EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF); Scientific Opinion on Flavouring Group Evaluation 13, Revision 2 (FGE.13 Rev2) Furfuryl and furan derivatives with and without additional side-chain substituents and heteroatoms from chemical group 14

EFSA Publication; Larsen, John Christian; Nørby, Karin Kristiane; Beltoft, Vibe Meister; Lund, Pia; Binderup, Mona-Lise

Link to article, DOI:
10.2903/j.efsa.2011.2313

Publication date:
2011

Document Version
Publisher's PDF, also known as Version of record

European Food Safety Authority

EFSA Journal 2011; 9(8):2313

SCIENTIFIC OPINION

Scientific Opinion on Flavouring Group Evaluation 13, Revision 2 (FGE.13Rev2):

Furfuryl and furan derivatives with and without additional side-chain substituents and heteroatoms from chemical group 14[1]

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

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

The Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids of the European Food Safety Authority was requested to evaluate 27 flavouring substances in the Flavouring Group Evaluation 13, Revision 2, using the Procedure in Commission Regulation (EC) No 1565/2000. Three of the substances [Fl-no: 13.125, 13.155 and 13.162] were considered to have genotoxic potential. The remaining 24 substances were evaluated through a stepwise approach (the Procedure) that integrates information on structure-activity relationships, intake from current uses, toxicological threshold of concern, and available data on metabolism and toxicity. The Panel concluded that the 24 substances do not give rise to safety concerns at their levels of dietary intake, estimated on the basis of the MSDI approach. Besides the safety assessment of these flavouring substances, the specifications for the materials of commerce have also been considered. Adequate specifications including complete purity criteria and identity for the materials of commerce have been provided for all 24 flavouring substances evaluated through the Procedure.

1 On request from the Commission, Question No EFSA-Q-2010-01555, EFSA-Q-2011-00041, EFSA-Q-2011-00815, EFSA-Q-2011-00816, EFSA-Q-2011-00859, EFSA-Q-2011-00860, EFSA-Q-2011-00861, adopted on 6 July 2011.

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

Suggested citation: EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF); Scientific Opinion on Flavouring Group Evaluation 13, Revision 2 (FGE.13 Rev2) Furfuryl and furan derivatives with and without additional side-chain substituents and heteroatoms from chemical group 14. EFSA Journal 2011;9(8):2313. [126 pp.]. doi:10.2903/j.efsa.2011.2313. Available online: www.efsa.europa.eu/efsajournal.htm

SUMMARY

The present revision of FGE.13, FGE.13Rev2, concerns, in total, the evaluation of 27 candidate flavouring substances; two more than in the previous revision of this FGE. New data included are additional data submitted on 5-hydroxymethylfurfural (5HMF) [FL-no: 13.139] in response to genotoxicity data requested in FGE.13, consideration of additional data on the stereoisomeric composition for two substances [FL-no: 13.185 and 13.199] and inclusion of two more candidate substances [FL-no: 13.130 and 13.155]. Also the evaluation of substance [FL-no: 13.135] has been re-considered.

Three of the 27 flavouring substances possesses a chiral centre [FL-no: 13.127, 13.185 and 13.199]. Industry has informed that the commercial substances are the racemates in all three cases. Two of the 27 substances can exist as geometrical isomers [FL-no: 13.011 and 13.129] and in both cases Industry has informed that the commercial substances are the (E)-isomers.

Eight of the candidate substances are classified into structural class II [FL-no: 13.122, 13.130, 13.136, 13.139, 13.145, 13.155, 13.125 and 13.162] and 19 candidate substances are classified into structural class III [FL-no: 13.011, 13.102, 13.108, 13.113, 13.114, 13.124, 13.127, 13.129, 13.132, 13.133, 13.135, 13.141, 13.143, 13.144, 13.146, 13.149, 13.178, 13.185 and 13.199].

Nineteen of the flavouring substances in the present group have been reported to occur naturally in a wide range of food items. For seven flavouring substances, no data on natural occurrence have been reported.

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

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

According to the default MSDI approach, the 27 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 volumes in Europe (MSDI approach), the total estimated combined intake of candidate and supporting substances would be 885 and 60 microgram/*capita*/day for structural class II and III, respectively. The total combined daily per capita intake of 885 microgram exceeds the threshold of concern of 540 microgram/person/day for structural class II substances. However, 50 % of the combined daily per capita intake of 885 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 established by EFSA. The total combined intake for the class III substances of 60 microgram/capita/day does not exceed the threshold for the structural class of 90 microgram/person/day.

Genotoxicity studies were only available on some of the candidate substances included in main group I or on related supporting substances. For subgroup Ia, 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 provided for one candidate substance [FL-no: 13.139] and for two of the supporting substances. For the candidate substance in group Ib, *in vitro* and *in vivo* genotoxicity data were available for one supporting substance. For subgroup Ic, data on *in vitro* genotoxicity were provided for two supporting substances. For one of these, also data on *in vivo* genotoxicity were provided. In addition, data for a supporting substance for subgroup Ib contribute to the evaluation of the genotoxic potential of substances in subgroup Ic due to structural similarity of metabolites of the Ic candidates with the substances in subgroup Ib.

In subgroup Ia the data available do not indicate a concern for genotoxicity. For the one candidate substance in subgroup Ib [FL-no: 13.155] and the two candidate substances in subgroup Ic [FL-no: 13.125 and 13.162], metabolism studies on closely related substances indicate a potential for DNA-binding of metabolites. In addition, in several *in vitro* studies with related substances, indications for genotoxic activity were obtained. These data preclude the evaluation through the Procedure of the three candidate flavouring substances in subgroup Ib and Ic.

No genotoxicity data were available on candidate substances or on structurally related substances in main group II. The lack of data on the sulphur-containing candidate substances from main group II, or on the related supporting substances, does not allow concluding on their genotoxicity, but would not preclude the evaluation of these substances through the Procedure.

The metabolism of non-sulphur-containing furfuryl alcohol derivatives in FGE.13 [FL-no: 13.127, 13.129, 13.130, 13.132 and 13.133] includes the formation of furfural, a known reactive aldehyde, that may lead to hepatotoxicity. Furfural will be further metabolised to furoic acid [Fl no: 13.136] and furanacrylic acid, the hydrolysis product of [FL-no: 13.011]. 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 Ia cannot be predicted to be metabolised to innocuous compounds. In addition, 5HMF [FL-no: 13.139] has a free aldehyde function (*cf.* furfural*)* and can be anticipated to be reactive. In addition, this substance may be bioactivated to reactive intermediates by sulphotransferases for which genotoxicity has been reported. However, the available genotoxicity and carcinogenicity data for this substance do not indicate a concern for this substance. Extensive ring-opening with formation of intermediates reactive towards DNA has been reported for 2-alkyl-substituted furans in subgroup 1c [FL-no: 13.125 and 13.162]. In addition, these compounds may be metabolised to ketones (subgroup 1b; [FL-no: 13.155]), for which genotoxicity may be anticipated.

Based on the metabolism of other sulphur-containing compounds, the candidate furfuryl and furan monosulphides are expected to be subject to oxidation mainly to sulphoxides and sulphones. Given the reactivity of thiol groups, whether free or from di- or trisulphides, 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 main group II can be predicted to be metabolised to innocuous substances.

Short-term and long-term toxicity studies are available for two candidate substances included in subgroup Ia, namely 2-furoic acid [FL-no: 13.136] and 5HMF [FL-no: 13.139] and for three related supporting substances. For the candidate substances structurally related to furfuryl alcohol or furfural, these studies indicate that the liver is the critical target for their toxicity. EFSA has established an ADI value of 0.5 mg/kg/d bw for furfural and the furfural component of furfural diethyl acetal. For 5-hydroxymethylfurfural [FL-no: 13.139], substance-specific data indicate that the kidney of the male mouse was the target organ, and for this substance a BMDL of 14.4 mg/kg bw/day could be derived, based on the results of a 13-weeks oral toxicity study.

No toxicity data are available on the sulphur-containing furan-derived candidate substances included in subgroups IIa-d. However, results from toxicity studies on 14 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 from 0.45 to 10 mg/kg bw/day.

It was considered on the basis of the default MSDI approach that for the 24 candidate substances [FL-no: 13.011, 13.102, 13.108, 13.113, 13.114, 13.122, 13.124, 13.127, 13.129, 13.130, 13.132, 13.133, 13.135, 13.136, 13.139, 13.141, 13.143, 13.144, 13.145, 13.146, 13.149, 13.178, 13.185 and 13.199], it could be concluded, at step B4 of the Procedure, that these 24 candidate substances do not pose a safety concern when used as flavouring substances at the estimated levels of intake based on the MSDI approach.

The Panel has reservations for three substances [FL-no: 13.125, 13.155 and 13.162] which could not be evaluated through the Procedure due to concern of genotoxicity *in vitro*. For these three substances additional date are required.

For three candidate substances [FL-no: 13.122, 13.136 and 13.139] in structural class II, evaluated through the Procedure, the mTAMDI range from 1400 to 3900 microgram/person/day, which are above the threshold of concern for this Cramer class. For one substance [FL-no: 13.145] the mTAMDI is 160 microgram/person/day, which is below the class threshold of 540 microgram/person/day. For substance [FL-no: 13.130], no use levels were provided, so no mTAMDI can be calculated.

The estimated intakes for 16 [FL-no: 13.011, 13.102, 13.108, 13.113, 13.114, 13.127, 13.129, 13.132, 13.133, 13.135, 13.141, 13.143, 13.146, 13.149, 13.178 and 13.185] of the 19 substances evaluated through the Procedure and assigned to structural class III, based on the mTAMDI, range from 160 to 3900 microgram/person/day, which are above the threshold of concern for structural class III substances of 90 microgram/person/day. For the remaining three substances from structural class III [FL-no: 13.124, 13.144 and 13.199] the mTAMDIs range from 49 to 78 microgram/person/day, which is below the threshold of concern.

Thus, for 20 of the candidate substances, evaluated using the Procedure, further information is required. This would include more reliable intake data and then, if required, additional toxicological data. 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 24 candidate substances evaluated through the Procedure can be applied to the material of commerce, it is necessary to consider the available specifications. Adequate specifications including complete purity criteria and identity for the materials of commerce have been provided for all 24 flavouring substances evaluated through the Procedure.

For these 24 candidate substances [FL-no: 13.011, 13.102, 13.108, 13.113, 13.114, 13.122, 13.124, 13.127, 13.129, 13.130, 13.132, 13.133, 13.135, 13.136, 13.139, 13.141, 13.143, 13.144, 13.145, 13.146, 13.149, 13.178, 13.185 and 13.199] the Panel concluded that they would be of "No safety concern at estimated levels of intake as flavouring substances" based on the MSDI approach.

KEYWORDS

Furfuryl, furan, flavourings, sulphur-substituted, disulphide, trisulphide, thioester, safety, FGE.13.

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BACKGROUND

Regulation (EC) No 2232/96 of the European Parliament and the Council (EC, 1996a) lays down a Procedure for the establishment of a list of flavouring substances the use of which will be authorised to the exclusion of all other substances in the EU. In application of that Regulation, a Register of flavouring substances used in or on foodstuffs in the Member States was adopted by Commission Decision 1999/217/EC (EC, 1999a), as last amended by Commission Decision 2009/163/EC (EC, 2009a). 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, 2000a), which is broadly based on the Opinion of the Scientific Committee on Food (SCF, 1999a). For the submission of data by the manufacturer, deadlines have been established by Commission Regulation (EC) No 622/2002 (EC, 2002b).

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

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

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

HISTORY OF THE EVALUATION

The first revision of FGE.13, FGE.13Rev1, included the assessment of seven additional candidate substances [FL-no: 13.125, 13.135, 13.141, 13.143, 13.162, 13.185 and 13.199]. For two of these substances [FL-no: 13.125 and 13.162] supporting toxicity data on a structurally related substance have been submitted. No other data have been provided. The No Observed Adverse Effect Level (NOAEL) for the structurally related substance [FL-no: 13.079] turned out to be taken from a study with a different substance. This necessitates an update of the evaluation of the flavouring substance [FL-no: 13.113], which depends on this NOAEL. For 12 flavouring substances in the original FGE.13, the classification according to Cramer et al, 1977 was revised. These revisions were necessary to create consistency with the evaluations in FGEs 65 and 67. For the substances involved, the final conclusions were not changed. In addition, the Panel noted that substance [FL-no: 13.178] is synonymous with [FL-no: 13.192], which is evaluated by the JECFA (JECFA no 1542) at its 69th meeting (JECFA, 2009a). For this substance in the JECFA evaluation an MSDI for Europe of 0.24 microgram *per capita* per day was given. This figure, which is higher and more recent than the exposure estimate in FGE.13 (0.0012 microgram *per capita* per day), was used in the first Revision of the FGE.

FGE	Opinion adopted by EFSA	Link	No. of candidate substances
FGE.13	28 April 2005	http://www.efsa.eu.int/science/afc/afc_opinions/1023_en.html	18
FGE.13Rev1	26 November 2009	http://www.efsa.europa.eu/en/scdocs/scdoc/1403.htm	25
FGE.13Rev2			27

The present revision of FGE.13, FGE.13Rev2 includes the consideration of newly submitted sub-chronic and chronic toxicity data as well as genotoxiciy data on 5-hydroxymethylfurfural [FL-no: 13.139]. For this substance additional genotoxicity data have been requested in FGE.13. The Panel has used the information from the new sub-chronic and chronic toxicity studies for a BMD derivation.

New information from Industry on the stereoisomeric composition of two candidate substances [FL-no: 13.185 and 13.199] has also been included.

