolic activation in the SOS *E. coli* chromotest (Mosier et al., 2003). In the same study, 2-acetyl-3-methylthiophene [FL-no: 15.037], thiophene-2-carbaldehyde [FL-no: 15.107] and 5-ethylthiophene-2-carbaldehyde [FL-no: 15.074] gave positive results without metabolic activation in the SOS *E. coli* chromotest. The concentrations tested were not reported for any of the substances subjected to the SOS *E. coli* chromotest (Mosier et al., 2003). The Panel considered the endpoint of this test inappropriate for the estimation of genotoxic potential. The supporting substance 5-methyl-2-thiophene-carboxaldehyde [FL-no: 15.004] was negative in a microbial mutagenicity assay and in the mouse lymphoma test.

#### **Subgroup A-II:**

2,4-Dimethylthiazole [FL-no: 15.062] was reported to be negative in microbial assays, using *S. typhimurium*, but only in strain TA100 and only in the absence of metabolic activation (Voogd et al., 1983). Two supporting substances, 4,5-dimethylthiazole [FL-no: 15.017] and 4-methylthiazole [FL-no: 15.035] were negative in microbial mutagenicity assays.



### **Subgroup A-III:**

2-Methyl-4,5-benzothiazole [FL-no: 15.088] was reported to be negative in an Ames test but only a summary report was available (Longfellow, 1998a). The supporting substance benzothiazole [FL-no: 15.016] was negative in microbial mutagenicity assay and in the mouse lymphoma test.

### **Subgroups B-I and B-II:**

No genotoxicity information was available for any candidate or supporting substances in these subgroups. However, considering the structural similarities between the thiazolines in subgroup B-II and the thiazolidines in subgroup B-III, the Panel also concluded that the thiazolines [FL-no: 15.060, 15.086 and 15.119] could not be evaluated through the Procedure (see Subgroup B-III below).

### **Subgroup B-III:**

The two candidate substances 2-methylthiazolidine [FL-no: 15.090] and 2-propylthiazolidine [FL-no: 15.099] as well as the structurally related ethyl, isopropyl, n-butyl and isobutyl thiazolidine have all been reported to be positive in the Ames tests (TA98 and TA100) (Mihara & Shibamoto, 1980). Owing to limited reporting, the data could not be properly evaluated. Nevertheless, these reports do raise the possibility of a genotoxic potential of these thiazolidines. Accordingly, it was concluded not to evaluate the candidate substances 2-methylthiazolidine and 2-propylthiazolidine through the Procedure.

### **Subgroups B-IV:**

No genotoxicity information was available for any candidate or supporting substance in this subgroup.

### **B-V:**

The two candidate substances 6-acetyl-2,3-dihydro-1,4-thiazine [FL-no: 15.114] (Register name: 5-acetyl-2,3-dihydro-1,4-thiazine) and 5-acetyl-2,3-dihydro-1,4-thiazine [FL-no: 15.133] are alpha,beta unsaturated ketones i.e. they have a structural alert for genotoxicity (EFSA, 2008b) and as there are no genotoxicity data available a concern for genotoxicity can not be ruled out.

### **B-VI:**

No genotoxicity information was available for any candidate or supporting substance in this subgroup.

### *Overall conclusion on genotoxicity:*

It is concluded that the genotoxicity data are limited and that genotoxicity could not be assessed adequately for the flavouring substances in FGE.21Rev1. However, except for the two dihydrothiazines, 6-acetyl-2,3-dihydro-1,4-thiazine [FL-no: 15.114] (Register name: 5-acetyl-2,3-dihydro-1,4-thiazine) and 5-acetyl-2,3-dihydro-1,4-thiazine [FL-no: 15.133], the two thiazolidines 2-methylthiazolidine [FL-no: 15.090] and 2-propylthiazolidine [FL-no: 15.099] and the three structurally related thiazolines 2-methyl-2-thiazoline [FL-no: 15.086], 2,4-dimethyl-3-thiazoline [FL-no: 15.060] and 2-isobutyl-3-thiazoline [FL-no: 15.119], the genotoxicity data available do not preclude the evaluation of the remaining 49 candidate substances using the Procedure.

## 9. Conclusions

The FGE.21Rev1 includes the assessment of two additional substances compared to FGE.21. The current evaluation deals with all 56 substances. All 56 substances are five- or six-member sulphur-containing heterocyclic compounds, some of which also contain nitrogen. They belong to chemical groups 29 and 30 and they can be divided into two main groups: (A) those with an aromatic ring (44 candidate substances) and (B) those with a non-aromatic ring structure (12 candidate substances). Except for thiophene, all are ring-substituted with one or more of the following substituents: alkyl, alkenyl, aryl, alcohol, keto, thio. For assessment purposes, the following further subdivision of groups (A) and (B) has been made:

Group (A): Aromatic: Subgroup A-Ia: thiophene. Subgroup A-Ib: thiophene derivatives with non-thiol-containing substituents. Subgroup A-Ic: thiophene derivatives with thiol-containing substituents. Subgroup A-II: thiazole derivatives. Subgroup A-III: benzothiazoles.

Group (B): Subgroup B-I: dihydrothiophenes. Subgroup B-II: thiazolines. Subgroup B-III: thiazolidines. Subgroup B-IV: dithiazine derivatives. Subgroup B-V: dihydrothiazines. Subgroup B-VI: thiadiazine derivatives.

Forty-six of the candidate substances belong to Cramer structural class II and 10 belong to structural class III.

Forty-two of the substances have been reported to occur naturally in a wide range of foods.

According to the default MSDI approach, the 49 flavouring substances in this group which have been evaluated using the Procedure, have intakes in Europe ranging from 0.0012 to 2.2 microgram/capita/day, which are below the thresholds of concern values for Cramer 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 (EFFA, 2004f), the combined estimated daily intakes of the candidate substances in subgroups, with more than one substance from a structural class (class), are: subgroup A-Ib (16 substances from class II) 3.6 microgram; subgroup A-Ic (three substances from class II) 0.14 microgram; subgroup A-II (23 substances from class II) 2.3 microgram; and subgroup B-IV (two substances from class III) 0.056 microgram/capita/day.

The 49 candidate substances are structurally related to 29 supporting substances, which were evaluated by JEFCA at its 59<sup>th</sup> JECFA meeting (JECFA, 2002c). The total combined daily per capita intakes (in Europe) of candidate and supporting substances in each of the four subgroups for which supporting substances were available are: subgroup A-Ib (18 substances from class II) 22 microgram; subgroup A-Ic (four substances from class III) 0.15 microgram; subgroup A-II (36 substances from class II) 500 microgram and subgroup A-III (two substances from class III) 1.2 microgram/capita/day.

For none of the subgroups did the combined intakes exceed the thresholds of concern for compounds belonging to Cramer structural class II (540 microgram/person/day) or to structural class III (90 microgram/person/day).

The genotoxicity data are limited and that genotoxicity could not be assessed adequately for the flavouring substances in FGE.21Rev1. However, the genotoxicity data available do not preclude the

evaluation of the remaining 49 candidate substances using the Procedure, except for seven substances: [FL-no: 15.114, 15.133, 15.090, 15.099, 15.086, 15.060 and 15.119].

The metabolism data available were insufficient to allow conclusions about the metabolic fate of the candidate substances, and accordingly the 49 candidate substances could not be anticipated to be metabolised to innocuous products. However, the Panel concluded that the evidence of binding to macromolecules from possible formation of electrophilic metabolites, (e.g. by either ring scission or S-oxidation) was not sufficiently strong to preclude the application of the Procedure.

Toxicological data on both candidate and supporting substances were limited and did not include information about chronic or reproductive toxicity. Valid toxicological data which could provide an adequate margin of safety compared to the intakes from use as flavouring substances were only available for the 26 candidate substances from subgroup A-Ic (thiophenes with thiol-containing ring substituents) and subgroup A-II (thiazoles).

For the remaining 23 candidate substances belonging to the subgroups of thiophene itself (A-Ia), thiophenes with non-thiol-containing ring substituents (A-Ib), benzothiazoles (A-III), dihydrothiophenes (B-I), dithiazines (B-IV) and thiadiazines (B-VI) the Panel concluded that there were insufficient data available to provide margins of safety from their use as flavouring substances and that additional toxicity data are needed.

The estimated intakes, based on the mTAMDI, for the 41 candidate substances assigned to structural class II and evaluated using the Procedure range from 78 to 160 microgram/person/day. For 40 candidate substances the mTAMDI values are below the threshold of concern of 540 microgram/person/day for structural class II. For one substance [FL-no: 15.129] the mTAMDI value is 4000 microgram/person/day, which is above the threshold of concern of 540 microgram/person/day for structural class II. The estimated intakes based on the mTAMDI of the eight candidate substances assigned to structural class III and evaluated through the Procedure ranged from 78 to 4000 microgram/person/day. For three of these eight candidate substances, [FL-no: 15.042, 15.055 and 15.088] the estimated intakes are above the threshold of concern for structural class III substances (90 microgram/capita/day), and further information is required. This would include more reliable intake data as well as the additional toxicological data already identified as a consequence of application of the Procedure.

Five of the 56 candidate substances possess one chiral centre [FL-no: 15.060, 15.077, 15.090, 15.099 and 15.119], two substances [FL-no: 15.054 and 15.129] possess two chiral centres and two substances [FL-no: 15.042 and 15.055] possess three chiral centres. For these nine substances the stereoisomeric composition has not been specified.

In order to determine whether the conclusion for the 49 candidate substances which have been evaluated using the Procedure can be applied to the materials of commerce, it is necessary to consider the available specifications. Adequate specifications including purity and identity for the materials of commerce have been provided for 43 of the 49 flavouring substances evaluated through the Procedure. Information on stereoisomerism is incomplete for five of the substances evaluated through the Procedure [FL-no: 15.042, 15.054, 15.055, 15.077, 15.129] and for one substance evaluated through the Procedure [FL-no: 15.129], an identity test has not been provided. Thus, the final evaluation of the materials of commerce cannot be performed for the five substances [FL-no: 15.042, 15.054, 15.055, 15.077 and 15.129], pending further information on stereoisomerism and/or identity test.

For 23 flavouring substances of the 49 flavouring substances evaluated through the Procedure [FL-no: 15.037, 15.040, 15.042, 15.043, 15.045, 15.054, 15.055, 15.064, 15.070, 15.072, 15.074, 15.076, 15.077, 15.088, 15.091, 15.092, 15.093, 15.094, 15.096, 15.097, 15.106, 15.107 and 15.129] the Panel considered that additional toxicity data are needed.

For the remaining 26 flavouring substances of the 49 flavouring substances evaluated through the Procedure the Panel considered there were valid toxicological data providing adequate margins of safety compared to the intakes from use as flavouring substances. Therefore, the following 26 flavouring substances evaluated using the Procedure would present no safety concern at their estimated levels of intake estimated on the basis of the MSDI approach: [FL-no: 15.038, 15.039, 15.044, 15.050, 15.051, 15.052, 15.058, 15.061, 15.062, 15.063, 15.067, 15.068, 15.069, 15.071, 15.078, 15.080, 15.082, 15.084, 15.085, 15.087, 15.089, 15.098, 15.108, 15.115, 15.116 and 15.118].

**TABLE 1: SPECIFICATION SUMMARY OF THE SUBSTANCES IN THE FLAVOURING GROUP EVALUATION 21, REVISION 1**

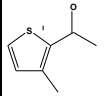
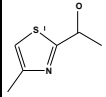
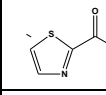
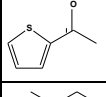
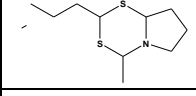
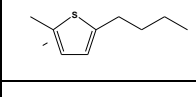
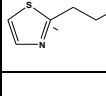
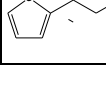
Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 21, revision 1								
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility in water 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
15.037	2-Acetyl-3-methylthiophene		11590 13679-72-6	Liquid C <sub>7</sub> H <sub>8</sub> OS 140.20	Practically insoluble or insoluble 1 ml in 1 ml	216 MS 95 %	1.558-1.564 1.130-1.136	
15.038	2-Acetyl-4-methylthiazole		11589 7533-07-5	Solid C <sub>6</sub> H <sub>7</sub> NOS 141.19	Practically insoluble or insoluble 1 ml in 1 ml	95 (16 hPa) 34 MS 95 %	n.a. n.a.	
15.039	2-Acetyl-5-methylthiazole		59303-17-2	Solid C <sub>6</sub> H <sub>7</sub> NOS 141.19	Practically insoluble or insoluble 1 ml in 1 ml	93 (16 hPa) 30 NMR 95 %	n.a. n.a.	
15.040	2-Acetylthiophene		11728 88-15-3	Solid C <sub>6</sub> H <sub>6</sub> OS 126.17	Practically insoluble or insoluble 1 ml in 1 ml	213 34 MS 98 %	1.563-1.569 1.164-1.171	
15.042	2-Butyl-4-methyl(4H)pyrrolidino[1,2d]-1,3,5-dithiazine (6)		132344-97-9	Solid C <sub>11</sub> H <sub>21</sub> NS <sub>2</sub> 231.42	Practically insoluble or insoluble 1 ml in 1 ml	304 164 NMR 95 %	n.a. n.a.	CASrn in Register does not specify isomer.
15.043	2-Butyl-5-ethylthiophene		11596 54411-06-2	Solid C <sub>10</sub> H <sub>16</sub> S 168.30	Practically insoluble or insoluble 1 ml in 1 ml	231 54 MS 95 %	n.a. n.a.	
15.044	2-Butylthiazole		11597 37645-61-7	Solid C <sub>7</sub> H <sub>11</sub> NS 141.23	Practically insoluble or insoluble 1 ml in 1 ml	212 79 NMR 95 %	n.a. n.a.	
15.045	2-Butylthiophene		1455-20-5	Liquid C <sub>8</sub> H <sub>12</sub> S 140.24	Practically insoluble or insoluble 1 ml in 1 ml	182 MS 95 %	1.504-1.510 0.951-0.957	

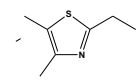
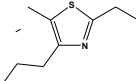
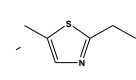
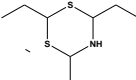
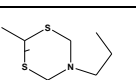
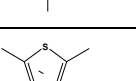
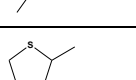
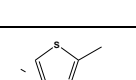
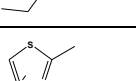
Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 21, revision 1								
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility in water 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
15.050	2,5-Diethyl-4-methylthiazole		41981-71-9	Solid C <sub>8</sub> H <sub>13</sub> NS 155.26	Practically insoluble or insoluble 1 ml in 1 ml	85 (20 hPa) 115 MS 95 %	n.a. n.a.	
15.051	2,5-Diethyl-4-propylthiazole		4276-68-0	Solid C <sub>10</sub> H <sub>17</sub> NS 183.31	Practically insoluble or insoluble 1 ml in 1 ml	72 (21 hPa) 139 NMR 95 %	n.a. n.a.	
15.052	2,5-Diethylthiazole		15729-76-7	Solid C <sub>7</sub> H <sub>11</sub> NS 141.23	Practically insoluble or insoluble 1 ml in 1 ml	187 92 MS 95 %	n.a. n.a.	
15.054	Dihydro-2,4,6-triethyl-1,3,5(4H)- dithiazine 6)		54717-17-8	Solid C <sub>9</sub> H <sub>19</sub> NS <sub>2</sub> 205.38	Practically insoluble or insoluble 1 ml in 1 ml	287 188 MS 95 %	n.a. n.a.	CASrn in Register does not specify isomer.
15.055	2,4-Dimethyl(4H)pyrrolidino[1,2c]- 1,3,5-dithiazine 6)		116505-60-3	Solid C <sub>8</sub> H <sub>15</sub> NS <sub>2</sub> 189.34	Practically insoluble or insoluble 1 ml in 1 ml	235 130 MS 95 %	n.a. n.a.	CASrn in Register refers to 4H- Pyrrolo[2,1-d]-1,3,5-dithiazine, tetrahydro-2,4-dimethyl-, [2S- (2a,4a,8ab)]-.
15.058	4,5-Dimethyl-2-ethylthiazole		873-64-3	Solid C <sub>7</sub> H <sub>11</sub> NS 141.23	Practically insoluble or insoluble 1 ml in 1 ml	185 104 MS 96 %	n.a. n.a.	
15.060	2,4-Dimethyl-3-thiazoline 6)		60755-05-7	Solid C <sub>5</sub> H <sub>9</sub> NS 115.19	Practically insoluble or insoluble 1 ml in 1 ml	52 (15 hPa) 102 MS 95 %	n.a. n.a.	CASrn in Register does not specify isomer.
15.061	2,5-Dimethyl-4-ethylthiazole		32272-57-4	Solid C <sub>7</sub> H <sub>11</sub> NS 141.23	Practically insoluble or insoluble 1 ml in 1 ml	185 104 MS 95 %	n.a. n.a.	
15.062	2,4-Dimethylthiazole		11605 541-58-2	Solid C <sub>5</sub> H <sub>7</sub> NS 113.18	Practically insoluble or insoluble 1 ml in 1 ml	145 69 MS 95 %	n.a. n.a.	











Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 21, revision 1								
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility in water 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
15.063	2,5-Dimethylthiazole		4175-66-0	Solid C <sub>5</sub> H <sub>7</sub> NS 113.18	Practically insoluble or insoluble 1 ml in 1 ml	152 69 MS 95 %	n.a. n.a.	
15.064	2,5-Dimethylthiophene		11609 638-02-8	Liquid C <sub>6</sub> H <sub>6</sub> S 112.19	Practically insoluble or insoluble 1 ml in 1 ml	135 MS 95 %	1.508-1.514 0.982-0.988	
15.067	4-Ethyl-2-methylthiazole		32272-48-3	Solid C <sub>6</sub> H <sub>9</sub> NS 127.20	Practically insoluble or insoluble 1 ml in 1 ml	91 (89 hPa) 80 MS 95 %	n.a. n.a.	
15.068	5-Ethyl-2-methylthiazole		19961-52-5	Solid C <sub>6</sub> H <sub>9</sub> NS 127.20	Practically insoluble or insoluble 1 ml in 1 ml	170 80 MS 95 %	n.a. n.a.	
15.069	4-Ethyl-5-methylthiazole		52414-91-2	Solid C <sub>6</sub> H <sub>9</sub> NS 127.20	Practically insoluble or insoluble 1 ml in 1 ml	174 80 MS 95 %	n.a. n.a.	
15.070	2-Ethyl-5-methylthiophene		40323-88-4	Liquid C <sub>7</sub> H <sub>10</sub> S 126.22	Practically insoluble or insoluble 1 ml in 1 ml	158 MS 97 %	1.503-1.509 0.963-0.970	
15.071	2-Ethylthiazole		15679-09-1	Liquid C <sub>5</sub> H <sub>7</sub> NS 113.18	Practically insoluble or insoluble 1 ml in 1 ml	148 MS 95 %	1.511-1.517 1.058-1.064	
15.072	2-Ethylthiophene		11614 872-55-9	Liquid C <sub>6</sub> H <sub>8</sub> S 112.19	Practically insoluble or insoluble 1 ml in 1 ml	133 MS 95 %	1.510-1.516 0.989-0.995	
15.074	5-Ethylthiophene-2-carbaldehyde		36880-33-8	Solid C <sub>7</sub> H <sub>8</sub> OS 140.20	Practically insoluble or insoluble 1 ml in 1 ml	104 (12 hPa) 62 MS 95 %	n.a. n.a.	
15.076	2-Hexylthiophene		11616 18794-77-9	Solid C <sub>10</sub> H <sub>16</sub> S 168.30	Practically insoluble or insoluble 1 ml in 1 ml	92 (13 hPa) 41 MS 95 %	n.a. n.a.	


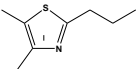
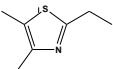

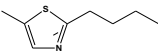




Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 21, revision 1								
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility in water 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
15.077	4-Hydroxy-2,5-dimethylthiophen-3(2H)-one 6)		26494-10-0	Solid C <sub>6</sub> H <sub>8</sub> O <sub>2</sub> S 144.19	Practically insoluble or insoluble 1 ml in 1 ml	296 133 MS 95 %	n.a. n.a.	CASrn in Register does not specify isomer.
15.078	2-Isobutyl-4,5-dimethylthiazole		11617 53498-32-1	Solid C <sub>9</sub> H <sub>13</sub> NS 169.29	Practically insoluble or insoluble 1 ml in 1 ml	267 112 MS 97 %	n.a. n.a.	
15.080	2-Isopropyl-4,5-dimethylthiazole		53498-30-9	Solid C <sub>8</sub> H <sub>13</sub> NS 155.26	Practically insoluble or insoluble 1 ml in 1 ml	244 101 MS 95 %	n.a. n.a.	
15.082	3-Mercaptothiophene		7774-73-4	Liquid C <sub>4</sub> H <sub>4</sub> S <sub>2</sub> 116.20	Slightly soluble 1 ml in 1 ml	171 MS 95 %	1.617-1.623 1.248-1.254	
15.084	5-Methyl-2-pentylthiazole		86290-21-3	Solid C <sub>9</sub> H <sub>15</sub> NS 169.26	Practically insoluble or insoluble 1 ml in 1 ml	262 114 NMR 95 %	n.a. n.a.	
15.085	4-Methyl-2-propionylthiazole		11622 13679-83-9	Solid C <sub>7</sub> H <sub>9</sub> NOS 155.21	Practically insoluble or insoluble 1 ml in 1 ml	86 (12 hPa) 142 NMR 95 %	n.a. n.a.	
15.086	2-Methyl-2-thiazoline		2346-00-1	Solid C <sub>4</sub> H <sub>7</sub> NS 101.17	Slightly soluble 1 ml in 1 ml	144 62 MS 95 %	n.a. n.a.	
15.087	2-Methyl-3-mercaptothiophene		2527-76-6	Solid C <sub>5</sub> H <sub>6</sub> S <sub>2</sub> 130.22	Slightly soluble 1 ml in 1 ml	73 (15 hPa) 27 MS 95 %	n.a. n.a.	
15.088	2-Methyl-4,5-benzothiazole		120-75-2	Liquid C <sub>8</sub> H <sub>7</sub> NS 149.21	Practically insoluble or insoluble 1 ml in 1 ml	238 MS 95 %	1.612-1.618 1.173-1.179	







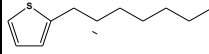
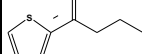
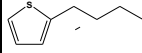



Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 21, revision 1								
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility in water 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
15.089	2-Methylthiazole		11626 3581-87-1	Liquid C <sub>4</sub> H <sub>5</sub> NS 99.15	Slightly soluble 1 ml in 1 ml	128 MS 95 %	1.511-1.517 1.109-1.116	
15.090	2-Methylthiazolidine 6)		24050-16-6	Liquid C <sub>4</sub> H <sub>7</sub> NS 103.18	Slightly soluble 1 ml in 1 ml	160 MS 95 %	1.522-1.528 1.519-1.525	CASrn in Register does not specify isomer.
15.091	2-Methylthiophene		11631 554-14-3	Liquid C <sub>5</sub> H <sub>6</sub> S 98.16	Practically insoluble or insoluble 1 ml in 1 ml	112 MS 97 %	1.516-1.523 1.013-1.022	
15.092	3-Methylthiophene		11632 616-44-4	Liquid C <sub>5</sub> H <sub>6</sub> S 98.16	Practically insoluble or insoluble 1 ml in 1 ml	115 MS 95 %	1.514-1.520 1.018-1.024	
15.093	2-Octylthiophene		880-36-4	Solid C <sub>12</sub> H <sub>20</sub> S 196.35	Practically insoluble or insoluble 1 ml in 1 ml	259 64 MS 95 %	n.a. n.a.	
15.094	2-Pentanoylthiophene		53119-25-8	Solid C <sub>9</sub> H <sub>12</sub> OS 168.26	Practically insoluble or insoluble 1 ml in 1 ml	257 80 MS 95 %	n.a. n.a.	
15.096	sec-Pentylthiophene		11634 4861-58-9	Liquid C <sub>9</sub> H <sub>14</sub> S 154.27	Practically insoluble or insoluble 1 ml in 1 ml	201 MS 95 %	1.495-1.501 0.940-0.946	Register name to be changed to 2-Pentylthiophene (EFFA).
15.097	2-Propionylthiophene		11635 13679-75-9	Solid C <sub>7</sub> H <sub>8</sub> OS 140.20	Practically insoluble or insoluble 1 ml in 1 ml	225 57 MS 95 %	n.a. n.a.	
15.098	2-Propylthiazole		17626-75-4	Solid C <sub>6</sub> H <sub>6</sub> NS 127.20	Practically insoluble or insoluble 1 ml in 1 ml	172 78 MS 95 %	n.a. n.a.	
15.099	2-Propylthiazolidine 6)		24050-10-0	Solid C <sub>6</sub> H <sub>13</sub> NS 131.24	Practically insoluble or insoluble 1 ml in 1 ml	75 (13 hPa) 84 MS 95 %	n.a. n.a.	CASrn in Register does not specify isomer



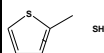
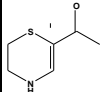
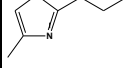
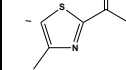
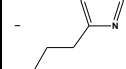
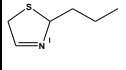
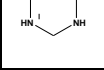

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 21, revision 1								
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility in water 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
15.106	Thiophene		11647 110-02-1	Liquid C <sub>4</sub> H <sub>4</sub> S 84.14	Practically insoluble or insoluble 1 ml in 1 ml	84 MS 95 %	1.525-1.531 1.062-1.068	
15.107	Thiophene-2-carbaldehyde		11874 98-03-3	Liquid C <sub>5</sub> H <sub>4</sub> OS 112.15	Slightly soluble 1 ml in 1 ml	198 MS 95 %	1.585-1.592 1.216-1.225	
15.108	2-Thiophenemethanethiol		6258-63-5	Liquid C <sub>5</sub> H <sub>6</sub> S <sub>2</sub> 130.22	Slightly soluble 1 ml in 1 ml	82 (16 hPa) MS 95 %	1.571-1.578 1.165-1.171	
15.114	5-Acetyl-2,3-dihydro-1,4-thiazine		101417-25-8	Solid C <sub>6</sub> H <sub>9</sub> NOS 143.2	Sparringly soluble 1 ml in 1 ml	242 126 NMR 95 %	n.a. n.a.	Name to be changed to 6-Acetyl-2,3-dihydro-1,4-thiazine.
15.115	2-Isobutyl-4-methyl thiazole		61323-24-8	Solid C <sub>8</sub> H <sub>13</sub> NS 155.26	Slightly soluble 1 ml in 1 ml	189 88 MS 95 %	n.a. n.a.	Name to be changed to 2-Isobutyl-4-methylthiazole.
15.116	2-Acetyl-4-ethylthiazole		233665-91-3	Solid C <sub>7</sub> H <sub>9</sub> NOS 155.22	Slightly soluble 1 ml in 1 ml	270 142 NMR 95 %	n.a. n.a.	
15.118	4-Butylthiazole		53833-33-3	Solid C <sub>7</sub> H <sub>11</sub> NS 141.23	Practically insoluble or insoluble 1 ml in 1 ml	212 79 MS 95 %	n.a. n.a.	
15.119	2-Isobutyl-3-thiazoline 6)		39800-92-5	Solid C <sub>7</sub> H <sub>13</sub> NS 143.25	Very slightly soluble 1 ml in 1 ml	197 80 MS 95 %	n.a. n.a.	CASrn in Register does not specify isomer.
15.129	Tetrahydro-2,4,6-trimethyl-1,3,5(2H)-thiadiazine 6)		53897-63-5	Solid C <sub>6</sub> H <sub>14</sub> N <sub>2</sub> S 146	Soluble Soluble	216-266 (1 hPa) 65-70 > 99 %	n.a. n.a.	ID 7).

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 21, revision 1								
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility in water 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
15.133	5-Acetyl-2,3-dihydro-1,4-thiazine		164524-93-0	Solid C <sub>6</sub> H <sub>9</sub> NOS 143	Soluble Soluble	289+/-30 760 67-69 >98%	n.a. n.a.	ID 7).

1) Solubility in water, if not otherwise stated

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

3) At 1013.25 hPa, if not otherwise stated

4) At 20°C, if not otherwise stated

5) At 25°C, if not otherwise stated

6) Stereoisomeric composition not specified

7) ID: Missing identification test

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





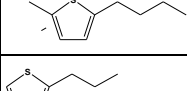




Table 2: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)							
FL-no	EU Register name	Structural formula	MSDI (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [ 4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
15.037	2-Acetyl-3-methylthiophene		0.18	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
15.038	2-Acetyl-4-methylthiazole		0.0049	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.039	2-Acetyl-5-methylthiazole		0.0024	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.040	2-Acetylthiophene		2.2	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
15.043	2-Butyl-5-ethylthiophene		0.0012	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
15.044	2-Butylthiazole		0.011	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.045	2-Butylthiophene		0.012	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
15.050	2,5-Diethyl-4-methylthiazole		0.012	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.051	2,5-Diethyl-4-propylthiazole		0.0012	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	

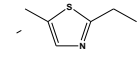
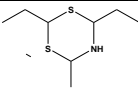

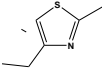



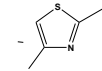
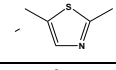



Table 2: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)							
FL-no	EU Register name	Structural formula	MSDI (µg/capita/day) <sup>1)</sup>	Class 2) Evaluation procedure path 3)	Outcome on the named compound [ 4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
15.052	2,5-Diethylthiazole		0.015	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.054	Dihydro-2,4,6-triethyl-1,3,5(4H)-dithiazine		0.0012	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
15.058	4,5-Dimethyl-2-ethylthiazole		0.015	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.061	2,5-Dimethyl-4-ethylthiazole		0.011	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.062	2,4-Dimethylthiazole		0.61	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.063 1758	2,5-Dimethylthiazole		0.0061	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.064	2,5-Dimethylthiophene		0.23	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
15.067	4-Ethyl-2-methylthiazole		0.0037	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.068	5-Ethyl-2-methylthiazole		0.0061	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.069	4-Ethyl-5-methylthiazole		0.012	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.070	2-Ethyl-5-methylthiophene		0.061	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
15.071	2-Ethylthiazole		0.028	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	


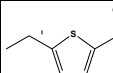
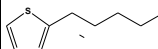
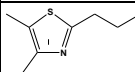
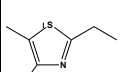
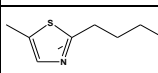




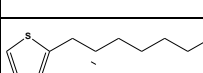
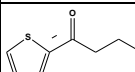
Table 2: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)							
FL-no	EU Register name	Structural formula	MSDI (µg/capita/day) <sup>1)</sup>	Class 2) Evaluation procedure path 3)	Outcome on the named compound [ 4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
15.072	2-Ethylthiophene		0.0012	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
15.074	5-Ethylthiophene-2-carbaldehyde		0.0012	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
15.076 1764	2-Hexylthiophene		0.12	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
15.078	2-Isobutyl-4,5-dimethylthiazole		0.12	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.080	2-Isopropyl-4,5-dimethylthiazole		0.012	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.084	5-Methyl-2-pentylthiazole		0.0037	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.085	4-Methyl-2-propionylthiazole		0.0037	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.089	2-Methylthiazole		0.018	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.091	2-Methylthiophene		0.019	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
15.092	3-Methylthiophene		0.12	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
15.093	2-Octylthiophene		0.012	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
15.094	2-Pentanoylthiophene		0.0012	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		

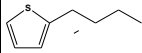



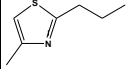
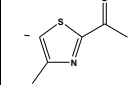
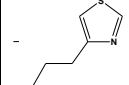




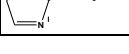
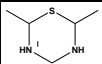
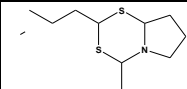
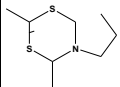






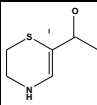

Table 2: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)							
FL-no	EU Register name	Structural formula	MSDI (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [ 4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
15.096	sec-Pentylthiophene		0.24	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
15.097	2-Propionylthiophene		0.12	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
15.098	2-Propylthiazole		0.085	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.107	Thiophene-2-carbaldehyde		0.21	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
15.115	2-Isobutyl-4-methylthiazole		0.011	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.116	2-Acetyl-4-ethylthiazole		0.024	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.118	4-Butylthiazole		1.3	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.060	2,4-Dimethyl-3-thiazoline		0.012	Class II No evaluation			a)
15.086	2-Methyl-2-thiazoline		0.24	Class II No evaluation			a)
15.090	2-Methylthiazolidine		0.024	Class II No evaluation			a)
15.099	2-Propylthiazolidine		0.012	Class II No evaluation			a)
15.119	2-Isobutyl-3-thiazoline		0.011	Class II No evaluation			a)

Table 2: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)							
FL-no	EU Register name	Structural formula	MSDI (µg/capita/day) <sup>1)</sup>	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
15.129	Tetrahydro-2,4,6-trimethyl-1,3,5(2H)-thiadiazine		0.61	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
15.042	2-Butyl-4-methyl(4H)pyrrolidino[1,2d]-1,3,5-dithiazine		0.0012	Class III B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
15.055 1763	2,4-Dimethyl(4H)pyrrolidino[1,2e]-1,3,5-dithiazine		0.055	Class III B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
15.077	4-Hydroxy-2,5-dimethylthiophen-3(2H)-one		0.12	Class III B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
15.082	3-Mercaptothiophene		0.011	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.087	2-Methyl-3-mercaptothiophene		0.12	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.088	2-Methyl-4,5-benzothiazole		0.0085	Class III B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
15.106	Thiophene		0.12	Class III B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
15.108	2-Thiophenemethanethiol		0.0073	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.114	6-Acetyl-2,3-dihydro-1,4-thiazine		0.012	Class III No evaluation			Genotoxic alert. (Register name 5-Acetyl-2,3-dihydro-1,4-thiazine, see Table 1).
15.133 1766	5-Acetyl-2,3-dihydro-1,4-thiazine		0.61	Class III No evaluation			Genotoxic alert.

