(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 18 flavouring substances in this group have intakes in Europe from 0.0012 to 37 microgram/*capita*/day which are below the threshold of concern value for both structural class II (540 microgram/person/day) and structural class III (90 microgram/person /day) substances.

The biotransformation of the four esters of furfuryl alcohol in the present Flavouring Group Evaluation leads to the formation of furfural, a reactive hepatotoxic aldehyde. Furfural is then oxidised to furoic acid, which can be conjugated with glycine yielding innocuous and readily excreted products. Also, the candidate substance ethyl furfuracrylate can be biotransformed to furoic acid. However, the furan ring of the candidate substance furoic acid and the furan moieties of the two candidate furoate esters may be completely oxidised to CO_2, with the opening of the furan ring and production of reactive intermediates. Therefore it cannot be predicted that these eight flavouring substances included in subgroup 1 are metabolised to innocous products.

In addition to the above mentioned pathways, 5-hydroxymethylfurfuraldehyde can be bioactivated to 5-[(sulfoxy)methyl] furfural, through sulfonation of its allylic hydroxyl functional group, catalyzed by sulfotransferases. The resulting ester has been demonstrated to induce genotoxic effects.

Based on the general knowledge on the metabolism of sulphur-containing compounds, the flavouring substances bearing a free thiol group can be considered reactive *per se* interacting with endogenous sulphur-containing substances, e.g. glutathione and proteins, thus triggering adverse effects. The candidate furfuryl and furan monosulfides are expected to undergo oxidation mainly to the corresponding sulfoxides and sulfones. Alternatively they can be conjugated with glutathione, giving rise to mixed disulfides, which can be oxidised to thiosulfinates or thiosulfones or reduced to free thiols. Similar metabolic pathways may be predicted for the candidate disulfides and very likely for the trisulfide. Given the reactivity of thiol groups, whether free or resulting from di(tri)sulfide, and their importance in cell physiology, it cannot be excluded that all the nine flavouring substances included in subgroup 2 of the present Flavouring Group Evaluation interfere with normal cell function and therefore, they cannot be predicted to be metabolised to innocuous substances.

Short-term and long-term toxicity studies are available for two flavouring substances included in subgroup 1, and for three related supporting substances, including furfural. They indicate that the liver is the critical target for their toxicity. Recently EFSA has established an ADI value of 0.5 mg/kg bw for furfural and the furfural component of furfural diethyl acetal.

No toxicity data are available on flavouring substances included in subgroup 2; however results from toxicity studies on 16 supporting substances have been reported. Many of the available studies were performed either with a single dose level or multiple dose levels that produced no effects; the dose producing no adverse effects ranged between 0.45 and 10 mg/kg/day.

Data on genotoxicity were available on two flavouring substances and on five structurally related substances. Overall, except for the flavouring substance 5-hydroxymethylfurfuraldehyde, the *in vitro* and *in vivo* data available do not give rise to concern with respect to genotoxicity of the remaining eight flavouring substances included in subgroup 1. Based on *in vitro* data on the mutagenic activity of a sulphate conjugate of 5-hydroxymethylfurfuraldehyde, there is sufficient evidence to raise concern about a genotoxic potential. Accordingly, the Procedure cannot be applied for this substance pending submission of *in vivo* genotoxicity data.

The lack of data on the sulphur-containing flavouring substances included in subgroup 2, or on the structurally related substances, does not allow to conclude on their genotoxicity.

It was considered that on the basis of the default MSDI approach the 17 flavouring substances which could be taken through the Procedure would not give rise to safety concerns at the estimated levels of intake arising from their use as flavouring substances.

When the estimated intakes were based on the mTAMDI, they ranged from 75 to 3700 microgram/person/day for the 16 flavouring substances from structural class II. Thus, the intakes for nine of the flavouring substances were above the threshold of concern for structural class II of 540 microgram/person/day. The estimated intakes of two flavouring substances assigned to structural class III, based on the mTAMDI are 150 microgram/person/day, which is above the threshold of concern for structural class III of 90 microgram/person/day.

Thus for 10 of the 17 flavouring substances taken through the Procedure, the intakes, estimated on the basis of the mTAMDI, exceed the relevant threshold for their structural class, to which the flavouring substance has been assigned. Therefore, for these 10 substances 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 this evaluation could be applied to the materials of commerce, it is necessary to consider the available specifications:

Adequate specifications including complete purity criteria and identity tests for the materials of commerce have been provided for the 17 flavouring substances which have been evaluated according to the Procedure, except that information on stereoisomerism is missing for three of the substances. Thus, the final evaluation of the materials of commerce cannot be performed for these three flavouring substances, pending further information.

KEYWORDS

Furfuryl, furan, flavourings, safety.

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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 2004/357/EC (EC, 2004). Furthermore, all substances are divided into 34 chemical groups. Substances within a group should have some metabolic and biological behaviour in common.

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

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

TERMS OF REFERENCE

EFSA is requested to carry out a risk assessment on flavouring substances prior to their authorisation and inclusion in a positive list according to Commission Regulation (EC) No 1565/2000 (EC, 2000).

ASSESSMENT

1. Presentation of the Substances in the Flavouring Group Evaluation 13

1.1. Description

The present Flavouring Group Evaluation, using the procedure as referred to in the Commission Regulation EC No 1565/2000 (EC, 2000) (The Procedure –shown in schematic form in Annex I), deals with 18 flavouring substances (candidate substances) from chemical group 14, Annex I of Commission Regulation (EC) No 1565/2000 (EC, 2000). All the candidate substances in FGE.13 are furan derivatives and can be divided into two subgroups, depending on the absence/ presence of sulphur-containing substituents.

The nine candidate substances in subgroup 1 are furfuryl alcohol derivatives such as esters of furfuryl alcohol [FL-no: 13.127, 13.129, 13.132, and 13.133] or furanacrylic acid [FL-no: 13.011], furoic acid [FL-no: 13.136] and its esters [FL-no: 13.102 and 13.122] and 5-hydroxymethylfurfuraldehyde [FL-no: 13.139].

The nine representatives of subgroup 2 are all sulphur-containing furan derivatives. The sulphur is present in the molecule as a free thiol group [FL-no: 13.108 and 13.149], as thioethers [FL-no: 13.114, 13.145, and 13.124], as disulfides [FL-no: 13.113, 13.144, and 13.178] or as trisulfide [FL-no: 13.146]. The candidate 4,5-dihydro-3-mercapto-2-methylfuran [FL-no: 13.108], the only compound containing a non aromatic ring and a free thiol, is also included in subgroup 2.

The 18 flavouring substances under consideration, as well as their chemical names, FLAVIS- (FL-no), Chemical Abstract Service- (CAS-) Council of Europe- (CoE-) and Flavor and Extract Manufactures Association- (FEMA-) numbers, structure and specifications, are listed in Table 1.

The 18 candidate substances are closely related structurally to 47 flavouring substances (supporting substances) evaluated at the 55th and 59th JECFA meetings (JECFA, 2001a; JECFA, 2002c) in the groups of "Furfuryl alcohol and related substances" and "sulphur substituted Furan derivatives". In addition, the implication for human health of furfural and furfuraldiethylacetal in the diet has recently been evaluated by the AFC Panel (EFSA, 2004j) and the risk assessment of furfural is under way in the framework of Existing Chemical Evaluation, according to EU Regulation 73/793/CE (EU-RAR, 2004b).

The candidate substances under consideration in the present evaluation are listed in Tables 1 and 2a, the hydrolysis products of candidate substances in Table 2b, and the supporting substances in 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.).

One of the 18 candidate substances possesses a chiral centre [FL-no: 13.127]. The substance has been presented without any indication whether the commercial flavouring substance was dominated by one optical isomer or the other.

Two of the 18 candidate substances can exist as geometrical isomers [FL-no: 13.011 and 13.129]. In both cases, no indication of the preponderance of either of the possible isomers in the commercial flavouring material has been given (see Table 1).

1.3. Natural Occurrence in Food

Sixteen of the 18 candidate substances have been reported to occur in milk, bread, coffee, cocoa, grain, a wide range of fruits and vegetables, meat, beer, various types of alcoholic beverages, soy protein, honey, and/or almond.

Quantitative data on the natural occurrence in food have been reported for nine of these substances.

These reports are:

- Butyl 2-furoate [FL-no: 13.102]: Less than 0.01 mg/kg in papaya.
- Ethyl 2-furoate [FL-no: 13.122]: Up to 0.05 mg/kg in beer, up to 0.3 mg/kg in brandy, less than 0.01 mg/kg in guava fruit, 0.0004 mg/kg in kiwi fruit, up to 0.05 mg/kg in papaya, 0.1 mg/kg in rum, trace amount in port, up to 0.04 mg/kg in wine.
- Ethyl furfuryl sulfide [FL-no: 13.124]: 0.01 mg/kg in coffee.
- Furfuryl but-2-enoate [FL-no: 13.129]: Up to 0.2 mg/kg in coffee.

- 2-Furoic acid [FL-no: 13.136]: 0.01 mg/kg in asparagus (cooked), up to 0.8 mg/kg in beer, up to 80 mg/kg in coffee, less than 0.05 mg/kg in guava fruit, less than 0.05 mg/kg in papaya fruit, 0.4 mg/kg in rum.
- 5-Hydroxymethylfurfuraldehyde [FL-no: 13.139]: 0.03 mg/kg in milk, up to 19.1 mg/kg in wheaten bread, up to 35 mg/kg in coffee, up to 8 mg/kg in beer, up to 11.6 mg/kg in brandy, up to 680 mg/kg in sherry, up to 10.1 mg/kg in whisky, up to 27.3 mg/kg in wine, up to 63 mg/kg in port, 9 mg/kg in almond (roasted), 0.2 mg/kg in cloudberry, up to 450 mg/kg in prune.
- Methyl 5-methylfurfuryl disulfide [FL-no: 13.144]: Up to 0.03 mg/kg in coffee.
- Methyl 5-methylfurfuryl sulfide [FL-no: 13.145]: Up to 0.2 mg/kg in coffee.
- 5-Methyl-2-furanmethanethiol [FL-no: 13.149]: Up to 0.2 mg/kg in coffee.

Two of the substances, (4,5-dihydro-3-mercapto-2-methylfuran [FL-no: 13.108], and (3-(furfuryldithio)-2-methylfuran [FL-no: 13.178]), have not been reported to occur naturally in any food items according to TNO (TNO, 2000).

2. Specifications

Purity criteria for the 18 candidate substances have been provided by the flavouring industry (EFFA, 2003g).

Judged against the requirements in Annex II of Commission Regulation EC No 1565/2000 (EC, 2000), the information is adequate for the 17 of the 18 candidate substances. However, information on chirality is needed for one candidate substance [FL-no: 13.127] and on geometrical stereoisomerism for two candidate substances [FL-no: 13.011 and 13.129] (see Section 1.2 and Table 1).

3. Intake Data

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

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

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

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

One of the alternatives is the "Theoretical Added Maximum Daily Intake" (TAMDI)-approach which is calculated on the basis of standard portions and upper use levels (SCF, 1995) for flavourable beverages and foods in general, with exceptional levels for particular foods. This method is regarded as a conservative estimate of the actual intake in most consumers because it is based on the assumption that the consumer regularly eats and drinks several food products containing the same flavouring substance at the upper use level.

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

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

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

Average *per capita* intake (MSDI) is estimated on the assumption that the amount added to food is consumed by 10 % of the EU population[1] (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 18 candidate substances for use as flavouring substances in Europe has been reported to be approximately 335 kg (EFFA, 2003g) and for 32 of the 47 supporting substances approximately 7000 kg (cited by (JECFA, 2001b; JECFA, 2003a)).

On the basis of the annual volumes of production reported for the 18 candidate substances, the daily *per capita* intakes for each of these flavourings have been estimated (Table 2a). More than 90% of the total annual volume of production for the candidate substances (EFFA, 2003g) is accounted for by one candidate substance: 4,5-Dihydro-3-mercapto-2-methylfuran [FL-no: 13.108]. The estimated daily *per capita* intake of this candidate substance from use as a flavouring substance is 37 microgram, and below 1 microgram for each of the remaining candidate substances.

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

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

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

For the 18 candidate substances information on food categories and normal and maximum use levels[2,3] were submitted by the Flavour Industry (EFFA, 2003g). The 18 candidate substances are used in flavoured food products divided into the food categories, outlined in Annex III of the

[1] *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*

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

[3] *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).*

Commission Regulation 1565/2000 (EC, 2000), as shown in Table 3.1. For the present calculation of mTAMDI, the reported normal use levels were used. In the case where different use levels were reported for different food categories the highest reported normal use level was used.

Table 3.1 Use of Candidate Substances

Food category	Description	Flavourings used
Category 1	Dairy products, excluding products of category 2	All 18
Category 2	Fats and oils, and fat emulsions (type water-in-oil)	All 18
Category 3	Edible ices, including sherbet and sorbet	All 18
Category 4.1	Processed fruits	All 18
Category 4.2	Processed vegetables (incl. mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds	None
Category 5	Confectionery	All 18
Category 6	Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery	All 18
Category 7	Bakery wares	All except [FL-no: 13.136]
Category 8	Meat and meat products, including poultry and game	All 18
Category 9	Fish and fish products, including molluscs, crustaceans and echinoderms	All 18
Category 10	Eggs and egg products	None
Category 11	Sweeteners, including honey	None
Category 12	Salts, spices, soups, sauces, salads, protein products etc.	All 18
Category 13	Foodstuffs intended for particular nutritional uses.	All 18
Category 14.1	Non-alcoholic ("soft") beverages, excl. dairy products	All 18
Category 14.2	Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts	None
Category 15	Ready-to-eat savouries	All 18
Category 16	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 1 – 15	All 18

According to the Flavour Industry the normal use levels for the 18 candidate substances are in the range of 0.1 - 20 mg/kg food, and the maximum use levels are in the range of 0.2 - 100 mg/kg (EFFA, 2003g).

The mTAMDI values for the 16 candidate substances from structural class II (see Section 5) range from 75 to 3700 microgram/person/day. For the remaining two candidate substances from structural class III the mTAMDI is 150 microgram/person/day.

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

4. Absorption, Distribution, Metabolism and Elimination

The candidate substances in FGE.13 are furan derivatives which can be divided into two different subgroups.

Subgroup 1

The nine candidate substances in subgroup 1 are furfuryl alcohol derivatives such as esters of furfuryl alcohol [FL-no: 13.127, 13.129, 13.132, 13.133], furoic acid [FL-no: 13.102, 13.122, 13.136], or furanacrylic acid [FL-no 13.011]. 5-Hydroxymethylfurfuraldehyde (5HMF) [FL-no: 13.139] with an additional hydroxymethyl side chain at C5 of the furan ring is also included in the

subgroup. For a number of candidate and supporting substances, including furfural, metabolism data are available.

The esters of furfuryl alcohol (i.e. candidate substances furfuryl hexanoate [FL-no: 13.132], furfuryl isobutyrate [FL-no: 13.133], furfuryl 2-methylbutyrate [FL-no: 13.127] and furfuryl but-2-enoate [FL-no: 13.129]) are expected to be hydrolysed to furfuryl alcohol and the corresponding aliphatic carboxylic acid based on hydrolysis data (See Annex III for a detailed description).

Furoates (i.e. candidate substances ethyl 2-furoate [FL-no: 13.122] and butyl 2-furoate [FL-no: 13.102]) are directly hydrolysed to the candidate substance 2-furoic acid [FL-no: 13.136], which is known as the major metabolite from furfural, and to the corresponding aliphatic alcohol. The candidate substance ethyl furfuracrylate [FL-no 13.011] is expected to be hydrolysed to furanacrylic acid, a known metabolite of furfural, and to ethanol. On the basis of the available results it can be anticipated that the substances in this subgroup or their hydrolysis products are rapidly absorbed after oral exposure.

In general, the non-furan containing component alcohols and carboxylic acids formed by ester hydrolysis of the candidate furfuryl alcohol esters and of the furoic acid esters participate in fatty acid oxidation and the citric acid cycle to yield CO_2 and water.

Furfuryl alcohol, furoic acid, furanacrylic acid, 5HMF and their derivatives participate in the same pathways as those involved in the detoxication of furfural in rodents. These pathways comprise as first step, the oxidation of the alcohol or aldehyde group of furfuryl alcohol and furfural, respectively, to furoic acid; furanacrylic acid can also be oxidised to furoic acid. The oxidation may be followed by conjugation with glycine, yielding 2-furoylglycine or 2-furanacryloylglycine, or by reaction with acetyl-CoA (leading to furanacryloyl-CoA) which can also further be followed by conjugation with glycine. The conjugates are readily excreted. However, there is some indication that in rats complete oxidation of furan to CO_2 can occur; the process requires the opening of the furan ring, with production of reactive intermediates. Although it seems that in humans this pathway is of minor importance, its presence in humans cannot be ruled out.

In addition to the above mentioned pathways, 5HMF has been shown to be bioactivated *in vitro* to 5-[(sulfoxy)methyl] furfural (SMF), through sulfonation of its allylic hydroxyl functional group, catalyzed by sulfotransferases. In the resulting ester, the sulfate is a good leaving group, thus producing a highly electrophilic allyl carbocation, which could be stabilized by distribution of charges on the furan ring. The subsequent interaction of this reactive intermediate with critical cellular nucleophiles (i.e. DNA, RNA and proteins) may result in toxic and mutagenic effects. The occurrence of this pathway *in vivo* cannot be ruled out.

Subgroup 2

The nine representatives of subgroup 2 are furan derivatives containing sulphur substituents as free thiol groups and as mono-, di- and tri-sulfides. 4,5-Dihydro-3-mercapto-2-methylfuran [FL-no: 13.108], the only candidate containing a non aromatic ring and a free thiol group, is also included in the subgroup.

Substances bearing a free thiol group [FL-no: 13.108 and 13.149] can directly react with endogenous sulphur-containing substances, e.g. glutathione and proteins. Metabolism data on candidate or supporting substances are not available, but the metabolic fate of these substances can be estimated from general knowledge of thiol metabolism. Free thiol groups can be metabolised by methylation after which the sulphur can be further oxidised to give sulfoxides or sulfones, which can be excreted unchanged in the urine. Alternatively, conjugation with glutathione may occur,

resulting in a mixed disulfide, which can be reduced to give free thiols, or oxidised to give thiosulfinates or thiosulfones (see Fig. III.3). They may also undergo thiol-disulfide exchange either with free thiol groups of proteins or with free thiol groups in other endogenous substances. The reaction with proteins may affect biochemical functions, thereby triggering adverse effects.

The thioethers [FL-no: 13.114, 13.124, and 13.145] may undergo sulphur oxidation reactions, similar to the methylether conjugates of free thiol groups; in addition, thiols may be formed (see Annex III).

Disulfides [FL-no: 13.113, 13.144, or 13.178] can be reduced to give the free thiols, or can be oxidised to give thiosulfinates or thiosulfones. For the one trisulfide candidate substance [FL-no: 13.146], no information on biotransformation was available, but it may be expected that this substance is metabolised *via* similar routes as disulfides.

Summary:

The metabolism of non-sulphur-containing furan derivatives in FGE.13 [FL-no: 13.127, 13.129, 13.132, 13.133, and 13.139] includes the formation of furfural, a known reactive aldehyde, that may lead to hepatotoxicity. In addition the two furoate esters [FL-no: 13.102 and 13.122] and furoic acid itself [FL-no: 13.136], which is the main metabolite of furfural, may be metabolised to CO_2, with the opening of the furan ring, producing reactive intermediates. Therefore, it is concluded that the candidate substances included in subgroup 1 cannot be predicted to be metabolised to innocuous compounds. In addition, 5HMF [FL-no: 13.139] may be bioactivated to reactive intermediates by sulfotransferases.

Based on the metabolism of other sulphur-containing compounds, the candidate furfuryl and furan monosulfides are expected to undergo oxidation mainly to sulfoxides and sulfones. Given the reactivity of thiol groups, whether free or from di(tri)sulfides, and the importance of thiol groups in cell physiology, it cannot be excluded that these substances interfere with normal cell function. Therefore, it is concluded that none of the candidate substances included in subgroup 2 can be predicted to be metabolised to innocuous substances.