Furthermore, one alpha,beta-unsaturated ketone [FL-no: 13.155] from FGE.221 has been included in a new subgroup, as well as seven supporting substances from the same FGE.221 have been included in the current FGE.13Rev2. EFSA concluded in November 2008 that the alpha,beta-unsaturated structure in conjugation with an aromatic ring system is comparable to the situation in acetophenone, i.e. no structural alert for genotoxicity. Industry has submitted additional genotoxicity data on the structurally related 2-furyl methyl ketone [FL-no: 13.054]. These data will be considered in this FGE as well.

In addition, this revision also includes one substance furfuryl butyrate [FL-no: 13.130] for which the JECFA did evaluate the specifications, but did not perform a safety evaluation. No toxicity or metabolism data were submitted for this substance.

In the previous versions of this FGE, substance [FL-no: 13.135] (1-(2-furfyrylthio)propanone) was incorrectly allocated to a subgroup of thioesters and evaluated as the thioester S-furfuryl-propanethioate. In the current revision of FGE.13 this candidate substance ([FL-no: 13.135]) is allocated to and re-evaluated in subgroup IIa, consisting of sulphides. Since there are no further thioester candidates substances left in this FGE, the respective subgroup has been deleted.

TERMS OF REFERENCE

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

In addition, in letter of 30 July 2010 the Commission requested EFSA to carry out an evaluation of flavouring substance 1-(2-furfyrylthio)propanone [FL-no: 13.135]:

"The European Commission requests the European Food Safety Authority to carry out a safety assessment on the new flavouring substances 5-Pentyl-3H-furan-2-one, 1-(2-furfyrylthio)propanone and 5-Ethyl 2,3-dimethyl pyrazine, in accordance with Commission Regulation (EC) No 1565/2000.

5-Pentyl-3H-furan-2-one [FL-no: 10.070] and 5-Ethyl 2,3-dimethyl pyrazine [FL-no: 14.170] are evaluated in FGE.10Rev3 and FGE17Rev3.

The evaluation programme has been finalised end of 2009. The deadline of the Terms of Reference in letter of 30 July 2010 was negotiated to end 2011.

ASSESSMENT

1. Presentation of the Substances in Flavouring Group Evaluation 13 Revision 2

1.1. Description

The present Flavouring Group Evaluation (FGE.13Rev2), using the Procedure as referred to in the Commission Regulation (EC) No 1565/2000 (EC, 2000a) (The Procedure – shown in schematic form in Annex I of this FGE), deals with 27 flavouring substances (candidate substances) from chemical group 14, Annex I of Commission Regulation (EC) No 1565/2000 (EC, 2000a). All candidate substances in FGE.13Rev2 are furan derivatives and can be divided into two main groups (I and II), depending on the absence/presence of sulphur-containing substituents. Within these two main groups a further differentiation in subgroups is introduced, depending on the nature of the ring substituents and

the number and position of the sulphur substituents. The subgrouping of the candidate substances is shown below. The candidate substances are structurally related to 53 flavouring substances (supporting substances) evaluated by the JECFA at their 55th, 59th, 65th and 69th meetings (JECFA, 2001a; JECFA, 2002c; JECFA, 2006d; JECFA, 2009a) or EFSA (EFSA, 2004c) [FL-no: 13.126]).

Main group I. Non-sulphur-containing Furan Derivatives

Subgroup Ia: Structurally related to furfuryl alcohol

The ten candidate substances in subgroup Ia are furfuryl alcohol derivatives such as esters of furfuryl alcohol [FL-no: 13.127, 13.129, 13.130, 13.132 and 13.133] or ethyl ester of 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]. These ten candidate substances are closely related to 14 supporting substances evaluated at the 55th JECFA meeting (JECFA, 2001a) in a group of "Furfuryl alcohol and related substances". The 14 supporting substances have subsequently been considered by EFSA in FGE.66 (EFSA, 2009ap). In addition, the implication for human health of dietary exposure to furfural and furfural diethyl acetal [FL-no: 13.126] (a fifteenth supporting substance for this group) has been evaluated by the AFC Panel (EFSA, 2004c) and a risk assessment report for furfural is available in the framework of Existing Chemical Evaluation, according to EU Regulation 73/793/CE (EU-RAR, 2004b).

Subgroup Ib: Alkoyl-substitutes furans

This subgroups contains only one candidate substance [FL no: 13.155], which is closely related to seven supporting substances evaluated at the 69th JECFA meeting (JECFA, 2009a) in a group of "Furan-substituted substances". These seven supporting substances will be considered by EFSA in FGE.67Rev1 (EFSA, 2011i).

Subgroup Ic: Alkyl-substituted furans

The two candidate substances in subgroup Ic are alkyl substituted furans [FL-no: 13.125 and 13.162] without any further functional groups. These two candidate substances are closely related to seven supporting substances evaluated at the 69th JECFA meeting (JECFA, 2009a) in a group of "Furan-substituted substances". These seven supporting substances have been considered by EFSA in FGE.67 (EFSA, 2009ao).

Main group II. Sulphur-containing Furan Derivatives

Subgroup IIa: Sulphides

The seven candidate substances in subgroup IIa are sulphides (or thioethers) [FL-no: 13.114, 13.124, 13.135, 13.141, 13.143, 13.145 and 13.199]. These seven candidate substances are closely related to 11 supporting substances evaluated at the 59th JECFA meeting (JECFA, 2002c) in a group of "Sulphur-substituted furan derivatives". These 11 supporting substances have been considered by EFSA in FGE.65 (EFSA, 2009an).

Subgroup IIb: Thiols

The two candidate substances in subgroup IIb are thiols [FL-no: 13.108 and 13.149]. Candidate substance [FL-no: 13.108] is a dihydrofuran derivative, rather than a furan derivative, but is included in this subgroup, nonetheless. The two candidate substances are closely related to five supporting substances evaluated at the 59th JECFA meeting (JECFA, 2002c) in a group of "Sulphur-substituted furan derivatives". These five supporting substances have been considered by EFSA in FGE.65 (EFSA, 2009an).

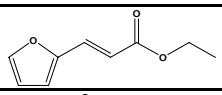
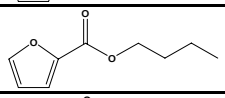
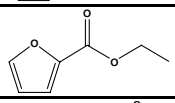
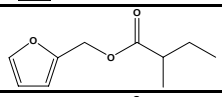
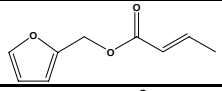
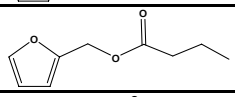
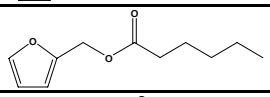
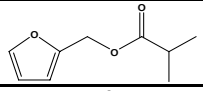
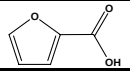
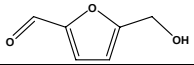
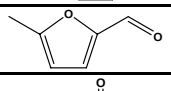
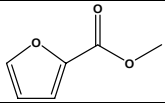
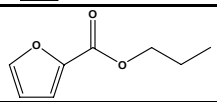
Subgroup IIc: Disulphides

The four candidate substances in subgroup IIc are disulphides [FL-no: 13.113, 13.144, 13.178 and 13.185]. These four candidate substances are closely related to seven supporting substances evaluated at the 59th JECFA meeting (JECFA, 2002c) in a group of "Sulphur-substituted furan derivatives". These seven supporting substances have been considered by EFSA in FGE.65 (EFSA, 2009an).

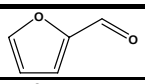
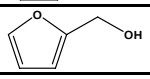
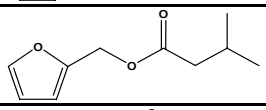
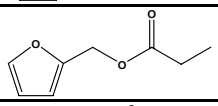
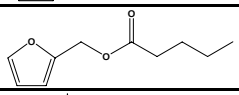
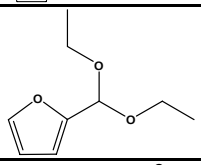
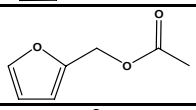
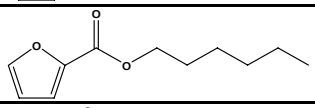
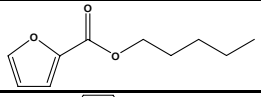
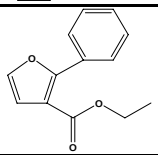
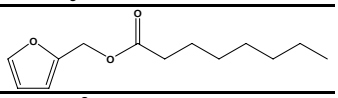
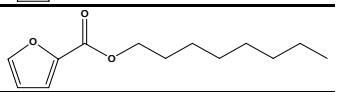
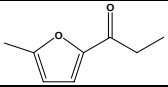
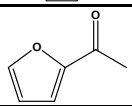
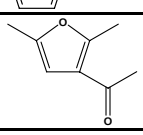
Subgroup IId: Polysulphides

The one candidate substance in subgroup IId is a trisulphide [FL-no: 13.146]. This candidate substance is closely related to a tetrasulphide supporting substance evaluated at the 59th JECFA meeting (JECFA, 2002c) in a group of "Sulphur-substituted furan derivatives". This supporting substance has been considered by EFSA in FGE.65 (EFSA, 2009an).

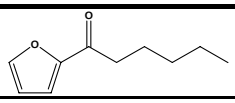
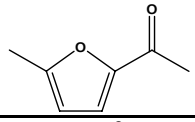
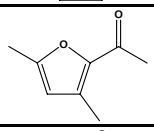
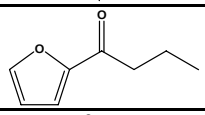
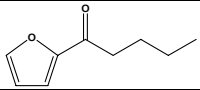
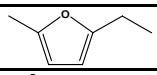
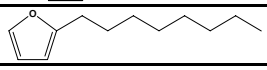
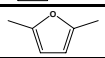
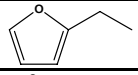
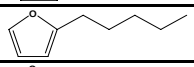
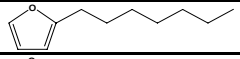
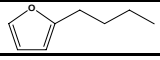
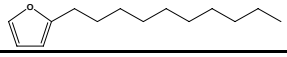
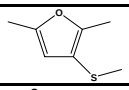
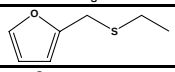
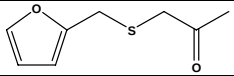
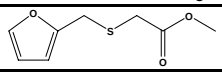
FGE.13Rev2 – Candidate and Supporting substances divided into subgroups of related chemical structures. Substances listed in bold are the candidate substances in this FGE. The supporting substances from the 55th, 59th, 65th or 69th JECFA and EFSA (EFSA, 2004c) are in normal type face.

FL-no JECFA-no	EU Register name	Structural formula
	Ia Structurally Related to Furfuryl Alcohol	
13.011	**Ethyl furfuracrylate**	
13.102	**Butyl 2-furoate**	
13.122	**Ethyl 2-furoate**	
13.127	**Furfuryl 2-methylbutyrate**	
13.129	**Furfuryl but-2-enoate**	
13.130	**Furfuryl butyrate**	
13.132	**Furfuryl hexanoate**	
13.133	**Furfuryl isobutyrate**	
13.136	**2-Furoic acid**	
13.139	**5-Hydroxymethylfurfuraldehyde**	
13.001 745	5-Methylfurfural	
13.002 746	Methyl 2-furoate	
13.003 747	Propyl 2-furoate	

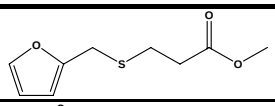
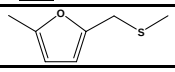
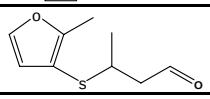
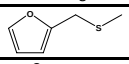
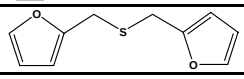
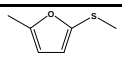
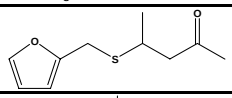
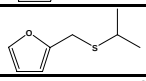
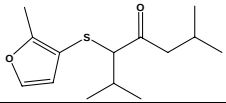
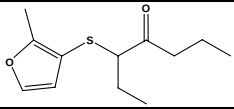
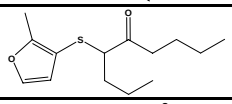
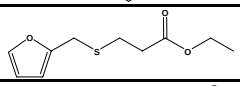
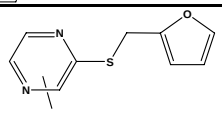
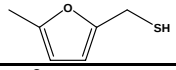
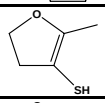
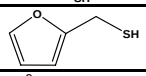
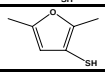
FGE.13Rev2 – Candidate and Supporting substances divided into subgroups of related chemical structures. Substances listed in bold are the candidate substances in this FGE. The supporting substances from the 55th, 59th, 65th or 69th JECFA and EFSA (EFSA, 2004c) are in normal type face.