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) *Genotoxic potential in vitro.*

**TABLE 3: SUPPORTING SUBSTANCES SUMMARY**

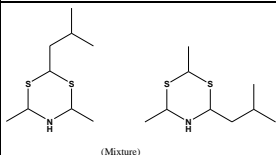
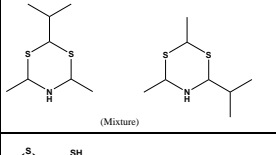

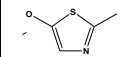
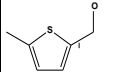
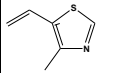
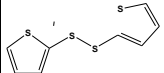
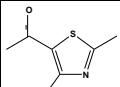
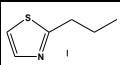
Table 3: Supporting Substances Summary							
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1 (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
	2-Isobutyl-4,6-dimethyl-dihydro-1,3,5-dithiazine and 4-isobutyl-2,6-dimethyl-dihydro-1,3,5-dithiazine (mixture)	 (Mixture)	3781 101517-87-7 101517-86-6	1046 JECFA specification (JECFA, 2002d)	0.1	No safety concern a)	Not in EU-Register.
	2-Isopropyl-4,6-dimethyl-2,6-dimethyl-dihydro-1,3,5-dithiazine and 4-isopropyl-2,6-dimethyl-dihydro-1,3,5-dithiazine (mixture)	 (Mixture)	3782 104691-41-0 104691-40-9	1047 JECFA specification (JECFA, 2002d)	ND	No safety concern a)	Not in EU-Register.
15.001	2-Mercaptothiophene		3062 478 7774-74-5	1052 JECFA specification (JECFA, 2002d)	0.012	No safety concern a) Deleted b)	
15.002	2-Methyl-5-methoxythiazole		3192 736 38205-64-0	1057 JECFA specification (JECFA, 2002d)	ND	No safety concern a) Category B b)	
15.004	5-Methyl-2-thiophenecarbaldehyde		3209 2203 13679-70-4	1050 JECFA specification (JECFA, 2002d)	0.73	No safety concern a) Deleted b)	
15.005	2,4-Dimethyl-5-vinylthiazole		3145 2237 65505-18-2	1039 JECFA specification (JECFA, 2002d)	ND	No safety concern a) Category B b)	
15.008	2-Thienyl disulfide		3323 2333 6911-51-9	1053 JECFA specification (JECFA, 2002d)	ND	No safety concern a) Category B b)	
15.011	5-Acetyl-2,4-dimethylthiazole		3267 2336 38205-60-6	1055 JECFA specification (JECFA, 2002d)	0.012	No safety concern a) Category B b)	
15.013	2-Isobutylthiazole		3134 11618 18640-74-9	1034 JECFA specification (JECFA, 2002d)	3	No safety concern a)	

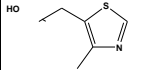
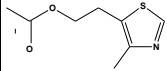
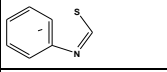
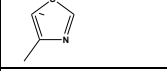
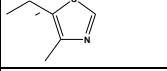
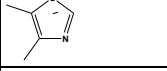
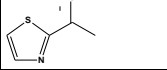
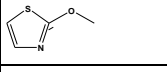
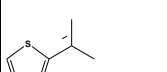
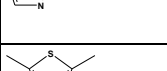
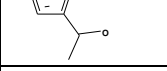
Table 3: Supporting Substances Summary							
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1 (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
15.014	5-(2-Hydroxyethyl)-4-methylthiazole		3204 11621 137-00-8	1031 JECFA specification (JECFA, 2002d)	150	No safety concern a)	
15.015	4-Methyl-5-(2-acetoxyethyl)thiazole		3205 11620 656-53-1	1054 JECFA specification (JECFA, 2002d)	8.6	No safety concern a)	
15.016	Benzothiazole		3256 11594 95-16-9	1040 JECFA specification (JECFA, 2002d)	1.2	No safety concern a)	
15.017	4,5-Dimethylthiazole		3274 11606 3581-91-7	1035 JECFA specification (JECFA, 2002d)	0.18	No safety concern a)	
15.018	4-Methyl-5-vinylthiazole		3313 11633 1759-28-0	1038 JECFA specification (JECFA, 2002d)	2.1	No safety concern a)	
15.019	2,4,5-Trimethylthiazole		3325 11650 13623-11-5	1036 JECFA specification (JECFA, 2002d)	0.61	No safety concern a)	
15.020	2-Acetylthiazole		3328 11726 24295-03-2	1041 JECFA specification (JECFA, 2002d)	9.7	No safety concern a)	
15.021	2-Ethoxythiazole		3340 11611 15679-19-3	1056 JECFA specification (JECFA, 2002d)	0.012	No safety concern a)	
15.022	2-(sec-Butyl)thiazole		3372 11598 18277-27-5	1033 JECFA specification (JECFA, 2002d)	0.024	No safety concern a)	JECFA evaluated 2-(1-methylpropyl)thiazole (CASrn as in Register). (R)- or (S)- enantiomer not specified by CASrn in Register..
15.024	3-Acetyl-2,5-dimethylthiophene		3527 11603 2530-10-1	1051 JECFA specification (JECFA, 2002d)	18	No safety concern a)	
15.026	2-Isopropyl-4-methylthiazole		3555 15679-13-7	1037 JECFA specification (JECFA, 2002d)	19	No safety concern a)	


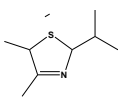
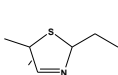
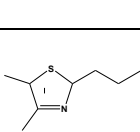


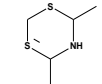
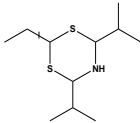
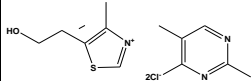
Table 3: Supporting Substances Summary							
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1 (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
15.027	2-Propionylthiazole		3611 43039-98-1	1042 JECFA specification (JECFA, 2002d)	ND	No safety concern a)	
15.029	2-(sec-Butyl)-4,5-dimethyl-3-thiazoline		3619 65894-82-8	1059 JECFA specification (JECFA, 2002d)	ND	No safety concern a)	JECFA evaluated 2-(2-Butyl)-4,5-dimethyl-3-thiazoline (CASrn as in Register). (R)- or (S)-enantiomer not specified by CASrn in Register.
15.030	4,5-Dimethyl-2-ethyl-3-thiazoline		3620 76788-46-0	1058 JECFA specification (JECFA, 2002d)	ND	No safety concern a)	JECFA evaluated 4,5-dimethyl-2-ethyl-3-thiazoline (CASrn as in Register). (R)- or (S)-enantiomer not specified by CASrn in Register.
15.032	4,5-Dimethyl-2-isobutyl-3-thiazoline		3621 65894-83-9	1045 JECFA specification (JECFA, 2002d)	0.012	No safety concern a)	JECFA evaluated 4,5-dimethyl-2-isobutyl-3-thiazoline (CASrn as in Register). (R)- or (S)-enantiomer not specified by CASrn in Register.
15.033	2-Ethyl 4-methylthiazole		3680 11612 15679-12-6	1044 JECFA specification (JECFA, 2002d)	3.2	No safety concern a)	
15.035	4-Methylthiazole		3716 11627 693-95-8	1043 JECFA specification (JECFA, 2002d)	0.097	No safety concern a)	
15.109	2,4,6-Trimethyldihydro-1,3,5(4H)-dithiazine		4018 11649 638-17-5	1049 JECFA specification (JECFA, 2002d)	ND	No safety concern a)	JECFA evaluated 2,4,6-trimethyldihydro-4H-1,3,5-dithiazine (CASrn as in Register). (R)- or (S)-enantiomer not specified by CASrn in Register.
15.113	5,6-Dihydro-2,4,6, tris(2-methylpropyl)4H-1,3,5-dithiazine		74595-94-1	1048 JECFA specification (JECFA, 2002d)	ND	No safety concern a)	JECFA evaluated 2,4,6-triisobutyl-5,6-dihydro-4H-1,3,5-dithiazine (CASrn as in Register). (R)- or (S)-enantiomer not specified by CASrn in Register.

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
16.027	Thiamine hydrochloride		3322 10493 67-03-8	1030 JECFA specification (JECFA, 2002d)	300	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, 2002c).

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, 2000a), named the "Procedure", is shown in schematic form in Figure I.1. The Procedure is based on the Opinion of the Scientific Committee on Food expressed on 2 December 1999 (SCF, 1999), which is derived from the evaluation procedure developed by the Joint FAO/WHO Expert Committee on Food Additives at its 44<sup>th</sup>, 46<sup>th</sup> and 49<sup>th</sup> 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.

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<sup>6</sup> (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<sup>7</sup> (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.

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<sup>6</sup> "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).

<sup>7</sup> "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).

### Procedure for Safety Evaluation of Chemically Defined Flavouring Substances

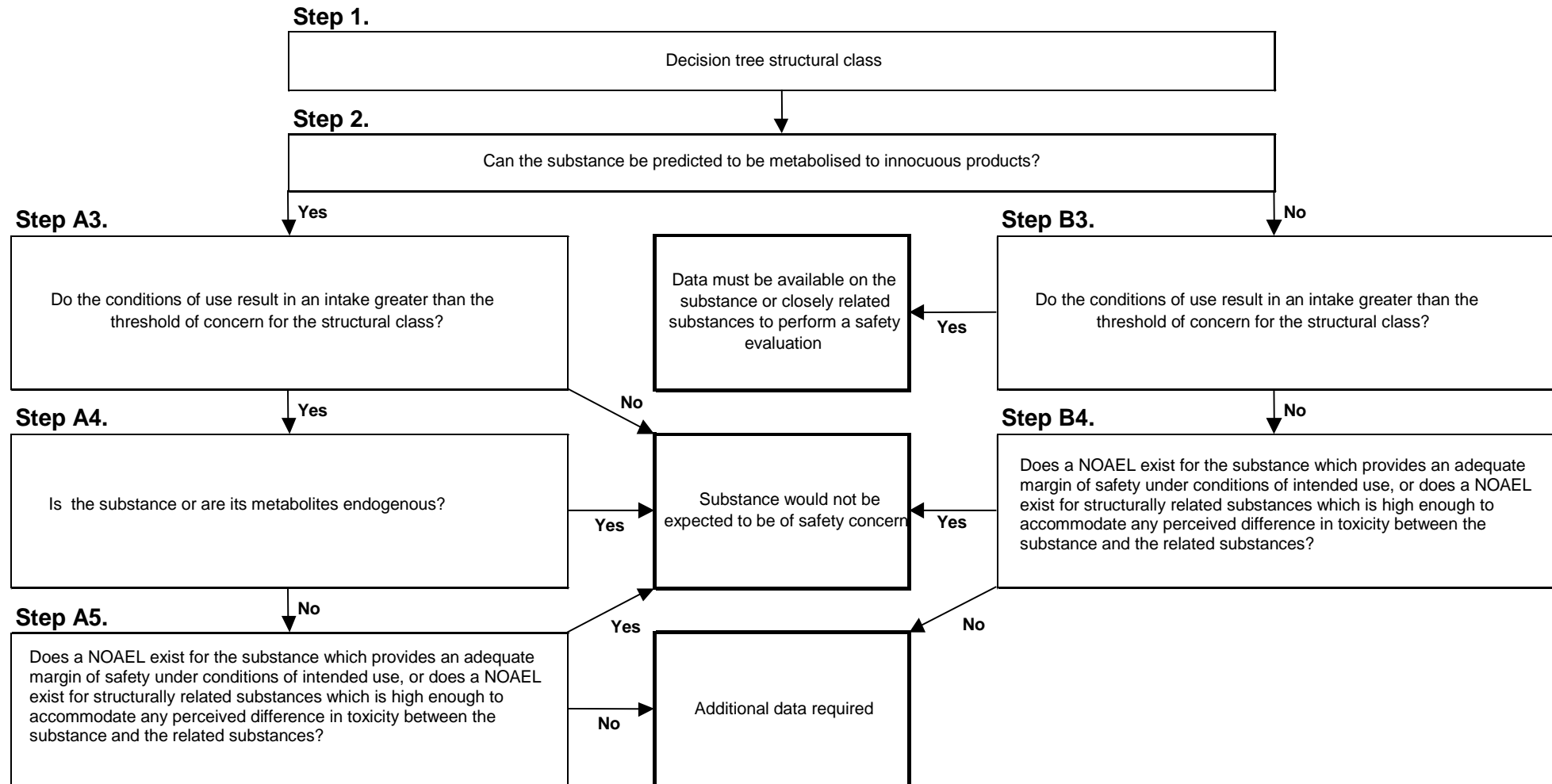


Figure I.1 Procedure for Safety Evaluation of Chemically Defined Flavouring Substances

## ANNEX II: USE LEVELS / mTAMDI

### II.1. Normal and Maximum Use Levels

For each of the 18 Food categories (Table II.1.1) in which the candidate substances are used, Flavour Industry reports a “normal use level” and a “maximum use level” (EC, 2000a; Flavour Industry, 2004-5). According to the Industry the ”normal use” is defined as the average of reported usages and ”maximum use” is defined as the 95<sup>th</sup> 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).

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

The “normal and maximum use levels” are provided by Industry for the 56 candidate substances in the present flavouring group (Table II.1.2).

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
15.037	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
15.038	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
15.039	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	-	-	-	0,2 1	0,4 2	0,2 1	0,4 2	1 5	0,2 1
15.040	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
15.042	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
15.043	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
15.044	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
15.045	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
15.050	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



**Table II.1.2 Normal and Maximum use levels (mg/kg) for candidate substances in FGE.21 (EFFA, 2004g; EFFA, 2004i; EFFA, 2007a; IFF, 2004)**

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
15.051	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
15.052	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
15.054	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
15.055	0,4 2	0,2 1	0,4 2	0,3 1,5	-	0,4 2	0,2 0,4	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
15.058	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	0,1 0,5	0,4 2	1 5	0,2 1
15.060	0,4 2	0,2 1	0,2 1	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
15.061	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
15.062	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	-	1 5	0,2 1
15.063	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
15.064	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
15.067	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
15.068	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	1 5	0,2 1	0,4 2	-	0,2 1
15.069	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
15.070	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
15.071	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
15.072	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
15.074	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
15.076	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
15.077	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
15.078	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
15.080	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
15.082	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
15.084	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
15.085	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
15.086	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
15.087	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
15.088	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
15.089	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	-	-	-	0,4 2	0,2 1	0,2 1	1 5	0,2 1
15.090	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
15.091	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
15.092	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
15.093	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
15.094	0,4 2	0,2 1	-	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
15.096	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
15.097	0,4	0,2	0,4	0,3	-	0,4	0,2	0,4	0,1	0,1	-	-	0,2	0,4	0,2	0,4	1	0,2

**Table II.1.2 Normal and Maximum use levels (mg/kg) for candidate substances in FGE.21 (EFFA, 2004g; EFFA, 2004i; EFFA, 2007a; IFF, 2004)**

FL-no	Food Categories																	
	Normal use levels (mg/kg)																	
	Maximum use levels (mg/kg)																	
	01.0	02.0	03.0	04.1	04.2	05.0	06.0	07.0	08.0	09.0	10.0	11.0	12.0	13.0	14.1	14.2	15.0	16.0
	2	1	2	1,5	-	2	1	2	0,4	0,4	-	-	1	2	1	2	5	1
15.098	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
15.099	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
15.106	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
15.107	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
15.108	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	0,2 1
15.114	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
15.115	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
15.116	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
15.118	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
15.119	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
15.129	0,1 2,5	0,1 2,5	1 25	0,1 2,5	0,1 2,5	10 250	2 50	1 25	0,1 2,5	0,1 2,5	-	-	0,1 2,5	-	10 250	10 250	0,1 2,5	0,1 2,5
15.133	0,1 0,5	0,1 0,5	1 5	0,1 0,5	0,1 0,5	10 50	2 10	1 5	0,1 0,5	0,1 0,5	-	-	0,1 0,5	-	10 50	10 50	0,1 0,5	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 consumes the amount of flavourable foods and beverages listed in Table II.2.1. These consumption estimates are then multiplied by the reported use levels in the different food categories and summed up.