A detailed description of the toxicokinetic features of the candidate substances in this FGE is reported in Annex 3.

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

In the present evaluation, one of the 18 candidate substances, 5-hydroxymethylfurfuraldehyde (5HMF) [FL-no: 13.139], may be metabolised to 5-[(sulfoxy)methyl]furfural (SMF), which shows genotoxic potential *in vitro*. Accordingly, the Procedure cannot be applied for this substance.

For the safety evaluation of the remaining 17 candidate substances from chemical group 14 the Procedure as outlined in Annex I was applied. The stepwise evaluations of the 17 substances are summarised in Table 2a.

The application of the Procedure is based on intakes estimated on the basis of the MSDI approach. For comparison of the intake estimations based on the MSDI approach and the mTAMDI approach, see Section 6.

Step 1

According to the decision tree approach by Cramer et al. (Cramer et al., 1978) 15 of the candidate substances are classified into structural class II and two candidate substances [FL-no: 13.108 and 13.178] into structural class III.

Step 2

Taking into account the metabolic pathways described in Section 4, none of the candidate substances is predicted to be metabolised to innocuous products. Therefore, the evaluation of the 17 candidate substances proceeds *via* the B-side of the evaluation scheme.

Step B3

The 15 candidate substances, which have been assigned to structural class II, have estimated European daily *per capita* intakes (MSDI) ranging from 0.0012 to 0.89 microgram (Table 2a). These intakes are below the threshold of concern of 540 microgram/person/day for structural class II. The estimated daily *per capita* intakes of the two candidate substances assigned to structural class III are 0.0012 and 37 microgram, respectively, which are also below the threshold of concern for the structural class of 90 microgram/person/day. Therefore, the safety evaluation proceeds to step B4 for all 17 candidate substances

Step B4

Considering that the seven candidate substances of subgroup 1 (non-sulphur-containing) are metabolised to yield furfural and furoic acid, the toxicity of the esters of furfuryl alcohol [FL-no: 13.127, 13.129, 13.132, and 13.133], furoic acid [FL-no: 13.102 and 13.122] and furanacrylic acid [FL-no: 13.011] is expected to be similar to that of the structurally related supporting substance furfural [FL-no:13.018] and of the candidate substance 2-furoic acid [FL-no: 13.136], which is the major metabolite of furfural. For furfural [FL-no:13.018] an ADI value of 0.5 mg/kg bw has recently been established by EFSA (EFSA, 2004j). The estimated daily *per capita* intakes based on the MSDI approach expressed in microgram/kg bw/day of candidate substances in subgroup 1 of the present FGE.13 are more than 500 fold below the ADI value.

Since no toxicity data are available on the eight sulphur-containing candidate substances in subgroup 2, the relevant NOAEL values originate from structurally related supporting substances:

The candidate substances ethyl furfuryl sulfide [FL-no: 13.124] and methyl 5-methylfurfuryl sulfide [FL-no: 13.145] are expected to participate in the same metabolic pathways as the supporting substance furfuryl isopropyl sulfide [FL-no: 13.032] and therefore to have same toxicological properties. Furfuryl isopropyl sulfide was tested in a single dose level (1.3 mg/kg bw/day) 90-day dietary study with rats (Posternak et al., 1969), giving no effects. Comparison of the only level tested with no effect taken as a NOAEL with the estimated daily *per capita* intakes based on the MSDI approach and expressed in microgram/kg bw/day of ethyl furfuryl sulfide [FL-no:13.124] and methyl 5-methylfurfuryl sulfide [FL-no: 13.145], provided a margin of safety > 10^5.

The candidate substance 5-methyl-2-furanmethanethiol [FL-no: 13.149] is structurally related to the supporting substance furfuryl mercaptan [FL-no: 13.026]. The NOAEL of furfuryl mercaptan in a multiple dose level 91-day oral gavage study with rats was 3 mg/kg bw/day (Phillips et al., 1977). Comparison of the NOAEL for furfuryl mercaptan with the estimated daily *per capita* intake based on the MSDI approach expressed in microgram/kg bw/day of 5-methyl-2-furanmethanethiol provided an adequate margin of safety > 4 x 10^5.

On the basis of general considerations on the metabolic pathways of di- and tri-sulfides (as described in Section 4 and in Annex III), the two candidate disulfides 2,5-dimethyl-3-(methyldithio)furan [FL-no: 13.113] and methyl 5-methylfurfuryl disulfide [FL-no: 13.144] and the candidate substance methyl furfuryl trisulfide [FL-no: 13.146] are expected to participate in the same metabolic pathways as the supporting substances furfuryl isopropyl sulfide [FL-no:13.032] and furfuryl mercaptan [FL-no: 13.026] and therefore to share also their toxicological properties. The NOAEL of furfuryl mercaptan in a multiple dose level 91-day oral gavage study with rats was 3 mg/kg bw/day (Phillips et al., 1977) whereas no effects were evidenced in a single dose level 90-day dietary study on rats with furfuryl isopropyl sulfide (1.3 mg/kg bw/day) (Posternak et al., 1969). Comparison of the lowest value with no effect taken as a NOAEL (1.3 mg/kg bw/day) with the estimated daily *per capita* intake based on the MSDI approach expressed in microgram/kg bw/day of methyl furfuryl trisulfide provided an adequate margin of safety > 3×10^6.

The candidate substance 3-(furfuryldithio)-2-methylfuran [FL-no: 13.178] is structurally related to the supporting substance bis(2-methyl-3-furyl) disulfide [FL-no: 13.016] which has been tested in two single-dose-level 90-day dietary studies with rats at 3.96 mg/kg bw/day and 0.45 mg/kg bw/day (Morgareidge & Oser, 1970a; Oser, 1970a). Integrating the results obtained in the two studies, which have been conducted by the same authors with the same strain of rats and the same methodology, it can be concluded that the NOAEL is 0.45 mg/kg bw/day. When this NOAEL was compared to the estimated daily *per capita* intake based on the MSDI approach expressed in microgram/kg bw/day of 3-(furfuryldithio)-2-methylfuran it provided an adequate margin of safety > 10^6.

The candidate substance 4,5-dihydro-3-mercapto-2-methylfuran [FL-no: 13.108] is structurally related to the supporting substance 2-methyl-3-thioacetoxy-4,5-dihydrofuran [FL-no: 13.086]. Several subchronic studies have been carried out with this supporting substance. The NOAEL has been derived in a multiple dose level 13 weeks dietary study with rats and was 1.4 mg/kg bw/day (Munday & Gellatly, 1973a). Comparison of the NOAEL for 2-methyl-3-thioacetoxy-4,5-dihydrofuran with the estimated daily *per capita* intake based on the MSDI approach expressed in microgram/kg bw/day of 4,5-dihydro-3-mercapto-2-methylfuran provided an adequate margin of safety of about 10^3.

In summary, it can be concluded at step B4 of the Procedure that the 17 candidate substances included in FGE.13 pose no safety concern when they are used as flavouring substances at the estimated levels of daily *per capita* intake based on the MSDI approach.

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

The estimated intakes for the 16 candidate substances in structural class II based on the mTAMDI range from 75 to 3700 microgram/person/day. For nine of the substances the mTAMDI is above the threshold of concern of 540 microgram/person/day. For comparison of the intake estimates based on the MSDI approach and the mTAMDI approach see Table 6.1.

The estimated intakes of the two substances assigned to structural class III, based on the mTAMDI, are 150 microgram/person/day, and thus 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.

For 11 candidate substances further information is required. This would include more reliable intake data and then, if required, additional toxicological data.

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

FL-no	EU Register name	MSDI (µg/*capita*/day)	mTAMDI (µg/person/day)	Structural class	Threshold of concern (µg/person/day)
13.011	Ethyl furfuracrylate	0.12	3700	Class II	540
13.102	Butyl 2-furoate	0.12	3700	Class II	540
13.113	2,5-Dimethyl-3-(methyldithio)furan	0.0012	150	Class II	540
13.114	2,5-Dimethyl-3-(methylthio)furan	0.0024	150	Class II	540
13.122	Ethyl 2-furoate	0.39	3700	Class II	540
13.124	Ethyl furfuryl sulfide	0.18	75	Class II	540
13.127	Furfuryl 2-methylbutyrate	0.73	3700	Class II	540
13.129	Furfuryl but-2-enoate	0.11	3700	Class II	540
13.132	Furfuryl hexanoate	0.58	3700	Class II	540
13.133	Furfuryl isobutyrate	0.89	3700	Class II	540
13.136	2-Furoic acid	0.013	1300	Class II	540
13.144	Methyl 5-methylfurfuryl disulfide	0.0024	75	Class II	540
13.145	Methyl 5-methylfurfuryl sulfide	0.0024	150	Class II	540
13.146	Methyl furfuryl trisulfide	0.0024	150	Class II	540
13.149	5-Methyl-2-furanmethanethiol	0.37	150	Class II	540
13.139	5-Hydroxymethylfurfuraldehyde	0.012	1600	Class II	540
13.108	4,5-Dihydro-3-mercapto-2-methylfuran	37	150	Class III	90
13.178	3-(Furfuryldithio)-2-methylfuran	0.0012	150	Class III	90

7. Considerations of Combined Intakes from use as Flavouring Substances

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

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

On the basis of the reported annual production volumes in Europe (EFFA, 2003g), the total estimated daily *per capita* intake as flavourings of the 16 candidate flavouring substances assigned to structural class II is 3.5 microgram, which does not exceed the threshold of concern for a compound belonging to structural class II of 540 microgram/person/day. The daily *per capita* intake as flavourings of the two candidate flavouring substances assigned to structural class III is 37 microgram, which does not exceed the threshold of concern for a compound belonging to structural class III of 90 microgram/person/day.

The 18 candidate substances are structurally related to 47 supporting substances evaluated by JEFCA at its 55th meeting (JECFA, 2001a) and 59th meeting (JECFA, 2002c)[4]. The total estimated combined intake of candidate and supporting substances (in Europe) would be 860 and 41 microgram/*capita*/day for structural class II and III, respectively. The combined daily *per capita* intake of 860 microgram exceeds the threshold of concern of 540 microgram/person/day for structural class II substances. However, more than 50% of the combined daily *per capita* intake of 860 microgram comes from furfural for which, together with the furfural component of furfural diethyl acetal, an ADI of 0.5 mg/kg bw has been allocated (EFSA, 2004j).

8. Toxicity

8.1. Acute Toxicity

Oral LD_{50} values in rodents have been reported for two candidate substances from subgroup 1 [FL-no: 13.102 and 13.139] and for five supporting substances for subgroup 1 and 13 supporting substances for subgroup 2 (see Annex IV-Table IV.1).

The reported oral LD_{50} values for the candidate substances are around 1500-2000 mg/kg bw both in rats and in mice, while the majority of reported rodent oral LD_{50} values for supporting substances falls between 100 and 250 mg/kg bw.

8.2. Subacute, Subchronic, Chronic and Carcinogenicity Studies

Short-term and long-term toxicity studies are available for two candidate substances included in subgroup 1, namely 2-furoic acid [FL-no: 13.136] and 5HMF [FL-no: 13.139] and for three related supporting substances, including furfural. They indicate that the liver is the critical target for their toxicity. Recently EFSA has established an ADI value of 0.5 mg/kg bw for furfural and the furfural component of furfural diethyl acetal (EFSA, 2004j).

No toxicity data are available on candidate substances included in subgroup 2, however, results from toxicity studies on 16 supporting substances have been reported.

Toxicity studies on supporting substances used for deriving the NOAEL used in the Procedure are briefly reported in the following.

Furfuryl mercaptan [FL-no: 13.026]

Groups of 15 Wistar rats of each sex were given daily doses of 0, 1, 3 or 30 mg/kg bw furfuryl mercaptan, dissolved in corn oil by stomach tube for 13 weeks. At the highest dose level, a decrease in body weight gain was associated with a reduced food intake. Significant differences in absolute and relative organ weights (i.e. brain, kidneys, stomach, heart, and liver) were reported for the 30 mg/kg bw group at 13 weeks. Haematological examination revealed increases in haemoglobin concentration and packed cell volume at the highest dose level at study termination. Histopathological examination showed no abnormalities related to the treatment. Based on organ weight changes evidenced at the highest dose group, 3 mg/kg bw/day can be considered as a NOAEL (Phillips et al., 1977).

[4] *Three of the JEFCA evaluated supporting substances are not used in Europe. European tonnage data are available for 32 of the remaining 44 supporting substances.*

Furfuryl isopropyl sulfide [FL-no: 13.032]

Groups of 16 Charles River CD rats of each sex were given *ad libitum* access to water and food. The test material (98% purity) was incorporated into the diet of the treatment group. The concentration of the test material in the diet was adjusted during the study to maintain constant levels of dietary intake of about 1.30 mg furfuryl isopropyl sulfide/kg bw/day (Posternak et al., 1969). The authors stated that no major differences were observed between groups of treated and control animals, based on measurements of growth, food intake, haematological and clinical chemistry parameters, organ weights and gross and histopathologic examinations. However, no numeric data were reported. The only level tested (1.34 mg/kg bw/day) has been taken as a NOAEL (Posternak et al., 1969).

Bis(2-methyl-3-furyl)disulfide [FL-no: 13.016]

Groups of 15 Wistar rats of each sex were administered bis(2-methyl-3-furyl)disulfide dissolved in acetone and blended into the diet to yield a daily dose of 3.96 mg/kg bw for 90 days. Control group received diet mixed with the vehicle only. Dietary acetone was evaporated before presentation to the animals. Body weight gain in treated animals was 37% and 15% less in males and females, respectively, than that for corresponding controls. Absolute liver and kidney weights were lower but their relative weights were significantly greater in treated animals than in control. Haematological examinations, blood chemical determinations, urine analysis, and gross and histopathological examinations, showed no differences between treated and control animals. The authors concluded that administration of 3.96 mg/kg bw/day of the test substance for 90 days was associated with retarded growth rates in both sexes accompanied by decreased efficiency of food utilization and elevated relative liver and kidney weights in males (Oser, 1970a).

Using the same protocol, the test material was tested in a 90-day study performed at a lower dose level (0.45 mg/kg/day). The results of this study indicated that there were no effects observed in the treatment group when compared to the control with respect to the above mentioned parameters. Therefore, 0.45 mg/kg bw/day can be considered the NOAEL for bis(2-methyl-3-furyl)disulfide (Morgareidge & Oser, 1970a).

2-Methyl-3-thioacetoxy-4, 5-dihydrofuran [FL-no: 13.086]

Groups of eight male and eight female Wistar rats were fed for 13 weeks on diets containing 2-methyl-3-thioacetoxy-4,5-dihydrofuran spray dried with maltodextrin, to give intakes of 1.4, 2.8, 5.55, 8.3, 13.85 or 27.7 mg/kg bw/day . Sixteen male and 16 female rats were fed a control diet. Parameters studied included body weight gain, food intake, food utilization, water intake, serum chemistry, haematology, organ weights, macroscopic appearance at post-mortem examination and histopathology.

Haemolysis of red blood cells as evidenced by reduced hematocrit values, generally accompanied by decreased haemoglobin levels, increased spleen weights and microscopic changes in the spleen and liver were observed. No other adverse effects were demonstrated. The no-effect level was established as 1.4 mg/kg bw/day (Munday & Gellatly, 1973a).

8.3. Developmental / Reproductive Toxicity Studies

Developmental and reproductive toxicity data are available for one candidate (2-furoic acid [FL-no: 13.136]) and one supporting substance (furfural [FL-no: 13.018]) included in subgroup 1. No data are available for subgroup 2. For more details see Table IV.3.

8.4. Genotoxicity Studies

All available genotoxicity studies were on candidate substances included in subgroup 1 or on their related supporting substances. Data on *in vitro* genotoxicity were provided for the two candidate substances, 5HMF [FL-no: 13.139] and furoic acid [FL-no: 13.136] as well as for five supporting substances. Data on *in vivo* genotoxicity were only provided on two of the supporting substances.

No data were available on candidate or supporting substances structurally related to sulphur-substituted furans (subgroup 2).

In the *in vitro* tests, 5HMF gave negative results in the traditional Ames test in strains TA 98, TA 100, TA 104, TA 1535 and TA 1537 in five and positive results in two studies; the positive response were in both cases higher in the absence of S9 (see Table IV.4). A positive result was obtained also in the *Umu* assay, although only at high concentrations, resulting in reduced cell viability (Janzowski et al., 2000) and in a Rec assay on *B. subtilis* (Shinohara et al., 1986). In V79 cells, 5HMF induced a small (although statistically significant) increase in chromosomal aberrations, a reduction in mitotic index and, only at high concentrations, resulting in reduced cell viability, also HPRT mutations (Janzowski et al., 2000). In TK6 human lymphoblast cells, 5HMF gave negative results in the HPRT and TK assay (Surh & Tannenbaum, 1994).

In an Ames test with TA 104 strain upon inclusion of PAPS, a sulfo-group donor, and rat liver cytosol into the experimental model, 5HMF gave a positive result, suggesting that it can be activated to reactive metabolites following sulfation, with formation of sulphate-ester (SMF). Indeed, the mutagenic effect could be partly suppressed by the addition of sulphotransferase inhibitors. In accordance, SMF in TA 104 was genotoxic in the absence of any metabolic system (cytotoxicity not specified); the effect was reduced by addition of GSH and GSH-transferases and restored when this latter enzyme was inhibited (Lee et al., 1995b).

The formation of SMF was supported by the detection of an unstable conjugate, which disappeared within 60 minutes, when 5HMF was incubated with ^{35}S-PAPS and liver cytosol. The exact nature of SMF was not elucidated, but its molecular mass was consistent with that of the sulphate-ester of 5HMF (Surh & Tannenbaum, 1994).

When the genotoxicity of chemically synthesised SMF was tested in Salmonella strain TM677 (8-AG-resistance), without any metabolic activation, a clear positive response was obtained at concentrations that reduced cell survival to < 60%. Genotoxicity was also observed with SMF in human lymphoblasts at the TK and HPRT loci, at concentrations ($\geq$ 40 microg/ml) reducing cell survival to $\geq$ 63%. No genotoxicity was observed with 5HMF, with its acetate ester or with the sulphation product of 2-methyl furfuryl alcohol, suggesting that the genotoxicity of SMF requires the presence of both a reactive sulphate group and a free aldehyde group.

An assay for primary DNA damage ("Comet assay") did not show an effect of 5HMF in V79 and Caco-2 cells up to cytotoxic concentrations (80 mM). 5HMF causes a slight but significant increase in DNA single strand breaks in primary rat hepatocytes at cytotoxic levels (40 – 100 mM), whereas in human colon biopsy material the same effect was seen in the absence of cytotoxicity. 5HMF at non cytotoxic concentrations induced a substantial concentration-related GSH depletion in V79, Caco-2 and rat liver cells. The effect of sulphate conjugation was not directly studied, but since this activity is present at least in primary hepatocytes, it might have contributed to the depletion of GSH and to induction of DNA strand breaks in these cells. However, this study was not considered appropriate to evaluate the possible mutagenic activity of SMF in mammalian cells and consequently of 5HMF *in vivo* (Janzowski et al., 2000).

To support the genotoxic potential of 5HMF, some indications for tumorigenic activities of 5HMF have been obtained with rats and mice. It has been reported that 5HMF may act as both an initiator and a promoter in the induction of colonic aberrant cryptic foci in rats (Archer et al., 1992; Bruce et al., 1993; Zang et al., 1993). In addition induction of skin papillomas has been described after topical application of doses of 10 or 25 micromol 5HMF to mice (Surh et al., 1994).

Furoic acid gave negative results in three studies in the Ames test in strains TA 98 and TA 100. Furoic acid was also negative in DNA repair test in E.coli and in a UDS assay using primary rat hepatocytes.