FL-no JECFA-no	EU Register name	Structural formula
13.018 450	Furfural	
13.019 451	Furfuryl alcohol	
13.057 743	Furfuryl isovalerate	
13.062 740	Furfuryl propionate	
13.068 741	Furfuryl valerate	
13.126	Furfuryl diethyl acetal	
13.128 739	Furfuryl acetate	
13.005 749	Hexyl 2-furoate	
13.025 748	Pentyl 2-furoate	
13.038 752	2-Phenyl-3-carbethoxyfuran	
13.067 742	Furfuryl octanoate	
13.073 750	Octyl 2-furoate	
	Ib Alkoyl-substituted furans	
13.155	**2-Methyl-5-propionylfuran**	
13.054 1503	2-Acetylfuran	
13.066 1506	3-Acetyl-2,5-dimethylfuran	

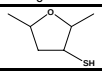
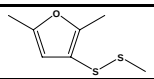
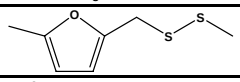
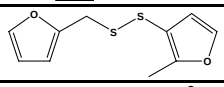
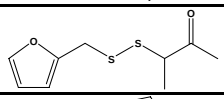
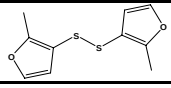
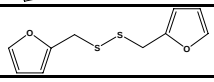
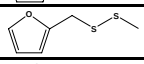
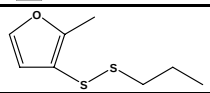
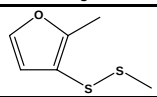
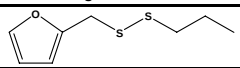
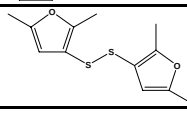
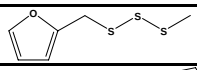
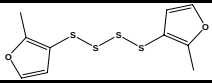
FGE.13Rev2 – Candidate and Supporting substances divided into subgroups of related chemical structures. Substances listed in bold are the candidate substances in this FGE. The supporting substances from the 55th, 59th, 65th or 69th JECFA and EFSA (EFSA, 2004c) are in normal type face.

FL-no JECFA-no	EU Register name	Structural formula
13.070 1512	2-Hexanoylfuran	
13.083 1504	2-Acetyl-5-methylfuran	
13.101 1505	2-Acetyl-3,5-dimethylfuran	
13.105 1507	2-Butyrylfuran	
13.163 1509	2-Pentanoylfuran	
	Ic Alkyl-substituted furans	
13.125	**2-Ethyl-5-methylfuran**	
13.162	**2-Octylfuran**	
13.029 1488	2,5-Dimethylfuran	
13.030 1487	2-Methylfuran	
13.092 1489	2-Ethylfuran	
13.059 1491	2-Pentylfuran	
13.069 1492	2-Heptylfuran	
13.103 1490	2-Butylfuran	
13.106 1493	2-Decylfuran	
	II Sulphur-substituted Furan Derivatives	
	IIa Sulphides	
13.114	**2,5-Dimethyl-3-(methylthio)furan**	
13.124	**Ethyl furfuryl sulfide**	
13.135	**1-(2-furfurylthio)-propanone**	
13.141	**Methyl (2-furfurylthio)acetate**	

FGE.13Rev2 – Candidate and Supporting substances divided into subgroups of related chemical structures. Substances listed in bold are the candidate substances in this FGE. The supporting substances from the 55th, 59th, 65th or 69th JECFA and EFSA (EFSA, 2004c) are in normal type face.

FL-no JECFA-no	EU Register name	Structural formula
13.143	**Methyl 3-(furfurylthio)propionate**	
13.145	**Methyl 5-methylfurfuryl sulfide**	
13.199	**3-[(2-methyl-3-furyl)thio]-butanal**	
13.053 1076	Methyl furfuryl sulfide	
13.056 1080	Difurfuryl sulfide	
13.065 1062	2-Methyl-5-(methylthio)furan	
13.152 1061	2-Methyl-3-(methylthio)furan	
13.196 1084	4-(Furfurylthio) pentan-2-one	
13.032 1077	Furfuryl isopropyl sulfide	
13.075 1086	2,6-Dimethyl-3-((2-methyl-3-furyl)thio)heptan-4-one	
13.077 1085	3-((2-Methyl-3-furyl)thio)heptan-4-one	
13.078 1087	4-((2-Methyl-3-furyl)thio)nonan-5-one	
13.093 1088	Ethyl 3-(2-furfurylthio)propionate	
13.151 1082	2-Methyl-3,5 and 6-(furfurylthio)pyrazine	 2 or 5 or 6 -Methyl-3-(furfurylthio)pyrazine
	IIb Thiols	
13.149	**5-Methyl-2-furanmethanethiol**	
13.108	**4,5-Dihydro-3-mercapto-2-methylfuran**	
13.026 1072	2-Furanmethanethiol	
13.055 1060	2-Methylfuran-3-thiol	
13.071 1063	2,5-Dimethylfuran-3-thiol	

FGE.13Rev2 – Candidate and Supporting substances divided into subgroups of related chemical structures. Substances listed in bold are the candidate substances in this FGE. The supporting substances from the 55th, 59th, 65th or 69th JECFA and EFSA (EFSA, 2004c) are in normal type face.

FL-no JECFA-no	EU Register name	Structural formula
13.160 1090	2-Methyltetrahydrofuran-3-thiol	
13.193 1091	2,5-Dimethyltetrahydro-3-furanthiol	
	IIc Disulphides	
13.113	**2,5-Dimethyl-3-(methyldithio)furan**	
13.144	**Methyl 5-methylfurfuryl disulfide**	
13.178	**3-(Furfuryldithio)-2-methylfurfuran**	
13.185	**2-Furfuryl 3-oxo-2-butyl disulphide**	
13.016 1066	bis-(2-Methyl-3-furyl) disulfide	
13.050 1081	Difurfuryl disulfide	
13.064 1078	Methyl furfuryl disulfide	
13.082 1065	Propyl 2-methyl-3-furyl disulfide	
13.079 1064	Methyl 2-methyl-3-furyl disulfide	
13.197 1079	Furfuryl propyldisulfide	
13.015 1067	bis-(2,5-Dimethyl-3-furyl) disulfide	
	IId Polysulphides	
13.146	**Methyl furfuryl trisulfide**	
13.017 1068	bis-(2-Methyl-3-furyl) tetrasulfide	

The 27 candidate flavouring substances under consideration, as well as their chemical Register 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.

A summery of the safety evaluation of the candidate substances under consideration in the present evaluation are listed in Table 2a.

The hydrolysis products of the candidate esters are listed in Table 2b.

The names and structures of the supporting substances are listed in Table 3, together with their evaluation status (CoE, 1992; JECFA, 2001a; JECFA, 2002c; JECFA, 2006b; JECFA, 2009c;SCF, 1995).

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.).

Three of the 27 candidate substances possess a chiral centre [FL-no: 13.127, 13.185 and 13.199]. For all three substances the Industry has informed that the commercial substance is the racemate.

Two of the 27 candidate substances can exist as geometrical isomers [FL-no: 13.011 and 13.129] and in both cases Industry has informed that the commercial substance is the (E)-isomer (see Table 1).

1.3. Natural Occurrence in Food

Nineteen of the 27 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 12 of these substances.

These reports include:

- 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.
- 2-Ethyl-5-methylfuran [FL-no: 13.125]: 0.011 mg/kg in shrimps.
- Furfuryl but-2-enoate [FL-no: 13.129]: Up to 0.2 mg/kg in coffee.
- Furfuryl butyrate [FL-no: 13.130]: up to 0.8 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.
- 2- Methyl-5-propionylfuran [FL-no: 13.155]: Up to 4.2 mg/kg in coffee.

Seven of the substances, have not been reported to occur naturally in any food items according to TNO (TNO, 2000; TNO, 2009), see Table 1.3.

1.3 Candidate substances not reported to occur in nature (TNO, 2000; TNO, 2009)

FL-no:	Name:
13.108	4,5-dihydro-3-mercapto-2-methylfuran
13.135	1-(2-furfurylthio)propanone
13.141	methyl (2-furfurylthio)acetate
13.143	methyl 3-(furfurylthio)propionate
13.178	3-(furfuryldithio)-2-methylfuran
13.185	2-furfuryl 3-oxo-2-butyl disulphide
13.199	3-[(2-methyl-3-furyl)thio]-butanal

2. Specifications

Purity criteria for the 27 candidate substances have been provided by the flavouring industry (EFFA, 2003g; EFFA, 2004y; Flavour Industry, 2009b; JECFA, 2001c).

Judged against the requirements in Annex II of Commission Regulation (EC) No 1565/2000 (EC, 2000a), the information is adequate for all 27 candidate substances.

For substance [FL-no: 13.185] the Register name and structure and for substance [FL no: 13.178] the Register name appeared to be incorrect. Corrected structure and names have been provided (written correspondence between EFSA and EFFA). The corrected names and structure have been given in Table 1.

3. Intake Data

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

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

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

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

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

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

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

The Maximised Survey-derived Daily Intake (MSDI (SCF, 1999a)) 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[4] (Eurostat, 1998). This is derived for candidate substances from estimates of annual volume of production provided by Industry and incorporates a correction factor of 0.6 to allow for incomplete reporting (60 %) in the Industry surveys (SCF, 1999a).

The total annual volume of production of the 27 candidate substances for use as flavouring substances in Europe has been reported to be approximately 357 kg (EFFA, 2003g; EFFA, 2004y; Flavour Industry, 2009b; EFFA, 2011b) and for 51 (for which european production volumes are available) of the 53 supporting substances approximately 7700 kg (cited by (JECFA, 2001b; JECFA, 2003a)).

On the basis of the annual volumes of production reported for the 27 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 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 1.2 microgram for each of the remaining candidate substances (Table 2a).

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

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

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

[4] EU figure 375 millions (Eurostat, 1998). This figure relates to EU population at the time for which production data are available, and is consistent (comparable) with evaluations conducted prior to the enlargement of the EU. No production data are available for the enlarged EU.

For 26 of the 27 candidate substances information on food categories and normal and maximum use levels[5,6,7] were submitted by the Flavour Industry (EFFA, 2003g; EFFA, 2004y; EFFA, 2007a; Flavour Industry, 2009b). The 26 candidate substances are used in flavoured food products divided into the food categories, outlined in Annex III of the Commission Regulation 1565/2000 (EC, 2000a), as shown in Table 3.1. For the present calculation of mTAMDI, the reported normal use levels were used. In the case where different use levels were reported for different food categories the highest reported normal use level was used. No normal and maximum use levels were provided for [FL-no: 13.130].

Table 3.1 Use of the 26 Candidate Substances for which use levels have been provided

Food category	Description	Flavourings used
01.0	Dairy products, excluding products of category 2	All 26
02.0	Fats and oils, and fat emulsions (type water-in-oil)	All 26
03.0	Edible ices, including sherbet and sorbet	All 26
04.1	Processed fruits	All 26
04.2	Processed vegetables (incl. mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds	Only [FL-no: 13.199]
05.0	Confectionery	All 26
06.0	Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery	All 26
07.0	Bakery wares	All 26 except [FL-no: 13.136]
08.0	Meat and meat products, including poultry and game	All 26
09.0	Fish and fish products, including molluscs, crustaceans and echinoderms	All 26
10.0	Eggs and egg products	None
11.0	Sweeteners, including honey	None
12.0	Salts, spices, soups, sauces, salads, protein products etc.	All 26
13.0	Foodstuffs intended for particular nutritional uses	All 26
14.1	Non-alcoholic ("soft") beverages, excl. dairy products	All 26
14.2	Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts	All 26
15.0	Ready-to-eat savouries	All 26
16.0	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 1 – 15	All 25

According to the Flavour Industry the normal use levels for the 26 of the 27 candidate substances are in the range of 0.001 - 20 mg/kg food, and the maximum use levels are in the range of 0.003 - 100 mg/kg food (EFFA, 2003g; EFFA, 2004y; Flavour Industry, 2009b).

The mTAMDI values for the seven candidate substances from structural class II (see Section 5) range from 160 to 3900 microgram/person/day. For 19 candidate substances from structural class III the mTAMDIs range from 49 to 3900 microgram/person/day.

[5] "Normal use" is defined as the average of reported usages and "maximum use" is defined as the 95th percentile of reported usages (EFFA, 2002i).
[6] 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).
[7] The use levels from food category 5 "Confectionery" have been inserted as default values for food category 14.2 "Alcoholic beverages" for substances for which no data have been given for food category 14.2 (EFFA, 2007a).

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

4. Absorption, Distribution, Metabolism and Elimination

The candidate substances in FGE.13Rev2 are furan derivatives which can be divided into subgroups based on their chemical structure (see Table 4.1).