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

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

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

- Beverages (SCF, 1995) correspond to food category 14.1 (EC, 2000a)

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

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

Key	Food categories according to Commission Regulation 1565/2000	Distribution of the seven SCF food categories		
		Food	Beverages	Exceptions
01.0	Dairy products, excluding products of category 02.0	Food		
02.0	Fats and oils, and fat emulsions (type water-in-oil)	Food		
03.0	Edible ices, including sherbet and sorbet	Food		
04.1	Processed fruit	Food		
04.2	Processed vegetables (incl. mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds	Food		
05.0	Confectionery			Exception a
06.0	Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery	Food		
07.0	Bakery wares	Food		
08.0	Meat and meat products, including poultry and game	Food		
09.0	Fish and fish products, including molluscs, crustaceans and echinoderms	Food		
10.0	Eggs and egg products	Food		
11.0	Sweeteners, including honey			Exception a
12.0	Salts, spices, soups, sauces, salads, protein products, etc.			Exception d
13.0	Foodstuffs intended for particular nutritional uses	Food		
14.1	Non-alcoholic ("soft") beverages, excl. dairy products		Beverages	
14.2	Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts			Exception c
15.0	Ready-to-eat savouries			Exception b
16.0	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 01.0 - 15.0	Food		

The mTAMDI values (see Table II.2.3) are presented for all 56 candidate substances in the present flavouring group, for which Industry has provided use and use levels (EFFA, 2004i; Flavour Industry, 2004-5). The mTAMDI values are only given for highest reported normal use levels (see Table II.2.3).

FL-no	EU Register name	mTAMDI (µg/person/day)	Structural class	Threshold of concern (µg/person/day)
15.037	2-Acetyl-3-methylthiophene	78	Class II	540
15.038	2-Acetyl-4-methylthiazole	160	Class II	540
15.039	2-Acetyl-5-methylthiazole	160	Class II	540
15.040	2-Acetylthiophene	78	Class II	540
15.043	2-Butyl-5-ethylthiophene	78	Class II	540
15.044	2-Butylthiazole	160	Class II	540
15.045	2-Butylthiophene	78	Class II	540
15.050	2,5-Diethyl-4-methylthiazole	160	Class II	540
15.051	2,5-Diethyl-4-propylthiazole	160	Class II	540
15.052	2,5-Diethylthiazole	160	Class II	540
15.054	Dihydro-2,4,6-triethyl-1,3,5(4H)-dithiazine	160	Class II	540

**Table II.2.3 Estimated intakes based on the mTAMDI approach**


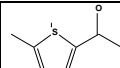
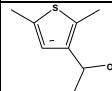
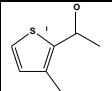
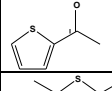
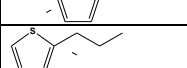
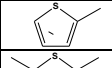
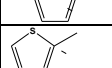
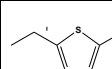
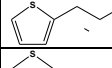
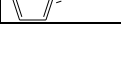

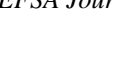
FL-no	EU Register name	mTAMDI (µg/person/day)	Structural class	Threshold of concern (µg/person/day)
15.058	4,5-Dimethyl-2-ethylthiazole	130	Class II	540
15.061	2,5-Dimethyl-4-ethylthiazole	160	Class II	540
15.062	2,4-Dimethylthiazole	140	Class II	540
15.063	2,5-Dimethylthiazole	160	Class II	540
15.064	2,5-Dimethylthiophene	78	Class II	540
15.067	4-Ethyl-2-methylthiazole	160	Class II	540
15.068	5-Ethyl-2-methylthiazole	220	Class II	540
15.069	4-Ethyl-5-methylthiazole	160	Class II	540
15.070	2-Ethyl-5-methylthiophene	78	Class II	540
15.071	2-Ethylthiazole	160	Class II	540
15.072	2-Ethylthiophene	78	Class II	540
15.074	5-Ethylthiophene-2-carbaldehyde	78	Class II	540
15.076	2-Hexylthiophene	78	Class II	540
15.078	2-Isobutyl-4,5-dimethylthiazole	160	Class II	540
15.080	2-Isopropyl-4,5-dimethylthiazole	160	Class II	540
15.084	5-Methyl-2-pentylthiazole	160	Class II	540
15.085	4-Methyl-2-propionylthiazole	160	Class II	540
15.089	2-Methylthiazole	150	Class II	540
15.091	2-Methylthiophene	78	Class II	540
15.092	3-Methylthiophene	78	Class II	540
15.093	2-Octylthiophene	160	Class II	540
15.094	2-Pentanylthiophene	160	Class II	540
15.096	sec-Pentylthiophene	160	Class II	540
15.097	2-Propionylthiophene	160	Class II	540
15.098	2-Propylthiazole	160	Class II	540
15.107	Thiophene-2-carbaldehyde	78	Class II	540
15.115	2-Isobutyl-4-methyl thiazole	160	Class II	540
15.116	2-Acetyl-4-ethylthiazole	160	Class II	540
15.118	4-Butylthiazole	160	Class II	540
15.060	2,4-Dimethyl-3-thiazoline	160	Class II	540
15.086	2-Methyl-2-thiazoline	160	Class II	540
15.090	2-Methylthiazolidine	160	Class II	540
15.099	2-Propylthiazolidine	160	Class II	540
15.119	2-Isobutyl-3-thiazoline	160	Class II	540
15.129	Tetrahydro-2,4,6-trimethyl-1,3,5(2H)-thiadiazine	4000	Class II	540
15.042	2-Butyl-4-methyl(4H)pyrrolidino[1,2d]-1,3,5-dithiazine	160	Class III	90
15.055	2,4-Dimethyl(4H)pyrrolidino[1,2e]-1,3,5-dithiazine	160	Class III	90
15.077	4-Hydroxy-2,5-dimethylthiophen-3(2H)-one	78	Class III	90
15.082	3-Mercaptothiophene	78	Class III	90
15.087	2-Methyl-3-mercaptothiophene	78	Class III	90
15.088	2-Methyl-4,5-benzothiazole	160	Class III	90
15.106	Thiophene	78	Class III	90
15.108	2-Thiophenemethanethiol	78	Class III	90
15.114	5-Acetyl-2,3-dihydro-1,4-thiazine	160	Class III	90
15.133	5-Acetyl-2,3-dihydro-1,4-thiazine	4000	Class III	90

### ANNEX III: METABOLISM


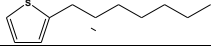
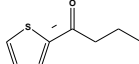
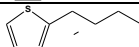



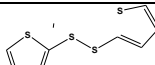



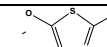

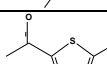
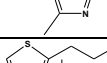
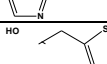
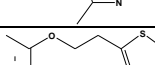




The 56 candidate substances in this FGE are structurally related to the 29 flavouring substances evaluated in “Safety Evaluations of Groups of Related Flavouring Agents: Sulphur-Containing Heterocyclic Compounds” (JECFA, 2002c).

The 56 candidate substances from EU chemical groups 29 and 30 in this FGE include five- and six-membered sulphur-containing aromatic and non-aromatic heterocycles, which have been arranged into 11 subgroups in order to facilitate comparisons of the data sets between the groups. This division was done on the basis of degree of aromaticity and according to the presence of other heteroatoms (i.e. nitrogen). Subgroup A-I has been further divided into three subgroups: A-Ia (thiophene), A-Ib (with alkyl, acyl or carbaldehyde ring substituents) and A-Ic with thiol-containing ring substituents.

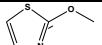
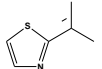
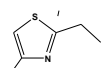
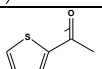
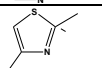
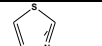
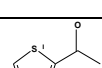
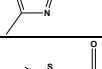
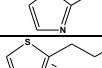
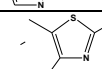
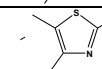
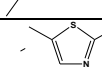
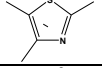
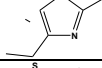
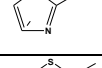
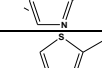
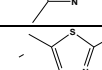
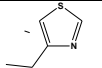
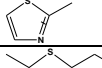
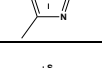
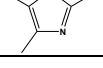

The substances and subgroups are presented in Table III.1:

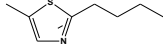
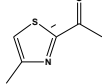


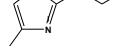
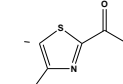
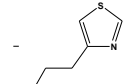
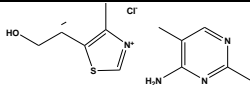



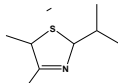
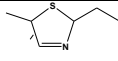
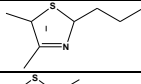


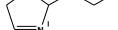

<b>Table III.1 Division of candidate and supporting substances into structurally related subgroups – supporting substances are listed in brackets</b>		
<b>FL-no</b>	<b>EU Register name</b>	<b>Structural formula</b>
<b>A-Ia: Thiophene</b>		
15.106	Thiophene	
<b>A-Ib: Thiophenes: (with non-thiol-containing ring substituents)</b>		
(15.004)	(5-Methyl-2-thiophenecarbaldehyde)	
(15.024)	(3-Acetyl-2,5-dimethylthiophene)	
15.037	2-Acetyl-3-methylthiophene	
15.040	2-Acetylthiophene	
15.043	2-Butyl-5-ethylthiophene	
15.045	2-Butylthiophene	
15.064	2,5-Dimethylthiophene	
15.070	2-Ethyl-5-methylthiophene	
15.072	2-Ethylthiophene	
15.074	5-Ethylthiophene-2-carbaldehyde	
15.076	2-Hexylthiophene	
15.091	2-Methylthiophene	

**Table III.1 Division of candidate and supporting substances into structurally related subgroups – supporting substances are listed in brackets**


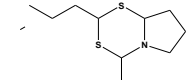
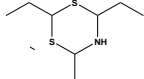
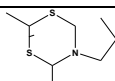
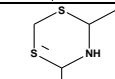
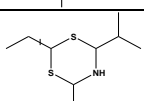
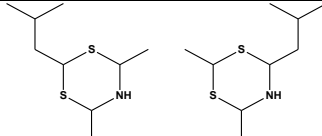
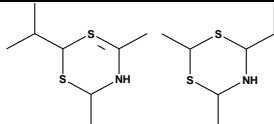



FL-no	EU Register name	Structural formula
15.092	3-Methylthiophene	
15.093	2-Octylthiophene	
15.094	2-Pentanoylthiophene	
15.096	sec-Pentylthiophene	
15.097	2-Propionylthiophene	
15.107	Thiophene-2-carbaldehyde	
<b>A-Ic: Thiophenes: (with thiol-containing ring substituents)</b>		
(15.001)	(2-Mercaptothiophene)	
(15.008)	(2-Thienyl disulfide)	
15.082	3-Mercaptothiophene	
15.087	2-Methyl-3-mercaptothiophene	
15.108	2-Thiophenemethanethiol	
<b>A-II: Thiazoles</b>		
(15.002)	(2-Methyl-5-methoxythiazole)	
(15.005)	(2,4-Dimethyl-5-vinylthiazole)	
(15.011)	(5-Acetyl-2,4-dimethylthiazole)	
(15.013)	(2-Isobutylthiazole)	
(15.014)	(5-(2-Hydroxyethyl)-4-methylthiazole)	
(15.015)	(4-Methyl-5-(2-acetoxyethyl)thiazole)	
(15.017)	(4,5-Dimethylthiazole)	
(15.018)	(4-Methyl-5-vinylthiazole)	
(15.019)	(2,4,5-Trimethylthiazole)	
(15.020)	(2-Acetylthiazole)	

**Table III.1 Division of candidate and supporting substances into structurally related subgroups – supporting substances are listed in brackets**

FL-no	EU Register name	Structural formula
(15.021)	(2-Ethoxythiazole)	
(15.022)	(2-(sec-Butyl)thiazole)	
(15.026)	(2-Isopropyl-4-methylthiazole)	
(15.027)	(2-Propionylthiazol)	
(15.033)	(2-Ethyl 4-methylthiazole)	
(15.035)	(4-Methylthiazole)	
15.038	2-Acetyl-4-methylthiazole	
15.039	2-Acetyl-5-methylthiazole	
15.044	2-Butylthiazole	
15.050	2,5-Diethyl-4-methylthiazole	
15.051	2,5-Diethyl-4-propylthiazole	
15.052	2,5-Diethylthiazole	
15.058	4,5-Dimethyl-2-ethylthiazole	
15.061	2,5-Dimethyl-4-ethylthiazole	
15.062	2,4-Dimethylthiazole	
15.063	2,5-Dimethylthiazole	
15.067	4-Ethyl-2-methylthiazole	
15.068	5-Ethyl-2-methylthiazole	
15.069	4-Ethyl-5-methylthiazole	
15.071	2-Ethylthiazole	
15.078	2-Isobutyl-4,5-dimethylthiazole	
15.080	2-Isopropyl-4,5-dimethylthiazole	

<b>Table III.1 Division of candidate and supporting substances into structurally related subgroups – supporting substances are listed in brackets</b>		
<b>FL-no</b>	<b>EU Register name</b>	<b>Structural formula</b>
15.084	5-Methyl-2-pentylthiazole	
15.085	4-Methyl-2-propionylthiazole	
15.089	2-Methylthiazole	
15.098	2-Propylthiazole	
15.115	2-Isobutyl-4-methylthiazole	
15.116	2-Acetyl-4-ethylthiazole	
15.118	4-Butylthiazole	
(16.027)	(Thiamine hydrochloride)	
<b>A-III: Benzothiazoles</b>		
(15.016)	(Benzothiazole)	
15.088	2-Methyl-4,5-benzothiazole	
<b>B-I : Dihydrothiophenes</b>		
15.077	4-Hydroxy-2,5-dimethylthiophen-3(2H)-one	
<b>B-II: Thiazolines</b>		
(15.029)	(2-(sec-Butyl)-4,5-dimethyl-3-thiazoline)	
(15.030)	(4,5-Dimethyl-2-ethyl-3-thiazoline)	
(15.032)	(4,5-Dimethyl-2-isobutyl-3-thiazoline)	
15.060	2,4-Dimethyl-3-thiazoline	
15.086	2-Methyl-2-thiazoline	
15.119	2-Isobutyl-3-thiazoline	
<b>B-III: Thiazolidines</b>		
15.090	2-Methylthiazolidine	



<b>Table III.1 Division of candidate and supporting substances into structurally related subgroups – supporting substances are listed in brackets</b>		
<b>FL-no</b>	<b>EU Register name</b>	<b>Structural formula</b>
15.099	2-Propylthiazolidine	
<b>B-IV: Dithiazines</b>		
15.042	2-Butyl-4-methyl(4H)pyrrolidino[1,2d]-1,3,5-dithiazine	
15.054	Dihydro-2,4,6-triethyl-1,3,5(4H)-dithiazine	
15.055	2,4-Dimethyl(4H)pyrrolidino[1,2e]-1,3,5-dithiazine	
(15.109)	(2,4,6-Trimethyldihydro-1,3,5(4H)-dithiazine)	
(15.113)	(5,6-Dihydro-2,4,6, tris(2-methylpropyl)4H-1,3,5-dithiazine)	
(Not in EU Register)	(2-Isobutyl-4,6-dimethyldihydro-1,3,5-dithiazine and 4-isobutyl-2,6-dimethyldihydro-1,3,5-dithiazine (mixture))	 <p>(Mixture)</p>
(Not in EU Register)	(2-Isopropyl-4,6-dimethyl 2,6-dimethyldihydro-1,3,5-dithiazine and 4-isopropyl-2,6-dimethyldihydro-1,3,5-dithiazine (mixture))	 <p>(Mixture)</p>
<b>B-V: Dihydrothiazines</b>		
15.114	6-Acetyl-2,3-dihydro-1,4-thiazine (Register name: 5-acetyl-2,3-dihydro-1,4-thiazine)	
15.133	5-Acetyl-2,3-dihydro-1,4-thiazine	
<b>B-VI: thiadiazines</b>		
15.129	Tetrahydro-2,4,6-trimethyl-1,3,5(2H)-thiadiazine	

The following is a description of the characteristic features of the 11 subgroups:

A: Aromatic group:

**Subgroup A-I: Thiophenes** (20 candidate substances and four supporting substances): aromatic heterocyclic five-membered ring substances with a sulphur atom at ring position 1. This group is further divided in three subgroups: A-Ia with thiophene, A-Ib with 16 ring-substituted thiophenes which have one or more alkyl, acyl or carbaldehyde substituents to the ring and subgroup A-Ic with three ring-substituted thiophenes which have a free thiol group in their ring-substituent group.