In vitro genotoxicity data were available for five supporting substances (furfuryl acetate, furfuryl alcohol, furfural, 5HMF, and methyl-2-furoate) and *in vivo* genotoxicity data for the two supporting substances furfuryl alcohol and furfural. Most studies were negative, although some positive results were reported. However, the genotoxicity of furfural has recently been re-evaluated by the AFC panel, which concluded that furfural did not induce gene mutations *in vivo*, on the basis of new studies with transgenic mice (EFSA, 2004j).
No genotoxicity data were available on the sulphur-containing candidate substances in subgroup 2, nor on their related supporting substances. Thus based on the lack of data the potential genotoxicity of the sulphur-containing candidate substances cannot be evaluated.

Overall, except for the candidate substance 5HMF [FL-no: 13.139], the genotoxicity data available on the candidate furoic acid and on supporting substances do not give rise to concern with respect to genotoxicity of the remaining eight candidate substances included in subgroup 1. Based on data on the mutagenic activity of a sulphate conjugate of 5HMF, there is sufficient evidence to raise concern about a genotoxic potential for this candidate.

The lack of data on the sulphur-containing candidate substances included in subgroup 2 or on related supporting substances does not allow to conclude on their potential for genotoxicity.

9. Conclusions

The 18 candidate substances are furfuryl and furan derivatives with and without additional side-chain substituents and heteroatoms from chemical group 14. They can be divided into two subgroups, consisting of nine candidates substances each, depending on the absence or the presence of sulphur-containing substituents.

One of the 18 flavouring substances possesses a chiral centre [FL-no: 13.127]. This substance has been presented without any indication that the commercial flavouring substance has dominance of one or the other enantiomer.

Two of the 18 substances can exist as geometrical isomers [FL-no: 13.011 and 13.129]. In both cases, no indication has been given that one of the possible isomers has preponderance in the commercial flavouring material.

Sixteen of the candidate substances are classified into structural class II, two candidate substances ([FL-no: 13.108 and 13.178]) are classified into structural class III.

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

According to the default MSDI approach, the 18 flavouring substances in this group have intakes in Europe from 0.0012 to 37 microgram/*capita*/day which are below the thresholds of concern for both

structural class II (540 microgram/person/day) and structural class III (90 microgram/person/day) substances.

On the basis of the reported annual production in Europe (MSDI approach), the combined intake of the 16 candidate substances belonging to structural class II and of the two candidate substances belonging to structural class III would result in a total intake of approximately 3.6 and 37 microgram/*capita*/day, respectively. These values are lower than the thresholds of concern for structural class II or class III substances. The total estimated combined intakes of the candidate and supporting substances (in Europe) are 860 and 41 microgram/*capita*/day for structural class II and III substances, respectively. The combined daily *per capita* intake of 860 microgram exceeds the threshold of concern of 540 microgram/person/day for structural class II substances. However, more than 50% of the combined daily *per capita* intake of 860 microgram comes from furfural for which, together with the furfural component of furfural diethyl acetal, an ADI of 0.5 mg/kg bw has been allocated.

The metabolism of the candidate substances involves hydrolysis of the esters and oxidation of the alcohol components to the corresponding aldehydes and carboxylic acids. These oxidations may be followed by conversion into conjugates which are readily excreted. Opening of the furan ring may give rise to the formation of reactive intermediates. Therefore, it cannot be predicted that the candidate substances included in subgroup 1 are metabolised to innocuous products. In addition, 5-hydroxymethylfurfuraldehyde (5HMF) [FL-no:13.139] has been shown to be bioactivated *in vitro* to 5-[(sulfoxy)methyl] furfural (SMF), through sulfonation of its allylic hydroxyl functional group, catalyzed by sulfotransferases. The resulting ester has been demonstrated to induce genotoxic effects *in vitro*.

Based on the general knowledge on the metabolism of sulphur-containing compounds, candidate substances bearing a free thiol group [FL-no: 13.108 and 13.149] can be considered reactive *per se* interacting with endogenous sulphur-containing substances, e.g. glutathione and proteins, thus potentially triggering adverse effects.

The candidate furfuryl and furan monosulfides are expected to undergo oxidation mainly to sulfoxides and sulfones, which are physiologically stable and excreted unchanged in the urine. Alternatively they can be conjugated with glutathione, giving rise to mixed disulfides, which can be oxidised to thiosulfinates or thiosulfones or reduced to free thiols. Similar metabolic pathways may be predicted for the candidate disulfides [FL-no: 13.113, 13.144, and 13.178] and very likely for the one trisulfide candidate substance [FL-no: 13.146]. Given the reactivity of thiol groups, whether free or resulting from di(tri)sulfide, and their importance in cell physiology, it cannot be excluded that the nine candidate substances included in subgroup 2 of FGE.13 interfere with normal cell function and therefore they cannot be predicted to be metabolised to innocuous substances.

Data on genotoxicity were available on two candidate substances in subgroup 1, on 5HMF [FL-no: 13.139] and 2-furoic acid [FL-no: 13.136], and on five supporting substances. Overall, except for the candidate substance 5HMF, the *in vitro* and *in vivo* data available do not give rise to concern with respect to genotoxicity of the remaining eight candidate substances included in subgroup 1. Based on data on the genotoxic activity of 5HMF mediated by its sulfate conjugation to its 5-hydroxymethyl group, there is sufficient evidence for a genotoxic potential *in vitro*. However, the lack of *in vivo* data does not allow a final evaluation and the substance cannot be taken through the Procedure.

The lack of data on the sulphur-containing candidate substances included in subgroup 2, or on the related supporting substances, does not allow to conclude on their genotoxicity.

Short-term and long-term toxicity studies are available for two candidate substances included in subgroup 1, namely 2-furoic acid [FL-no: 13.136] and 5HMF [FL-no: 13.139] and for three related supporting substances, including furfural. They indicate that the liver is the critical target for their toxicity. Recently EFSA has established an ADI value of 0.5 mg/kg bw for furfural and the furfural component of furfural diethyl acetal.

No toxicity data are available on candidate substances included in subgroup 2; however results from toxicity studies on 16 supporting substances have been reported. Many of the available studies were performed either with a single dose level or multiple dose levels that produced no effects; the doses producing no adverse effects ranged between 0.45 and 10 mg/kg/day.

It was considered that on the basis of the default MSDI approach the remaining 17 candidate substances would not give rise to safety concerns at the estimated levels of intake arising from their use as flavouring substances.

When the estimated intakes were based on the mTAMDI they ranged from 150 to 3700 microgram/person/day for the 16 flavouring substances from structural class II. Thus, the intakes were above the threshold of concern for structural class II of 540 microgram/person/day for nine flavouring substances [FL-no: 13.011, 13.102, 13.122, 13.127, 13.129, 13.132, 13.133, 13.136 and 13.139] and below the threshold of concern for seven flavouring substances [FL-no: 13.113, 13.114, 13.124, 13.144, 13.145, 13.146, and 13.149]. The estimated intakes of the two flavouring substances assigned to structural class III, based on the mTAMDI, were 150 microgram/person/day, which are above the threshold of concern for structural class III of 90 microgram/person/day. Thus for 10 of the 17 flavouring substances taken through the Procedure, the intakes, estimated on the basis of the mTAMDI, exceed the relevant threshold for their structural class, to which the flavouring substance has been assigned. Therefore, for these 10 substances 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 candidate substances can be applied to the materials of commerce, it is necessary to consider the available specifications:

Provided that information on geometrical isomerism/chirality is submitted by Industry for the substances [FL-no: 13.011, 13.127, and 13.129] then adequate specifications including complete purity criteria and identity tests for the materials of commerce have been provided for the 17 flavouring substances which have been evaluated according to the Procedure. The final evaluation of the material of commerce cannot be performed for the three substances [FL-no: 13.011, 13.127, and 13.129] pending further information.

TABLE 1: SPECIFICATION SUMMARY OF THE SUBSTANCES IN THE FLAVOURING GROUP EVALUATION 13

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 13

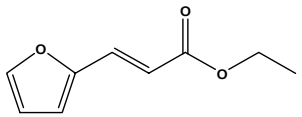
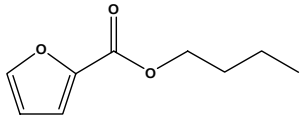
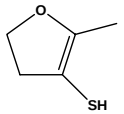
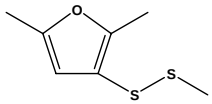
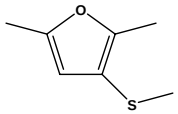
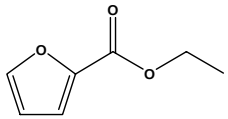
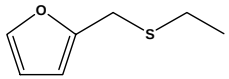
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility in water 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
13.011	Ethyl furfuracrylate 6)		545 623-20-1	Liquid $C_9H_{10}O_3$ 166.18	Practically insoluble or insoluble 1 ml in 1 ml	229 14 MS 95 %	1.544-1.550 1.092-1.098	(Z) or (E) isomer not specified by CAS no reported
13.102	Butyl 2-furoate		583-33-5	Liquid $C_9H_{12}O_3$ 168.19	Practically insoluble or insoluble 1 ml in 1 ml	233 MS 95 %	1.469-1.475 1.052-1.058	
13.108	4,5-Dihydro-3-mercapto-2-methylfuran		26486-13-5	Liquid C_5H_8OS 116.18	Slightly soluble 1 ml in 1 ml	160 MS 95 %	1.497-1.503 1.047-1.053	
13.113	2,5-Dimethyl-3-(methyldithio)furan		61197-06-6	Solid $C_7H_{10}OS_2$ 174.28	Practically insoluble or insoluble 1 ml in 1 ml	284 45 MS 95 %	n.a. n.a.	
13.114	2,5-Dimethyl-3-(methylthio)furan		63359-63-7	Liquid $C_7H_{10}OS$ 142.22	Practically insoluble or insoluble 1 ml in 1 ml	63 (13 hPa) MS 95 %	1.503-1.509 1.042-1.048	
13.122	Ethyl 2-furoate		10588 614-99-3	Solid $C_7H_8O_3$ 140.14	Practically insoluble or insoluble 1 ml in 1 ml	196 36 MS 99 %	n.a. n.a.	
13.124	Ethyl furfuryl sulfide		2024-70-6	Liquid $C_7H_{10}OS$ 142.22	Slightly soluble 1 ml in 1 ml	73 (13 hPa) MS 95 %	1.509-1.515 1.047-1.053	

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 13

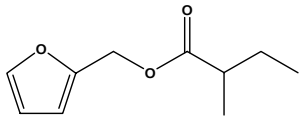
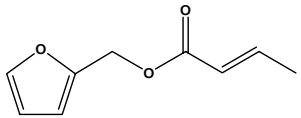
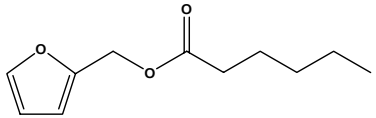
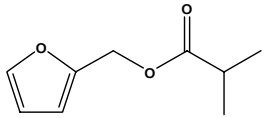
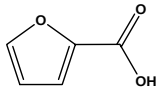
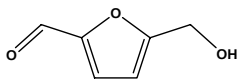
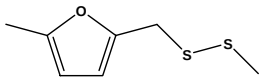
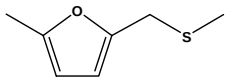
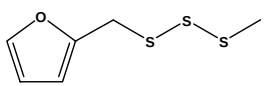
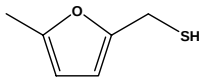
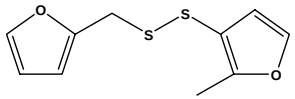
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility in water 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
13.127	Furfuryl 2-methylbutyrate 6)		10643 13678-61-0	Liquid $C_{10}H_{14}O_3$ 182.22	Practically insoluble or insoluble 1 ml in 1 ml	263 MS 95 %	1.455-1.461 1.009-1.015	(R) or (S) enantiomer not specified by CAS no reported
13.129	Furfuryl but-2-enoate 6)		59020-84-7	Liquid $C_9H_{10}O_3$ 166.17	Practically insoluble or insoluble 1 ml in 1 ml	245 NMR 95 %	1.491-1.497 1.034-1.040	(Z) or (E) isomer not specified by CAS no reported
13.132	Furfuryl hexanoate		39252-02-3	Liquid $C_{11}H_{16}O_3$ 196.25	Practically insoluble or insoluble 1 ml in 1 ml	224 MS 98 %	1.452-1.458 1.002-1.014	SG range > 0.01
13.133	Furfuryl isobutyrate		10641 6270-55-9	Liquid $C_9H_{12}O_3$ 168.19	Practically insoluble or insoluble 1 ml in 1 ml	85 (20 hPa) MS 95 %	1.497-1.503 1.028-1.034	
13.136	2-Furoic acid		10098 88-14-2	Solid $C_5H_4O_3$ 112.08	Slightly soluble 1 ml in 1 ml	231 132 MS 95 %	n.a. n.a.	
13.139	5-Hydroxymethylfurfuraldehyde		11112 67-47-0	Solid $C_6H_6O_3$ 126.11	Slightly soluble 1 ml in 1 ml	154 (16 hPa) 34 MS 95 %	n.a. n.a.	
13.144	Methyl 5-methylfurfuryl disulfide		78818-78-7	Solid $C_7H_{10}OS_2$ 174.28	Practically insoluble or insoluble 1 ml in 1 ml	279 32 NMR 95 %	n.a. n.a.	
13.145	Methyl 5-methylfurfuryl sulfide		11522 13679-60-2	Liquid $C_7H_{10}OS$ 142.22	Slightly soluble 1 ml in 1 ml	84 (20 hPa) NMR 95 %	1.509-1.515 1.048-1.054	
13.146	Methyl furfuryl trisulfide		66169-00-4	Solid $C_6H_8OS_3$ 192.32	Practically insoluble or insoluble 1 ml in 1 ml	320 43 NMR 95 %	n.a. n.a.	

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 13

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
13.149	5-Methyl-2-furanmethanethiol		59303-05-8	Liquid C_6H_8OS 128.19	Slightly soluble 1 ml in 1 ml	62 (17 hPa) MS 95 %	1.523-1.529 1.041-1.047	
13.178	3-(Furfuryldithio)-2-methylfuran		109537-55-5	Solid $C_{10}H_{10}O_2S_2$ 226.32	Practically insoluble or insoluble 1 ml in 1 ml	398 122 NMR 95 %	n.a. n.a.	

1) Solubility in water, if not otherwise stated

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

3) At 1013.25 hPa, if not otherwise stated

4) At 20°C, if not otherwise stated

5) At 25°C, if not otherwise stated

6) Stereoisomeric composition not specified

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

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

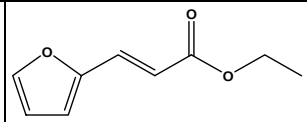
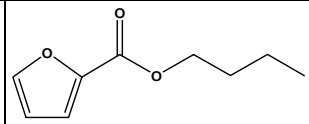
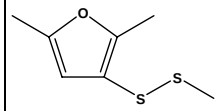
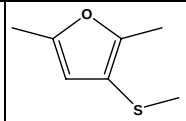
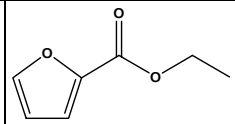
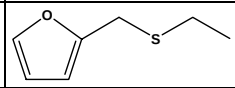
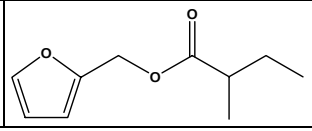
FL-no	EU Register name	Structural formula	MSDI 1) (μg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
13.011	Ethyl furfuracrylate		0.12	Class II B3: Intake below threshold, B4: Adequate NOEL exists	4)	7)	
13.102	Butyl 2-furoate		0.12	Class II B3: Intake below threshold, B4: Adequate NOEL exists	4)	6)	
13.113	2,5-Dimethyl-3-(methyldithio)furan		0.0012	Class II B3: Intake below threshold, B4: Adequate NOEL exists	4)	6)	
13.114	2,5-Dimethyl-3-(methylthio)furan		0.0024	Class II B3: Intake below threshold, B4: Adequate NOEL exists	4)	6)	
13.122	Ethyl 2-furoate		0.39	Class II B3: Intake below threshold, B4: Adequate NOEL exists	4)	6)	
13.124	Ethyl furfuryl sulfide		0.18	Class II B3: Intake below threshold, B4: Adequate NOEL exists	4)	6)	
13.127	Furfuryl 2-methylbutyrate		0.73	Class II B3: Intake below threshold, B4: Adequate NOEL exists	4)	7)	

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

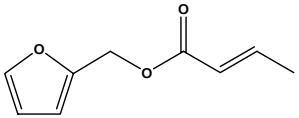
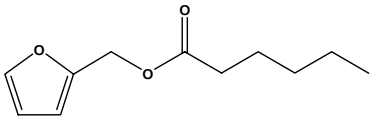
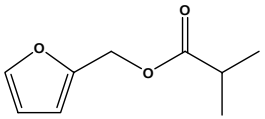
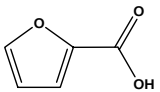
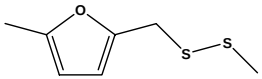
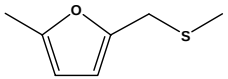
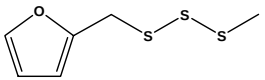
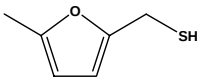
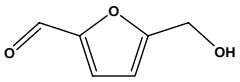
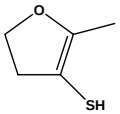
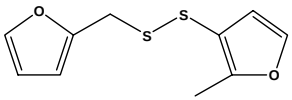
FL-no	EU Register name	Structural formula	MSDI 1) (μg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
13.129	Furfuryl but-2-enoate		0.11	Class II B3: Intake below threshold, B4: Adequate NOEL exists	4)	7)	
13.132	Furfuryl hexanoate		0.58	Class II B3: Intake below threshold, B4: Adequate NOEL exists	4)	6)	
13.133	Furfuryl isobutyrate		0.89	Class II B3: Intake below threshold, B4: Adequate NOEL exists	4)	6)	
13.136	2-Furoic acid		0.013	Class II B3: Intake below threshold, B4: Adequate NOEL exists	4)	6)	
13.144	Methyl 5-methylfurfuryl disulfide		0.0024	Class II B3: Intake below threshold, B4: Adequate NOEL exists	4)	6)	
13.145	Methyl 5-methylfurfuryl sulfide		0.0024	Class II B3: Intake below threshold, B4: Adequate NOEL exists	4)	6)	
13.146	Methyl furfuryl trisulfide		0.0024	Class II B3: Intake below threshold, B4: Adequate NOEL exists	4)	6)	
13.149	5-Methyl-2-furanmethanethiol		0.37	Class II B3: Intake below threshold, B4: Adequate NOEL exists	4)	6)	
13.139	5-Hydroxymethylfurfuraldehyde		0.012	Class II No evaluation			a)

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

FL-no	EU Register name	Structural formula	MSDI 1) (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
13.108	4,5-Dihydro-3-mercapto-2-methylfuran		37	Class III B3: Intake below threshold, B4: Adequate NOEL exists	4)	6)	
13.178	3-(Furfuryldithio)-2-methylfuran		0.0012	Class III B3: Intake below threshold, B4: Adequate NOEL exists	4)	6)	

1) 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.