Table 4.1. Candidate substances divided into subgroups of related chemical structures

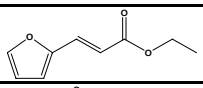
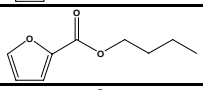
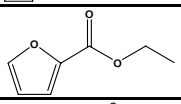
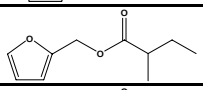
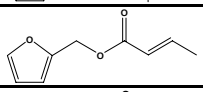
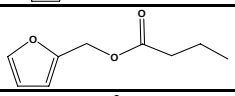
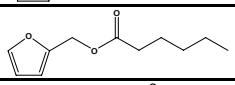
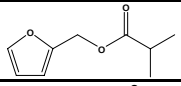
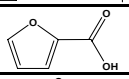
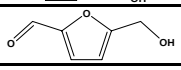
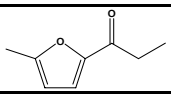
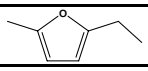
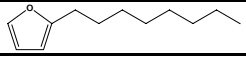
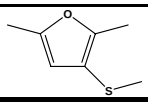
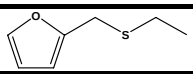
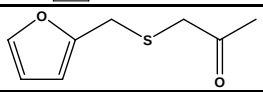
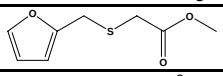
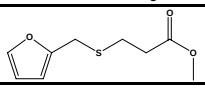
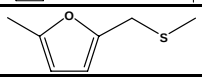
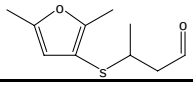
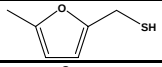
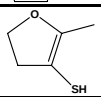
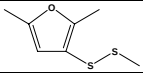
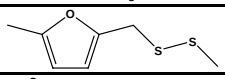
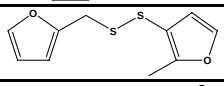
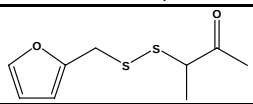
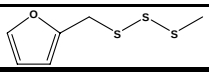
FL-no	EU Register name	Structural formula	Structural class
	1a Structurally Related to Furfuryl Alcohol		
13.011	Ethyl furfuracrylate		III
13.102	Butyl 2-furoate		III
13.122	Ethyl 2-furoate		II
13.127	Furfuryl 2-methylbutyrate		III
13.129	Furfuryl but-2-enoate		III
13.130	Furfuryl butyrate		II
13.132	Furfuryl hexanoate		III
13.133	Furfuryl isobutyrate		III
13.136	2-Furoic acid		II
13.139	5-Hydroxymethylfurfuraldehyde		II
	Ib Alkoyl-substituted furans		
13.155	2-Methyl-5-propionylfuran		II
	Ic Alkyl-substituted furans		
13.125	2-Ethyl-5-methylfuran		II
13.162	2-Octylfuran		II
	II Sulphur-substituted Furan Derivatives		
	IIa Sulphides		
13.114	2,5-Dimethyl-3-(methylthio)furan		III

Table 4.1. Candidate substances divided into subgroups of related chemical structures

FL-no	EU Register name	Structural formula	Structural class
13.124	Ethyl furfuryl sulfide		III
13.135	1-(2-Furfurylthio)propanone		III
13.141	Methyl (2-furfurylthio)acetate		III
13.143	Methyl 3-(furfurylthio)propionate		III
13.145	Methyl 5-methylfurfuryl sulfide		II
13.199	3-[(2-methyl-3-furyl)thio]-butanal		III
	IIb Thiols		
13.149	5-Methyl-2-furanmethanethiol		III
13.108	4,5-Dihydro-3-mercapto-2-methylfuran		III
	IIc Disulphides		
13.113	2,5-Dimethyl-3-(methyldithio)furan		III
13.144	Methyl 5-methylfurfuryl disulfide		III
13.178	3-(Furfuryldithio)-2-methylfurfuran		III
13.185	2-Furfuryl 3-oxo-2-butyl disulphide		III
	IId Polysulphides		
13.146	Methyl furfuryl trisulfide		III

4.1. Main Group I

4.1.1. Subgroup Ia

The ten candidate substances in subgroup Ia are furfuryl alcohol derivatives such as esters of furfuryl alcohol [FL-no: 13.127, 13.129, 13.130, 13.132 and 13.133], ethyl ester of furanacrylic acid [FL-no 13.011], furoic acid [FL-no: 13.136] and two esters [FL-no: 13.102 and 13.122] of furoic acid. 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 butyrate [FL-no: 13.130], 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 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 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 furfural and then 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.

Also for 5HMF, data have shown that for this substance the principal route of metabolism in mice and rats is oxidation to the furoic acid derivative followed by glycine conjugation and rapid elimination in the urine. However, in addition to this pathway, 5HMF has been shown to be bioactivated *in vitro* to 5-[(sulphoxy)methyl] furfural (SMF), through sulphonation of its allylic hydroxyl functional group, catalyzed by sulfotransferases. In the resulting ester, the sulphate 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. After intravenous administration of 5HMF to mice, SMF can be detected in the blood. This is an unstable substance with a half-life of only 4.2 minutes.

4.1.2. Subgroup Ib

The candidate substance in subgroup Ib [FL-no: 13.155] is an alkoyl-substituted furan which has a keto function at the carbon atom directly adjacent to the furan ring (i.e. the C_1'-carbon of the ring substituent). It is considered that the alpha,beta-unsaturated structure in this substance is structurally related and comparable to the structure in acetophenone (EFSA, 2008am). It is anticipated that the ketone in the C_1 position of the sidechain will undergo reduction to the corresponding secondary alcohol which subsequently is excreted after conjugation (JECFA, 2006d). For this substance also ring oxidation followed by ring opening may be anticipated (see subgroup Ic).

4.1.3. Subgroup Ic

The candidate substances in subgroup Ic [FL-no: 13.125 and 13.162] are substituted furans which in contrast to the candidate substances in subgroup Ia do not bear any functional (carbonyl) groups in the side chain. Based on the limited data availabe, also for these substances absorption from the GI-tract may be anticipated, similar to the subgroup Ia substances. Mono-alkyl furans, such as the candidate substance 2-octylfuran [FL-no: 13.162], may be subject to oxidation (possibly epoxidation of the

unsubstituted double bond in the furan ring) followed by ring opening and rearrangement to alkyl-substituted dialdehydes. For several 2-alkyl-substituted furans reactivity of dialdehydes towards proteins and DNA has been demonstrated, resulting in toxicity to liver and kidneys. Oxidation of the C_1'-carbon of the alkyl substituent may result in the formation of an α,β-unsaturated ketone with the carbonyl group connected to the aromatic double bonds in the furan ring, similar to acetophenone (see also subgroup Ib). No data on metabolism of 2,5-dialkylfurans were available, but for these substances oxidation of the side chains may be anticipated. It is not clear if such substances would be subject to ring-opening, and in absence of data, this metabolic option should be anticipated.

4.2. Main group II

The 14 representatives of subgroup II are all sulphur-containing furan derivatives. The sulphur is present in the molecule either as:

- sulphides (subgroup IIa): [FL-no: 13.114, 13.124, 13.135, 13.141, 13.143, 13.145 and 13.199]
- a free thiol group (subgroup IIb): [FL-no: 13.108 and 13.149]
- disulphides (subgroup IIc): [FL-no: 13.113, 13.144, 13.178 and 13.185]
- trisulphide (subgroup IId): [FL-no: 13.146]

4.2.1. Subgroup IIa

Sulphides [FL-no: 13.114, 13.124, 13.135, 13.141, 13.143, 13.145 and 13.199]

The sulphides [FL-no: 13.114, 13.124, 13.135, 13.145 and 13.199] may undergo sulphur oxidation reactions; free thiol groups may also be formed. In addition to this, the sulphides [FL-no: 13.141 and 13.143] carrying an esterified carboxylic acid group may also be anticipated to be subject to ester hydrolysis in the GI-tract or shortly after absorption in e.g. the liver (see Annex III).

4.2.2. Subgroup IIb

Thiols [FL-no: 13.108 and 13.149]

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 this 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 sulphoxides or sulphones, which can be excreted unchanged in the urine. Alternatively, conjugation with glutathione may occur, resulting in a mixed disulphide, which can be reduced to give free thiols, or oxidised to give thiosulphinates or thiosulphones (see Annex III, Fig. III.3). They may also undergo thiol-disulphide 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.

4.2.3. Subgroup IIc

Disulphides [FL-no: 13.113, 13.144, 13.178 and 13.185] can be reduced to give the free thiols, or can be oxidised to give thiosulphinates or thiosulphones.

2-Furfuryl 3-oxo-2-butyl disulphide [FL-no: 13.185] which also carries a keto group in the side chain might be subject to keto-reduction after which the resulting hydroxyl group could be conjugated. However, there is no data to support this observation.

4.2.4. Subgroup IId

Trisulphide [FL-no: 13.146]

For the one trisulphide candidate substance [FL-no: 13.146] no direct information on metabolism was available. However, it has been described that tri and higher sulphides may be cleaved upon reaction with GSH, resulting in the formation of reactive perthiols [EFSA, 2010w].

An extensive description of the information on metablism of the candidate and supporting substances including references has been presented in Annex III.

4.3. Summary

The metabolism of non-sulphur-containing furfuryl alcohol derivatives in FGE.13 [FL-no: 13.127, 13.129, 13.130, 13.132 and 13.133] includes the formation of furfural, a known reactive aldehyde, that may lead to hepatotoxicity. Ethyl furfuracrylate [FL-no 13.011] is expected to be hydrolysed to furanacrylic acid, a known metabolite of furfural, and to ethanol. 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. In addition to the above mentioned oxidation pathway of the alcohol function, 5HMF [FL-no: 13.139] has been shown to be bioactivated *in vitro* to a reactive intermediate, SMF, by sulphotransferases. The same metabolism has been demonstrated *in vivo* after intravenous administration, but the SMF has a short half-life. This metabolite raises a concern for genotoxicity and therefore additional data on genotoxicity of 5HMF need to be present to overcome this concern. Extensive ring-opening with formation of intermediates reactive towards DNA has been reported for 2-alkyl-substituted furans and the two candidate substances in subgroup Ic are two examples of these. The same metabolic conversion can be anticipated for the candidate substance in subgroup Ib. Therefore, it is concluded that the candidate substances included in main group I cannot be predicted to be metabolised to innocuous compounds.

It is anticipated that the predominant metabolic attack of the sulphur-substituated furans (main group II) will be on the sulphur atom(s), and ring opening is not considered to be a major metabolic route. Based on the metabolism of other sulphur-containing compounds, the candidate furfuryl and furan monosulphides are expected to undergo oxidation mainly to sulphoxides and sulphones. Given the reactivity of thiol groups, whether free or from di- or tri-sulphides, 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 main group II 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 III.

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

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

In Section 4 it has been indicated that the candidate substance 5-hydroxymethylfurfuraldehyde (5HMF) [FL-no: 13.139] from subgroup Ia may be metabolised to 5-[(sulphoxy)methyl]furfural (SMF), which shows genotoxic potential *in vitro*. Sufficient data have been provided to mitigate this concern with respect to genotoxic potential *in vivo*.

Based on genotoxicity data for the substance 2-acetylfuran [FL-no: 13.054] supporting to the candidate substance in subgroup Ib [FL-no: 13.155], a concern for genotoxicity is raised for candidate substance [FL-no: 13.155].

For the two substances (2-ethyl-5-methylfuran [FL-no: 13.125] and 2-octylfuran [FL-no: 13.162] from subgroup Ic, genotoxicity may be anticipated based on formation of DNA-reactive metabolites and based on information available for the candidate substance in subgroup Ib [FL-no: 13.155].

In absence of sufficient experimental data on genotoxicity on these or structurally related substances, the Procedure cannot be applied to the candidate substance in subgroup Ib and the two candidate substances in subgroup Ic. A further extensive discussion on the genotoxicity of the candidate substances has been presented in Section 8.4.

Thus, the Procedure for the safety evaluation of flavouring substances as outlined in Annex I has been applied to 24 candidate substances from chemical group 14. The stepwise evaluations of the 24 substances are summarised in Table 2a.

Step 1

Five [FL-no: 13.122, 13.130, 13.136, 13.139 and 13.145] of the 24 candidate substances evaluated via the Procedure are classified into structural class II and 19 [FL-no: 13.011, 13.102, 13.108, 13.113, 13.114, 13.124, 13.127, 13.129, 13.132, 13.133, 13.135, 13.141, 13.143, 13.144, 13.146, 13.149, 13.178, 13.185 and 13.199] are classified into structural class III according to the decision tree approach by Cramer et al. (Cramer et al., 1978), see Table 2a.

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 24 candidate substances proceeds *via* the B-side of the evaluation scheme.

Step B3

The five candidate substances, which have been assigned to structural class II, have estimated European daily *per capita* intakes (MSDI) ranging from 0.0024 to 0.39 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 19 candidate substances assigned to structural class III range from 0.0012 to 37 microgram, 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 24 candidate substances.

Step B4

Subgroup Ia structurally related to furfurylalcohol [FL-no: 13.011, 13.102, 13.122, 13.127, 13.129, 13.130, 13.132, 13.133, 13.136 and 13.139]:

Considering that the ten candidate substances of subgroup Ia are metabolised to yield furfural and furoic acid or furanacrylic acid, the toxicity of the esters of furfuryl alcohol [FL-no: 13.127, 13.129, 13.130, 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/d bw has been recently established by EFSA (EFSA, 2004c). The estimated daily *per capita* intakes based on the MSDI approach expressed in microgram/kg body weight (bw)/day of candidate substances in subgroup Ia of the present FGE.13Rev2 are more than 30.000 fold below the ADI value.

For 5HMF [FL-no: 13.139] a substantial amount of substance-specific data are available, including 13-week subchronic studies and chronic studies in $B6C3F_1$ mice and F344/N rats (NTP, 2010c), see Section 8.2). The carcinogenicity study in mice demonstrated that 5HMF may induce liver tumours, but these are considered irrelevant for humans. In contrast, no carcinogenic responses have been reported in the study with rats. The data have shown that the critical effect is cytoplasmic alterations in renal proximal tubule epithelium in mice, observed in the 13-weeks study with mice at 188 mg/kg bw for 5 days (d)/week (w) and above with an intermittent dose regimen of five days per week. For this effects a BMDL of 20.2 bw for 5d/w can be derived, which would be equivalent to 14.4 mg/kg bw/day, when corrected for continuous daily administration (see Section 8.2 and Annex V). When this BMDL of 14.4 mg/kg bw/day derived from the 13-weeks study in mice is compared to the MSDI of 0.39 microgram/*capita*/day for this substance, a margin of safety of 2.2×10^6 can be calculated. From this it is concluded that 5HMF [FL-no: 13.139] does not raise a safety concern as a flavouring substance, at its current level of use in foods.

Since no toxicity data are available on the sulphur-containing candidate substances in main group II, the relevant No Observed Adverse Effect Level (NOAEL) values originate from structurally related supporting substances.