**Subgroup A-II: Thiazoles** (23 candidate substances and 17 supporting substances): five-membered *aromatic* heterocycles containing one sulphur and one nitrogen atom in the 1- and 3-ring positions, respectively. To this heterocyclic ring one or more alkyl- or acyl-substituents may be attached.

**Subgroup A-III: Benzothiazoles** (one candidate substance and one supporting substance): a bicyclic molecule consisting of a five-membered *aromatic* heterocycle, containing one sulphur and one nitrogen atom in the 1- and 3-ring positions, respectively, with a fused benzene ring at the 4- and 5-ring positions.

B: Non-aromatic group:

**Subgroup B-I: Dihydrothiophenes** (one candidate substance and no supporting substances): heterocyclic five-membered ring substance with a sulphur atom at ring position 1. Only one double bond is present in the ring. The ring bears several substituents.

**Subgroup B-II: Thiazolines** (three candidate substances and three supporting substances): five-membered heterocycles containing a sulphur and a nitrogen atom in the 1- and 3-ring positions, respectively. In the ring, one double bond is present (in contrast to the thiazoles, which have two double bonds).

**Subgroup B-III: Thiazolidines** (two candidate substances and no supporting substances): five-membered heterocycles containing a sulphur and a nitrogen atom in the 1- and 3-ring positions, respectively. In the ring no double bonds are present (*cf.* thiazoles and thiazolines).

**Subgroup B-IV: Dithiazines** (three candidate substances and two supporting and two structurally related substances): six-membered non-aromatic heterocycles containing two sulphur atoms, and one nitrogen atom, with various ring substituents, including a fused second ring in two of these.

**Subgroup B-V: Dihydrothiazines** (two candidate substances and no supporting substances): a six-membered heterocyclic ring, containing a sulphur and a nitrogen atom in para positions, and one double bond. The ring bears an acetyl substituent group.

**Subgroup B-VI: Thiadiazines** (one candidate substance and no supporting substances): six-membered non-aromatic heterocycles containing one sulphur atoms, and two nitrogen atoms in para-positions, with three methyl substituent groups.

### III.1. Absorption, Distribution, and Excretion

Only limited data were submitted for the evaluation of the absorption, distribution and elimination of the candidate flavouring substances in this group. The only candidate substance for which

absorption and elimination were studied is thiophene [FL-no: 15.106]. Data were also submitted for a few more or less related substances. Because the information is so fragmentary, it is not useful to present it (sub)group-wise. In addition, some of the studies are related to oral exposure and some others to parenteral dosing, and the relevance of the latter may be limited.

### ***Candidate substances***

#### ***Thiophene [FL-no: 15.106 ]***

Single doses of 33 mg/kg bw [2,5-<sup>14</sup>C]-thiophene were administered to mice via oral and rectal intubation. At 48 h after the oral gavage dose, 78 % of the radioactivity was found in the urine, while 5 % was found in both faeces and expired air. At 24 h after the rectal dose, 43 % was found in the urine, but 40 % was found in the expired air. The difference in the elimination pattern after oral vs. rectal administration was attributed to an extensive first-pass effect after the oral dose. Also, the oral dose resulted in 10 times higher levels of radioactivity in the liver as compared to the rectal dose. With thin-layer chromatography two urinary metabolites were observed. However, none of these metabolites and none of the radioactivity in tissues or excreta were further identified (Chanal et al., 1974).

Female rats (200 – 250 g) and rabbits (2 – 3 kg) were administered thiophene as single oral gavage doses of 60 mg and 450 mg, respectively. Urine was collected over a 24-hour period following dosing. Table III.2 provides a summary of the quantitative determinations of metabolites in this study.

**Table III.2 Summary of excretion of metabolites of thiophene in rabbits and rats**

<b>Metabolites</b>	<b>Rabbit (N=6)</b>	<b>Rat (N=3)</b>
(Pre)-mercapturic acids in urine	38 (32 – 49)	40 (36 – 45)
Free thiophene expired	Not determined	32 (29 – 35)
Free thiophene in faeces	Not determined	0.48 (0.25 – 0.68)

Tabulated data are percentage of administered dose (%)

Other urinary metabolites, in particular conjugates of 2-hydroxy- or 3-hydroxythiophene, any acid-hydrolysable products of thiophene or free thiophene were not found (Bray et al., 1971).

When male Sprague-Dawley rats were injected intraperitoneally with a 200 mg/kg bw dose of tritiated (at C2 and C5 positions) thiophene in corn oil, approximately 31 % of the radioactivity was excreted between 0 and 15 hours while 4 % of the radioactivity was excreted between 15 and 50 hours. More than 94 % of the urinary radioactivity was accounted for by a single metabolite, the 2-mercapturic acid derivative of 1,4-dihydrothiophene-S-oxide (Dansette et al., 1992).

In rats, a single subcutaneous dose of <sup>35</sup>S-thiophene was rapidly absorbed into the blood stream. Thiophene produced a peak blood concentration within 30 minutes and peak levels in the brain within three hours. Radioactivity was excreted in the urine at 61.1 % of the dose within 34 hours and 74.4 % of the dose within three days (Bikbulatov & Nigmatullina, 1976; O'Donoghue, 2000).

### Non-candidate substances:

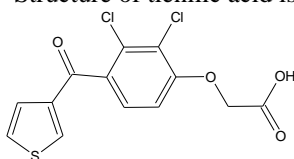
In two male Sprague-Dawley rats, approximately 20 % of the radioactivity of an intraperitoneal injection of 30 mg/kg bw of the 3-arylthiophene (tienilic acid isomer 1)<sup>8</sup> (<sup>14</sup>C at the keto position) is recovered in the urine within 24 hours. Approximately 15 % of urinary radioactivity could be accounted for as a mixture of two diastereoisomers of the mercapturic acid conjugate of the 3-aryl-4,5-dihydrothiophene. The study was designed to identify these two metabolites. The rest of the urinary radioactivity was identified as parent substance (*ca.* 17 % of the dose) and an unidentified metabolite (*ca.* 1 % of the dose), presumably a derivative of one or both of the already identified mercapturates (Valadon et al., 1996).

When the benzothiazole derivative fostedil<sup>9</sup> was administered to dogs via intravenous infusion or orally as solution, suspension or as encapsulated substance, mean relative bioavailabilities for the parent substance after oral dosing could be calculated to be 71, 64 or 42 %, respectively. The substance was cleared from the plasma with a terminal half-life of 6-9 hrs and based on high biliary excretion, enterohepatic circulation has been suggested. When the substance was administered with a radiolabel (no further details) 80 % of the radioactivity was found in the faeces (Thomas, 1984; Thomas & Bopp, 1984).

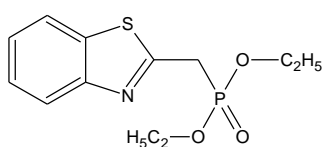
In a previous FGE.24 (EFSA 2006) data on the absorption and elimination of indole<sup>10</sup> have been discussed. As this substance bears some resemblance to the benzothiazoles in subgroup A-III, these data are also briefly mentioned here. After oral dosing of 2-<sup>14</sup>C-indole to rats 62 – 82 % of the administered radioactivity was excreted via urine or expired air within 48 – 72 hrs post-dosing.

The substances in subgroup B-IV (the dithiazines with FL-no: 15.042, 15.054 and 15.055) may be regarded as cyclic thioacetals, which could be subject to acid hydrolysis in the stomach, similar to oxygen-containing acetals. However, thioacetals are more resistant to hydrolysis than oxygen acetals. In addition, chances of hydrolysis would be reduced by their cyclic character even more (Smith & March, 2001; EFSA, 2004d). It is thus anticipated that these substances may be expected to reach the intestinal lumen intact and may also be absorbed as such.

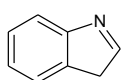
<sup>8</sup> Structure of tienilic acid isomer 1:



<sup>9</sup> Structure of fostedil:



<sup>10</sup> Structure of indole:



## III.2. Metabolism

In the sections below, the metabolism data available have been presented according to the division of the candidate flavouring substances in this FGE into subgroups.

### III.3.1. Aromatic candidate substances

#### ***Subgroup A-Ia thiophene and A-Ib thiophenes with non-thiol-containing ring substituents***

(candidate substances [FL-no: 15.037, 15.040, 15.043, 15.045, 15.064, 15.070, 15.072, 15.074, 15.076, 15.091, 15.092, 15.093, 15.094, 15.096, 15.097, 15.106 and 15.107])

#### ***Candidate substances:***

Thiophene [FL-no: 15.106]

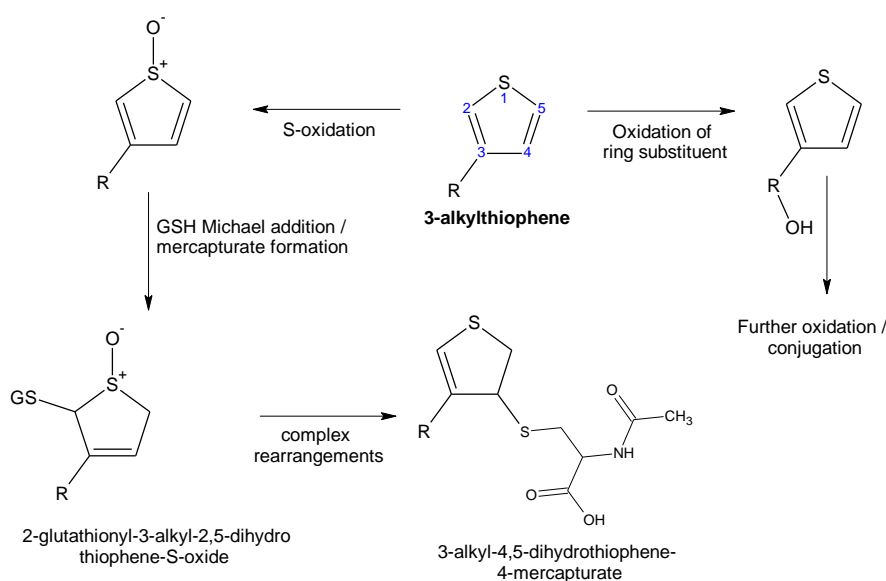
Thiophene was administered to female rats (200 – 250 g) and rabbits (2 – 3 kg) as single oral gavage doses of 60 mg and 450 mg, respectively. Urine was collected over a 24-hour period following dosing. The predominant metabolites excreted in the urine were a mercapturic acid and a premercapturic acid, which according to the study authors could result from the conjugation of glutathione with an intermediate 2,3-thiophene-epoxide. 2-Thienylmercapturic acid was excreted in relatively small quantities and 3-hydroxy-2,3-dihydro-2-thienyl-mercapturic acid was the major metabolite excreted in urine. The metabolites were identified by IR spectroscopy, GLC and MS techniques and further by characteristic chemical reactions of thiophene derivatives (desulphuration following treatment of the premercapturic acid with Raney nickel resulting in the formation of 2-butanol) (Bray et al., 1971).

More recently, male Wistar rats (190 – 400 g) were injected intraperitoneally with 0.2 mmol (= ~ 17 mg) thiophene in peanut oil, and mercapturic acids were not found to be important thioethers (i.e. comprising not more than about 1 % of the dose) excreted in the urine (Hickman et al., 1992), contrary to the results of Bray et al. (1971). It is noted that 2-thienyl mercapturic acid was also identified as a less important metabolite by Bray et al. (1971) and that the two studies differ in route of administration. The oral route of administration (Bray et al., 1971) results in higher metabolic conversion as compared to the intraperitoneal one (Hickman et al., 1992). In addition, identification of thioethers by Hickman et al. (1992) was only done by reaction with Ellman's reagent following extraction and hydrolysis of the urine samples. No attempts were made to identify any other thiophene metabolite in urine samples.

When male Sprague-Dawley rats were injected (intraperitoneal) with 200 mg/kg bw [2,5-<sup>3</sup>H]-labelled thiophene, greater than 94 % of the urinary radioactivity was accounted for by the 2-mercapturic acid derivative of 2,5-dihydrothiophene-S-oxide, as identified by NMR, IR and Mass spectroscopical techniques. The authors concluded that its formation occurred by S-oxidation yielding the very reactive thiophene sulfoxide, which undergoes Michael-type addition of glutathione at the 2-position. In subsequent reactions the glutathione moiety of this metabolite is converted into an N-acetyl cysteine group. The final metabolite is subsequently excreted via urine, and may be converted spontaneously into 2-thienyl-mercapturic acid. (Dansette et al., 1992). The authors considered the formation of the epoxide intermediate, as suggested by Bray et al. (1971) highly unlikely.

### Non-candidate substances:

In two male Sprague-Dawley rats injected i.p. with the 3-arylthiophene tienilic acid isomer 1 (see above), 15 % of urinary radioactivity (~ 2 % of the dose) could be accounted for as a mixture of two diastereoisomers of the mercapturic acid conjugate of 3-aryl-4,5-dihydrothiophene. In the ultimate urinary metabolite, the *N*-acetylcysteine residue (mercapturate) is found in to the 4-position of the thiophene ring. This residue may be introduced at that position after initial Michael-type addition of glutathione to the primary thiophene sulphoxide metabolite (see Figure III.1) at the ring position 2 (i.e. the C next to the S atom) and several rearrangements, including ring opening / closing and reaction with second GSH molecules. Results of *in vitro* experiments with rat liver microsomes indicate that the thiophene sulphoxide is a reactive intermediate in the conversion of the 3-arylthiophene to the dihydromercapturic acid metabolite (Valadon et al., 1996).



**Figure III.1.** Proposed metabolism of 3-alkylthiophenes

### Subgroup A-Ic: Thiophenes with a thiol group in the ring substituent

Candidate substances [FL-no: 15.082, 15.108 and 15.087]

There is no specific information on any of the candidate substances in this subgroup, or on structurally related thiophene derivatives. It may be anticipated that some of the metabolic conversions described above for subgroups A-Ib and c may also apply to this subgroup.

In addition, the mercapto-group (thiol) in the ring-substituent chain may undergo *S*-methylation to produce the corresponding methyl thioether or sulphide, with further oxidation to the corresponding sulphoxide and sulphone. They also may react with glutathione or other endogenous thiol substances to form mixed disulphides, which may undergo reduction to thiols, or oxidative desulphuration. Alternatively, they may undergo enzymatic oxygenation resulting in the formation of the corresponding sulphinic or sulphonic acid. All these metabolites are expected to be excreted in the urine. A more detailed discussion on the metabolism of sulphur compounds may be found in an other EFSA-AFC opinion on other sulphur-containing flavouring substances (EFSA, 2005c).