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

a) Genotoxic in vitro

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

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

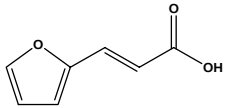
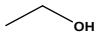
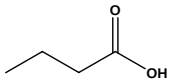
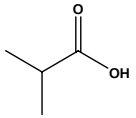
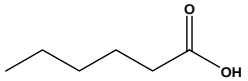
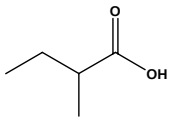
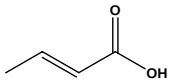
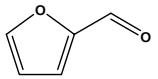
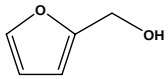
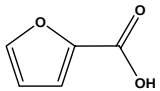
FL-no	EU Register name JECFA no	Structural formula	SCF status 1) JECFA status 2) CoE status 3)	Structural class 4) Procedure path (JECFA) 5)	Comments
	2-Furanacrylic acid		Not evaluated as flavour		Not in EU register
02.078	Ethanol 41		Category 1 a)	No evaluation	
08.005	Butyric acid 87		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake above threshold, A4: Endogenous	
08.006	2-Methylpropionic acid 253		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake below threshold	
08.009	Hexanoic acid 93		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake above threshold, A4: Endogenous	
08.046	2-Methylbutyric acid 255		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake below threshold	
08.072	But-2-enoic acid (cis and trans) 1371		No safety concern (JECFA, 2005)	No evaluation	JECFA evaluated (E)-isomer
13.018	Furfural 450		Category 4 a) No safety concern d) Category B c)	Class II B3: Intake below threshold, B4: Adequate NOEL exists	EFSA opinion, (EFSA, 2004j)
13.019	Furfuryl alcohol 451		No safety concern d) Category B c)	Class II B3: Intake below threshold, B4: Adequate NOEL exists	

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

FL-no	EU Register name JECFA no	Structural formula	SCF status 1) JECFA status 2) CoE status 3)	Structural class 4) Procedure path (JECFA) 5)	Comments
13.136	2-Furoic acid			Class II B3: Intake below threshold, B4: Adequate NOEL exists	

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

2) No safety concern at estimated levels of intake

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

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

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

a) (SCF, 1995)

b) (JECFA, 1999b)

c) (CoE, 1992)

d) (JECFA, 2001a)

TABLE 3: SUPPORTING SUBSTANCES SUMMARY

Table 3: Supporting Substances Summary

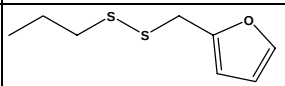
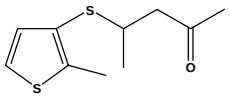
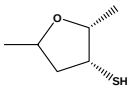
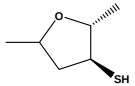
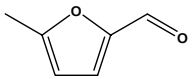
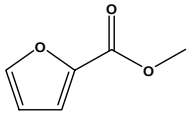
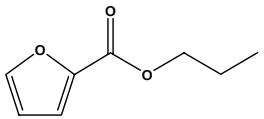
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/*capita*/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
	Furfuryl propyl disulfide		3979 252736-36-0	1079 JECFA specification (JECFA, 2002d)	ND	No safety concern c)	Not in EU-Register
	4-[(2-furanmethyl)thio]-2-pentanone		3840 180031-78-1	1084 JECFA specification (JECFA, 2002d)	ND	No safety concern c)	Not in EU-Register
	cis- and trans-2,5-dimethyl-3-tetrahydrofuranthiol	 cis-2,5-dimethyl-3-tetrahydrofuranthiol  trans-2,5-dimethyl-3-tetrahydrofuranthiol	3971 26486-21-5	1091 JECFA specification (JECFA, 2002d)	ND	No safety concern c)	Not in EU-Register
13.001	5-Methylfurfural		2702 119 620-02-0	745 JECFA specification (JECFA, 2000d)	140	No safety concern a) Category B b)	
13.002	Methyl 2-furoate		2703 358 611-13-2	746 JECFA specification (JECFA, 2000d)	30	No safety concern a) Deleted b)	Deleted CoE: the CoE Committee of Experts had no information as to the real use in foodstuffs and/or for which insufficient technicological and/or toxicological information was available (CoE, 1992)
13.003	Propyl 2-furoate		2946 359 615-10-1	747 JECFA specification (JECFA, 2000d)	ND	No safety concern a) Deleted b)	Deleted CoE: the CoE Committee of Experts had no information as to the real use in foodstuffs and/or for which insufficient technicological and/or toxicological information was available (CoE, 1992)

Table 3: Supporting Substances Summary

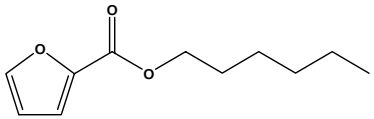
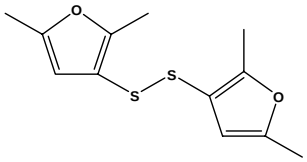
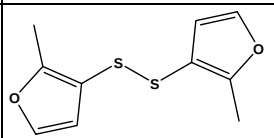
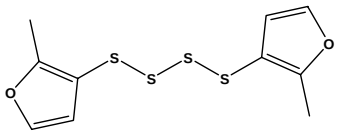
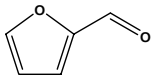
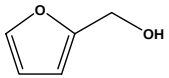
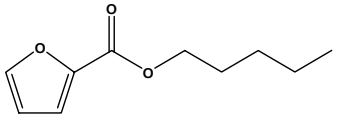
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (μg/*capita*/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
13.005	Hexyl 2-furoate		2571 361 39251-86-0	749 Tentitative JECFA spec. (JECFA, 2000d)	ND	No safety concern a) Deleted b)	Deleted CoE: the CoE Committee of Experts had no information as to the real use in foodstuffs and/or for which insufficient technicological and/or toxicological information was available (CoE, 1992)
13.015	bis-(2,5-Dimethyl-3-furyl) disulfide		3476 722 28588-73-0	1067 JECFA specification (JECFA, 2002d)	0.012	No safety concern c) Category A b)	
13.016	bis-(2-Methyl-3-furyl) disulfide		3259 723 28588-75-2	1066 JECFA specification (JECFA, 2002d)	0.27	No safety concern c) Category A b)	
13.017	bis-(2-Methyl-3-furyl) tetrasulfide		3260 724 28588-76-3	1068 JECFA specification (JECFA, 2002d)	ND	No safety concern c) Category A b)	
13.018	Furfural		2489 2014 98-01-1	450 JECFA specification (JECFA, 2001a)	440	Category 4 d) No safety concern a) Category B b)	EFSA opinion, 2004
13.019	Furfuryl alcohol		2491 2023 98-00-0	451 JECFA specification (JECFA, 2001a)	180	No safety concern a) Category B b)	
13.025	Pentyl 2-furoate		2072 2109 1334-82-3	748 Tentitative JECFA spec. (JECFA, 2000d)	ND	No safety concern a) Deleted b)	Deleted CoE: the CoE Committee of Experts had no information as to the real use in foodstuffs and/or for which insufficient technicological and/or toxicological information was available (CoE, 1992)

Table 3: Supporting Substances Summary

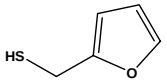
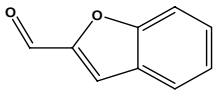
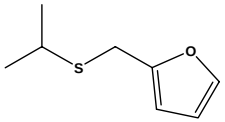
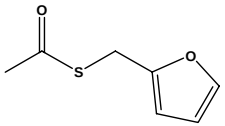
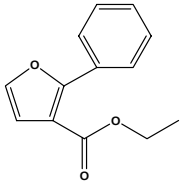
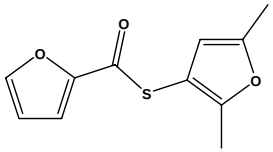
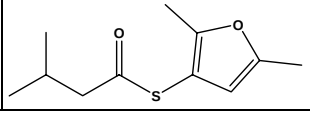
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (μg/*capita*/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
13.026	2-Furanmethanethiol		2493 2202 98-02-2	1072 JECFA specification (JECFA, 2002d)	29	No safety concern c) Category A b)	
13.031	2-Benzofurancarboxaldehyde		3128 2247 4265-16-1	751 JECFA specification (JECFA, 2000d)	ND	No safety concern a) Category B b)	
13.032	Furfuryl isopropyl sulfide		3161 2248 1883-78-9	1077 JECFA specification (JECFA, 2002d)	0.0012	No safety concern c) Category B b)	
13.033	S-Furfuryl acetothioate		3162 2250 13678-68-7	1074 JECFA specification (JECFA, 2002d)	0.43	No safety concern c) Category B b)	
13.038	2-Phenyl-3-carbethoxyfuran		3468 2309 50626-02-3	752 Tentitative JECFA spec. (JECFA, 2000d)	0.012	No safety concern a) Category B b)	
13.040	2,5-Dimethyl-3-thiofuroylfuran		3481 2323 65505-16-0	1071 JECFA specification (JECFA, 2002d)	ND	No safety concern c) Category B b)	
13.041	2,5-Dimethyl-3-(isopentylthio)furan		3482 2324 55764-28-8	1070 JECFA specification (JECFA, 2002d)	ND	No safety concern c) Category B b)	

Table 3: Supporting Substances Summary

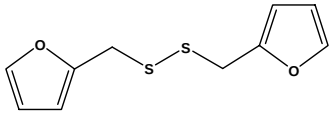
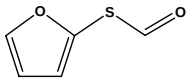
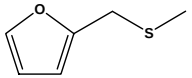
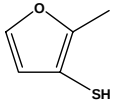
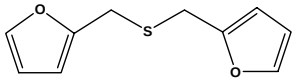
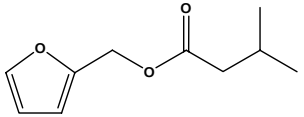
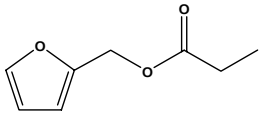
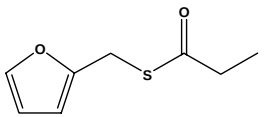
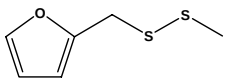
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (μg/*capita*/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
13.050	Difurfuryl disulfide		3146 11480 4437-20-1	1081 JECFA specification (JECFA, 2002d)	3.3	No safety concern c)	
13.051	2-Furfuryl thioformate		3158 11770 59020-90-5	1073 JECFA specification (JECFA, 2002d)	1.3	No safety concern c)	
13.053	Methyl furfuryl sulfide		3160 11482 1438-91-1	1076 JECFA specification (JECFA, 2002d)	0.97	No safety concern c)	
13.055	2-Methylfuran-3-thiol		3188 11678 28588-74-1	1060 JECFA specification (JECFA, 2002d)	0.52	No safety concern c)	
13.056	Difurfuryl sulfide		3238 11438 13678-67-6	1080 JECFA specification (JECFA, 2002d)	0.73	No safety concern c)	
13.057	Furfuryl isovalerate		3283 10642 13678-60-9	743 Tentitative JECFA spec. (JECFA, 2000d)	0.024	No safety concern a)	
13.062	Furfuryl propionate		3346 10646 623-19-8	740 Tentitative JECFA spec. (JECFA, 2000d)	1.7	No safety concern a)	
13.063	S-Furfuryl propanethioate		3347 11484 59020-85-8	1075 JECFA specification (JECFA, 2002d)	0.012	No safety concern c) -	
13.064	Methyl furfuryl disulfide		3362 11513 57500-00-2	1078 JECFA specification (JECFA, 2002d)	0.85	No safety concern c)	

Table 3: Supporting Substances Summary

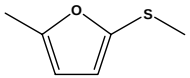
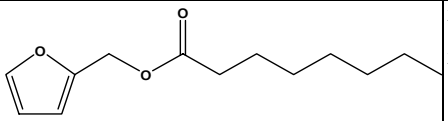
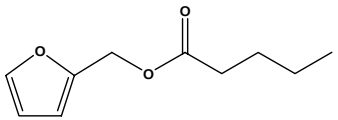
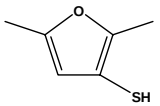
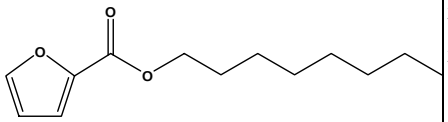
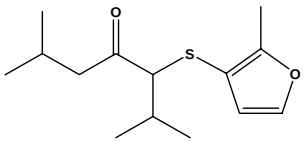
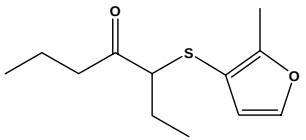
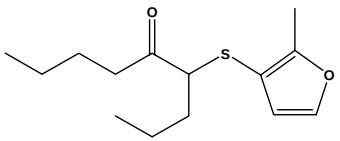
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (μg/*capita*/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
13.065	2-Methyl-5-(methylthio)furan		3366 11550 13678-59-6	1062 JECFA specification (JECFA, 2002d)	1.1	No safety concern c)	
13.067	Furfuryl octanoate		3396 10645 39252-03-4	742 Tentitative JECFA spec. (JECFA, 2000d)	0.012	No safety concern a)	
13.068	Furfuryl valerate		3397 10647 36701-01-6	741 Tentitative JECFA spec. (JECFA, 2000d)	0.24	No safety concern a)	
13.071	2,5-Dimethylfuran-3-thiol		3451 11457 55764-23-3	1063 JECFA specification (JECFA, 2002d)	0.024	No safety concern c)	
13.073	Octyl 2-furoate		3518 10864 39251-88-2	750 Tentitative JECFA spec. (JECFA, 2000d)	2.2	No safety concern a)	
13.075	2,6-Dimethyl-3-((2-methyl-3-furyl)thio)heptan-4-one		3538 11915 61295-51-0	1086 JECFA specification (JECFA, 2002d)	ND	No safety concern c)	
13.077	3-((2-Methyl-3-furyl)thio)heptan-4-one		3570 11922 61295-41-8	1085 JECFA specification (JECFA, 2002d)	ND	No safety concern c) -	
13.078	4-((2-Methyl-3-furyl)thio)nonan-5-one		3571 11923 61295-50-9	1087 JECFA specification (JECFA, 2002d)	ND	No safety concern c)	

Table 3: Supporting Substances Summary

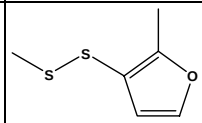
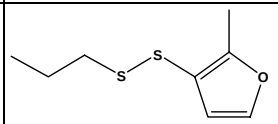
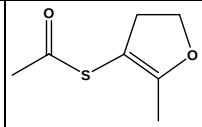
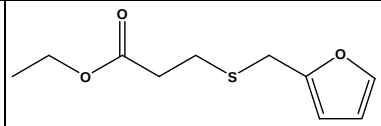
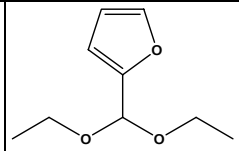
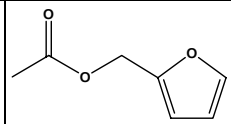
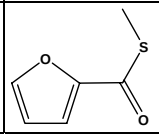
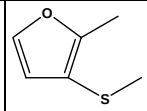
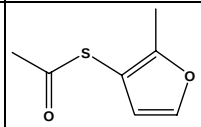
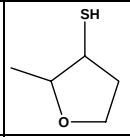
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/*capita*/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
13.079	Methyl 2-methyl-3-furyl disulfide		3573 11924 65505-17-1	1064 JECFA specification (JECFA, 2002d)	0.73	No safety concern c)	
13.082	Propyl 2-methyl-3-furyl disulfide		3607 61197-09-9	1065 JECFA specification (JECFA, 2002d)	ND	No safety concern c)	
13.086	4,5-Dihydro-2-methyl-3-thioacetoxyfuran		3636 26486-14-6	1089 JECFA specification (JECFA, 2002d)	ND	No safety concern c)	
13.093	Ethyl 3-(2-furfurylthio)propionate		3674 94278-27-0	1088 JECFA specification (JECFA, 2002d)	0.012	No safety concern c)	
13.126	Furfural diethyl acetal		13529-27-6		0.085		
13.128	Furfuryl acetate		2490 2065 623-17-6	739 JECFA specification (JECFA, 2000d)	16	No safety concern a) Category B b)	
13.142	S-Methyl 2-furanthiocarboxylate		3311 11547 13679-61-3	1083 JECFA specification (JECFA, 2002d)	0.37	No safety concern c)	

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
13.152	2-Methyl-3-(methylthio)furan		3949 63012-97-5	1061 JECFA specification (JECFA, 2002d)	1.2	No safety concern c)	
13.153	2-Methyl-3-furyl thioacetate		 55764-25-5	1069 JECFA specification (JECFA, 2002d)	0.012	No safety concern c)	
13.160	2-Methyltetrahydrofuran-3-thiol		3787 57124-87-5	1090 JECFA specification (JECFA, 2002d)	3.5	No safety concern c)	

1) 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, 2001a)

b) (CoE, 1992)

c) (JECFA, 2002c)

d) (SCF, 1995)

ND No data available

ANNEX I: PROCEDURE FOR THE SAFETY EVALUATION

The approach for a safety evaluation of chemically defined flavouring substances as referred to in Commission Regulation EC No 1565/2000 (EC, 2000), named the "Procedure", is shown in schematic form in Figure I.1. The Procedure is based on the opinion of the Scientific Committee on Food expressed on 2 December 1999 (SCF, 1999), which is derived from the evaluation procedure developed by the Joint FAO/WHO Expert Committee on Food Additives at its 44th, 46th and 49th meetings (JECFA, 1995; JECFA, 1996a; JECFA, 1997a; JECFA, 1999b).

The Procedure is a stepwise approach that integrates information on intake from current uses, structure-activity relationships, metabolism and, when needed, toxicity. One of the key elements in the procedure is the subdivision of flavourings into three structural classes (I, II, III) for which thresholds of concern (human exposure thresholds) that are not considered to present a safety concern 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[5] (Step 2)?
- do their exposures exceed the threshold of concern for the structural class (Step A3 and B3)?
- are the flavourings or their metabolites endogenous[6] (Step A4)?
- does a NOAEL exist on the flavourings or on structurally related substances (Step A5 and B4)?

In addition to the data provided for the flavouring substances to be evaluated (candidate substances), toxicological background information available for compounds structurally related to the candidate substances is considered (supporting substances), in order to assure that these data are consistent with the results obtained after application of the Procedure.

The Procedure is not to be applied to flavourings with existing unresolved problems of toxicity. Therefore, the right is reserved to use alternative approaches if data on specific flavourings warranted such actions.

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

[6] *"Endogenous substances": Intermediary metabolites normally present in human tissues and fluids, whether free or conjugated; hormones and other substances with biochemical or physiological regulatory functions are not included (JECFA, 1997a).*

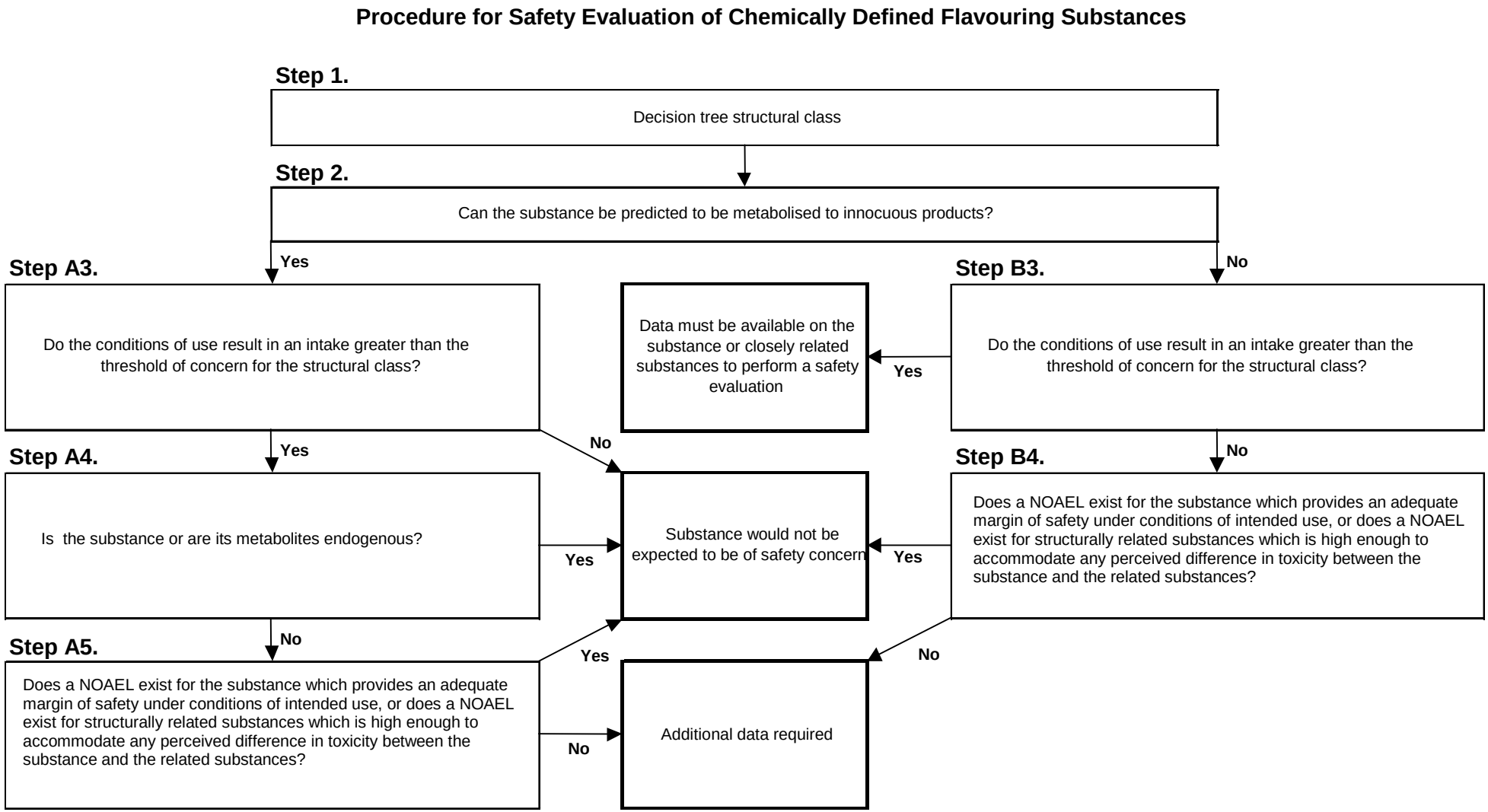


Figure I.1 *Procedure for Safety Evaluation of Chemically Defined Flavouring Substances*

ANNEX II: USE LEVELS / MTAMDI

II.1 Normal and Maximum Use Levels

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

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

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

The "normal and maximum use levels" are provided by Industry for the 18 candidate substances in the present flavouring group (Table II.1.2).