Subgroup IIa sulphides [FL-no: 13.114, 13.124, 13.135, 13.141, 13.143, 13.145 and 13.199]:

The candidate substances ethyl furfuryl sulfide [FL-no: 13.124], methyl 5-methylfurfuryl sulfide [FL-no: 13.145] and 2,5-dimethyl-3-(methylthio)furan [FL-no: 13.114] 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. No effects were observed for furfuryl isopropyl sulfide in a 90-day dietary study with rats at a single dose level (1.34 mg/kg bw/day) (Posternak et al., 1969). Comparison of the only level tested with no effect taken as a no oberved adverse effect level (NOAEL) with the estimated daily *per capita* intakes based on the MSDI approach and expressed in microgram/kg bw/day for ethyl furfuryl sulfide [FL-no: 13.124], methyl 5-methylfurfuryl sulfide [FL-no: 13.145] and 2,5-dimethyl-3-(methylthio)furan [FL-no: 13.114] provides adequate margins of safety > 10^5.

After ester hydrolysis, the candidate substances methyl (2-furfurylthio)acetate and methyl 3-furfurylthio)propionate [FL-no: 13.141 and 13.143] are anticipated to be metabolised and to have toxicological properties similar to the supporting substance ethyl-3-(2-furfurylthio) propionate [FL-no: 13.093]. For this substance an NOAEL of 5.78 mg/kg bw/day has been identified in a 90-day study (Bio-Research Laboratory, 1980). Comparison of this NOAEL with the estimated daily *per capita* intakes based on the MSDI approach and expressed in microgram/kg bw/day of methyl (2-furfurylthio)acetate and methyl 3-furfurylthio) propionate [FL-no: 13.141 and 13.143] provides an adequate margin of safety of 3.2×10^7 for both substances.

Candidate substances 1-(2-furfurylthio)propanone [FL no: 13.135] and 3-[(2-methyl-3-furyl)thio]-butanal [FL-no: 13.199] may be evaluated by comparison of their exposure estimates with the NOAEL from supporting substance 3-[(2-methyl-3-furyl)thio]-4-heptanone [FL-no: 13.077]. 3-[(2-Methyl-3-furyl)thio]-4-heptanone was tested in rats at a single dose level of 3.76 mg/kg bw/day in the diet for 90 days without treatment-related effects (Gallo et al., 1976b). Comparison of the estimated daily *per capita* intake based on the MSDI approach for 3-[(2-methyl-3-furyl)thio]-butanal [FL-no: 13.199] with the NOAEL of 3.76 mg/kg bw/day for the supporting substance provided an adequate margin of safety of 1.9×10^5. Comparison of the estimated daily *per capita* intake based on the MSDI approach for 1-(2-furfurylthio)propanone [FL-no: 13.135] with the NOAEL of 3.76 mg/kg bw/day for the supporting substance provides an adequate margin of safety of 2.1×10^7.

Subgroup IIb thiols [FL-no: 13.108 and 13.149]:

The candidate substances 5-methyl-2-furanmethanethiol [FL-no: 13.149] is structurally related to the supporting substance 2-furanmethanethiol [FL-no: 13.026]. The NOAEL of 2-furanmethanethiol 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 2-furanmethanethiol with the estimated daily *per capita* intake based on the MSDI approach expressed in microgram/kg bw/day of 5-methyl-2-furanmethanethiol [FL-no: 13.149] provides an adequate margin of safety of 3.7×10^5.

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] from subgroup IIe (of FGE13Rev1). Several subchronic studies have been carried out with this supporting substance. A NOAEL of 1.4 mg/kg bw/day has been derived in a multiple dose level 13 weeks dietary study with rats (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 [FL-no: 13.108] provided an adequate margin of safety of 2.3×10^3.

Subgroup IIc disulphides [FL-no: 13.113, 13.144, 13.178 and 13.185]:

In the previous version of this FGE, the candidate substance 2,5-dimethyl-3-(methyldithio)furan [FL-no: 13.113] was evaluated against a NOAEL which turned out to belong to a structurally unrelated substance. Therefore this evaluation was not valid and thus substance [FL-no: 13.113] had to be reconsidered. It may be anticipated that this disulphide will be subject to fission of the disulphide bridge. The resulting furan-containing fragment, which is more reactive than the disulphide itself, could be evaluated by comparison with the toxicity of 2-methyl-3-furanthiol [FL-no: 13.055] from subgroup IIb. The NOAEL of 2-methyl-3-furanthiol in a multiple dose level 90-day oral gavage study with rats was 5 mg/kg bw/day (Oser, 1970b). When the NOAEL for 2-methyl-3-furanthiol is compared with the estimated daily *per capita* intake based on the MSDI approach expressed in microgram/kg bw/day for 2,5-dimethyl-3-(methyldithio)furan [FL-no: 13.113] an adequate margin of safety of 25×10^7 can be calculated.

For the candidate substances methyl 5-methylfurfuryl disulfide [FL-no: 13.144] and 2-furfuryl 3-oxo-2-butyl disulphide [FL-no: 13.185] a NOAEL for a comparable substance is not available. However, after fission of the disulphide bridge the resulting furan-containing fragment, which is more reactive than the disulphide itself, could be evaluated by comparison with the toxicity of furfuryl mercaptan [FL-no: 13.026] from subgroup IIb. 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). When the NOAEL for furfuryl mercaptan is compared with the estimated daily *per capita* intakes based on the MSDI approach expressed in microgram/kg bw/day for methyl 5-methylfurfuryl disulfide [FL-no: 13.144] and 2-furfuryl 3-oxo-2-butyl disulphide [FL-no: 13.185], adequate margins of safety of 75×10^6 and 16×10^6, respectively, can be calculated.

The Panel noted that the candidate substance 3-(furfuryldithio)-2-methylfuran [FL-no: 13.178] is identical to [FL-no: 13.192]. The latter substance has been assigned the JECFA number 1524 in the report of the 69th meeting (JECFA, 2009a). For this substance, in the JECFA evaluation, an MSDI for Europe of 0.24 microgram *per capita* per day was given. This figure, which is higher and more recent than the exposure estimate in the previous version of this FGE (0.0012 microgram *per capita* per day), will be used in the current revision of this FGE. 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 5 mg/kg bw/day and 0.45 mg/kg bw/day, respectively (Oser, 1970a; Morgareidge and Oser, 1970e). Treatment-related effects were seen at the intake level of 5.0 mg/kg bw/day, but the intake level of 0.45 mg/kg bw/day was determined to be a NOAEL. The disulphide bridge fission products are related to [FL-no: 13.026] (of subgroup IIb), for which a NOAEL of 3 mg/kg bw/day has been derived. When the estimated daily *per capita* intake based on the MSDI approach expressed in microgram/kg bw/day of 3-(furfuryldithio)-2-methylfuran [FL-no: 13.178] is compared to this NOAEL an adequate margins of safety of 7.5×10^5 can be calculated for [FL-no: 13.178].

Alternatively, the two fission products may be considered separately. These fission products are [FL no: 13.055] and [FL-no: 13.026], for which NOAELs of 5 mg/kg bw/day and 3 mg/kg bw/day, respectively, have been derived (Oser, 1970b; Phillips et al., 1977). Exposure to [FL-no: 13.178] at the level of its MSDI would correspond to exposures to [FL-no: 13.026] and [FL-no: 13.055] of 0.12 microgram per person per day for both fragments. Comparison of these exposure estimates to the NOAELs for these two fragments provides adequate margins of safety of 1.5×10^6 and 2.5×10^6 for [FL-no: 13.026] and [FL-no: 13.055], respectively.

Subgroup IId polysulphide [FL-no: 13.146]:

The one candidate flavouring substance in this subgroup methyl furfuryl trisulphide [FL-no: 13.146] is a trisulphide which may be anticipated to release perthiols upon metabolism. Similar reactive products may be anticipated for bis-(2-methyl-3-furyl)tetrasulphide [FL-no: 13.017] for which a NOAEL of 0.56 mg/kg bw/day in a 90-day study has been derived (Morgareidge and Oser, 1970f). Comparison of this NOAEL with the estimated daily *per capita* intake of methyl furfuryl trisulphide [FL-no: 13.146] based on the MSDI approach expressed in microgram/kg bw/day of 0.0024 microgram provides an adequate margin of safety of 14×10^6.

Summary:

For the ten, seven, two, four and one substances in subgroups Ia [FL-no: 13.011, 13.102, 13.122, 13.127, 13.129, 13.130, 13.132, 13.133, 13.136 and 13.139], IIa [FL-no: 13.114, 13.124, 13.135, 13.141, 13.143, 13.145 and 13.199], IIb [FL-no: 13.108 and 13.149], IIc [FL-no: 13.113, 13.144, 13.178 and 13.185] and IId [FL-no: 13.146], respectively, which have been evaluated through the Procedure, it can be concluded at step B4 of the Procedure that these 24 candidate substances do not pose a safety concern when used as flavouring substances at the estimated levels of intake based on the MSDI approach.

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

For three candidate substances [FL-no: 13.122, 13.136 and 13.139] in structural class II, evaluated through the Procedure, the mTAMDI range from 1400 to 3900 microgram/person/day, which is above the threshold of concern for this Cramer class. For one substance [FL- no: 13.145] the mTAMDI is 160 microgram/person/day, which is below the class threshold of 540 microgram/person/day. For substance [FL-no: 13.130] no use levels were provided and no mTAMDI can be calculated.

The estimated intakes for 16 [FL-no: 13.011, 13.102, 13.108, 13.113, 13.114, 13.127, 13.129, 13.132, 13.133, 13.135, 13.141, 13.143, 13.146, 13.149, 13.178 and 13.185] of the 19 substances evaluated

through the Procedure and assigned to structural class III, based on the mTAMDI, range from 160 to 3900 microgram/person/day, which are above the threshold of concern for structural class III substances of 90 microgram/person/day. For the remaining three substances from structural class III [FL no: 13.124, 13.144 and 13.199] the mTAMDIs range from 49 to 78 microgram/person/day, which is below the threshold of concern.

Thus, for 20 of the candidate substances, evaluated using the Procedure, further information is required. This would include more reliable intake data and then, if required, additional toxicological data. For comparison of the MSDI and mTAMDI values, see Table 6.1.

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

FL-no	EU Register name	MSDI (µg/*capita*/day)	mTAMDI (µg/person/day)	Structural class	Threshold of concern (µg/person/day)
13.122	Ethyl 2-furoate	0.39	3900	Class II	540
13.130	Furfuryl butyrate	0.24		Class II	540
13.136	2-Furoic acid	0.013	1400	Class II	540
13.139	5-Hydroxymethylfurfuraldehyde	0.39	1600	Class II	540
13.145	Methyl 5-methylfurfuryl sulfide	0.0024	160	Class II	540
13.155	2-Methyl-5-propionylfuran	0.011	420	Class II	540
13.125	2-Ethyl-5-methylfuran	0.011	420	Class II	540
13.162	2-Octylfuran	0.011	420	Class II	540
13.011	Ethyl furfuracrylate	0.12	3900	Class III	90
13.102	Butyl 2-furoate	0.12	3900	Class III	90
13.108	4,5-Dihydro-3-mercapto-2-methylfuran	37	160	Class III	90
13.113	2,5-Dimethyl-3-(methyldithio)furan	0.0012	160	Class III	90
13.114	2,5-Dimethyl-3-(methylthio)furan	0.0024	160	Class III	90
13.124	Ethyl furfuryl sulfide	0.18	78	Class III	90
13.127	Furfuryl 2-methylbutyrate	0.73	3900	Class III	90
13.129	Furfuryl but-2-enoate	0.11	3900	Class III	90
13.132	Furfuryl hexanoate	0.58	3900	Class III	90
13.133	Furfuryl isobutyrate	0.89	3900	Class III	90
13.135	1-(2-Furfurylthio)propanone	0.61	780	Class III	90
13.141	Methyl (2-furfurylthio)acetate	0.011	400	Class III	90
13.143	Methyl 3-(furfurylthio)propionate	0.011	420	Class III	90
13.144	Methyl 5-methylfurfuryl disulfide	0.0024	78	Class III	90
13.146	Methyl furfuryl trisulfide	0.0024	160	Class III	90
13.149	5-Methyl-2-furanmethanethiol	0.37	160	Class III	90
13.178	3-(Furfuryldithio)-2-methylfuran	0.24	160	Class III	90
13.185	2-Furfuryl 3-oxo-2-butyl disulphide	0.011	420	Class III	90
13.199	3-[(2-Methyl-3-furyl)thio]-butanal	1.2	49	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 evaluated through the Procedure.

On the basis of the reported annual production volumes in Europe (EFFA, 2003g; EFFA, 2004y), the combined estimated daily *per capita* intake as flavourings of the five candidate substances assigned to structural class II, and evaluated through the Procedure, is 1 microgram, which does not exceed the threshold of concern of 540 microgram/person/day for substances belonging to structural class II. The combined daily *per capita* intake as flavourings of the 19 candidate substances assigned to structural class III is 42 microgram, which does not exceed the threshold of concern of 90 microgram/person/day for substances belonging to structural class III.

The 24 candidate substances evaluated through the Procedure, are structurally related to 39[8] supporting substances evaluated by the JEFCA at their 55th, 59th and 69th meetings (JECFA, 2001a; JECFA, 2002c; JECFA, 2009c) or by EFSA (EFSA, 2004c). The total combined intake of candidate and supporting substances (in Europe) would be 885 and 60 microgram/*capita*/day for structural class II and III, respectively.

The total combined daily per capita intake of 885 microgram exceeds the threshold of concern of 540 microgram/person/day for structural class II substances. However, 50 % of the combined daily per capita intake of 885 microgram comes from furfural for which, together with the furfural component of furfural diethyl acetal, an ADI of 0.5 mg/kg bw/day has been established by EFSA (EFSA, 2004c). The total combined intake for the class III substances of 60 microgram/capita/day does not exceed the threshold for the structural class of 90 microgram/person/day.