### Additional considerations for the candidate substances in Subgroup A-I

No information was submitted that would indicate whether side chain oxidation of the alkyl-substituted thiophenes or keto-reduction of the acyl-substituted thiophenes could occur. Such reactions may be anticipated (e.g. omega or omega-1 oxidations). Similar reactions have been discussed for alkylated furane and pyrazines in previous opinions (EFSA, 2005c; EFSA, 2005d). In addition, the two aldehyde candidate substances [FL-no: 15.107 and 15.074] may be expected to be oxidised to the corresponding carboxylic acids. Where applicable, conjugation with amino acids (e.g. glycine) or glucuronic acid may also be expected to occur (EFSA, 2005c; EFSA, 2005d; EFSA, 2005e).

Rance (1989) has indicated that *unsubstituted* thiophene may be subject to S-oxidation and ring substitution. It was stated that S-oxides of thiophene are highly reactive. In this review several studies have been quoted with C2 substituted thiophene derivatives (in particular the pharmaceuticals tienilic acid, morantel and pyrantel) for which it was demonstrated that ring C5 hydroxylation of these substituted thiophenes may occur to a considerable extent. In contrast C2, C5 disubstituted thiophenes are less susceptible to ring hydroxylation (Rance, 1989).

### Subgroup A-II: Thiazoles

Candidate substances [FL-no: 15.089, 15.071, 15.098, 15.044, 15.118, 15.062, 15.063, 15.068, 15.067, 15.069, 15.052, 15.084, 15.058, 15.061, 15.050, 15.051, 15.115, 15.078, 15.080, 15.038, 15.039, 15.116 and 15.085]

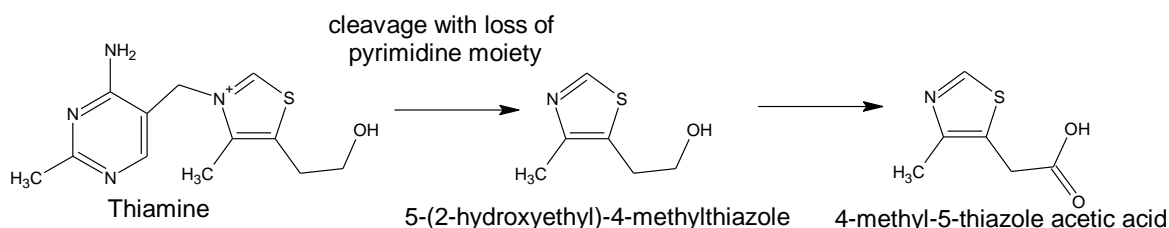
No specific information was available for any of the candidate substances in this subgroup.

### Non-candidate substances:

Thiamine hydrochloride [FL-no: 16.027] and 5-(2-hydroxyethyl)-4-methylthiazole [FL-no: 15.014]

Thiamine (see Figure III.2) is excreted in the urine as unchanged substance but also in the form of 25 – 30 metabolites, some of which still contain the pyrimidine moiety, while other metabolites are generated as a result of molecular cleavage.

In fish and micro-organisms, breakdown of thiamine is catalysed by two enzymes, namely thiaminase I and thiaminase II (Tanphaichitr, 1976; Tietz, 1986a). Based on comparative studies in which rodents were given thiamine intravenously or orally, it has been demonstrated that in the gastrointestinal tract thiamine may already be split into a pyrimidine and a thiazole derivative, namely 5-(2-hydroxyethyl)-4-methylthiazole, which together with its oxidation product (4-methylthiazole-5-acetic acid) and parent thiamine have been identified in urine. The fate of the pyrimidine derivative will not be further discussed. The relevance of the intraintestinal cleavage may be different among various animal species (Informatics Inc., 1974).



**Figure III.2.** Metabolism of thiamine and 5-(2-hydroxyethyl)-4-methylthiazole

### *Chlormethiazole*

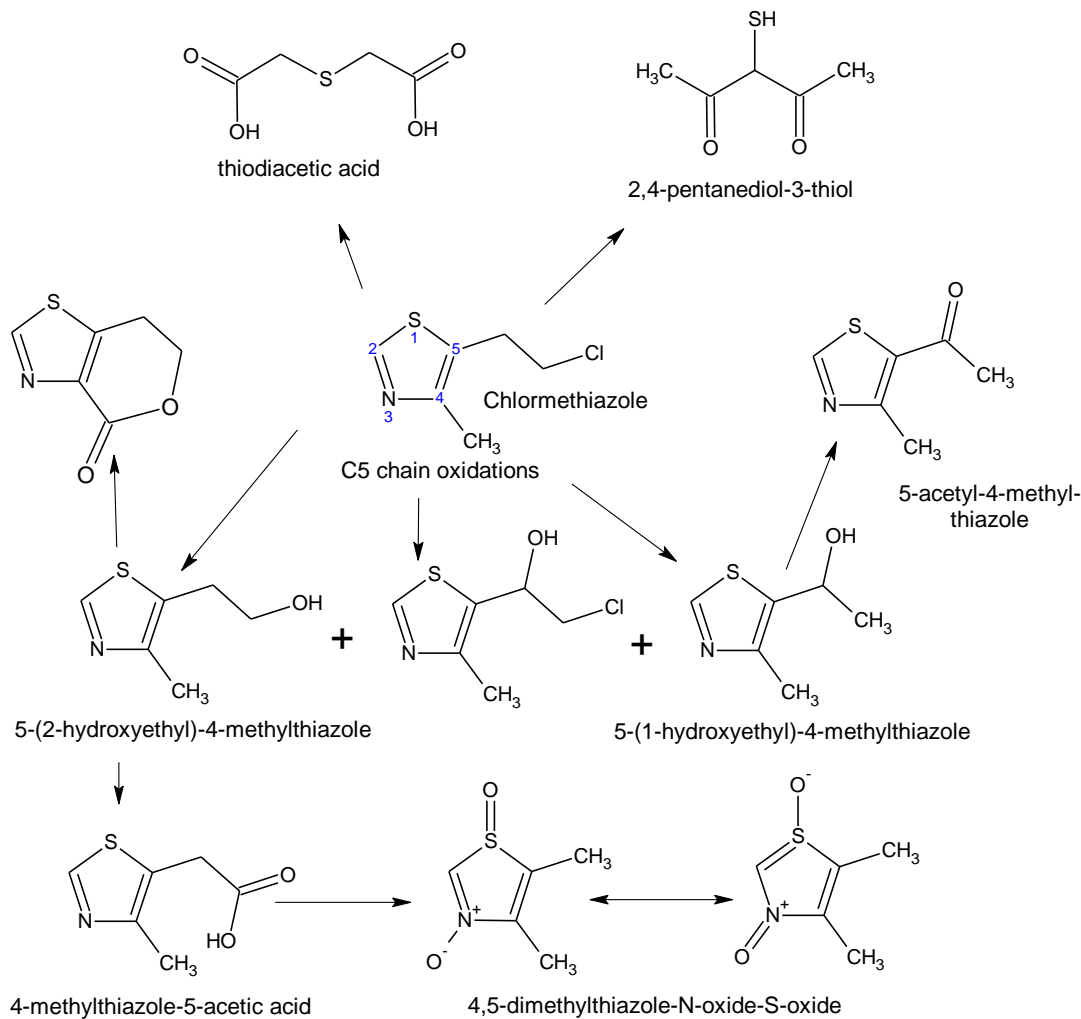
The therapeutic agent chlormethiazole (5-(2-chloroethyl)-4-methylthiazole; see Figure III.3), is an alkyl- and chloroalkyl-substituted thiazole derivative that is metabolised by C-oxidation of the alkyl- and chloroalkyl-substituents and by S- and N-oxidation. In male humans, a 1000 mg oral dose of chlormethiazole is metabolised *via* side-chain C-oxidation of the ethyl C1-position to yield 5-(1-hydroxyethyl)-4-methylthiazole, 5-acetyl-4-methylthiazole, 5-(1-hydroxy-2-chloroethyl)-4-methylthiazole, 4,5-dimethylthiazole and 4-methylthiazole-5-acetic acid, which is the major C-oxidation urinary metabolite (see Figure III.3). Presumably, the 5-methyl derivative is formed *via* decarboxylation of the 5-acetic acid metabolite. Additionally, oxidation of the methyl group at C4 in 5-(2-hydroxyethyl)-4-methylthiazole yields 5-(2-hydroxyethyl)-4-thiazolecarboxylic acid lactone.

Separate ring sulphoxide and ring N-oxide products were not identified, but a combined ring S- and N-oxide of a side-chain oxidised metabolite was also eliminated in the 12-hour urine of volunteers. This was the first reported example of a sulphoxidation of a thiazole sulphur and simultaneous oxidation of two different heteroatoms in the same heteroaromatic ring *in vivo* (Offen et al., 1985).

Moore et al. (1975) reported the excretion of urinary metabolites of chlormethiazole after oral administration of this anticonvulsant drug (in the form of its unstable chlormethiazole ethanedisulphonate derivative) to humans. The substance was given in capsules to three volunteers (sex unknown) in an amount of 768 mg. Urinary samples were collected for up to 36 hours post-dosing and processed for the identification and quantification of metabolites by GS-MS techniques. Results of urinalysis were compared to results obtained with reference substances. After oral administration about 16 % of the dose could be identified in the 36 hr urine as 5 substances; parent chlormethiazole (< 0.01 % of the dose), 5-acetyl-4-methylthiazole, 5-(1-hydroxyethyl)-4-methylthiazole (free and as unspecified conjugate) and 4-methylthiazole-5-acetic acid (> 75 % of all metabolites identified). 5-(2-Hydroxyethyl)-4-methylthiazole was only found in trace amounts. In addition, a metabolite was tentatively identified as 4-methyl-5-thiazole-acetaldehyde. The authors noted that at that time 80 % of the dose was still unaccounted for, which might indicate extensive metabolism of the substance in humans (Moore et al., 1975).

It has also been reported that chlormethiazole undergoes nucleophilic attack at C2, which would ultimately result in ring opening. The exact mechanism for the formation of ring opening products has not been well-described/identified, but the products are found in human urine nonetheless. It was suggested that N-oxidation followed by glutathione conjugation and subsequent glutathione break-down would yield the 2-thiomethyl derivative of chlormethiazole, whereas hydroxide ion attack would yield 2,4-pentanedione-3-thiol and thiodiacetic acid. The authors suggest that the N-oxidation would make the C2 prone to nucleophilic attack (Grupe & Spiteller, 1982; Pal & Spiteller, 1982). According to data from Grupe & Spiteller (1982), urinary excretion of thiodiacetic acid may comprise up to about 5 % of the dose of chlormethiazole in humans. For the other metabolites (see Figure III.3) no quantitative data were provided.





**Figure III.3.** Metabolism of Chlormethiazole.

Herbertz et al. (1973) studied the metabolism of (2-<sup>14</sup>C)-chlormethiazole-ethane disulphonate in isolated recycling-perfused rat liver. After 2 h, 35 % of the system's radioactivity was found in the bile, which activity was associated with a polar metabolite, not observed in previous *in vivo* studies in urine. This metabolite was identified as a mixture of N,4-dimethyl-5-thiazole-acetic acid, 2-hydroxy-N,4-dimethyl-5-thiazole-acetic acid and the glycine conjugate of the latter. Other substances in the perfusate were chlormethiazole, 2-hydroxy-4-methyl-5-(2-chloroethyl)thiazole, 4-methyl-5-(2-hydroxyethyl)thiazole, 4-methyl-5-thiazole-acetic acid, 2-hydroxy-4-methyl-5-thiazole-acetic acid and its glycine conjugate (Herbertz et al., 1973).

*In vivo* the urine was identified as the primary route of elimination (70 % of the dose within 3 hrs; in a study by Allgen et al., 1963; cited by Herbertz et al., 1973), with no formation of N-methylated products. Hence, it was speculated that *in vivo* enterohepatic recycling of chlormethiazole-metabolites would occur, in particular of the N-methylated substances, which would be demethylated in the gastro-intestinal tract and subsequently be excreted via the kidneys. It was noted that the difference between the observations *in vivo* and the perfused system may have occurred because of the build-up of (secondary) metabolite levels in the (closed) perfused system (Herbertz et al., 1973).

Mizutani et al. (1993) investigated the relationship between the toxicological profile of several thiazole derivatives and the toxicity of the ring cleavage products in particular the thioamide metabolites (see Figure III.4). The various thiazoles were nephrotoxic *in vivo* in GSH-depleted mice, which toxicity decreased upon substitution at the ring 4 and 5 carbons. The larger the substituents the less nephrotoxic the substances appeared to be. In addition to being nephrotoxic, some of the thiazoles studied were also hepatotoxic. In the ring C4- and C5-substituted derivatives, ring substitution at the 2 position carbon (the one between the S and the N atom) markedly reduced the nephrotoxicity, but the hepatotoxicity was maintained, except when the substituted was a hydroxyl group (e.g. 2-hydroxy-4-methylthiazole). It was also demonstrated that thioformamide was both nephrotoxic and hepatotoxic, but only in GSH-depleted animals. Thioamides from ring C2 substituted thiazoles appeared to be only hepatotoxic, both after GSH-depletion and in normal animals, although in the normal animals the hepatotoxicity was less pronounced (Mizutani et al., 1993). Thus the toxicity appears to be associated with GSH-depletion, which is a high-dose phenomenon.

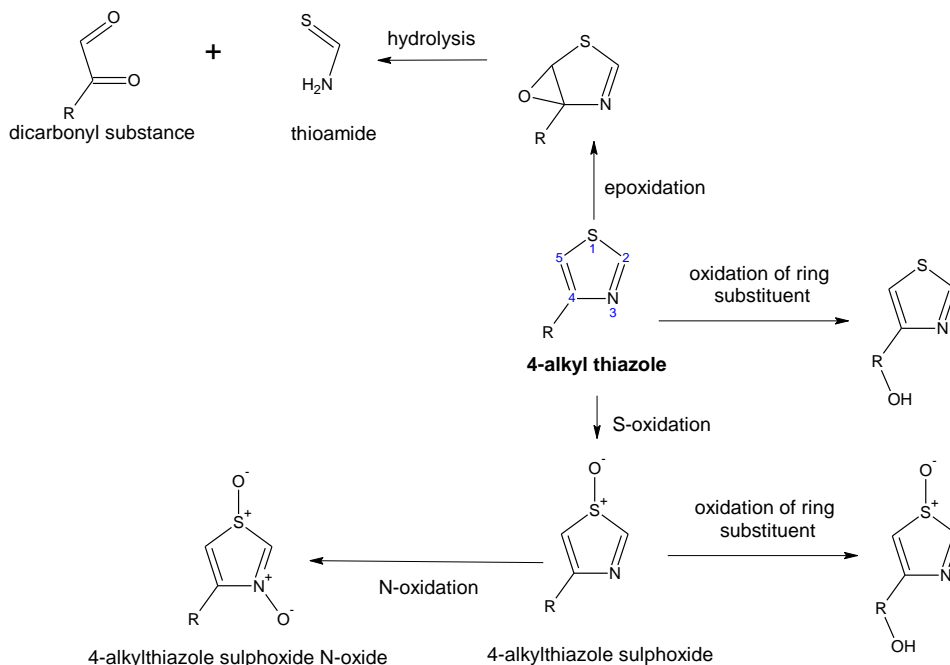
In additional studies again with mice, administration of another non-flavouring thiazole derivative with known hepatotoxic and nephrotoxic properties, 4-*t*-butyl-2-methylthiazole, at a dose of 312 mg/kg bw by gavage resulted in limited ring C-oxidation. Only 0.25 % of the administered dose was recovered as a ring fragmentation product (3,3-dimethyl-2-oxobutanal; an alpha-dicarbonyl metabolite) in the urine within 24 hours. In addition a thioamide metabolite (thioacetamide) was found; but this was not quantified. Other metabolites were not studied. Based on the structure of the dicarbonyl fragment, it was postulated that the 4,5-double bond of the thiazole ring undergoes epoxidation followed by hydrolysis to yield 4,5-diol. The diol then undergoes hydrolytic cleavage to yield the corresponding carbonyl derivatives and thioacetamide. Similar thiazole ring opening has also been observed with thiabendazole (= 2-(thiazol-4-yl)benzimidazole) and with 2-(*p*-methoxyphenyl)-4-methylthiazole (Mizutani et al., 1994).