Table II.1.2.Normal and Maximum use levels (mg/kg) for candidate substances in FGE.13 (EFFA, 2003g)

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**
13.011	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	- -	20 100	5 25
13.102	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	- -	20 100	5 25
13.108	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
13.113	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
13.114	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	- -	1 5	0,2 1
13.122	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	- -	20 100	5 25
13.124	0,2 1	0,1 0,5	0,2 1	0,2 1	- -	0,2 1	0,1 0,5	0,2 1	0,1 0,2	0,1 0,2	- -	- -	0,1 0,5	0,2 1	0,1 0,3	- -	0,4 2	0,1 0,5
13.127	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	- -	20 100	5 25
13.129	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	- -	20 100	5 25
13.132	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	- -	20 100	5 25
13.133	7 35	5 25	10 50	7 35	- -	10 50	5 25	10 50	2 10	2 10	- -	- -	5 25	10 50	5 25	- -	20 100	5 25
13.136	3 15	2 10	3 15	2 10	- -	5 25	2 10	- -	1 5	1 5	- -	- -	2 10	3 15	2 10	- -	5 25	2 10

Table II.1.2.Normal and Maximum use levels (mg/kg) for candidate substances in FGE.13 (EFFA, 2003g)

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**
13.139	3 15	2 10	3 15	2 10	- -	4 20	2 10	5 25	1 5	1 5	- -	- -	2 10	3 15	2 10	- -	5 25	2 10
13.144	0,2 1	0,1 0,5	0,2 1	0,2 1	- -	0,2 1	0,1 0,5	0,2 1	0,1 0,2	0,1 0,2	- -	- -	0,1 0,5	0,2 1	0,1 0,3	- -	0,4 2	0,1 0,5
13.145	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
13.146	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
13.149	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
13.178	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

II.2 mTAMDI Calculations

The method for calculation of modified Theoretical Added Maximum Daily Intake (mTAMDI) values is based on the approach used by SCF up to 1995 (SCF, 1995). The assumption is that a person may consume the amount of flavourable foods and beverages listed in Table II.2.1. These consumption estimates are then multiplied by the reported use levels in the different food categories and summed up.

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

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

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

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

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

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

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

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

The mTAMDI values are presented for each of the 18 flavouring substances in the present flavouring group, for which Industry has provided use and use levels (EFFA, 2003g). The mTAMDI values are only given for highest reported use levels (see Table II.2.3).

Table II.2.3 mTAMDI (µg/person/day) and MSDI (µg/*capita*/day) for substances allocated to structural class II and III. (Threshold of concern for structural class II: 540 µg/person/day and structural class III: 90 µg/person/day)

FL-no	EU Register name	MSDI (µg/*capita*/day)	mTAMDI (µg/person/day)	Structural class	Threshold of concern (µg/person/day)
13.011	Ethyl furfuracrylate	0.12	3700	Class II	540
13.102	Butyl 2-furoate	0.12	3700	Class II	540
13.113	2,5-Dimethyl-3-(methyldithio)furan	0.0012	150	Class II	540
13.114	2,5-Dimethyl-3-(methylthio)furan	0.0024	150	Class II	540
13.122	Ethyl 2-furoate	0.39	3700	Class II	540
13.124	Ethyl furfuryl sulfide	0.18	75	Class II	540
13.127	Furfuryl 2-methylbutyrate	0.73	3700	Class II	540
13.129	Furfuryl but-2-enoate	0.11	3700	Class II	540
13.132	Furfuryl hexanoate	0.58	3700	Class II	540
13.133	Furfuryl isobutyrate	0.89	3700	Class II	540
13.136	2-Furoic acid	0.013	1300	Class II	540
13.144	Methyl 5-methylfurfuryl disulfide	0.0024	75	Class II	540
13.145	Methyl 5-methylfurfuryl sulfide	0.0024	150	Class II	540
13.146	Methyl furfuryl trisulfide	0.0024	150	Class II	540
13.149	5-Methyl-2-furanmethanethiol	0.37	150	Class II	540
13.139	5-Hydroxymethylfurfuraldehyde	0.012	1600	Class II	540
13.108	4,5-Dihydro-3-mercapto-2-methylfuran	37	150	Class III	90
13.178	3-(Furfuryldithio)-2-methylfuran	0.0012	150	Class III	90

ANNEX III: METABOLISM

III.1. Introduction

The candidate substances in FGE.13 are furan derivatives which can be divided into two subgroups. The nine candidate substances in subgroup 1 are furan derivatives, which contain only carbon, oxygen and hydrogen atoms.

The nine representatives of subgroup 2 are furan derivatives, containing sulphur substituents as free thiol groups, mono-, di- and tri-sulfides, except for 4,5-dihydro-3-mercapto-2-methylfuran [FL-no: 13.108], the only candidate containing a non aromatic ring.

Further details on the structural properties of the substances in these two subgroups are presented in the respective Sections of this annex.

III.2. Subgroup I – Furan derivatives without sulphur substitution

The candidate substances in subgroup I are furan derivatives such as esters of furfuryl alcohol, 2-furoic acid or furanacrylic acid. One substance 5-hydroxymethylfurfuraldehyde (5HMF) [FL-no: 13.139] is a furfural derivative with a hydroxymethyl side chain at C5 position of the furan ring. Several candidate substances are alpha-beta unsaturated alcohols, aldehydes or ketones, or precursors thereof. These are: esters of furfuryl alcohol [FL-no: 13.127, 13.129, 13.132, 13.133], and 5-hydroxymethylfurfuraldehyde [FL-no: 13.139]. For a number of candidate or supporting substances, among which furfural itself, metabolism data are available. An evaluation of the metabolism of several supporting substances can be found in JECFA/WHO (JECFA, 2001b).

III.2.1. Hydrolysis of esters

Four of the candidate substances in subgroup 1 [FL-no: 13.127, 13.129, 13.132, and 13.133] are esters of furfuryl alcohol [FL-no: 13.019] and two are esters of 2-furoic acid [FL-no: 13.122 and 13.102]. One candidate substance is an ester of furanacrylic acid [FL-no: 13.011].

The furfuryl alcohol esters (i.e. candidate substances furfuryl hexanoate [FL-no: 13.132], furfuryl isobutyrate [FL-no: 13.133], furfuryl 2-methylbutyrate [FL-no: 13.127] and furfuryl but-2-enoate [FL-no: 13.129]) are expected to be hydrolysed to furfuryl alcohol and the corresponding aliphatic carboxylic acid. Furoate esters (i.e. the candidate substances ethyl 2-furoate [FL-no: 13.122] and butyl 2-furoate [FL-no: 13.102]) are hydrolysed to candidate substance 2-furoic acid [FL-no: 13.136] and the corresponding saturated aliphatic alcohol. Upon hydrolysis, candidate substance [FL-no: 13.011] will produce furanacrylic acid and ethanol.

Hydrolysis is catalysed by classes of enzymes recognised as carboxylesterases or esterases, the most important of which are the B-esterases. In mammals, B-esterases occur in most tissues throughout the body, but predominate in the hepatocytes (Heymann, 1980).

While no hydrolysis data have been provided for the candidate esters of the present group of flavourings, there are *in vitro* hydrolysis data for some structurally related esters. Also some indirect evidence from an *in vivo* study is available.

Concentrations of 27 microlitre/L isoamyl furylpropion-1-ate or 40 microlitre/L ethyl furylpropion-1-ate were reported to be completely hydrolysed within two hours by pancreatin (Grundschober, 1977). Furoylglycine, the glycine conjugate of 2-furoic acid [FL-no: 13.136], has been reported to

be the major metabolite in the urine of rats given a 50 to 66 mg/kg oral dose of furfuryl diacetate, furfuryl propionate or 3-furanacrylate methyl ester which demonstrates that these esters are hydrolysed *in vivo* to form furfuryl alcohol, the metabolic precursor of 2-furoic acid [FL-no: 13.136]. After giving 3-furanacrylate methylester orally to rats, some parent substance was detected in the urine, which may indicate that for this substance the hydrolysis is not complete (Paul et al., 1949).

In summary:

Based on the data on supporting substances, it can be expected that the seven candidate esters included in this evaluation will be hydrolysed in humans, possibly prior to gastrointestinal absorption or otherwise during their passage through the liver, to their corresponding acids and alcohols within a relatively short time. The expected hydrolysis products for these esters and their evaluation status, when used as flavouring substances, are shown in table 2b to the main text of this opinion.

III.2.2. Absorption, distribution and elimination

Since enteric bacterial strains are capable of converting furfural to furfuryl alcohol under both aerobic and anaerobic conditions, both furfuryl alcohol and furfural are anticipated to be present in the gastrointestinal tract of animals given furfural. Enteric bacterial strains are also capable of reducing 5-hydroxymethylfurfuraldehyde (5HMF) [FL-no: 13.139] to a compound postulated to be 5-hydroxymethylfurfuryl alcohol under both aerobic and anaerobic conditions during an 8-hour incubation period (Boopathy et al., 1993). Therefore, 5HMF and 5-hydroxymethylfurfuryl alcohol are anticipated to be present in the gastrointestinal tract of animals given 5HMF. Biotransformation of 5HMF was accomplished by co-metabolism in the presence of glucose and peptone as main substrates (Boopathy et al., 1993). However, these enteric bacteria did not transform 2-furoic acid [FL-no: 13.136] under the experimental conditions.

At doses in the range from 0.1 mg/kg bw to 200 mg/kg bw, furfuryl alcohol and furfural are rapidly absorbed from the gastrointestinal tract, metabolised and excreted primarily in the urine (Nomeir, 1992; Parkash & Caldwell, 1994).

More than 86% of 0.275, 2.75 or 27.5 mg [carbonyl-^{14}C]-furfuryl alcohol/kg bw or 0.127, 1.15 or 12.5 mg [carbonyl-^{14}C]-furfural/kg bw given to F344 rats (4/group) by gavage in corn oil is rapidly absorbed from the gastrointestinal tract and excreted. At all dose levels, 83 – 89% of the dose was excreted in the urine and 2 – 4% in the faeces. The majority of radioactivity was excreted within the first 24 hours following dosing. Approximately 7% of the 12.5 mg/kg bw dose of furfural was exhaled as $^{14}CO_2$. Other ^{14}C-containing volatile substances in the exhaled air could not be demonstrated. At 72 hours following administration, residual radioactivity was distributed primarily to the liver and kidney, with tissue radioactivity generally proportional to the dose. Total radioactivity in the carcass comprised about 0.5% of the dose at 72 hrs (Nomeir, 1992).

[Carbonyl-^{14}C]-furfural was given in single oral doses of 0.1, 10 and 60 mg /kg bw to F344 rats or at 1, 20 and 200 mg [^{14}C]furfural/kg bw to CD1 mice. For both species both sexes were studied. More than 90% of the dose was recovered within 72 hours. The major route of elimination was the urine (>76% in rats and >60% in mice within 24 hours). Faecal elimination (1 – 7% in 72 hours for all dose groups) and expired CO_2 (5% in high dose male mice and 4% in low dose female mice after 24 hours; no other CO_2 measurements were taken) constituted minor routes of excretion (Parkash & Caldwell, 1994). Only minor differences in elimination were observed between the two sexes.

Results of inhalation and dermal absorption studies indicate that humans efficiently and rapidly absorb furfural through the lungs and skin. Dermal absorption of vapours corresponds to

approximately 20 – 30% of the dose retained by the lungs. The biological half-life of furfural in humans is 2 – 2.5 hours, and mean pulmonary retention is approximately 80%. Pulmonary retention is independent of the concentration of furfural vapour or the duration of exposure. About 1% of the amount of furfural retained after inhalatory exposure was eliminated *via* expiration, while no free furfural could be demonstrated in the urine. Urinary elimination of metabolites was complete within 24 hrs after the beginning of the exposure to furfural vapours (Flek & Sedivec, 1978).

A similar pattern of absorption, distribution and excretion is reported for 5HMF [FL-no: 13.139]**.** Groups of male F344 rats and male B6C3F1 mice were administered 5, 10, 100 or 500 mg [U-^{14}C]5HMF/kg bw *via* oral gavage. In both species, 5HMF-derived radioactivity was rapidly cleared from all major tissues, with no evidence of accumulation in any tissue, but some covalent binding could be demonstrated in liver, kidney and possibly the GI-tract at 8 and 24 hrs. Tissue concentrations varied with dose in both species at most time points. Within 48 hours, 70 – 82% of the administered dose was excreted in the urine of rats, while 8 – 12% was excreted in the faeces. In mice, 61 – 77% was excreted in the urine and 15 – 26% in the faeces within the same time period. In both species 80 - 100% of the total amount of radioactivity excreted *via* the urine was recovered within the first 24 hrs post dosing. It was stated that $^{14}CO_2$ was not exhaled (Godfrey et al., 1999).

Fasted male Sprague-Dawley rats were administered single oral gavage doses of [U-^{14}C]-5HMF [FL-no: 13.139] at 0.08, 1.3, 13 or 330 mg/kg. After 24 hours, less than 1% of the radioactivity was retained in the body-cavity organs and faeces. Over the dose range studied, 85% of the radioactivity was eliminated after 8 hours and 95 – 100% of the radioactivity was eliminated after 24 hours, almost exclusively *via* urine. No significant differences in the rate of urinary elimination of [U-^{14}C]-5HMF were observed over the administered dose range. Distribution of [U-^{14}C]-5HMF and its metabolites was determined by whole-body autoradiography of rats fasted overnight. One hour after oral administration, radioactivity was observed mainly in the kidney and bladder, as well as the liver. After 24 hours, no accumulation of radioactivity was observed in the body indicating essentially complete elimination of the test substances and its metabolites (Germond et al., 1987).

III.2.3. Biotransformation

Furan-containing moieties:

The glycine conjugate of 2-furoic acid [FL-no: 13.136] was detected in rat urine, collected for six hours after a single oral dose of 50 – 66 mg/kg bw of furfuryl alcohol, furfural, 2-furoic acid, furfuryl diacetate, furfuryl propionate, furanacrylic acid or 3-furanacrylate methyl ester (Paul et al., 1949).

In F344 male rats (4/group) oral doses of 0.275, 2.75 or 27.5 mg [^{14}C]-furfuryl alcohol [FL-no: 13.019]/kg bw or 0.127, 1.15 or 12.5 mg [^{14}C]-furfural [FL-no: 13.018]/kg bw (radiolabel on carbonyl C) are oxidised to 2-furoic acid [FL-no: 13.136], which is excreted mainly in the urine either free (1 − 6%) or as the glycine conjugate (73 − 80%). In addition, the glycine conjugate of furanacrylic acid (3 − 8%) was found, while up to 1.5% of the dose was excreted as an unidentified urinary metabolite. In rats receiving 12.5 mg furfural/kg bw, 7% was exhaled as CO_2. Carbon dioxide measurements were not taken for any other dose group. The authors stated that the percentage of furfural or furfuryl alcohol exhaled as CO_2 is unlikely to vary significantly with the dose, based on the argument that over the dose ranges studied, the extent of metabolism and relative amounts of metabolites were independent on the dose level, while rates of excretion were linear to dose (Nomeir, 1992).

Single oral doses of 0.1, 10 and 60 mg [^{14}C]furfural [FL-no: 13.018]/kg bw given to male and female F344 rats or 1, 20 and 200 mg [^{14}C]furfural/kg bw given to male and female CD1 mice were

metabolised to the glycine conjugates of 2-furoic acid [FL-no: 13.136] (76 − 84% in rats and 65 − 89% in mice within 24 hours) and 2-furanacrylic acid (16 − 24% in rats and 11 − 35% in mice within 24 hours). About 5% of the radiolabel was expired as $^{14}CO_2$ in the high-dose group (males) and 4% was expired in the low dose group (female) of mice. Expired $^{14}CO_2$ was not determined in any other dose group. In urine from all male rat dose groups and in the high-dose male mice 1.5 – 3% of the dose was recovered as an unidentified, polar metabolite, not conjugated to sulfate or glucuronide. The absorption, tissue distribution, extent of metabolism, and rates of excretion in rodents were linear over the dose ranges investigated (0.1 mg/kg bw to 200 mg/kg bw) (Parkash & Caldwell, 1994).

It was suggested by the authors that 2-furanacrylic acid is generated from 2-furoic acid by condensation with acetyl-CoA, rather than that it is the product of direct aldol condensation of furfural with acetate as suggested in other papers e.g. (Nomeir, 1992). According to Parkash and Caldwell it is more likely that the thioester-CoA derivatives of furanacrylic acid and furoic acid are in a dynamic equilibrium favouring the CoA-thioester of 2-furoic acid. This is supported by the following observations:

- 2-Furoic acid is excreted in the urine of rats or dogs given 2-furanacrylic acid (Paul et al., 1949; Friedman, 1911), which shows that the reverse reaction (reverse to furanacrylic acid formed out of furoic acid) does also occur, thereby suggesting that these two acids are interconvertible.
- Analogous conversions have been shown for other aromatic carboxylic acids (e.g. benzoic acid and cinnamic acid) (Nutley et al., 1994).

Parkash and Caldwell (Parkash & Caldwell, 1994) suggested that an observed decrease in the extent of glycine conjugation would result in an increase in the percentage of the dose of furfural, that was excreted as free furoic or free furanacrylic acids. The small shift in the urinary metabolite pattern would suggest that, similar to what has been observed for benzoic acid, glycine conjugation may be capacity limited, probably by the supply of endogenous glycine (Gregus et al., 1993), but for furfural, the shift was limited to a few percents of the dose if any, even at dose levels up to 60 mg/kg bw in rats or 200 mg/kg bw in mice.

When human volunteers were exposed to furfural vapour at concentrations of 15 to 31 mg/m^3 for 8 hours, the following metabolites could be identified in the urine: 2-furoylglycine (major) and 2-furanacryloylglycine (minor). In contrast to what has been seen in animals, no free 2-furoic acid was observed, and the authors suggested that this might be due to the low level of furfural to which the volunteers had been exposed (max. ~ 2 mg/kg bw) (Flek & Sedivec, 1978).