8. Toxicity

8.1. Acute Toxicity

Oral LD_{50} values in rodents have been reported for three candidate substances [FL-no: 13.102, 13.122 and 13.139] and for five supporting substances for main group I. No acute oral toxicity data were available for any of the candidate substances in main group II, but oral LD_{50} values were found for 12 supporting substances in this group.

The reported oral LD_{50} values for the candidate substances are around 1500 - 2000 mg/kg body weight (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.

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

8.2. Subacute, Subchronic, Chronic and Carcinogenicity Studies

Main group I

Short-term and long-term toxicity studies are available for two candidate substances included in main group I, namely 2-furoic acid [FL-no: 13.136] and 5-hydroxymethylfurfural (5HMF [FL-no: 13.139]) and for three supporting substances and one structurally related substance. The data for these

[8] Of the 53 EFSA considered supporting substances 14 substances have not been considered using the Procedure and for two of these no use has been reported for Europe.

substances indicate that the liver is the target for their toxicity. EFSA has established an ADI value of 0.5 mg/kg bw/d for furfural and the furfural component of furfural diethyl acetal (EFSA, 2004c). This ADI will be used for the evaluation of the nine furfural-related candidate flavouring substances in subgroup Ia [FL-no: 13.011, 13.102, 13.122, 13.127, 13.129, 13.130, 13.132, 13.133 and 13.136].

For candidate substance [FL-no: 13.139] specific toxicity data are available in a NTP study report (NTP, 2010c) which will be used for the evaluation of this substance. The reported 13-weeks and chronic bioassays have been described below, and the results of these studies have been analysed using BMDL dose-response modelling (EFSA, 2009bc; EFSA, 2011g); see Annex V for details).

Data on 5-hydroxymethylfurfuraldehyde (*5HMF) [FL- no: 13.139]*

Studies in mice (NTP, 2010c):

Subchronic study in mice

Groups of 10 male and 10 female mice ($B6C3F_1$) were administered 0, 47, 94, 188, 375 or 750 mg 5HMF/kg bw for 5 days (d)/week (w) in deionised water by gavage for 3 months. No treatment-related undercurrent death was observed. At term, animals were examined for changes in hematology, clinical chemistry, sperm count and motility, vaginal cytology and CNS neuropathological examination. In addition, full histopathology was carried out on control and highest does group. Mean body weight and body weight gain of 750 mg/kg males was significantly less (< 15 % less) than those of the vehicle control group. Female body weights were not affected. There were no treatment-related changes in hematology or clinical chemistry parameters or organ weights.

The absolute kidney weights of 188 mg/kg or greater males and the relative kidney weight of 375 mg/kg males were significantly less than those of the vehicle controls. The absolute weights of the heart and liver were significantly decreased in 750 mg/kg males, and the relative testis weight of 750 mg/kg males and relative lung weight of 375 mg/kg females were significantly increased, NTP did not consider any of these organ weight changes chemical-related.

The relative epididymis weight of the 750 mg/kg males was significantly increased, but 5HMF did not cause any biologically significant changes in the sperm parameters. There were no significant differences between dosed and vehicle control groups in vaginal cytology parameters in females. There were no significant changes in the histopathology of the reproductive organs for male or female mice.

The incidences of minimal to mild cytoplasmic alterations of the kidney were significantly increased in males administered 188 mg/kg or greater. Cytoplasmic alterations were characterized by the variable depletion of large, clear cytoplasmic vacuoles in the proximal tubule epithelial cells that were readily observed in the proximal tubule epithelial cells of vehicle controls; depletion of cytoplasmic vacuoles was most pronounced in 750 mg/kg males. These cytoplasmic vacuoles are typical in the kidneys of $B6C3F_1$ mice. No other histopathological changes in any other tissue were observed that could be attributed to treatment with 5HMF. This effect has been used for dose-response modelling. A BMDL of 20.2 mg/kg bw for 5d/w was derived (see Annex V).

Chronic study in mice

Groups of $B6C3F_1$ mice (50/sex/dose) were administered 0, 188, 375 or 750 mg 5HFM/kg bw for, five days per week for 104 weeks via aqueous gavage. Survival of both males and female mice in the 750 mg/kg group was significantly less than that of vehicle control. Mean body weights of 750 mg/kg were 14 % less than those of the vehicle controls after week 26. Mean body weights of 375 and 750 mg/kg females were 9 % and 30 % less, respectively, than those of the vehicle controls after week 36. The following histopathological findings were reported:

The incidences hepatocellular adenoma in the 188 and 375 mg/kg female mice were significantly increased two-fold up to 53 % and 52 % and exceeded the historical control ranges for water gavage studies. However, the incidences were within the historical ranges for all routes of administration. No increase was observed in the incidence of hepatocellular carcinomas. Conversely, the incidences of hepatocellular adenoma and hepatocellular adenoma or carcinoma (combined) were significantly decreased in 750 mg/kg males; these decreased incidences of age-related neoplasms were due to early and high mortality in this dosed group rather than a direct chemical effect. Because of the reduced survival (30 % reduction) of the groups receiving 750 mg 5HMF/kg bw per day, NTP did not include the results for these dose groups in the evaluation of carcinogenic potential.

Incidences of mononuclear cell infiltration and eosinophilic foci were significantly increased in the livers of 188 mg/kg females, and the incidences of basophilic foci were significantly increased in livers of 188 and 375 mg/kg females. However, the degree of severity of the mononuclear cell infiltration was lower in the exposed groups as compared to the control group and the changes in incidences of basophilic and eosinophilic foci were not dose related. No such changes were observed in the 750 mg/kg dose group. Also, no changes were reported in the livers of male mice. The non-cancerous histological responses in livers were not dose-related and were only observed in one sex. Therefore the Panel is of the opinion that these changes have no toxicological significance.

A spectrum of non-neoplastic nasal lesions considered to be related to administration of 5HMF was observed. The following effects extended into the low dose group in either or both sexes:

- Olfactory epithelial metaplasia (significantly increased in all dosed groups of males and in 375 and 750 mg/kg females). Metaplasia consisted of focal, multifocal, or diffuse replacement of the segments of the normal pseudostratified olfactory epithelium by tall, ciliated, columnar epithelium resembling normal respiratory epithelium.
- Chronic active olfactory epithelium inflammation (significantly increased in all dosed groups of males and in 375 and 750 mg/kg females) consisted of mixed inflammatory cell infiltrates of macrophages, lymphocytes and neutrophils in varying numbers in the lamina propria of the dorsal aspects of Levels II and III.
- Bowman gland hyperplasia (was significantly increased in all dosed groups of females and in 375 and 750 mg/kg males). Bowman's glands were tortuous with increased numbers of acinar profiles (glandular hyperplasia) within the lamina propria.
- Bowman gland dilatation in all dosed groups of females and in 375 and 750 mg/kg males.
- Chronic active inflamation of Bowman glands (significantly increased in all dosed groups of males and in 375 and 750 mg/kg females). Glandular lumens contained varying numbers of macrophages, lymphocytes and neutrophils and degenerate cellular debris with often hypertrophic epithelial acinar cells and with focally multilayered and disorganized epithelium.

These effects have been used for dose-response modelling. A BMDL of 62.5 mg/kg bw for 5d/w was derived for chronic active inflammation of Bowman glands in males (see Annex V).

Studies in rats (NTP, 2010c):

Sub-chronic study in rats

Groups of 10 male and 10 female rats (F344/N) were administered 0, 94, 188, 375, 750, or 1,500 mg 5HMF/kg bw for 5 d/w in deionised water by gavage for 3 months. One male and three female rats administered 1,500 mg/kg died before the end of the study; the male died as a result of gavage trauma. At term, animals were examined for changes in hematology, clinical chemistry, sperm count and motility, vaginal cytology and CNS neuropathological examination. In addition, full histopathology was carried out on control and highest does group. Mean body weights of 750 and 1,500 mg/kg males were significantly less (< 10 % less) than those of the vehicle control group. There were no treatment-related changes in hematology or clinical chemistry parameters or biologically relevant changes in absolute organ weights.

There were no relevant changes in organ weights except for relative epididymus weight in the high dose group, but there were no changes in sperm parameters and no histopathological changes in the male sex organs were found.

Based on the NTP report, it seems that the effects on oestrus cyclicity are the most critical in this study. Female rats (only 0, 375, 750 and 1500 mg/kg groups were examined) had elongated estrous cycles in the 750 and 1500 mg/kg females (not statistically significant) and fewer females in these groups had regular cycles. Statistical significance for the proportion of regular cycling females was only reached for the 1500 mg/kg dose group, but this may be related to the fact that estrous cycle length was unclear in more animals from the high dose group than in females from the control group.

Females in the 375, 750 and 1500 mg/kg groups had a significantly increased probability of extended dioestrus. The Panel noted that determination of stage in oestrus cycle appeared problematic in several animals in particular in the control group. The "% time in dioestrus" parameter was *ca.* 20 – 40 % less in the control group females than the treatment groups females. However, for the control the diagnoses of estrous stage was unclear in *ca*. 20 % of the cycle time, while this percentage was much less in the treatment groups (down to 0 % in the high dose group). There were no histopathological changes in the female sex organs.

No other histopathological changes in any other tissue were observed that could be attributed to treatment with 5HMF.

NTP considered the toxicity of 5HMF as "minimal" except for the high dose group females (three animals had died). The Panel noted that statistically significant changes in estrous cyclicity were only observed in the 1500 mg/kg dose group. Given the reported problems with the diagnosis of estrous cyclicity in the females and the absence of any further histopathological changes, the Panel questions the relevance of the reported findings. Since in the other studies carried out by NTP effects were observed at lower dose levels, the Panel did not consider these changes useful for further examination using dose-response modelling.

Chronic study in rats

Groups of F344/N rats (50/sex/dose) were administered 0 (vehicle control), 188, 375 and 750 mg 5HMF/kg bw per day, five days per week for 104 weeks via aqueous gavage. Survival of the 188 and 750 mg/kg per day male groups was higher than that of vehicle controls. Survival in the 375 mg/kg males and in all female dose groups was comparable to the respective vehicle control groups. Mean body weights of all test groups were comparable to vehicle controls throughout the study. The following histopathological findings were reported:

Two cases of hepatocellular adenoma occurred in 375 mg/kg males, but none in the high dose group. Males of the 188 and 375 mg/kg per day males showed increased incidences of C-cell adenoma or carcinoma (combined) of the thyroid gland, but without clear dose-response relationship. No other carcinogenic effects were observed (NTP, 2010c).

Incidences of olfactory epithelium degeneration were increased in all dosed groups of male and female rats and were significantly increased in 750 mg/kg males (incidence: 29/49; grade 1.5) and in 188 and 375 mg/kg females (incidences 35/49 and 35/49, respectively; grade 1.2 for both groups), but not in the high dose females (incidence 28/49, grade 1.3). Degeneration of the olfactory epithelium was graded minimal to mild in all groups (grade 1.1 to 1.5 in a scale of 1 to 4). A similar degree of olfactory epithelium degeneration (grade 1.1) was observed in the control group, but at a lower incidence (males: 18/50, grade 1.1; females: 21/50, grade 1.2). In the male and female high dose groups (750 mg/kg) a variety of other histological changes in the nose were observed (e.g. increased incidences of mild respiratory epithelium, squamous metaplasia, increase incidences of suppurative inflammation and chronic active inflammation of the nose).

The incidence of clear cell foci was significantly increased in the livers of 750 mg/kg bw/day males. Incidences of minimal chronic active inflammation of the liver were increased in all dosed groups of males (incidences 25/50, 34/50, 30/50 and 38/50 in 0, 188, 375 and 750 mg/kg groups, respectively) and the increase in 750 mg/kg bw /day males was statistically significant. The Panel noted that the dose response was not very strong.

Based on the minimal changes in respiratory epithelium and the reported changes in the livers of male rats, the Panel concluded that the effects in the high dose group may be considered as adverse, resulting in an NOAEL of 375 mg/kg bw for 5d/w. Since in other studies (e.g. the mouse studies) effects were reported to occur at lower dose levels, the data from the NTP chronic rat bioassay have not been used for dose-response modelling.

Evaluation of NTP's subchronic and chronic bioassays with 5HMF in rodents

carcinogenicity

According to NTP, in the above described chronic bioassays with rats and mice, there was no evidence of carcinogenic activity of 5HMF in male or female F344/N rats administered 188, 375 or 750 mg/kg bw for 5d/w under the conditions of the 2-year gavage study. There was no evidence of carcinogenic activity in male $B6C3F_1$ mice administered 188 or 375 mg/kg bw for 5d/w. There was some evidence of carcinogenic activity of 5HMF in female $B6C3F_1$ mice, based on increased incidences of hepatocellular adenoma in the 188 and 375 mg/kg groups. The Panel considered, the about two-fold increase (not dose-related) of these benign hepatocellular tumours observed in $B6C3F_1$ female mice to be irrelevant for humans, in view of the recognized high genetic susceptibility of this strain to hepatocarcinogenesis and the known high back-ground incidence of these tumours in this mouse strain. Therefore, these studies do not give rise to concern with respect to the carcinogenic potential of 5HMF.