#### *Additional information for candidate substances in Subgroup A-II*

In an extensive review on the metabolism of heterocyclic substances, Rance (1989) indicated that several alternative metabolic pathways are available to thiazoles. One involves oxidation of S to form the corresponding sulphoxide and sulphone. Electrophilic attack at the ring N (resulting in quaternisation, e.g. N-methylation) is stated to be even more preferential than S-oxidation. The C2 carbon atom is electron deficient and substituents at this position are reactive and subject to nucleophilic displacement, in particular if the ring N is quaternised. Another pathway involves electrophilic attack at ring carbons, preferably C5. The C5 hydroxythiazoles exist as two tautomeric forms. C5-hydroxylation, however, is quantitatively unimportant because thiazoles do not readily undergo electrophilic substitution reactions (Rance, 1989).

Alkyl- and acyl-substituted thiazole derivatives can be metabolised via side-chain oxidation and ring S- and N-oxidation (Dalvie et al., 2002). The major metabolites are readily excreted in the urine either free or as glutathione conjugates. In addition, it may be expected that the presence of alkyl- and acyl-substituents at the thiazole ring increases the number of metabolic options. It has also been demonstrated that to a very small extent ring C-oxidation may be followed by ring cleavage to yield alpha-diketone and thioamide fragments (see Figure III.4). The acetyl-substituted thiazoles [FL-no: 15.038, 15.039 and 15.116] may also be expected to undergo keto-reduction, possibly followed by conjugation, similar to acetyl derivatives of furane (EFSA, 2005c) and pyrazine (EFSA, 2005d). However, there is very limited information to evaluate the extent of metabolism of ring substituent

groups, and it is also very difficult, based on the information available to assess how the presence of ring substituents and their positions at the ring might influence the metabolism of the ring itself.



**Figure III.4.** Metabolism of 4-alkylthiazols

### Subgroup A-III: Benzothiazoles

#### 2-Methyl-4,5-benzothiazole [FL-no: 15.088]

No studies are available on the candidate substance in this group. However, there are some data on another (more complex) benzothiazole derivative and on benzothiazole [FL-no: 15.016]. The data are presented below:

Benzothiazole is primarily metabolised in guinea pigs by thiazole ring cleavage. A 30 mg/kg bw dose administered by intraperitoneal injection is metabolised to free or conjugated forms of *o*-aminophenyl methyl sulphide, *o*-aminophenyl methyl sulfoxide, and *o*-aminophenyl methyl sulphone. Small amounts of the *N*-hydroxy derivatives of the sulfoxide and sulphone were also detected (Wilson et al., 1991).

If substitution is present at the 2-position of the thiazole ring, depending on the nature of the substituent, hydroxylation of the benzene ring may be observed. In dogs, the calcium channel blocker fostedil (i.e. (4-(2-benzothiazolyl)phenyl) methylphosphonic acid diethyl ester), a 2-substituted benzothiazole, has been claimed to undergo benzene ring hydroxylation mainly at C5, C6 and C7, but experimental details were not available (Thomas, 1984). It is not clear if benzene ring hydroxylation would also apply to the one candidate substance in this group.

### III.3.2. Non-aromatic candidate substances

#### ***Subgroup B-I: Dihydrothiophenes***

*4-Hydroxy-2,5-dimethylthiophen-3(2H)-one [FL-no: 15.077]*

No data were available for the non-aromatic dihydrothiophene candidate substance [FL-no: 15.077].

Rance (1984) has drafted some general rules for the prediction of metabolism of sulphur-heterocycles. One of these rules is that with increasing level of ring saturation, the possibilities for S-oxygenation leading to sulphoxides and sulphones, will increase due to the lower effect of aromatic stabilisation, which might be relevant for the prediction of the metabolism of this candidate flavouring substance (Rance, 1989).

#### ***Subgroups B-II, B-III, B-IV, B-V and B-VI: Thiazoline, Thiazolidine, Dithiazine, Dihydrothiazine and Thiadiazine derivatives***

*Candidate substances [FL-no: 15.086, 15.060 and 15.119] (thiazolines from subgroup B-II),*

*Candidate substances [FL-no: 15.090 and 15.099] (thiazolidines from subgroup B-III),*

*Candidate substances [FL-no: 15.054, 15.055 and 15.042] (dithiazines from subgroup B-IV),*

*Candidate substances [FL-no: 15.114 and 15.133] (dihydrothiazines from subgroup B-V)*

*Candidate substance [FL-no: 15.129] (a thiadiazine from subgroup B-VI)*

No specific information was available to evaluate the metabolism of any of the candidate substances mentioned in these four subgroups (B-II, B-III, B-IV, B-V and B-VI).

It may be speculated that the substances in these chemical groups which contain a partially or completely reduced thiazole, a dithiazine or a reduced thiazine ring, are metabolised primarily by oxidation of the ring S or via N-oxidation, similar to the aromatic thiazole compounds. In addition, metabolism of the ring substituents is likely to occur.

### **III.3. Conclusions**

The 56 candidate substances in this FGE are structurally related to 29 flavouring substances evaluated by JECFA in "Safety Evaluations of Groups of Related Flavouring Agents: Sulphur-Containing Heterocyclic Compounds". The 56 candidate and 29 supporting substances and two structurally related substances in this group were subdivided into 11 main subgroups based on the nature of the ring (aromatic (clustered in subgroups A-I to A-III) vs. non-aromatic (clustered in subgroups B-I to B-VI)), type and number of ring heteroatoms (sulphur or sulphur with nitrogen), and the degree of saturation in the non-aromatic rings.

For the evaluation of the metabolism of the candidate substances in this group, only very limited data were available. These were confined to a few references on thiophene, and some thiophene-, thiazole- and benzothiazole-derivatives (i.e. only directly relevant for the evaluation of subgroups-A; the aromatic candidate substances). Other information was found in several review papers. Virtually no data were found on the possible metabolism of the non-aromatic ring structures in the B-subgroups.

### III.3.3. A-subgroups (substances with aromatic ring structures)

Very few data are available on absorption, distribution and excretion. Some of the studies are related to oral exposure and some others to parenteral dosing, and the relevance of the latter may be limited. In mice, a thiazolobenzimidazole derivative was rapidly absorbed after oral dosing, *S*-oxidised, and eliminated from plasma. The candidate substance thiophene has been shown to be absorbed rapidly from the gastro-intestinal tract and eliminated *via* urine as metabolites, or *via* the lungs. Also the thiazole derivative fostedil is absorbed from the gastro-intestinal tract (42-71 % of the dose), metabolised and eliminated with a plasma half-life of about 7-8 hrs in dogs. Hence, from the data on absorption and elimination that are available, for the aromatic candidate substances in this FGE, it may be speculated that most of them will be fairly well absorbed and eliminated after biotransformation. Some volatile substances may also be eliminated *via* the lungs. For the non-aromatic candidate substances no information is available.

From the available metabolism data, it may be anticipated that the sulphur- and nitrogen containing heterocyclic and heteroaromatic derivatives participate in metabolic pathways, principally involving side-chain *C*-oxidation, oxidation of the ring *S* and ring *N* to yield sulphoxide or sulphones and *N*-oxides, respectively. Sulphoxide metabolites may conjugate with glutathione. As far as studied, ring *C*-oxidation may to a very minor extent be accompanied by heterocyclic ring cleavage, which for thiazoles could result in the formation of reactive thioamide intermediates. In addition to this cleavage of the heterocyclic ring, oxidation of the fused benzene ring has been reported for some structural analogues of the one candidate substance 2-methyl-4,5-benzothiazole [FL-no: 15.088] in subgroup A-III benzothiazoles.

Additional alkyl and acyl substituents in the thiazole ring may increase the alternative metabolic pathways available to thiazole derivatives as was demonstrated for chlormethiazole. Alkyl- and acyl-substituted thiazole derivatives are primarily metabolised *via* side-chain oxidation and ring *S*- and *N*-oxidation. The major metabolites are readily excreted in the urine either free or as glutathione conjugates. Metabolites of the side-chain oxidation pathways may be expected to be conjugated e.g. to glucuronic acid. To a very small extent ring *C*-oxidation may be followed by ring cleavage to yield alpha-diketone and thioamide fragments. The acetyl-substituted thiazoles [FL-no: 15.038, 15.039, and 15.116] may also be expected to undergo keto-reduction, possibly followed by conjugation, similar to acetyl derivatives of furanes (EFSA, 2005c) and pyrazines (EFSA, 2005d).

Thiophene (subgroup A-Ia) and the ring-substituted thiophenes (subgroups A-Ib and A-Ic) undergo *S*-oxidation and glutathione conjugation. Also from some studies with thiazole derivatives, it can be seen that their metabolites may react spontaneously with glutathione, and it is likely that they have also reactivity towards protein thiols, which may result in toxicity. E.g. for the thiazole-ring cleavage products (thioamide-intermediates) a relationship with nephrotoxicity and hepatotoxicity has been established, especially after GSH depletion, even though this is only a very minor metabolite.

No information was submitted that would indicate whether side chain oxidation of the alkyl- or acyl-substituted thiophenes (subgroups A-Ib and A-Ic) or thiazoles (subgroup A-II) could also occur. Such reactions may be anticipated (e.g. omega or omega-1 oxidations). Similar reactions have been discussed for alkylated pyrazines (EFSA, 2005d). In addition the two candidate substances with aldehyde groups [FL-no: 15.107 and 15.074] may be expected to be oxidised to the corresponding carboxylic acids. Where applicable, conjugation with amino acids (e.g. glycine) or glucuronic acid may also be expected to occur (EFSA, 2005c; EFSA, 2005d; EFSA, 2005e).

Based on the MSDI exposure estimates (see Table 6.1 of the main text) it seems reasonable to assume that saturation of the metabolic pathways for the candidate substances in this FGE is unlikely, given these low levels of exposure to the candidate substances from their use as flavouring substances.

Overall there is insufficient quantitative information to evaluate the extent of metabolism of ring substituent groups, and it is also very difficult, based on the data available to assess how the presence of ring substituents and their positions at the ring influences metabolism of the rings itself, for any of the subgroups within this FGE. With respect to sulphhydryl reactivity in thiophenes (subgroup A-Ic), this property was a reason to consider such substances, e.g. FGE.13, as not being metabolised through pathways leading to innocuous metabolites. In concordance with this and based on the information presented above, it is concluded that for all candidate flavouring substances in A-subgroups of the present FGE, it also cannot be anticipated that they are metabolised to innocuous metabolites.

#### III.3.4. B-subgroups (substances with non-aromatic ring structures):

No specific information was available on the metabolism of the dihydrothiophene-, thiazoline-, thiazolidine-, dithiazine-, dihydrothiazine- or thiadiazine-derivatives or of related substances for any of the (non-aromatic) substances in the B-subgroups. It may be speculated that the substances in these chemical groups are metabolised primarily by oxidation of the ring S which indeed was reported for the thiazolidine derivative already mentioned, or, if applicable, via N-oxidation, similar to the aromatic thiazole and thiophene compounds. In addition, metabolism of the ring substituents is likely to occur. Due to the lack of metabolism data on the substances in the B-subgroups, it cannot be concluded that the candidate flavouring substances in the respective B-subgroups will be metabolised to innocuous products.

## ANNEX IV: TOXICITY

Oral acute toxicity data are available for two candidate substances of the present flavouring group evaluation from chemical group 29 or 30, and for 17 supporting substances evaluated by the JECFA at the 59<sup>th</sup> 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 <sub>50</sub> (mg/kg bw)	Reference	Comments
Thiophene [15.106]	Mouse	M	Oral	1902	(O'Donoghue, 1979)	
	Mouse	F	Oral	> 500	(Eli Lilly & Co., 1992)	
	Rat	M	Oral	1131	(O'Donoghue, 1979)	
	Rat	M, F	Gavage	3120	(Younger, 1964)	
Thiophene-2-carbaldehyde [15.107]	Mouse	NR	Oral	565	(Sharp, 1979)	
	Rat	NR	Oral	915 (950)	(Sharp, 1979)	
(2-Thienyl disulfide [15.008])	Mouse	M, F	Gavage	400	(Moran et al., 1980)	
(2-Methyl-5-methoxythiazole [15.002])	Rat	NR	Oral	1250	(Posternak et al., 1975)	
(2,4-Dimethyl-5-vinylthiazole [15.005])	Mouse	M, F	Oral	400 – 800	(Moran et al., 1980)	
(5-Acetyl-2,4-dimethylthiazole [15.011])	Mouse	M, F	Gavage	975	(Moran et al., 1980)	
	Mouse	M, F	Gavage	M: 610, F: 620	(Shellenberger, 1971d)	
(5-(2-Hydroxyethyl)-4-methylthiazole [15.014])	Mouse	M, F	Oral	4800	(Calvary & Nelson, 1944)	
(4-Methyl-5-vinylthiazole [15.018])	Mouse	M, F	Gavage	400 – 800	(Oser, 1970c)	
(2-Propionylthiazole [15.027])	Mouse	M, F	Gavage	2113	(Moran et al., 1980)	
(4-Methylthiazole [15.035])	Mouse	F	Gavage	0.36 ml/kg (360 mg/kg, based on an assumed specific gravity of 1.0 g/ml)	(O'Neal et al., 1978)	
	Rat	M, F	Gavage	930	(Mondino, 1981b)	
(4,5-Dimethylthiazole [15.017])	Rat	M, F	Gavage	964.9	(Piccirillo et al., 1982a)	
(2-Ethoxythiazole [15.021])	Rat	M, F	Gavage	910.9	(Piccirillo et al., 1982b)	
(2-Isopropyl-4-methylthiazole [15.026])	Rat	M, F	Gavage	0.83 ml/kg (832 mg/kg based on a specific gravity of 1.003 g/ml)	(Griffiths & Babish, 1977b)	
(2-Ethyl-4-methylthiazole [15.033])	Rat	M, F	Oral	540	(Moreno et al., 1981b)	
(Thiamine hydrochloride [16.027])	Rat	M	Gavage	3710	(Sprince et al., 1974)	
(Benzothiazole [15.016])	Mouse	M, F	Gavage	900	(Moran et al., 1980)	
	Rat	M, F	Oral	380	(Birch, 1976a)	
	Rat	M, F	Gavage	492	(Younger, 1964)	
	Rat	M, F	Gavage	479	(Reddy & Mayhew, 1992)	

<b>Table IV.1: ACUTE TOXICITY</b>						
<b>Chemical Name [FL-no]</b>	<b>Species</b>	<b>Sex</b>	<b>Route</b>	<b>LD<sub>50</sub> (mg/kg bw)</b>	<b>Reference</b>	<b>Comments</b>
	Rat	M, F	Oral	257 (M), 177 (F)	(Loeser, 1982a; Loeser, 1982b)	
(2-(2-Butyl)-4,5-dimethyl-3-thiazoline [15.029])	Mouse	M, F	Gavage	2827	(Moran et al., 1980)	
(4,5-Dimethyl-2-ethyl-3-thiazoline [15.030])	Mouse	M, F	Gavage	1265	(Moran et al., 1980)	
(4,5-Dimethyl-2-isobutyl-3-thiazoline [15.032])	Mouse	M, F	Gavage	3067	(Moran et al., 1980)	



Subacute / Subchronic toxicity data are available for two candidate substance of the present flavouring group evaluation from chemical group 29 or 30 and for 11 supporting substances evaluated by the JECFA at the 59<sup>th</sup> meeting. No chronic or carcinogenicity studies were available. 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	Duration (days)	NOAEL (mg/kg bw/day)	Reference	Comments
Thiophene [15.106]	Rat; M 5	Gavage	0, 50, 100, 500, 1000 mg/kg bw/day	Up to 19 days	100	(O'Donoghue, 1979)	Inadequately reported study of short duration.
Thiophene-2-carbaldehyde [15.107]	Rat; sex not reported 5	Diet	0, 0.1, and 1.0 % in diet equal to 0, 95 and 840 mg/kg bw/day	11 days	95	(Sharp, 1979)	Reduced food consumption and reduced absolute kidney weight . Inadequately reported study of short duration.
(5-Methyl-2-thiophene-carbaldehyde [15.004])	Rat; M, F 5/sex/group	Diet	10 mg/kg bw/day	14 days	10	(Gill & Van Miller, 1987b)	One dose level, GLP study, comprehensively reported, but no haematology or clinical chemistry carried out, only a limited number of organs examined at autopsy and only liver and kidney examined histopathologically. Short duration, limited study design.
(3-Acetyl-2,5-dimethylthiophene [15.024])	Rat; M, F 5/sex/group	Diet	10 mg/kg bw/day	14 days	10	(Gill & Van Miller, 1987b)	One dose level, GLP study, described as a dietary Minimum Toxicity Screen (MTS), comprehensively reported, but no haematology or clinical chemistry carried out, only a limited number of organs examined at autopsy and only liver and kidney examined histopathologically. Minor body weight changes in both sexes and organ weight changes in males only .Short duration, limited study design.
(2-Thienyl disulfide [15.008])	Rat; M, F 15/sex	Diet	0.29 mg/kg bw/day	90 days	0.29	(Morgareidge & Oser, 1970g)	Study carried out at a one low dose level. Standard toxicological parameters were monitored (body weight, food consumption, haematology, urinalysis, histopathology). Parameters assessed were reasonably comprehensive. Study considered valid.
(5-Acetyl-2,4-dimethylthiazole [15.011])	Rat; M, F 23/sex	Diet	Approx. 25 mg/kg bw/day.	90 days	M: 25.2 F:24.4	(Shellenberger, 1971d)	Old study carried out at a one dose level. Standard toxicological parameters were monitored (body weight, food consumption, haematology, urinalysis, histopathology). Parameters assessed were reasonably comprehensive. Study considered valid.
(2-Acetylthiazole [15.020])	Rat; M 5	Diet	5000 and 10,000 mg/kg in diet equivalent to 250 and 500 mg/kg bw/day	28 days	< 250	(Wheldon et al., 1970)	Preliminary dose-range finding study for the 90-day study described below, very limited experimental details provided.