The formation of labelled CO_2 from radioactive furfural or furfuryl alcohol in rats and mice may occur *via* decarboxylation of 2-furoic acid; not directly at the C2 position but rather after ring opening. Koenig and Andreesen (Koenig & Andreesen, 1990) have shown that the microorganism *Pseudomonas putida* is capable of hydroxylation of 2-furoic acid (or rather 2-furoyl-CoA) at the C5 position followed by ring opening to yield the citric acid cycle intermediate alpha-ketoglutaric acid.

Using 2-methylfuran in rat lung and liver microsomal preparations, ring opening of the furan ring was inferred from the formation of the reactive intermediate 4-keto-pent-2-enal (= acetylacrolein) (Ravindranath & Boyd, 1985). This is a conversion similar to the one described above for the metabolism of 2-furoic acid in bacteria, but whether in the bacterial pathway an aldehyde is also formed as intermediate is not clear.

However, there is no evidence of ring oxidation and opening in humans at low levels of exposure. Essentially all absorbed furfural (*ca*. 2 mg/kg bw) in a human inhalation study was excreted in the

urine as furoylglycine and furanacrylic acid. Because of a virtually complete mass balance between the amount of metabolites in the urine and the amount absorbed *via* the lungs, it was concluded that no important elimination routes had been missed, which may be interpreted in the way that in humans, in contrast to animals, no evidence of furfural decarboxylation was obtained (Flek & Sedivec, 1978). However, the evidence is rather indirect and based on a small number of individuals.

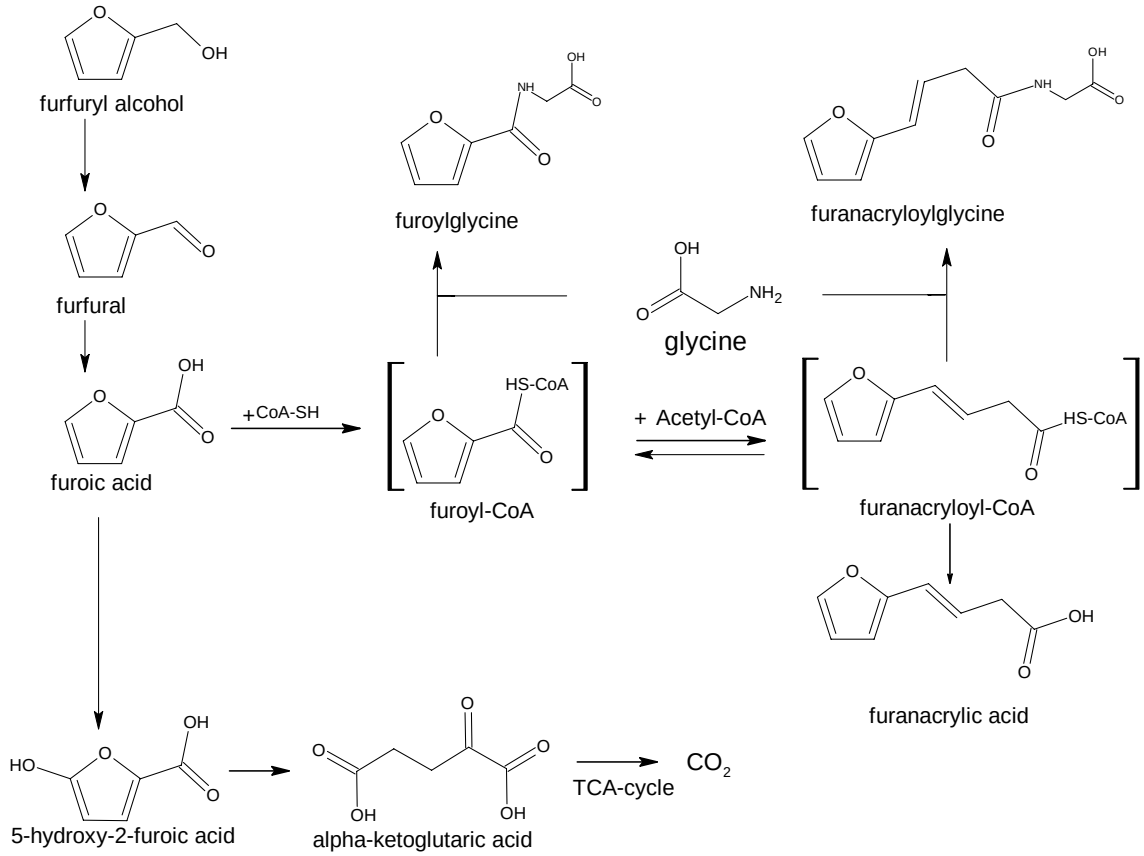


Figure III.1. *Biotransformation of furfuryl alcohol and furfural. Formation of glycine conjugates are major pathways in mammals. Formation of carbon dioxide also occurs in mammals. The pathway via alpha-ketoglutaric acid has been described for micro-organisms, and might occur in mammals, as well.*

In rats, 5-methylfurfural [FL-no: 13.001] exhibits a metabolic fate similar to that of furfural. Two urinary metabolites were identified when rats were given an oral dose of an aqueous solution containing 80, 120 or 160 mg 5-methylfurfural/kg bw. The principal metabolite was the glycine conjugate of 5-methylfuroic acid (>40% of the dose) accompanied by about 7 - 8% of 5-methylfuryl methyl ketone. 5-Methylfuryl methyl ketone was also a urinary metabolite of 5-methylfuroic acid, but not of 2-methylfuran, suggesting that both side chains to the furan ring are essential and that the ketone is formed from the acid (Jodynis-Liebert, 1985; Jodynis-Liebert, 1988). From this observation, it may be inferred that similar to the formation of 2-furanacrylic acid from furfural, the CoA-thioester of 5-methylfuroic acid condenses with acetyl-CoA to form a beta-ketothioester (corresponding intermediate not shown in Figure III.1), which sequentially undergoes hydrolysis and decarboxylation to form 5-methylfuryl methyl ketone.

Male Sprague-Dawley rats were fasted overnight and then administered single oral gavage doses of 5HMF [FL-no: 13.139] at 0.08, 1.3, 13 or 330 mg/kg, which contained 8 microCi [U-^{14}C]-5HMF. After 24 hours, less than 1% of the radioactivity was exhaled as $^{14}CO_2$. Over the dose range studied, 85% of the radioactivity was eliminated within 8 hours and 95 – 100% of the radioactivity was eliminated within 24 hours, almost exclusively *via* urine. Two major urinary metabolites were identified as 5-hydroxymethyl-2-furoic acid (HMFA) and its glycine conjugate [*N*-(5-hydroxymethyl-2-furoyl)glycine, HMFG]. A third, minor polar metabolite was not further identified.

The ratio of HMFA to HMFG concentrations increased at higher doses of [U-^{14}C]5HMF. Up to a dose of 1.3 mg/kg of [U-^{14}C]-5HMF, the HMFA-to-HMFG ratio was about 1, increasing to a maximum ratio of 15 – 20 in the first two hours after a dose of 330 mg/kg of [U-^{14}C]-5HMF, but then falling to about 5 after 8 hours. When a 3 g/kg bw supplementation with glycine was given shortly before the high dose of 5HMF, the increase in the HMFA/HMFG was much less pronounced, indicating that the availability of free glycine may limit the rate of conjugation at higher doses of 5HMF. Distribution of [U-^{14}C]-5HMF and its metabolites was determined by whole-body autoradiography of rats fasted overnight. One hour after oral administration, radioactivity was observed mainly in the kidney and bladder, as well as the liver. After 24 hours, no accumulation of radioactivity was observed in the body indicating essentially complete elimination of the test substances and its metabolites (Germond et al., 1987).

In a more recent study, the disposition of uniformly labelled candidate substance 5HMF (i.e. [U-^{14}C]-5HMF [FL-no: 13.139]) was investigated in male F344 rats and B6C3F1 mice following oral gavage administration of 5, 100 or 500 mg (rats) or 10, 100 or 500 mg (mice) [U-^{14}C]-5HMF/kg at approximately 10 μCi/animal. Total radioactivity recovered in excreta in the 48 hours following administration was 82 - 91% in rats and 80 - 92% in mice. No radioactivity was exhaled. In both species the urinary excretion of metabolites accounted for the major part of the elimination. At a dose of 5 mg [U-^{14}C]-5HMF/kg to rats, the cumulative excretion of radioactivity at 24 hours as percent of initial dose was 73.0% in urine and 6.7% in faeces. In mice relatively more radioactivity was excreted in faeces (approximately 13% at 24 h) than in rats.

5-Hydroxymethyl-2-furoic acid (HMFA) was the major urinary metabolite in both species and accounted for 78 − 85% of the recovered radioactivity of each administered dose. Excretion of the glycine conjugate, *N*-(5-hydroxymethyl-2-furoyl)-glycine (HMFG) accounted for 5 - 8% of the dose in the mice. In rats the excreted amount of this metabolite was inversely proportional to dose, possibly due to glycine depletion (6% at the low dose and 1.3% at the high dose). 2,5-Furan dicarboxylic acid (FDCA) accounted for only 2 − 4% of the recovered radioactivity in mice and 4 − 6% in rats. The identified metabolites result from initial oxidation of the aldehyde followed by either conjugation of the resulting carboxylic acid (major pathway) or oxidation of the alcohol group (minor pathway) (Godfrey et al., 1999).

The pathways involved in the metabolism of 5HMF to animals have been presented in Figure III.2.

In humans, furoylglycine and 2,5-furandicarboxylic acid can be found in the urine, and it has been demonstrated that these are derived from precursors in food. Heat-sterilised aqueous solutions of fructose and glucose may be rich in e.g. 5HMF, which after i.v. administration may be excreted as 5-hydroxymethyl-2-furoic acid or as 2,5-furandicarboxylic acid (Jellum et al., 1973; Pettersen & Jellum, 1972).

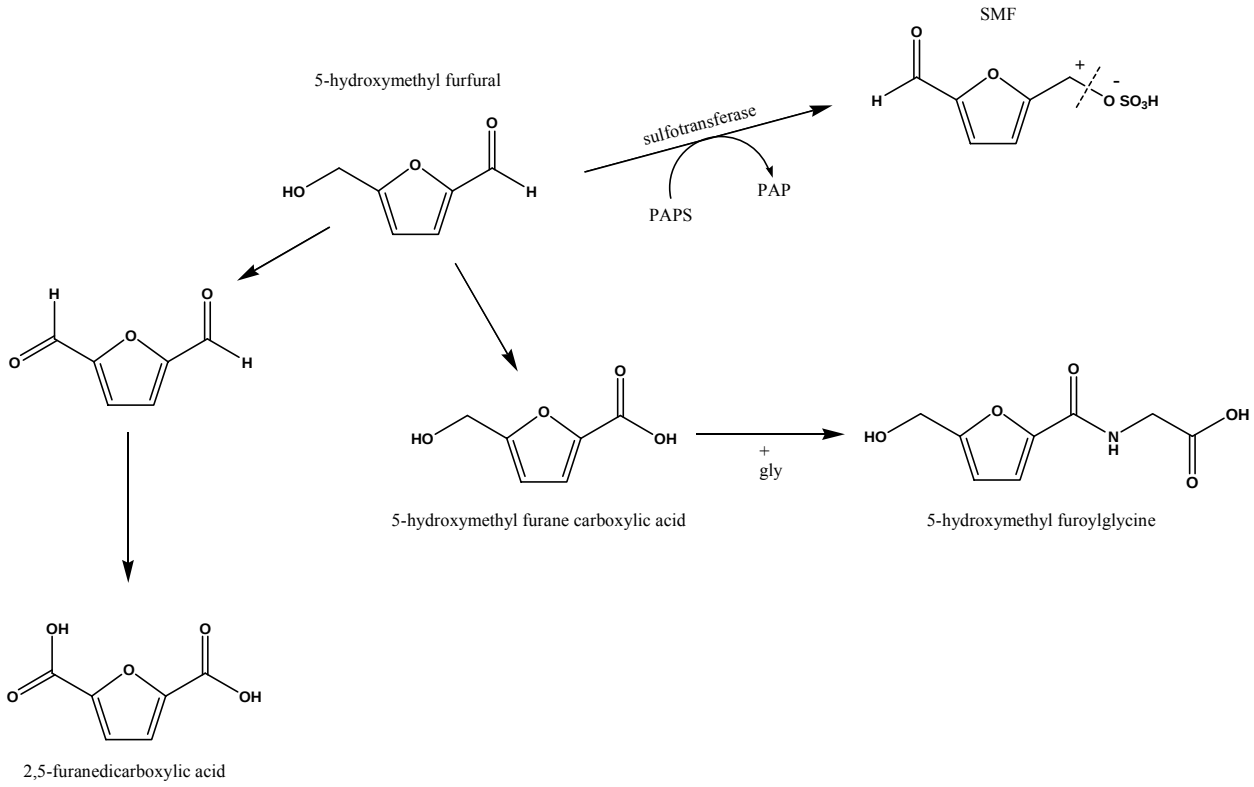


Figure III.2 *Metabolic pathways for 5-hydroxymethylfurfuraldehyde. Glycine conjugation will require transient coupling to CoA (see Figure III.1)*

In addition to the above mentioned pathways, 5HMF has been shown to be bioactivated *in vitro* to 5-[(sulfoxy)methyl] furfural (SMF), through sulfonation of its allylic hydroxyl functional group, catalyzed by sulfotransferases. In the resulting ester, the sulfate is a good leaving group, thus producing a highly electrophilic allyl carbocation, which could be stabilized by distribution of charges on the furan ring. The subsequent interaction of this reactive intermediate with critical cellular nucleophiles (i.e. DNA, RNA and proteins) may be result in toxic and mutagenic effects. This sulphate conjugate has not been observed *in vivo,* since it appears to be too unstable to allow its excretion as such and detection in urine. Indeed, when 5HMF was incubated with ^{35}S-PAPS, a sulfo-group donor, and liver cytosol an unstable conjugate was formed, which disappeared within 60 minutes. The time dependent decline in the amount of the reaction product appears to be associated with its hydrolysis in an aqueous environment (Surh & Tannenbaum, 1994). Moreover, 5HMF was mutagenic in tests with *Salmonella typhimurium* in the presence of rat hepatic cytosol enriched with the sulfo-group donor PAPS; the effect was markedly lessened by sulphotransferase inhibitors, clearly suggesting that 5HMF can be metabolically bioactivated to an allylic sulphate with genotoxic potential (Lee et al., 1995b).

Summary

Furan-containing moieties:

Following hydrolysis of furfuryl esters, furfuryl alcohol is oxidised to furfural, which is subsequently oxidised to 2-furoic acid. In the major metabolic detoxication pathway, the CoA-thioester of 2-furoic acid is either conjugated with glycine and excreted in the urine, or condensed with acetyl-CoA to form the CoA thioester of 2-furanacrylic acid. This compound, 2-furanacryloyl CoA, is also conjugated with glycine and excreted primarily in the urine. At high dose levels, the

availability of glycine might limit the rate of conjugation, resulting in the excretion of free furoic acid. The same detoxication pathways have been demonstrated to be present in humans. In a minor pathway that has been reported in rodents, CO_2 is produced presumably *via* oxidation and opening of the furan-ring, in which a reactive intermediate may be involved. Apart from the formation of CO_2 similar reactions have been observed with 5HMF, which can be additionally bioactivated to an allylic sulphuric acid ester with genotoxic potential.

Moieties without a furan ring:

In general, the non-furan containing component alcohols and carboxylic acids (C2 – C8) formed by ester hydrolysis of the candidate furfuryl esters and furoic acid esters participate in fatty acid β-oxidation and the citric acid cycle to eventually yield CO_2 and H_2O (Voet & Voet, 1990). The metabolism of aliphatic alcohols and carboxylic acids has been discussed extensively in previous Flavouring Group Evaluations e.g. FGE.01, 02, 03, and 05 and will not be discussed here any further.

III.3. Subgroup II – Furan derivatives with sulphur substitution

The candidate substances in subgroup 2 are sulphur-substituted furan derivatives. Among them 4,5-dihydro-3-mercapto-2-methylfuran [FL-no: 13.108], is the only compound containing a non aromatic ring. The sulphur is present, either:

- as free thiol group: [FL-no: 13.108 and 13.149],
- as thioethers [FL-no: 13.114, 13.145, and 13.124],
- as disulfides [FL-no: 13.113, 13.144, and 13.178],
- as trisulfides [FL-no: 13.146].

In candidate substance [FL-no: 13.178] the disulfide bridge forms a link between two furan rings. In the other members of this subgroup only one furan ring is present.

For none of the candidate substances, absorption, distribution, metabolism or elimination studies were found in the published or available unpublished literature. An evaluation of the metabolism of several supporting substances can be found in JECFA/WHO (JECFA, 2003a). For two of the supporting substances, some data on hydrolysis and oxidation in the gastro-intestinal tract were found. These data are presented below.

III.3.1 Hydrolysis and spontaneous oxidation in the gastro-intestinal tract

Approximately 96% ester hydrolysis was observed when 3-(furfurylthio)propionic acid ethyl ester [FL-no: 13.093] was incubated in simulated intestinal fluid for one hour (Bio-Research Laboratory, 1980), while only 14% was hydrolysed after six hours of incubation in simulated gastric fluid. However, it is noted that this supporting substance is a normal ester, which are not represented in the group of candidate substances. This ester hydrolysis is thus of little relevance.

When 2-methyl-3-tetrahydrofuranthiol [FL-no: 13.160] was incubated in intestinal fluid for four hours, as a result of oxidation, 18% bis-(2-methyl-3-tetrahydrofuryl)disulfide was formed (Salzer, 1991).

III.3.2 Biotransformation

Although no *in vivo* studies are available for the candidate substances in this group, thiofurans and thiofurfuryl derivatives are likely metabolised *via* reactions of the divalent sulphur atom, similar to

other substances containing a sulphur substituent. An extensive review of the possible reactions for a number of different sulphur-containing chemicals (mainly pharmaceuticals) has been presented by Damani (Damani, 1987). An overview of the possible metabolic conversions of organic thiols and sulfides is given in Figure III.3.

Metabolism of free thiol groups:

Thiols are highly reactive *in vivo* mainly because most thiols exist in the ionised form at physiologic pH. Thiols are oxidised to unstable sulfenic acids, which are further oxidised to the corresponding sulfinic and sulfonic acids (Damani, 1987). Methylation of thiols primarily by methyl-S-transferases, which require S-adenosyl methionine as a co-factor, yields methyl sulfides, which then are readily oxidised to sulfoxides and sulfones. Substances which contain free thiol groups may also react with endogenous thiol- containing compounds such as proteins or glutathione to form mixed disulfides or, alternatively, with glucuronic acid to give thio-beta-D-glucuronide conjugates (Dutton & Illing, 1972; Maiorino et al., 1989; McBain & Menn, 1969; Richardson et al., 1991). Sulfoxides and sulfones are physiologically stable and are excreted unchanged in the urine (Nickson & Mitchell, 1994; Nickson et al., 1995; McBain & Menn, 1969).

The oxidation to sulfoxides is mainly catalysed by two enzyme systems, cytochromes P_{450} and Flavine-containing MonoOxygenase (FMO) (Renwick, 1989; Nnane & Damani, 1995). Any organosulphur compound may be a substrate for both the enzyme systems, although with different affinity, essentially dependent on the electromolecular environment in which the sulphur is located: the more nucleophilic divalent sulphur are primarily oxidised by FMO and to a lesser extent by P_{450}. This is the case for simple aliphatic (e.g. diethyl sulfide), alicyclic (e.g. thiolane) and aromatic (e.g., ethyl p-tolyl sulfide) sulfides (Damani, 1987; Hoodi & Damani, 1984). Moreover, another important determinant is the tissue-specific distribution of the two different enzymatic systems, especially in extrahepatic tissues, as well as the differential presence of single isoforms, with different catalytic activities.