Non-neoplastic lesions

Administration of 5-hydroxymethylfurfuraldehyde (5HMF) [FL- no: 13.139] produced a mild toxic response in mice. The only histologic lesion related to chemical administration were cytoplasmic alterations in the renal proximal tubule epithelium at 188 mg/kg or above in male mice in a sub-chronic bioassay. Although this effect was not observed in the chronic study in mice, or in the sub-chronic or chronic studies in rats, the Panel was not convinced that this result could be dismissed as being non-adverse. In addition, in this assay also kidney weight changes were observed at dose levels of 188 mg/kg bw and above. In other tissues (liver, heart, testes, lungs) also weight changes were observed but at higher dose levels. Although the study authors (NTP 2010c) considered these weight chages of little relevance, the Panel noted a dose-relationship and decided not to dismiss these observations. The Panel used the data on the histopathological changes in the kidneys as a basis for dose-response modelling, since for this effect the dose-response in the data was the most pronounced. A BMDL of 20.2 mg/kg bw for 5d/w was derived (see Annex V).

Considerations were made by the Panel on the occurence of histopathological changes in the upper respiratory tract (i.e. nose) observed in both mouse and rat. Similar changes are frequently observed in animal species following administration of inhaled products but less common in studies performed by other routes of exposure. The Panel has derived a BMDL of 62.5 mg/kg bw for 5d/w for the nasal effets in the mouse. However, extraplotation to humans of these injuries is complicated by the complexity and diversity of this upper airway among animal species. Though important local bioactivations are known to occur in the olfactory mucosa, in absence of characteristic, site-specific, distibution pattern of the lesion, the predominantly chronic inflammatory description of the finding is rather suggestive of a mild irritant effect of the substance that could have rich significant concentration in the upper airways (e.g. regurgitation or presence of potential irritant substances from metabolism in the faces or urine in the cage or in the litter). Although the effect is adverse in rodent species, the Panel considered it unlikely to be of human relevance (Harkema et al, 2006).

For the evaluation of 5HMF the Panel has used the BMDL of 20.2 mg/kg bw for 5d/w based on renal histopathology in the mouse. The Panel noted that this BMDL is sufficiently low to cover also the organ weight changes in kidneys and other tissues at dose levels of 188 mg/kg bw and above.

Main group II

No toxicity data are available on candidate substances included in main group II, however, results from toxicity studies on 14 supporting substances and one structurally related substance have been reported.

Toxicity studies on supporting substances used for deriving the NOAELs 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).

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 give an intended daily dose of 3.96 mg/kg bw for 90 days. The actual mean intake calculated over the period of the study was 5.0 mg/kg bw/day. 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 author concluded that administration of 5.0 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 (mean intake over the period of the study was 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 and Oser, 1970e).

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. Separate groups of six animals per sex were dosed and monitored similarly, but were killed at week 6. Treatment and dose-related haemolysis of red blood cells was observed in animals receiving 2.8 mg/kg bw/day and above, particularly in females, as evidenced by significantly 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 haemolytic changes were not apparent in animals receiving 1.4 mg/kg bw/day, other than a significant decrease in haemoglobin in the female rats, which was not seen at the next higher dose level of 2.8 mg/kg bw/day. The report concluded that the no-effect level was 1.4 mg/kg bw/day (Munday & Gellatly, 1973a).

Ethyl 3-(furfurylthio)propionate [FL-no: 13.093]

Ethyl 3-(furfurylthio) propionate was added to the diet of groups of 10 male and 10 female rats (strain not given) at concentrations calculated to result in an average daily intake of 5.8 or 17 mg/kg bw for 90 days. Body weight, food intake and food use efficiency were recorded weekly. No significant difference was found in these parameters between treated animals at either dose and controls (no numeric data were provided, nor for the haematological, biochemical and organ weight results described in the following text). Minor variations in haematological parameters at weeks 7 and 13 were judged by the study authors to be of no toxicological significance. Blood chemistry showed a significant (but less than 2-fold) increase in aspartate aminotransferase (AST (SGOT)) after 13 weeks in high dose females, while blood urea nitrogen (BUN) was statistically increased in females (presumed both dose levels) at 6 weeks but not at 12 weeks. Urine analyses conducted at weeks 7 and 13 gave normal values. The weights of the liver and kidneys at necropsy were normal, and gross and microscopic examination revealed no dose-related effects, other than an increased incidence of histiocytic myocarditis with occasional concomitant myocardial degeneration in high dose males and females. The cardiac changes were seen at lower incidence in the lower dose animals and in controls, and the authors concluded that this was due to a treatment-related exacerbation of a pre-existing endemic cardiac infection (Bio-Research Laboratory, 1980). Although the changes in AST and the reported cardiac changes may be linked and may not be truly adverse, as it appears that they are linked to a pre-existing inflammatory condition, the level of 5.78 mg/kg bw/day has been taken as a NOAEL for this study (Bio-Research Laboratory, 1980).

3[(2-Methyl-3-furyl)thio]-4-heptanone [FL-no: 13.077]

Groups of 15 male and 15 female weanling Wistar rats were administered 3[(2-methyl-3-furyl)-thio]-4-heptanone dissolved in acetone and blended into a basal laboratory diet to give a daily dose approximating to 3.76 mg/kg bw for 90 days. Controls received the basal laboratory diet mixed with acetone. The acetone was evaporated from the diets before presentation to the animals. Samples of each diet were taken weekly to assess the stability and concentration of the test material. Daily observations of appearance, behaviour, appetite, elimination, gross signs of adverse effects and mortality showed no differences among test and control animals. Weekly measurement of body weight and food consumption also showed no significant differences between test animals and controls. Haematological, blood chemical and urine analyses performed during weeks 6 and 12 on eight males and eight females from each group gave normal values. No differences in absolute or relative weights of the liver or kidneys were found at necropsy. No evidence of gross or histological alteration was seen in tissues from major organs of eight male and eight female rats or in the livers and kidneys of the remaining seven animals. The only level tested (3.76 mg/kg bw/day) has been taken as a NOAEL (Gallo et al., 1976b).

2-Methyl-3-furanthiol [FL-no: 13.055]

Groups of 15 male and 15 female weanling Wistar rats were administered the test substance 2-methyl-3-furanthiol [FL no: 13.055] dissolved in acetone and blended into a basal laboratory diet to give a daily dose approximating to an intake of 5.0 mg/kg bw/day for 90 days. Controls received the basal laboratory diet admixed with acetone. The acetone was evaporated from the diets before presentation to the animals. Samples of each diet were taken weekly to assess the stability and concentration of the test material. Daily observations of appearance, behaviour, appetite, elimination, gross signs of adverse effects and mortality showed no differences among test and control animals. Weekly measurement of body weight and food consumption also showed no significant differences between test animals and controls. Haematological, blood chemical and urine analyses performed during weeks 6 and 12 on eight males and eight females from each group gave normal values. No differences in absolute or relative weights of the liver or kidneys were found at necropsy. No evidence of gross or histological alteration was seen in tissues from major organs of eight male and eight female rats or in the livers and kidneys of the remaining seven animals. The only level tested (5.0 mg/kg bw/day) has been taken as a NOAEL (Oser, 1970b).

bis(2-Methyl-3-furyl) tetrasulphide [FL-no: 13.017]

Groups of 15 male and 15 female weanling Wistar rats were administered the test substance bis(2-methyl-3-furyl) tetrasulphide dissolved in acetone and blended into a basal laboratory diet to give a daily dose approximating to 0.56 mg/kg bw for 90 days. Controls received the basal laboratory diet admixed with acetone. The acetone was evaporated from the diets before presentation to the animals. Daily observations of appearance, behaviour, appetite, elimination, gross signs of adverse effects and mortality showed no differences among test and control animals. Weekly measurement of body weight and food consumption also showed no significant differences between test animals and controls. Haematological, blood chemical and urine analyses performed during weeks 6 and 12 on eight males and eight females from each group gave normal values. No differences in absolute or relative weights of the liver or kidneys were found at necropsy. No treatment-related gross abnormalities were seen in tissues from major organs of eight male and eight female rats or in the livers and kidneys of the remaining seven animals. Although histopathological examination of these tissues was reported to be carried out, the test report did not include these findings, and the absence of treatment-related histopathological changes could not be confirmed. However, bis(2-methyl-3-furyl) tetrasulphide had no significant effect on organ weights and the structurally related supporting substance bis(2-methyl-3-furyl)disulfide [FL-no: 13.016] did not produce treatment-related histopathological changes in two comparable 90-day studies providing intakes of 5.0 mg/kg bw/day and 0.45 mg/kg bw/day respectively. The only level tested (0.56 mg/kg bw/day) has been taken as a NOAEL (Morgareidge and Oser, 1970f).

Repeated dose toxicity data are summarised in Annex IV, Table IV.2.

8.3. Developmental / Reproductive Toxicity Studies

Developmental and reproductive toxicity data are available for one candidate substance (2-furoic acid [FL-no: 13.136]) and one supporting substance (furfural [FL-no: 13.018]) included in main group I. No data are available for main group II.

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

8.4. Genotoxicity Studies

Genotoxicity studies were available only on some of the candidate substances included in main group I or on their related supporting substances. For subgroup Ia, 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 from subgroup Ia. New genotoxicity data on the candidate substance 5HMF have become available and will be considered in this revision of FGE.13.

For the one candidate substance [FL-no: 13.155] in subgroup Ib no genotoxicity data are available, but *in vitro* and *in vivo* genotoxicity data are available for the supporting substance 2-acetylfuran [FL-no: 13.054].

For subgroup Ic, data on *in vitro* genotoxicity were provided for two supporting substances. Data on *in vivo* genotoxicity were only provided for one of the two supporting substances [FL-no: 13.029 and 13.030]. Since the supporting substance for subgroup Ib gives information on genotoxic properties of putative metabolites of the candidate substances in subgroup Ic, the information given for the evaluation of subgroup Ib is also relevant for subgroup Ic.

No genotoxicity data were available on candidate- or on structurally related substances in main group II.

Subgroup Ia

Candidate substances:

5-Hydroxymethyl furfural [FL-no: 13.139]

In the *in vitro* tests, 5HMF gave negative results in the traditional Ames test in strains TA98, TA100, TA104, TA1535 and TA1537 in five and positive results in two studies. The validity of these two studies could not be assessed. In one of these two studies (Omura et al, 1983) the positive response was observed in strain TA100, but not in TA 98 and the mutagenic potential was higher in the absence of S9 than in the presence of S9. In the other study (Shinohara et al., 1986) mutagenicity was only observed in strain TA100 in the presence of metabolic activation (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 TA104 strain upon inclusion of PAPS, a sulpho-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 sulphation, with formation of sulphate-ester (SMF). Indeed, the mutagenic effect could be partly suppressed by the addition of sulphotransferase inhibitors. In accordance, SMF in TA104 was genotoxic in the absence of any metabolic system (cytotoxicity not specified); the effect was reduced by addition of Glutathione (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 microgram/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).

Newly submitted data on 5-hydroxymethylfurfural[9](Table IV.5 and IV.7)

Weak mutagenic activity was reported in *S. typhimurium* TA100 strain in the absence of metabolic activation, while no mutagenicity was observed in strains TA97, TA98, TA102 and TA1535 in a range of concentrations of 100 - 10,000 micrograms/plate; however, negative results were reported in another study with TA98 and TA100 strains and *E. coli* WP2 uvrA/pKM101 in a range of concentrations of 1,500 - 10,000 micrograms/plate (NTP, 2010c).

At the end of a 3-month toxicity study, peripheral blood samples were obtained from male and female $B6C3F_1$ mice receiving 0, 47, 188, 375 or 750 mg/kg bw/day of 5HMF via gavage. Slides were scanned to determine the frequency of micronuclei in 1000 normochromatic erythrocytes (NCEs) in 10 animals per sex per treatment group. In addition, the percentage of polychromatic erythrocytes (PCE) in a population of 1,000 erythrocytes was determined as a measure of bone marrow toxicity. No increases in the frequency of micronucleated erythrocytes were observed; in addition, no significant dose-related changes in the percentage of immature PCE were observed, suggesting that the chemical did not exhibit bone marrow toxicity (NTP, 2010c).

The DNA-damaging potential of 5HMF was tested *in vitro* in the Comet assay with the following five cell lines with various degree of SULT1A1 expression (Durling et al., 2009): two human lines (Caco-2, no detectable 1A1 activity; HEK293, high 1A1 activity); two cell lines from Chinese hamster (V79, no detectable 1A1 activity and V79-hp-PST, high 1A1 activity) and a one mouse lymphoma line (L5178Y, no detectable activity). The cell lines were incubated with 0, 2.5, 7.5, 25, 50 or 100 mM (ca. 0, 0.3, 1.0, 3.3 6.3 or 12.6 mg/ml) of 5HMF for three hours and subjected to a Comet assay to study DNA damage.

DNA damage was observed at the highest concentration (100 mM) in all cell lines, with significant reduction in cell viability (from 11 to 30 %), The concentration of 100 mM is ten times higher than the highest concentration (10 mM or 5000 micrograms/ml) recommended by OECD guidelines for *in vitro* testing with mammalian cells. 100 mM was the lowest effective concentration for three cell lines: Caco-2, HEK293 and L5178Y. In the V79 (lowest SULT1A1) and V79-hp-PST (highest SULT1A1) DNA damage was induced also at lower concentrations (lowest effective concentration: 25 mM or 3193 micrograms/ml), without a reduction in cell viability. Surprisingly, the positive control (HMP, 0.01 mM) induced significant damage in Caco-2, V79 and V79-hp-PST cells, but not in HEK293. The

[9] An *in vivo* micronucleus test in mouse bone marrow with neofuraneol was also submitted, but an adequate identification of the substance studied was not possible due to incomplete reporting. The study did not show an effect of neofuraneol on the occurrence of micronuclei. Since no target organ toxicity was seen, this evidence provided by this study is of very limited relevance. For these two reasons the study is not further discussed.

authors (Durling et al., 2009) concluded that DNA damage induced in all cell lines was unrelated to the expression of SULT1A1 but they mentioned that the SULT1A1 activities in these three cell lines (Caco-2, HEK293 and L5178Y) were much lower than those that can be found in human gut and liver. The possibility was left open that SULT1A1 activity was too low to efficiently bioactivate 5HMF also in the cell line with highest SULT1A1 activity. In V79 cells without SULT1A1 activity and in V79-hp-PST with SULT1A1 activity at the same level as in human gut and liver, no difference in extent of DNA-damage could be observed. This would indicate absence of a significant contribution of sulphate conjugation in the DNA-damaging activity of 5HMF.