Table IV.2: Subacute / Subchronic / Chronic / Carcinogenicity Studies							
Chemical Name [FL-no]	Species; Sex No./Group	Route	Dose levels	Duration (days)	NOAEL (mg/kg bw/day)	Reference	Comments
	Rat; M 10	Diet	0, 100, 1000, 10,000 mg/kg in diet equivalent to 0.5, 50, 500 mg/kg bw/day	90 days	50	(Wheldon et al., 1970)	Study considered to be of limited validity, since no clinical chemistry was undertaken, limited number of organs weighed, number of organs taken for histopathology was limited, and histopathological review restricted to 5 animals/sex/group.
(2-Methyl-5-methoxythiazole [15.002])	Rat; M, F 10-16/sex	Diet	approx. 9 mg/kg bw/day	90 days	M: 8.83 F: 8.63	(Posternak et al., 1975)	Toxicity data reported as part of a summary publication on a large number of flavouring substances. Body weight, food utilisation, haematological and histopathology examination undertaken, clinical chemistry restricted to blood urea nitrogen, organ weight changes restricted to liver and kidney. Due to reporting limitations data quality could not be assessed.
(2,4-Dimethyl-5-vinylthiazole [15.005])	Rat; M, F 10-16/sex	Diet	approx. 1 mg/kg bw/day	90 days	M: 0.92 F: 1.0	(Posternak et al., 1969)	Toxicity data reported as part of a summary publication on a large number of flavouring substances. Body weight, food utilisation, haematological and histopathology examination undertaken, clinical chemistry restricted to blood urea nitrogen, organ weight changes restricted to liver and kidney. Due to reporting limitations data quality could not be assessed.
(Benzothiazole [15.016])	Rat; M, F 15/sex	Oral	5.1 mg/kg bw/day	90 days	5.1	(Morgareidge, 1971c)	Body weight, food utilisation, haematology and limited clinical chemistry parameters were evaluated, and a range of tissues was examined microscopically. No treatment-related effects were seen. Only the histopathological report was available for the current evaluation. The study is considered valid.
2-(2-Butyl)-4,5-dimethyl-3-thiazoline [15.029])	Rat; M, F 15/sex	Oral	1.21(M) /1.26 (F) mg/kg bw/day	90 days	M: 1.21 F: 1.26	(Babish & Re, 1978)	Old study carried out at a one dose level. Standard toxicological parameters were monitored (body weight, food consumption, haematology, urinalysis, histopathology) Study considered valid.
(2- Isobutyl-4, 6-dimethyldihydro-1,3,5-dithiazine and 4-isobutyl-2,6-dimethyldihydro-1,3,5-dithiazine (mixture))	Rat, M, F 5/sex	Oral	10 mg/kg bw/day	14 days	M: 11.5 F: 11.1	(Rush, 1989a)	One dose level study of short duration, no haematological or clinical chemistry parameters were examined and histopathological examinations were restricted to liver and kidney.
(2- Isopropyl-4, 6-dimethyldihydro-1,3,5-dithiazine and 4- isopropyl-2, 6-dimethyldihydro-1,3,5-dithiazine (mixture))	Rat, M, F 5/sex	Oral	10 mg/kg bw/day	14 days	M: 11.8 F: 11.1	(Rush, 1989b)	One dose level study of short duration, no haematological or clinical chemistry parameters were examined and histopathological examinations were restricted to liver and kidney.

**TABLE IV.3: DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES**

No developmental and reproductive toxicity data are available for the candidate substances of the present flavouring group evaluation from chemical group 29 or 30, nor for the supporting substance evaluated by the JECFA at the 59<sup>th</sup> meeting.

*In vitro* mutagenicity/genotoxicity data are available for 12 candidate substances of the present flavouring group evaluation from chemical group 29 or 30 and for four supporting substances evaluated by the JECFA at the 59<sup>th</sup> meeting and four related supporting substances. Supporting substances are listed in brackets.

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

Table IV.4: GENOTOXICITY ( <i>in vitro</i> )						
Chemical Name	Test System	Test Object	Concentration	Result	Reference	Comments
<b>Subgroup A-Ia</b>						
Thiophene [15.106]	Ames assay (plate incorporation method)	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537	3 µmol/plate (all strains) (252 µg/plate)	Negative (±S9)	(Florin et al., 1980)	Published non-GLP study. Qualitative screening in a spot-test with three strains, quantitative study (4 doses, 0.03, 0.3, 3, 30 µmol/plate) with TA 100 only. Limited report of experimental details and results. Insufficient quality, study not considered adequate for the evaluation of mutagenic activity.
	Ames assay (preincubation method)	<i>S. typhimurium</i> TA97;TA98; TA100; TA1535; TA1537	up to 10,000 µg/plate	Negative (±S9) <sup>1</sup>	(Zeiger et al., 1987)	Non-GLP study roughly in accordance with OECD guideline 471. The study is considered valid.
	Ames assay (preincubation method)	<i>S. typhimurium</i> TA98; TA100; TA102	0.01-1.2 mmol/plate (100,968 µg/plate)	Negative (±S9)	(Aeschbacher et al., 1989)	Greatest effects are quantified by "mutation factor," no numbers are given for negative results. Limited quality (only 3 strains used), but otherwise acceptable study.
	Ames assay (plate incorporation method)	<i>S. typhimurium</i> TA98; TA100	Up to 100 µmol/plate (8414 µg/plate)	Negative (±S9)	(Lee et al., 1994a)	Only two strains used but otherwise acceptable study
<b>Subgroup A-Ib</b>						
2-Methylthiophene [15.091]	Ames assay (preincubation method)	<i>S. typhimurium</i> TA98; TA100; TA102	0.00001- 1.0 mmol/plate (98,170 µg/plate)	Negative (±S9)	(Aeschbacher et al., 1989)	Greatest effects are quantified by "mutation factor," no numbers are given for negative results. Limited quality (only 3 strains used), but otherwise acceptable study.
	Ames assay (plate incorporation method)	<i>S. typhimurium</i> TA98; TA100	Up to 100 µmol/plate (9817 µg/plate)	Negative (±S9)	(Lee et al., 1994a)	Only two strains used but otherwise acceptable study
3-Methylthiophene [15.092]	Ames assay (preincubation method)	<i>S. typhimurium</i> TA98; TA100; TA102	0.01-1.0 mmol/plate (98,170 µg/plate)	Negative (±S9)	(Aeschbacher et al., 1989)	Greatest effects are quantified by "mutation factor," no numbers are given for negative results. Limited quality (only 3 strains used), but otherwise acceptable study.
	Ames assay (plate incorporation method)	<i>S. typhimurium</i> TA98; TA100	Up to 100 µmol/plate (9817 µg/plate)	Negative (±S9)	(Lee et al., 1994a)	Only two strains used but otherwise acceptable study
2,5-Dimethylthiophene [15.064]	Ames assay (plate incorporation method)	<i>S. typhimurium</i> TA98; TA100	Up to 100 µmol/plate (11,219 µg/plate)	Negative (±S9)	(Lee et al., 1994a)	Only two strains used but otherwise acceptable study

<b>Table IV.4: GENOTOXICITY (<i>in vitro</i>)</b>						
<b>Chemical Name</b>	<b>Test System</b>	<b>Test Object</b>	<b>Concentration</b>	<b>Result</b>	<b>Reference</b>	<b>Comments</b>
2-Acetylthiophene [15.040]	Ames assay (plate incorporation method)	<i>S. typhimurium</i> TA98; TA100	Up to 100 µmol/plate (12,618 µg/plate)	Negative (±S9)	(Lee et al., 1994a)	Only two strains used but otherwise acceptable study
	SOS Chromotest	<i>E. coli</i>	NR	Negative with rat S9, positive without rat S9	(Mosier et al., 2003)	Study endpoint inappropriate for the estimation of genotoxic potential
2-Acetyl-3-Methylthiophene [15.037]	SOS Chromotest	<i>E. coli</i>	NR	Negative with rat S9, positive without rat S9	(Mosier et al., 2003)	Study endpoint inappropriate for the estimation of genotoxic potential.
Thiophene-2-carbaldehyde [15.107]	SOS Chromotest	<i>E. coli</i>	NR	Negative with rat S9, positive without rat S9	(Mosier et al., 2003)	Study endpoint inappropriate for the estimation of genotoxic potential.
5-Ethylthiophene-2-carbaldehyde [15.074]	SOS Chromotest	<i>E. coli</i>	NR	Negative with rat S9, positive without rat S9	(Mosier et al., 2003)	Study endpoint inappropriate for the estimation of genotoxic potential.
(5-Methyl-2-thiophenecarbaldehyde [15.004])	Ames assay (plate incorporation method)	<i>S. typhimurium</i> TA98; TA100	Up to 100 µmol/plate (12,618 µg/plate)	Negative (±S9)	(Lee et al., 1994a)	Only two strains used but otherwise acceptable study.
<b>Subgroup A-II</b>						
2,4-Dimethylthiazole [15.062]	Ames assay (plate incorporation method)	<i>S. typhimurium</i> TA100	9.3 and 94 mmol/l top agar (10,639 µg/ml)	Negative (-S9)	(Voogd et al., 1983)	Insufficient quality (one test strain as well as without metabolic activation only).
(4,5-Dimethylthiazole [15.017])	Ames assay (plate incorporation method)	<i>S. typhimurium</i> TA98; TA100	Up to 100 µmol/plate (11,318 µg/plate)	Negative (±S9)	(Lee et al., 1994a)	Only two strains used but otherwise acceptable study.
(4-Methylthiazole [15.035])	Ames assay (plate incorporation method)	<i>S. typhimurium</i> TA98; TA100	Up to 100 µmol/plate (9916 µg/plate)	Negative (±S9)	(Lee et al., 1994a)	Only two strains used but otherwise acceptable study.
<b>Subgroup A-III</b>						
2-Methyl-4,5-benzothiazole [15.088]	Ames assay (plate incorporation method)	<i>S. typhimurium</i> TA98; TA100; TA102; TA1535; TA1537	100-10,000 µg/plate	Negative (±S9) <sup>1</sup>	(Longfellow, 1998a)	Summary report of NCI-short-term test program, results not given in detail.
(Benzothiazole [15.016])	Ames assay	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537	Up to 5000 µg/plate	Negative (±S9)	(Bayer, 1991)	Summary in IUCLID data set only. According to this summary, the assay was in compliance with GLP; accordance with OECD guideline 471 not stated.
	Mouse lymphoma assay	Mouse L5178Y tk+/- cells	10-250 µg/ml	Negative (±S9)	(Longfellow, 1997)	Summary report of NCI-short-term test program, results not given in detail.
<b>Subgroup B-III</b>						
2-Propylthiazolidine [15.099]	Ames assay	<i>S. typhimurium</i> TA98; TA100	1, 10, 100 µg/ml	1 and 10 µg/ml: positive in TA100 (±S9); 100 µg/ml: positive in TA98 and TA100.(±S9)	(Mihara & Shibamoto, 1980)	The results were stated to be positive, however, the magnitude and a positive dose effect relationship could not be assessed (no numbers are given).

<b>Table IV.4: GENOTOXICITY (<i>in vitro</i>)</b>						
<b>Chemical Name</b>	<b>Test System</b>	<b>Test Object</b>	<b>Concentration</b>	<b>Result</b>	<b>Reference</b>	<b>Comments</b>
2-methylthiazolidine [15.090]	Ames assay	<i>S. typhimurium</i> TA98; TA100	1, 10, 100 µg/ml	1 and 10 µg/ml: positive in TA100; (±S9) 100 µg/ml: positive in TA98 and TA100 (±S9)	(Mihara & Shibamoto, 1980)	The results were stated to be positive, however, the magnitude and a positive dose effect relationship could not be assessed (no numbers are given)..
(2-ethylthiazolidine)	Ames assay	<i>S. typhimurium</i> TA98; TA100	1, 10, 100 µg/ml	1 µg/ml: positive in TA100 (±S9) and TA98 (-S9); 10 µg/ml: positive in TA100 (±S9); 100 µg/ml: positive TA98 and TA100. (±S9)	(Mihara & Shibamoto, 1980)	The results were stated to be positive, however, the magnitude and a positive dose effect relationship could not be assessed (no numbers are given).
(2-isopropylthiazolidine)	Ames assay	<i>S. typhimurium</i> TA98; TA100	1, 10, 100 µg/ml	1 and 10 µg/ml: positive in TA100 (±S9); 100 µg/ml: positive in TA100 (±S9) and TA98 (-S9)	(Mihara & Shibamoto, 1980)	The results were stated to be positive, however, the magnitude and a positive dose effect relationship could not be assessed (no numbers are given).
(2-butylthiazolidine)	Ames assay	<i>S. typhimurium</i> TA98; TA100	1, 10, 100 µg/ml	1 µg/ml: positive in TA100 (+S9); 10 µg/ml: positive in TA100 (±S9); 100 µg/ml: positive in TA100 (±S9) and TA98 (-S9)	(Mihara & Shibamoto, 1980)	The results were stated to be positive, however, the magnitude and a positive dose effect relationship could not be assessed (no numbers are given).
(2-isobutylthiazolidine)	Ames assay	<i>S. typhimurium</i> TA98; TA100	1, 10, 100 µg/ml	1 µg/ml: positive in TA98 and TA100 (+S9); 10 µg/ml: positive in TA98 and TA100 (±S9); 100 µg/ml: positive in TA98 and TA100 (±S9)	(Mihara & Shibamoto, 1980)	The results were stated to be positive, however, the magnitude and a positive dose effect relationship could not be assessed (no numbers are given).

NR: Not Reported

<sup>1</sup> With and without rat and hamster S9 metabolic activation.

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

No *in vivo* mutagenicity/genotoxicity data are available for the candidate substance of the present flavouring group evaluation from chemical group 29 or 30, nor for the supporting substances evaluated by the JECFA at the 59<sup>th</sup> meeting.

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**ABBREVIATIONS:**

ADI	Acceptable Daily Intake
CAS	Chemical Abstract Service
CEF	Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CHO	Chinese hamster ovary (cells)
CoE	Council of Europe
DNA	Deoxyribonucleic acid
EC	European Commission
EFSA	The European Food Safety Authority
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FEMA	Flavor and Extract Manufacturers Association
FGE	Flavouring Group Evaluation
FLAVIS (FL)	Flavour Information System (database)
GSH	Glutathione
ID	Identity
IOFI	International Organization of the Flavour Industry
IR	Infrared spectroscopy
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
LD <sub>50</sub>	Lethal Dose, 50%; Median lethal dose
MS	Mass spectrometry
MSDI	Maximised Survey-derived Daily Intake
mTAMDI	Modified Theoretical Added Maximum Daily Intake
No	Number
NOAEL	No observed adverse effect level
NOEL	No observed effect level
NTP	National Toxicology Program
SCE	Sister chromatid exchange
SCF	Scientific Committee on Food
TAMDI	Theoretical Added Maximum Daily Intake
WHO	World Health Organisation