Metabolism of thioethers:

The major reactions by which simple sulfides can be metabolised involve oxidation of the S to give sulfoxides, which can be further converted to sulfones. Alternatively, sulfides can undergo oxidation of the carbon alpha to the -S- resulting in the formation of an unstable hydroxyalkyl intermediate, which can be split to give an aldehyde and a free thiol. The aldehyde can be oxidised to its corresponding acid. This reaction may also occur with a free thiol group, and in that case the sulphur is finally released as sulfate (see Figure III.3, panel A) (Damani, 1987; Richardson et al., 1991). For dipropyl sulfide it was demonstrated that the sulfoxide was the most important metabolite. Formation of dipropyl sulfone was much less important and complete oxidation, resulting in excretion of free sulfate accounted for less than 3% of the dose (Nickson & Mitchell, 1994). These authors also indicated that with diethyl and dimethyl sulfides, sulphur oxidation to the sulfones was more important than for dipropylsulfide. No metabolism occurred in other parts of the molecule.

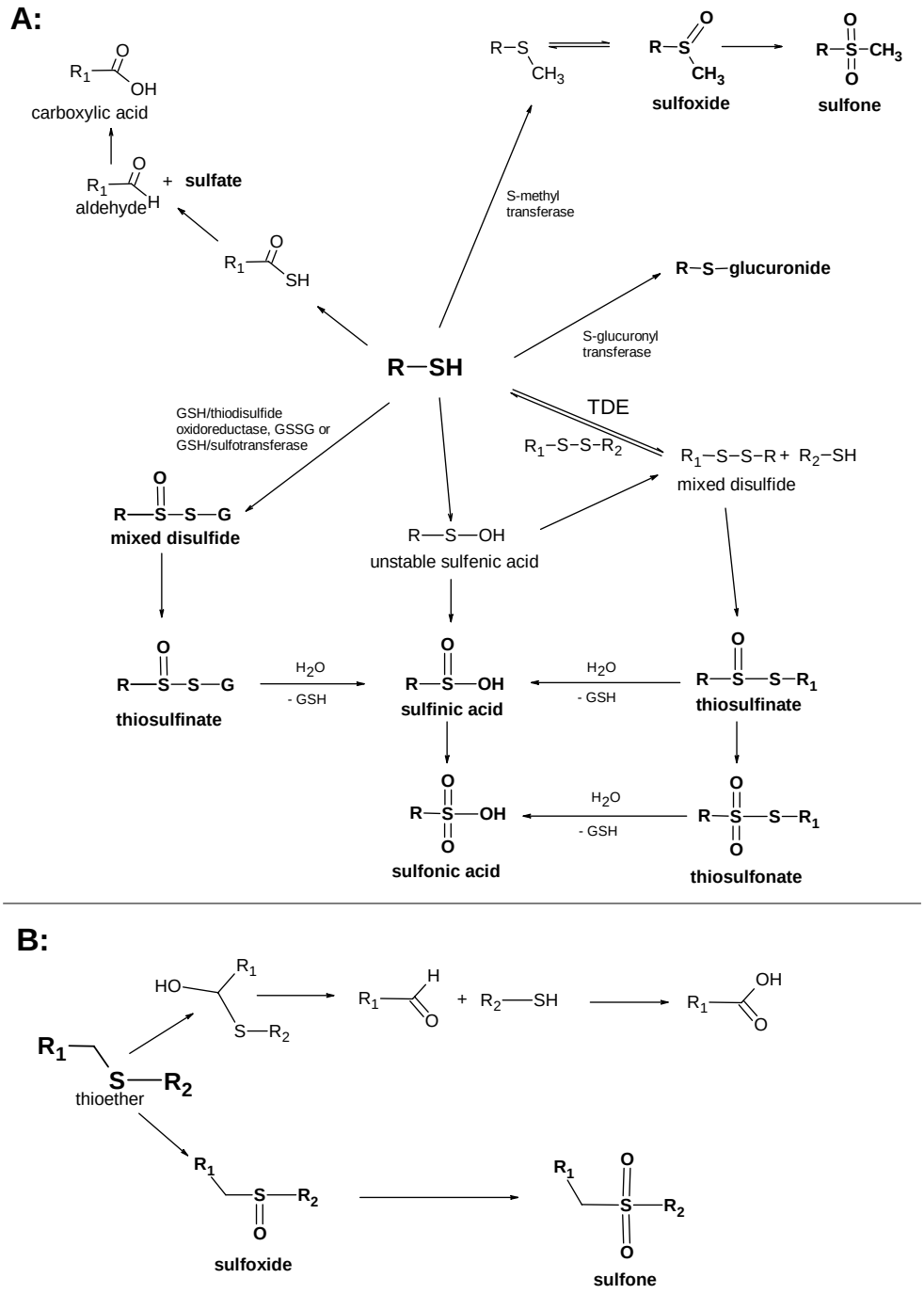


Figure III.3 *Metabolism of organic sulphur compounds. A panel: metabolism of thiols and disulfides. B panel: metabolism of thioethers. Structures in bold are major excretion products.*

TDE = Thiol-Disulfide Exchange

Metabolism of di- and tri-sulfides:

The labile nature of the S-S bond also presents a variety of metabolic options for detoxication. Disulfides can be either symmetrical (i.e. the two moieties at either side of the S-S bridge are the same) or mixed (i.e. the two moieties on both sides of the S-S bridge are different). The disulfide bond may be rapidly reduced to yield the corresponding thiol or thiols (i.e. mercaptans) in a

reversible reaction *in vivo*. Therefore, the metabolic options available to thiols are also available to disulfides.

Thiol-disulfide exchange (TDE) reactions occur *in vivo* and result from nucleophilic substitution by sulphur. TDE reactions with endogenous cellular thiols (GSH) and disulfides (GSSG) produce mixed disulfides that may also undergo reduction. In these TDE reactions, also –SH groups from proteins may be involved and in many cases such interactions affect the biological function of the proteins involved. Under normal conditions, TDE reactions control the cellular concentrations of endogenous thiols (e.g. GSH) and disulfides (i.e. GSSG) and maintenance of an adequate GSH/GSSG ratio is essential for cell survival and function (Cotgreave et al., 1989; Brigelius, 1985; Sies et al., 1987).

No information about the metabolism of trisulfides has been located. However, as these substances may be considered as a special case of disulfides, it may be assumed that some of the reduction and oxidation reactions described above may also apply to them.

Summary

The nine representatives of subgroup 2 are all furan derivatives containing sulphur substituents as free thiol groups, mono-, di- and tri-sulfides. Substances bearing a free thiol group, can directly react with endogenous sulphur-containing substances, e.g. glutathione and proteins. These free thiol groups can be metabolised by methylation, after which the sulphur can be further oxidised to give sulfoxides or sulfones, which can be readily excreted. Alternatively, conjugation with glutathione may occur resulting in a mixed disulfide, which can be reduced to give the free thiols. These can be oxidised to give thiosulfinates or thiosulfones or can undergo thiol-disulfide exchange either with free thiol groups of proteins or with free thiol groups in endogenous substances. The reactions with proteins may affect their biochemical functions, thus triggering adverse effects. The simple thioethers may undergo sulphur oxidation reactions, similar to the methylether conjugates of free thiol groups; in additions thiols may be formed. Disulfides can be reduced to give the free thiols, or can be oxidised to give thiosulfinates or thiosulfones. For the one trisulfide candidate substance, no information on biotransformation was available, but it may be expected that this substance is metabolised *via* similar routes.

III.4. Summary

The candidate substances in FGE.13 are all furan derivatives which can be divided into two subgroups.

The nine candidate substances in subgroup 1 are furfuryl alcohol derivatives such as esters of furfuryl alcohol, furoic acid, or furanacrylic acid; in addition 5-hydroxymethylfurfuraldehyde, a furfural with an additional hydroxymethyl side chain at C5 of the furan ring is included.

The esters of furfuryl alcohol [FL-no: 13.127, 13.129, 13.132, and 13.133] are expected to be hydrolysed to furfuryl alcohol and the corresponding saturated aliphatic carboxylic acid. Furoate esters [FL-no: 13.102 and 13.122] are directly hydrolysed to candidate substance 2-furoic acid [FL-no: 13.136] and the corresponding saturated aliphatic alcohol. The candidate substance [FL-no: 13.011] is expected to be hydrolysed to furanacrylic acid (a known metabolite of furfural) and ethanol. It can be anticipated that the substances in this subgroup or there hydrolysis products are rapidly absorbed after oral exposure.

In general, the non-furan containing component alcohols and carboxylic acids formed by ester hydrolysis of the candidate furfuryl alcohol and furoic acid esters participate in fatty acid β-oxidation and the citric acid cycle to yield CO_2 and water.

Furfuryl alcohol, furanacrylic acid, 5HMF and their derivatives in rodents participate in pathways involved in the detoxication of furfural, a known reactive aldehyde, that may lead to hepatotoxicity. The oxidation of the alcohol or aldehyde group to the candidate 2-furoic acid [FL-no: 13.136] can be followed by conjugation with glycine or acetyl-CoA (leading to furanacryloyl,-CoA) which are readily excreted in the urine. The same detoxication pathways have been demonstrated to be present in humans. In a minor pathway which has been reported in rodents, the two furoate esters [FL-no: 13.102 and 13.122] and 2-furoic acid itself [FL-no: 13.136], which is the main metabolite of furfural, may be metabolised to CO_2 presumably *via* oxidation and opening of the furan-ring, in which production of reactive intermediate(s) may be involved.

In addition to the above mentioned pathway, 5HMF [FL-no: 13.139] *in vitro* has been shown to be bioactivated to reactive genotoxic intermediates by sulfotransferases.

The nine representatives of subgroup 2 are all sulphur-substituted furan derivatives, containing sulphur substituents as free thiol groups, mono-, di- and tri-sulfides, except for 4,5-dihydro-3-mercapto-2-methylfuran [FL-no: 13.108], the only candidate containing a non aromatic ring.

Substances bearing a free thiol group can directly react with endogenous sulphur-containing substances, e.g. glutathione and proteins. Metabolism data on candidate- or supporting substances are not available, but their metabolic fate can be estimated from general knowledge of thiol metabolism. Free thiol groups can be metabolised by methylation after which the sulphur can be further oxidised to give sulfoxides or sulfones, which can be readily excreted. Alternatively, conjugation with glutathione may occur resulting in a mixed disulfide, which can be reduced to give the free thiols, or which can be oxidised to give thiosulfinates or thiosulfones. They may also undergo thiol-disulfide exchange either with free thiol groups in endogenous molecules such as proteins possibly affecting biochemical functions and triggering adverse effects.

The simple thioethers may undergo sulphur oxidation reactions, similar to the methylether conjugates of free thiol groups. Disulfides can be reduced to give the free thiols, or can be oxidised to give thiosulfinates or thiosulfones. For the one trisulfide candidate substance, no information on biotransformation was available, but it may be expected that this substance is metabolised *via* similar routes.

III.5. Conclusion

Based on the available information, it is concluded that the candidate substances included in subgroup 1 [FL-no: 13.127, 13.129, 13.132, 13.133, and 13.139] as far as their metabolism includes formation of furfural, a known hepatotoxic reactive aldehyde, cannot be predicted to be metabolised to innocuous or endogenous compounds.

The two furoate esters [FL-no: 13.102 and 13.122] and 2-furoic acid itself [FL-no: 13.136], which is the main metabolite of furfural, may be metabolised in rodents to CO_2, with the opening of the furan ring, producing reactive intermediates. Data available do not allow to rule out the presence of this pathway in humans.

Therefore it cannot be predicted that these candicate substances are metabolised to innocous products.

In addition to the above mentioned pathways, 5HMF [FL-no: 13.139] has been shown to be bioactivated *in vitro* to reactive genotoxic intermediates by sulfotransferases.

Given the reactivity of thiol groups, whether free or as obtained from the metabolism of di-(tri)sulfides, and the importance of thiol groups in cell physiology, it cannot be excluded that these substances interfere with normal cell function. Therefore it is concluded that the candidate substances [FL-no: 13.144, 13.108, 13.113, 13.114, 13.124, 13.145, 13.146, 13.149, and 13.178] included in subgroup 2 cannot be predicted to be metabolised to innocuous products.

ANNEX IV: TOXICITY

Oral acute toxicity data are available for three candidate substances of the present flavouring group evaluation from chemical group 14, and for 17 supporting substances evaluated by JECFA at the 55th and 59th meetings. The supporting substances are listed in brackets.

TABLE IV.1: ACUTE TOXICITY

Table IV.1: ACUTE TOXICITY

Chemical Name [FL-no:]	Species	Sex	Route	LD50 (mg/kg bw)	Reference	Comments
Subgroup I – Structurally Related to Furfuryl Alcohol						
(Furfural [13.018])	Rat	M, F	Gavage	M: 145 – 204; F: 90 - 119	(Brown, 1982)	
5-Hydroxymethylfurfuraldehyde [13.139]	Rat	NR	Oral	3100	(Simonyan, 1969)	Article in Russian
	Rat	M, F	Gavage	2500	(Warf Institute, 1977)	
	Mouse	NR	Oral	1910	(Simonyan, 1969)	Article in Russian
	Mouse	NR	Oral	> 2000	(Czok, 1970)	
(5-Methylfurfural [13.001])	Rat	NR	Oral	2200	(Moreno, 1978g)	
(Methyl-2-furoate [13.002])	Rat	M, F	Gavage	300	(Great Lakes Chemical Corp., 1998)	
	Rat	NR	I.p. injection	100	(Phatak & Emerson, 1936)	
Ethyl 2-furoate [13.122]	Rat	NR	I.p. injection	75 – 100	(Phatak & Emerson, 1936)	Questionable route of exposure
(Propyl 2-furoate [13.003])	Rat	NR	I.p. injection	75 – 100	(Phatak & Emerson, 1936)	
Butyl 2-furoate [13.102]	Mouse	NR	Oral	1500	(Stasenkova & Shchirskaya, 1967)	
	Rat	NR	I.p. injection	100 – 150	(Phatak & Emerson, 1936)	Questionable route of exposure
(Amyl 2-furoate [13.025])	Rat	NR	I.p. injection	250 – 500	(Phatak & Emerson, 1936)	
Subgroup II – Structurally Related to Sulphur-substituted Furan Derivatives						
(2-Methyl-3-furanthiol [13.055])	Mouse	M, F	Gavage	100	(Oser, 1969a)	
	Mouse	M, F	Gavage	100	(Moran et al., 1980)	
(Methyl 2-methyl-3-furyl disulfide [13.079])	Mouse	M, F	Gavage	142	(Moran et al., 1980)	
(Propyl 2-methyl-3-furyl disulfide [13.082])	Mouse	M, F	Gavage	284	(Moran et al., 1980)	
(Bis(2-methyl-3-furyl) disulfide [13.016])	Mouse	M, F	Gavage	106	(Oser, 1969b)	
(2,5-Dimethyl-3-furanthiol [13.071])	Mouse	M, F	Gavage	< 544	(Fogleman & Suppers, 1973a)	
	Mouse	M, F	Gavage	360	(Moran et al., 1980)	
(Furfuryl mercaptan [13.026])	Mouse	M	I.p. injection	100 – 200	(Doull et al., 1962)	
(2-Methyl-3-tetrahydrofuranthiol [13.160])	Mouse	NR	Gavage	1860	(Oser, 1970c)	
(Bis(2-methyl-3-furyl) tetrasulfide [13.017])	Mouse	M, F	Gavage	220	(Oser, 1970c)	
	Mouse	M, F	Gavage	220	(Moran et al., 1980)	
(2,5-Dimethyl-3-furyl thioisovalerate [13.041])	Mouse	M, F	Gavage	580	(Fogleman & Suppers, 1974a)	
	Mouse	M, F	Gavage	720	(Fogleman & Suppers, 1973b)	
	Mouse	M, F	Gavage	580	(Moran et al., 1980)	
(2,5-Dimethyl-3-thiofuroylfuran [13.040])	Mouse	M, F	Gavage	625	(Fogleman & Suppers, 1974b)	

Table IV.1: ACUTE TOXICITY

Chemical Name [FL-no:]	Species	Sex	Route	LD50 (mg/kg bw)	Reference	Comments
	Mouse	M, F	Gavage	625	(Fogleman & Suppers, 1973a)	
	Mouse	M, F	Gavage	540	(Moran et al., 1980)	
(3-[(2-Methyl-3-furyl)thio]-4-heptanone [13.077])	Mouse	M, F	Gavage	425	(Moran et al., 1980)	
(Ethyl 3-(furfurylthio)propionate [13.093])	Mouse	M, F	Oral	> 5000	(Griffiths & Babish, 1977a)	

NR = Not reported.

M = Male; F = Female.

Subacute / subchronic / chronic / Carcinogenic toxicity data are available for two of the candidate substances of the present flavouring group evaluation from chemical group 14 and for 18 supporting substances evaluated by JECFA at the 55th and 59th meetings. The supporting substances are listed in brackets.

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

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

Chemical Name [FL-no:]	Species; Sex No./Group	Route	Dose levels mg/kg bw/day	Duration	NOAEL (mg/kg /day)	Reference	Comments
Subgroup I – Furfuryl Alcohol and Furan derivatives							
(Furfural [13.018])	Mouse; M, F 10	Gavage	0, 25, 50, 100, 200, 400	16 days	400	(NTP, 1990a)	
	Mouse; M, F 20	Gavage	0, 75, 150, 300, 600, 1200	13 weeks	M: 75 F: < 75	(NTP, 1990a)	
	Mouse; M, F 100	Gavage	0, 50, 100, 175	2 years	50	(NTP, 1990a)	
	Rat; M, F 10	Gavage	0, 15, 30, 60, 120, 200	16 days	120	(NTP, 1990a)	
	Rat; M, F 20	Gavage	0, 11, 22, 45, 90, 180	13 weeks	45	(NTP, 1990a)	
	Rat; M 6	Diet	0, 20-30 [7]	150 days	< 30	(Shimizu et al., 1989)	
	Rat; M 48	Diet	0, 20-30-40 (ml/kg/d)[8]	105 days	< 40 ml/kg	(Shimizu & Kanisawa, 1986)	
	Rat; M, F 20	Diet[1]	0, 30, 60, 90, 180	13 weeks	60	(Jonker, 2000b)	
	Rat; M, F 100	Gavage	0, 30, 60	2 years	30	(NTP, 1990a)	
	Hamster; M, F 70	Oral	0, 25	36 weeks	25[2]	(Feron, 1972)	
5-Hydroxymethylfurfuraldehyde [13.139]	Rat; NR NR	Diet	0, 1000	100 days	< 1%[3] (1000)	(Archer et al., 1992)	Meeting Abstract. Due to lack of experimental details the validity could not be evaluated
	Rat; NR NR	Drinking water	0, 0.16, 1.6	6 months	1.6[2]	(Simonyan, 1969)	Article in Russian (only the abstract in english) Due to lack of experimental details the validity could not be evaluated
	Rat; M 19	Diet	0, 250	40 weeks	250[2]	(Lang et al., 1970)	
	Rat; NR NR	Gavage	0, 40, 80, 160	11 months	80	(Zaitzev et al., 1975)	Article in Russian (only the abstract in english) Due to lack of experimental details the validity could not be evaluated
2-Furoic acid [13.136]	Rat; M NR	Orally	0, 20	14 days	20[4]	(Hall et al., 1993)	Only the hypolipodemic effects have been tested. No other end-point has been considered

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

Chemical Name [FL-no:]	Species; Sex No./Group	Route	Dose levels mg/kg bw/day	Duration	NOAEL (mg/kg /day)	Reference	Comments
	Mouse; M NR	Orally	0, 20, 40, 80	14 days	20	(Hall et al., 1993)	The study was not extensively reported but can be considered valid
(2-Benzofurancarboxaldehyde [13.031])	Rat; M, F 32	Diet	0, 25	90 days	M: 25[2] F: 27[2]	(Posternak et al., 1969)	Poorly reported study
(2-Phenyl-3-carbethoxy furan [13.038])	Rat; M, F 30	Diet	0, 13	90 days	13[2]	(Posternak et al., 1969)	Poorly reported study
Subgroup II – Sulphur-containing Furan Derivatives							
(2-Methyl-3-furanthiol [13.055])	Rat; M, F 30	Diet	0, 5	90 days	5[2]	(Oser, 1970b)	
(Bis(2-methyl-3-furyl) disulfide [13.016])	Rat; M, F 30	Diet	0, 0.45	90 days	0.45[6]	(Morgareidge & Oser, 1970e)	Good quality study
	Rat; M, F 30	Diet	0, 3.96	90 days	Not established	(Oser, 1970a)	Good quality study
(Furfuryl mercaptan [13.026])	Rat; M, F 30	Gavage	0, 1, 3, 30	91 days	3	(Phillips et al., 1977)	Good quality study
(Furfuryl thioacetate [13.033])	Rat; M, F 32	Diet	0, 0.831 (M); 0.812 (F)	90 days	M: 0.831[2] F: 0.812[2]	(Posternak et al., 1969)	Poorly reported study
(4-[(2-Furanmethyl)thio]-2-pentanone)	Rat; M, F 10	Diet	0, 54.2 (M); 50.8(F)	14 days	50.8[2]	(Wnorowski, 1997d)	
(2-Methyl-3-tetrahydrofuranthiol [13.160])	Rat; M, F 10	Diet	0, 12.5 (M): 11.0 (F)	14 days	M: 12.5 F: 11.0	(Rush, 1991)	
(2,2'-(Thiodimethylene)difuran [13.056])	Rat; M, F 10	Diet	0, 10	14 days	10	(Gill & Van Miller, 1987b)	
(Bis(2-methyl-3-furyl) tetrasulfide [13.017])	Rat; M, F 30	Diet	0, 0.56	90 days	0.56[2]	(Morgareidge & Oser, 1970f)	
(2,5-Dimethyl-3-furyl thioisovalerate [13.041])	Rat; M, F 30	Diet	0, 0.73	90 days	0.73[2]	(Morgareidge et al., 1974a)	
(2,5-Dimethyl-3-thiofuroylfuran [13.040])	Rat; M, F 30	Diet	0, 0.74	90 days	0.74[2]	(Morgareidge et al., 1974b)	
(Furfuryl isopropyl sulfide [13.032])	Rat; M, F 32	Diet	0, 1.34 (M): 1.31 (F)	90 days	M: 1.34[2] F: 1.31[2]	(Posternak et al., 1969)	Poorly reported study
(2-Methyl-3-, 5-, or 6-(furfurylthio)pyrazine [13.151])	Rat; M, F 32	Diet	0, 1.66 (M): 1.64 (F)	90 days	M: 1.66[2] F: 1.64[2]	(Posternak et al., 1975)	Poorly reported study
(3-[(2-Methyl-3-furyl)thio]-4-heptanone [13.077])	Rat; M, F 30	Diet	0, 3.8	90 days	3.8[2]	(Gallo et al., 1976b)	
(Ethyl 3-(furfurylthio)propionate [13.093])	Rat; M, F 20	Diet	0, 5.78, 17.24	90 days	17.24[2]	(Bio-Research Laboratory, 1980)	
(2-Methyl-3-thioacetoxy-4,5-dihydrofuran [13.086])	Rat; M, F 6	Diet	0, 2.5, 6.5, 12.5, 25, 37.5, 50	3 weeks	2.5	(Munday & Gellatly, 1972)	
	Rat; M, F 16	Diet	0, 1.4, 2.8, 5.5, 8.3, 13.85, 27.70	13 weeks	1.4	(Munday & Gellatly, 1973a)	Good quality study
	Rat; M, F 16	Diet	0, 13.85, 27.70	16 weeks	Not established	(Munday & Gellatly, 1973b)	
(2-Methyl-3-thioacetoxy-4,5-dihydrofuran [13.086]) continued	Rat; M, F 16	Diet	Changing during the study	1 year	8.3[5]	(Munday & Gellatly, 1974)	

M = Male; F = Female.

[1] *Furfural was administered in the diet in microencapsulated form.*

[2] *This study was performed at either a single dose level or multiple dose levels that produced no adverse effects.*

[3] *Previously initiated animals administered 1% 5-hydroxymethylfurfuraldehyde in the diet were found to have double the occurrence of microadenoma in the colon as compared to controls.*

[4] *NOEL not reported. Dose concentrations of 20 mg/kg day reduced serum cholesterol and triglyceride levels by 50 and 42%, respectively.*

[5] *For the first 16-weeks, the animals were maintained on diets containing 1.4, 2.8, 5.55 or 8.3 mg/kg bw of test material. , then they received 8.3 for the rest of the study The authors gave no clear explanation for theabsence of effects measured at 13 and 16 weeks*

[6] *The study was conducted with the same methodology, test item and rat strain as the study by Oser 1970a , in order to establish a NOAEL.*

[7] *For the first 30 days animals received 20 mg/kg/day; then the dose as increased to 30 mg/kg/day*

[8] *The animals received 20 ml/kg/d during the 1st week, 30 ml/kg/d during the 2nd week and 40) ml/kg/d for the rest of the study*

Developmental and reproductive toxicity data are available for one candidate substance of the present flavouring group evaluation from chemical group 14 and for one supporting substance evaluated by JECFA at the 55th meeting. Supporting substance listed in brackets.

TABLE IV.3: DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

Table IV.3: Developmental and Reproductive Toxicity Studies

Chemical Name [FL-no:]	Study type Duration	Species/Sex No/group	Route	Dose levels mg/kg bw/day	NOAEL (mg/kg/day) Including information on possible maternal toxicity	Reference	Comments
Subgroup I – Structurally Related to Furfuryl Alcohol							
(Furfural [13.018])	Developmental toxicity Gestation days 6-15	Rat; F 8	Gavage	0, 10, 50, 100, 500, 1000	Maternal: 100 Foetal: ND	(Nemec, 1997a)	
	Developmental toxicity Gestation days 6-15	Rat; F 8	Gavage	0, 10, 50, 100, 150, 250, 350	Maternal: 100 Foetal: 150	(Nemec, 1997a)	
	Developmental toxicity Gestation days 6-15	Rat; F 25	Gavage	0, 50, 100, 150	Maternal: <50 Foetal: 100	(Nemec, 1997b)	
2-Furoic acid [13.136]	3 weeks[1]	Mouse; F 6	Oral	0, 20, 50, 100	Reproductive: 50	(Hall et al., 1993)	Very few details reported. No statistical analysis performed. Poor quality data.

ND: Not determined

[1]Animals were exposed for three weeks prior to mating and for three weeks during mating.

In vitro mutagenicity/genotoxicity data are available for two candidate substances of the present flavouring group evaluation from chemical group 14 and for five supporting substances evaluated by JECFA at the 55th meeting. Supporting substances are listed in brackets.

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

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

Chemical Name [FL-no:]	Test system	Test Object	Concentration	Result	Reference	Comments
Subgroup I – Structurally Related to Furfuryl Alcohol						
(Furfuryl alcohol [13.019])	Ames test	*S. typhimurium* TA98, TA100, TA1535 and TA1537	294 µg/plate	Negative[1]	(Florin et al., 1980)	
	Ames test	*S. typhimurium* TA98, TA100, TA1535 and TA1537	10000 µg/plate	Negative[1]	(Mortelmans et al., 1986) (NTP, 1999)	
	Ames test	*S. typhimurium* TA100	2500 - 12500 µg/ml	Negative[1]	(Stich et al., 1981a)	
	Ames test	*S. typhimurium* TA98, TA100 and TA102	198000 µg/plate	Negative[1]	(Aeschbacher et al., 1989)	
	Ames test	*S. typhimurium* TA98 and TA100	81 - 323 µg/plate	Negative[1]	(Shinohara et al., 1986)	
	Modified Ames test	*S. typhimurium* TA1535, TA100 and TA1537	200000 µg/ml	Positive[1]	(McGregor et al., 1981)	
	Rec assay	*B. subtilis*	2000 - 20000 µg/disk	Positive[1]	(Shinohara et al., 1986)	
	Sister chromatid exchange	CHO cells	245 µg/ml	Positive[1]	(Stich et al., 1981a)	
	Sister chromatid exchange	CHO cells	500 µg/ml	Positive/weakly positive[2] Negative[3]	(NTP, 1999)	
	Sister chromatid exchange	Human Lymphocytes	196 µg/ml	Negative	(Jansson et al., 1986)	
	Sister chromatid exchange	Human Lymphocytes	970 µg/ml	Negative	(Gomez-Arroyo & Souza, 1985)	
	Chromosomal aberration	CHO cells	2000 µg/ml	Positive	(Stich et al., 1981a)	
	Chromosomal aberration	CHO cells	1600 µg/ml	Negative[1]	(NTP, 1999)	
	SHE test	Syrian hamster embryo cells	NR	Negative[3]	(Kerckaert et al., 1996)	
	Gene Conversion Assay	*S. cerevisiae* strain D7	13500 -16000 µg/ml	Positive[2]	(Stich et al., 1981b)	
	Mammalian cell assay	Mouse embryo fibroblast cells (T1)	10 µg/ml	Negative[2]	(Kowalski et al., 2001)	
	p53 – induction assay	Mouse embryo fibroblast cells (NCTC 929)	50 µg/ml	Negative[2]	(Duerksen-Hughes et al., 1999)	
(Furfuryl acetate [13.128])	Ames test	*S. typhimurium* TA1535, TA98 and TA100	33 - 666 µg/plate	Positive[2]	(Mortelmans et al., 1986)	
(Furfural [13.018])	Ames test	*S. typhimurium* TA 1535, TA100, TA1537, TA1538, TA98	0.1 – 1000 µg/ml	Negative[1]	(McMahon et al., 1979)	
	Ames test	*S. typhimurium* TA100, TA98 and TA1535	Up to 3460 µg/plate 5766 µg/plate	Negative[1] Positive[2] (weak)	(Loquet et al., 1981)	
	Ames test	*S. typhimurium* TA100, TA98 and TA102	Up to 115320 µg/plate	Negative[1]	(Aeschbacher et al., 1989)	
	Ames test	*S. typhimurium* TA100 and TA98	15 – 63 µg/plate	Negative[1]	(Shinohara et al., 1986)	
	Ames test	*S. typhimurium* TA104	5 – 500 µg/plate	Positive[3]	(Shane et al., 1988)	
	Ames test	*S. typhimurium* TA100 and TA102	5 – 500 µg/plate	Negative[3]	(Shane et al., 1988)	
	Ames test	*S. typhimurium* TA104 and TA102	96 µg/plate	Negative	(Marnett et al., 1985a)	
	Ames test	*S. typhimurium* TA98, TA100 and TA1535	Up to 6667 µg/plate	Negative[1]	(Mortelmans et al., 1986)	
(Furfural [13.018]) continued	Ames test	S.*typhimurium* TA98, TA100	Up to 1000 µg	Negative[2]	(Osawa & Namiki, 1982)	

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

Chemical Name [FL-no:]	Test system	Test Object	Concentration	Result	Reference	Comments
	Ames test	S.*typhimurium* TA98, TA100, TA1535, TA1537	33 – 6666 µg/plate	Negative[1] TA100 equivocal[2]	(NTP, 1990a)	
	Ames test	*S. typhimurium* TA100	8000 µg/plate	Positive[1]	(Zdzienicka et al., 1978)	
	Ames test	*S. typhimurium* TA98	8000 µg/plate	Negative[1]	(Zdzienicka et al., 1978)	
	Ames test	*S. typhimurium* TA100, TA102	100 - 10000 µg/plate	Negative[2]	(Dillon et al., 1998)	
	Ames test	*S. typhimurium* TA104	100 - 10000 µg/plate	Equivocal[2]	(Dillon et al., 1998)	
	Ames test	*S. typhimurium* TA102, TA104	100 – 10000 µg/plate	Negative[3]	(Dillon et al., 1998)	
	Ames test	*S. typhimurium* TA100	100 – 10000 µg/plate	Equivocal[3]	(Dillon et al., 1998)	
	Modified Ames test	*S. typhimurium* TA100	426 µg/plate	Negative	(Kim et al., 1987)	
	Modified Ames test	*S. typhimurium* TA100, TA1535 and TA1537	200000 µg/ml	Negative	(McGregor et al., 1981)	
	Modified Ames test	*E. coli* WP2 and WP2 uvrA	0.1 – 1000 µg/ml	Negative[1]	(McMahon et al., 1979)	
	SOS induction	S.*typhimurium* TA1535/ pSK1002	1932 µg/ml	Negative[1]	(Nakamura et al., 1987)	
	Rec-assay	B.*subtilis* H17 & M45	Up to 1000 µg	Negative	(Osawa & Namiki, 1982)	
	Rec-assay	B.*subtilis* H17 & M45	0.6 ml	Negative[1]	(Matsui et al., 1989)	
	Rec-assay	*B. subtilis* strains H17 & M45	1700 - 17000 µg/disk	Positive[1]	(Shinohara et al., 1986)	
	Forward mutation assay	L5178Y tk+/- Mouse Lymphoma Cells	25 - 100 µg/ml 200 µg/ml	Negative[2] Positive[2]	(McGregor et al., 1988b)	
	Sister chromatid exchange	CHO cells	2500 – 4000 µg/ml	Positive[1]	(Stich et al., 1981a)	
	Sister chromatid exchange	CHO cells	Up to 1170 µg/ml	Positive[1]	(NTP, 1990a)	
	Sister chromatid exchange	Human Lymphocytes	Up to 0.035 mM[4] 0.07 – 0.14 Mm[4]	Negative[1] Positive[1]	(Gomez-Arroyo & Souza, 1985)	
	Chromosomal aberration	CHO cells	500 µg/ml 1000-2000 µg/ml	Negative Positive	(Nishi et al., 1989)	
	Chromosomal aberration	CHO cells	Up to 40 mM (3,840 mg)	Positive[1]	(Stich et al., 1981a)	
	Chromosomal aberration	CHO cells	3000 µg/ml	Positive	(Stich et al., 1981b)	
	Chromosomal aberration	CHO cells	375 µg/ml[2] 750 µg/ml[3]	Positive	(Gudi & Schadly, 1996)	
	Chromosomal aberration	CHO cells	Up to 1,230 µg/ml	Positive[1]	(NTP, 1990a)	
	Unscheduled DNA Synthesis	Human liver slices	0.005 – 10 mM	Negative	(Adams et al., 1998b)	
	DNA-protein cross-links	EBV- human Burkitt's lymphoma cells	25 mM	Positive[5]	(Costa, 1997)	
5-Hydroxymethyl-furfuraldehyde [13.139]	Ames test	*S. typhimurium* TA98; TA100	0.2-1 µmol/plate	Negative	(Surh et al., 1994)	The study is considered valid Purity 99%
	Ames test	*S. typhimurium* TA98; TA100	0.2-2.0 µg/plate	Positive[3]	(Omura et al., 1983)	Posisitive dose related respons inTA 100 only, most potent without S9. Purity and other experimental details not reported. The validity of the study can not be evaluated
	Ames test	*S. typhimurium* TA98; TA100	0.17- 0.66 µmol/plate	Positive[3]	(Shinohara et al., 1986)	Positive results only obtained in TA 100 with S9. Reverse dose-respons relationship. Experimental details are lacking.
5-Hydroxymethyl-furfuraldehyde [13.139] continued	Ames test	*S. typhimurium* TA104	0.1 - 0.8 mM	Negative[2] Positive	(Lee et al., 1995b)	Positive result was obtained by inclusion of PAPS and rat liver cytosol in the assay. The study is considered valid
	Ames test	*S. typhimurium* TA98; TA100;	1 - 50 µl/plate3	Negative[1]	(Aeschbacher et al., 1981)	The study is considered valid
	Ames test	*S. typhimurium* TA100	4.44 µM/plate	Negative[2]	(Kim et al., 1987)	Single dose only

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

Chemical Name [FL-no:]	Test system	Test Object	Concentration	Result	Reference	Comments
	Ames test	*S. typhimurium* TA98; TA100; TA1535; TA1537	3 µmol/plate	Negative[1]	(Florin et al., 1980)	Spot test. The study is considered valid
	Ames test	*S. typhimurium* TA100	10 µg/plate	Negative[2]	(Majeska & McGregor, 1992)	The study is considered valid
	Umu assay	*S. typhimurium* TA1535	20 mM	Positive[9]	(Janzowski et al., 2000)	Positive results were only obtained at high concentrations resulting in reduced cell viability and growth. The study is considered valid but interpretation of data is questionable
	Rec assay	*B. subtilis* H 17 rec+; M 45 rec-	0.25 - 12.5 mg/disk	Positive[1]	(Shinohara et al., 1986)	Experimental details are lacking. The validity of the study can not be evaluated
	Chromosomal aberration	Chinese hamster V79 cells	Up to 2000 µg/ml	Positive[10]	(Nishi et al., 1989)	Weak positive response were only obtained at high concentrations. The study is considered valid.
	Comet assay	V79, Caco-2, primary human colon cells and primary rat hepatocytes	Up to 80 mM	Negative[2]	(Janzowski et al., 2000)	The study is considered valid but interpretation of data is questionable
	HPRT assay	V79 cells	Up to 140 mM	Positive[1, 11]	(Janzowski et al., 2000)	Positive response were only obtained at high concentrations resulting in reduced cell viability and growth. The study is considered valid but interpretation of data is questionable.
	HPRT and tk assay	TK6 human lymphoblast cells	20 - 75 µg/ml	Negative	(Surh & Tannenbaum, 1994)	The study is considered valid
(5-Methylfurfural [13.001])	Ames test	*S. typhimurium* TA1537, TA100 and TA1535	288 µg/plate	Negative[1]	(Florin et al., 1980)	
	Ames test	*S. typhimurium* TA98, TA100 and TA102	96100 µg/plate	Negative[1]	(Aeschbacher et al., 1989)	
	Ames test	*S. typhimurium* TA98 and TA100	79 - 316 µg/plate	Negative[1]	(Shinohara et al., 1986)	
	Rec-assay	*B. subtilis* strains H17 & M45	0.55 – 5500 µg/disk	Positive[1]	(Shinohara et al., 1986)	
	Sister chromatid exchange	CHO cells	2200 – 4070 µg/ml	Positive[1]	(Stich et al., 1981a)	
2-Furoic acid [13.136]	Ames test	*S. typhimurium* TA98; TA100	25 -100 µg/plate	Negative[2]	(Ichikawa et al., 1986b)	The study is considered valid
	Ames test	*S. typhimurium* TA100		Negative	(Soska et al., 1981)	Dose not reported. The validity of the study can not be evaluated.
	Ames test	*S. typhimurium* TA100	1000 µg/plate	Negative	(Kitamura et al., 1978)	The study is considered valid
	DNA repair test	*E. coli* WP2l WP2 uvrA; WP67; WP100; CM 561; CM 571; CM 611	1000 µg/disk	Negative	(Soska et al., 1981)	The study is considered valid
	Unscheduled DNA synthesis	Primary rat hepatocytes	1000 µg/ml	Negative[10, 12]	(Aaron et al., 1989)	Study performed in accordance with GLP. The study is considered valid
(Methyl-2-furoate [13.002])	Ames test	*S. typhimurium* TA98; TA100	100 µg/plate	Negative[10]	(Ichikawa et al., 1986b)	

NR=Not Reported

[1] *With and without S9 metabolic activation.*

[2] *Without S9 metabolic activation.*

[3] *With S9 metabolic activation.*

[4] *Concentration that was added to the culture.*

[5] *Significant increases in % DNA-protein cross-links occurred only when cell viability was 40% or less (i.e. high incidence of cell death).*

[6] *TA98 with S9 metabolic activation; TA100 without S9 metabolic activation.*

[7] *5-Hydroxymethylfurfuraldehyde with 0.05 mol L-tryptophan without the presence of nitrite treatment.*

[8] *5-Hydroxymethylfurfuraldehyde with 0.05 mol L-tryptophan treated with nitrite.*

[9] *At concentrations of 12 mmol and greater, positive results were obtained without S9 metabolic activation. The dose dependent results were noted at concentrations known to be cytotoxic.*

[10] *Metabolic activation not reported.*

[11] *Effects occurred at concentrations inhibiting cellular growth.*

[12] *Dose levels above 300 microgram/ml were cytotoxic.*