These results are in conflict with the results of Glatt et al. (Glatt et al., 2005) who reported induction of SCE in 5HMF-exposed genetically modified V79 cells expressing high levels of human CYP2E1 and SULT1A1. They are also in conflict with the observations by Sommer et al. (Sommer et al., 2003) reporting the mutagenicity of 5HMF in a *S. typhimurium* strain genetically modified and expressing human SULT1A1. According to Durling et al. (2009), the reasons of these discrepancies are unknown; one possibility is the different sensitivity of the COMET assay compared to other systems. Durling et al. (Durling et al., 2009) concluded that other important mechanisms for the observed DNA damage should be investigated, but that under the conditions of the test, 5HMF is a rather weak DNA-damaging agent.

In a new publication by Severin et al. (2010), a dose dependent increase in DNA damage was observed in an COMET assay with HepG2 cells exposed to 5HMF (0, 5.35, 7.87, 11.57, 17, 25, 36.6 mM) for 20 hours, with a significant increase from 7.87 to 36.6 mM 5HMF. Cytotoxicity was observed at the two highest doses (25 and 36.6 mM), with estimated IC_{50} of 38 mM. HepG2 cells express both CYP and SULT enzymes. In the same publication no effect of 5HMF was found in an *in vitro* micronucleous assay in the same cell line exposed to similar doses of 5HMF (20 hours). 5HMF was also tested in an Ames test performed according to the OECD guidelines 471. No increase in mutants was observed in *S. typhimurium* strains TA 98, TA 100, TA1535 and TA 1537 exposed to 5HMF at 0.5 µg/mL up to 5000 µg/mL with or without metabolic activation (S9). However, no additional PAPS was added to the test system (Severin et al., 2010).

However, while 5HMF was unable to induce micronuclei *in vivo*, in the NTP 3-months study in mice by gavage, and *in vitro*, using the Hep-G2 human cell line expressing both CYP and SULT enzymes, its metabolite SMF has been reported to induce micronuclei in peripheral erythrocytes in mice (Dahlberg, 2004 as cited by Glatt and Sommer, 2006 (no further data were available).

According to Glatt and Sommer, incubation of DNA with SMF in a cell-free system led to the formation of DNA adducts that could be detected by the 32P-postlabelling technique. No adducts were formed in incubations with 5HMF instead of SMF. In subsequent experiments, the authors searched for these adducts in mammalian and bacterial cells treated with SMF and in SULT-proficient cells treated with 5HMF. Although mutations were induced, adducts were not seen in these cells under the same conditions (no data are available to be listed in the genotoxicity table). The authors hypothesized that the lack of DNA adducts might be due to technical problems, since generally DNA adducts are a more sensitive endpoint than mutations as observed with many other compounds (Glatt and Sommer, 2006).

In conclusion, with respect to the genotoxicity 5HMF, taking into account additional data on metabolism, the following picture emerges. The substance is negative in the conventional Ames test. Mutagenicity is observed only upon inclusion of PAPS, a sulpho-group donor and liver cytosol into the metabolic system, suggesting the formation of a sulphate-ester (SMF). In accordance, SMF was mutagenic in the absence of any metabolic activation system. In an *in vitro* assay, 5HMF induced dose-dependent increase in DNA damage (Comet assay), but this study has major drawbacks and inconsistencies and has to be considered of limited validity. A major limitation is the use of too high concentrations that can produce unpredictable effects, not related to the real genotoxic potential of 5HMF, and this is particularly true for a test like the Comet assay. Furthermore, as also stated by the authors, DNA damage was unrelated to the expression of SULT1A1 activity. Also in another Comet

assay in HepG2 cells, able to express both CYP and SULT enzymes, indications for DNA damage were observed, but the substance did not induce clastogenic or aneugenic effects (micronucleus assay) in the same cell system. *In vivo*, a non-standard micronucleus assay in peripheral blood erythrocytes associated to a sub-chronic study in mice, provided no indication of a genotoxic potential, but this study has limited validity since no bone marrow cell toxicity was observed.

Metabolic studies indicate that *in vivo*, in mice $B6C3F_1$ and rats, the principal route of metabolism is oxidation of 5HFM to 5-hydroxymethylfuroic acid, followed by glycine conjugation and rapid elimination in the urine. However, a recent pharmacokinetic study in FVB/N mice has shown that SMF has been detected in plasma from animals given 5HMF, intravenously. This indicates that there is a competition for the substrate 5HMF between the oxidation pathway leading to the furoic acid derivative and the sulphonation pathway leading to the SMF metabolite. The Panel noted that SMF is very hydrophilic and therefore will have problems crossing the cell membrane and entering cells. Therefore SMF is more likely to induce mutation at the site of formation, mainly the liver. In addition, the half life was reported to be 4.2 minutes, and it is not likely that this metabolite will manage to reach the bone marrow and give any positive effect in an *in vivo* micronucleous test, taking into account that SMF will most likely be formed in the liver. However, 5HMF has been found unable to induce micronuclei also *in vitro*, using the HepG2 human cell line, expressing both CYP and SULT enzymes. In the rodent bioassays no carcinogenic response was observed and from this it may be concluded that the formation of the SMF metabolite is too low to result in a carcinogenic response. Assuming that in humans the ratio between the two competing pathways is not more favourable for the formation of SMF than in rodents, no gentoxicity or carcinogenicity is expected in humans either.

Furoic acid [FL-no: 13.136]

Furoic acid gave negative results in three studies in the Ames test in strains TA98 and TA100. Fluoric acid was also negative in DNA repair test in *E. coli* and in a UDS assay using primary rat hepatocytes.

Supporting substances:

In vitro genotoxicity data were available for five supporting substances (furfuryl acetate, furfuryl alcohol, furfural, 5-methylfurfural 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 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, 2004c).

Overall, the genotoxicity data available on the candidate furoic acid and on supporting substances do not give rise to concern with respect to genotoxicity of nine candidate furfural-related candidate substances included in subgroup Ia [FL-no: 13.011, 13.102, 13.122, 13.127, 13.129, 13.130, 13.132, 13.133 and 13.136]. Based on newly submitted data on the mutagenic activity of 5HMF [FL-no: 13.139] the concern for genotoxicity which was raised because of genotoxic properties of one of its metabolites (SMF) is overcome. Thus there are no further concerns for genotoxicity of the candidate substances in subgroup Ia, which could preclude their evaluation through the Procedure.

Subgroup Ib

No data are available for the one candidate substance in subgroup Ib ([FL no: 13.155]). However, several studies have been carried out with a structurally related flavouring substance, 2-acetylfuran [FL-no: 13.054] (2-furyl methyl ketone).

In vitro studies

For the supporting substance 2-acetylfuran [FL-no: 13.054] data were found showing an increased mutation frequency in a bacterial reverse gene mutation test in *S. typhimurium* TA98 with metabolic activation, but not in TA100. The study has limited validity. The increase was not dose-related and no clear data on cytotoxicity were presented, but a decrease in the number of revertants was observed at the highest concentrations, which could indicate cytotoxicity. A second trial was not performed (Shinohara et al., 1986). Also with this substance a positive result was obtained in the *rec*-assay (Shinohara et al., 1986) and in an SOS-chromo test for bacterial DNA-repair (Eder et al., 1993), but the predictive value of these test systems is considered to be limited. With [FL-no: 13.054] also chromosomal abberations in chinese hamster ovary cells have been reported in a limited study by Stich et al. (Stich et al., 1981b).

2-Acetylfuran [FL-no: 13.054] was evaluated for induction of unscheduled DNA synthesis (UDS) in human hepatocytes following OECD guidelines. Human (sex not given) hepatocytes from two batches purchased from a commercial provider were incubated with concentrations of the compound (purity not given) of between 2.19 and 280 µg/ml for 16 hours, and UDS was measured autoradiographically. No UDS was elicited, in contrast to the positive control, 2-acetylaminofluorene (Durward, 2007a)

In vivo studies

2-Acetylfuran [FL-no: 13.054] was also evaluated for induction of sister chromatid exchanges (SCE) in bone marrow of female Swiss albino mice. Groups of two per dose per exposure regimen were administered compound (99 % pure) at 0, 1000, 2000 or 3000 mg/l via gavage either once or for 5 consecutive days. 5-Bromodeoxyuridine was injected intraperitoneally to label chromatids. The mice were sacrificed at 12, 24 or 48 hours after receiving the last dose, and slides of bone marrow were prepared and processed for differential staining. A dose-related increase up to about 2-fold in SCE was observed for the 12- and 24-hours groups of both the single-dose regimen and the multiple-dose regimen (Sujatha, 2007). This study was considered valid. In an earlier study by the same group (Sujatha et al., 1993) this substance was reported to cause chromosomal aberrations in mouse bone marrow at oral dose levels up to 60 mg/kg bw/day. Also this study was consided valid.

2-Acetylfuran was evaluated for induction of UDS in hepatocytes isolated from livers of dosed male Sprague-Dawley rats. The assay was conducted according to GLP and OECD guidelines. In a preliminary range-finding toxicity study, lethality was observed at 30 mg/kg bw and greater, and signs of toxicity were observed at 20 mg/kg bw. No sex differences were observed, and therefore only males were used in the main study. Groups of four rats were administered compound (purity not given) at 0, 7 or 21 mg/kg bw via gavage. In experiment 1, the hepatocytes were isolated 16 hours post-dosing; in experiment 2, hepatocytes were isolated 2 hours post-dosing and cultured for autoradiographic measurement of UDS. No UDS was observed in either experiment (Durward, 2007b).

The candidate and supporting substance in this subgroup are alpha,beta-unsaturated ketones. This stuctural characteristic has been considered as an additional reason for concern for genotoxic potential of these substances. However, due to structural similarity with acetophenone (i.e. the alpha,beta unsaturated double bond is part of an aromatic system and therefore less reactive) the concern for genotoxicity, resulting from the formation of such alpha,beta-unsaturated ketones has been lifted. Nevertheless, the experimentally obtained genotoxicity data indicate that the supporting substance may give rise to DNA damage, which may result in chromosomal aberrations rather than gene mutations. Also from chapter 4 and Annex III, the formation of DNA-reactive metabolites may be anticipated. In combination with this, the available genotoxicity data are sufficiently strong to raise a concern, which would preclude the evaluation of the candidate substance in subgroup Ib through the Procedure.

Subgroup Ic

No data are available on the genotoxic properties of the two candidate substances in this subgroup.

Several studies were found with the supporting substances 2-methylfuran [FL-no: 13.030] and 2,5-dimethylfuran [FL-no: 13.029]. Negative results were obtained in a limited bacterial reverse gene mutation test with *S. Typhimurium* (TA97 and TA100 strains only, no data on cytotoxicity, no duplicate trial (Shinohara et al., 1986)). However, a clear dose-related positive response with limited validity (e.g. no clear data on cytotoxicity; no clear description of scoring criteria) was obtained with both substances in a chromosome aberration test in Chinese hamster ovary cells with and without metabolic activation in presence or absence of metabolic activation (Stich et al., 1981b). Both substances also gave a positive response in a *rec*-assay for bacterial DNA-repair (Shinohara et al., 1986), but the predictive value of this test system is considered to be limited.

For 2-alkyl- and 2,5-dialkyl-substituted furans, formation of reactive ring opening products cannot be excluded (see chapter 4 and Annex III). These reactive intermediates can bind covalently to DNA, which might result in genotoxic events. In an alternative metabolic pathway, these flavouring substances may also be converted to ketones which are structurally related to the substances in subgroup Ib and for these substances a concern for genotoxicity has been identified. Therefore, owing to the anticipated metabolism of the two candidate substances in subgroup Ic into possible genotoxic metabolites a concern for genotoxicity cannot be excluded. For the two candidate substances in subgroup Ic [FL-no: 13.125 and 13.162] this concern for genotoxicity would preclude their evaluation through the procedure.

Main group II

No genotoxicity data were available on any of the 14 sulphur-containing candidate substances in main group II, nor on their related supporting substances. As it is anticipated that the predominant metabolic attack for these substances will be on the sulphur atom(s), for the candidate substances in main group II, ring opening is not considered to be a major metabolic route. The lack of data on the 14 sulphur-containing candidate substances included in main group II or on related supporting substances does not allow to conclude on their potential for genotoxicity. However, this would not preclude the evaluation of these 14 candidate substances from subgroup II using the Procedure.

Genotoxicity data are summarised in Annex IV, Table IV.4 to Table IV.7.

9. Conclusions

The present revision of FGE.13, FGE.13Rev2, concerns the evaluation, in total, of 27 candidate flavouring substances; two more than in the previous revision of this FGE. New data included are additional data submitted on 5-hydroxymethylfurfural (5HMF) [FL-no: 13.139] in response to genotoxicity data requested in FGE.13, consideration of additional data on the stereoisomeric composition for two substances [FL-no: 13.185 and 13.199] and inclusion of two more candidate substances [FL-no: 13.130 and 13.155]. Also the evaluation of substance [FL-no: 13.135] has been re-considered.

Three of the 27 flavouring substances possess a chiral centre [FL-no: 13.127, 13.185 and 13.199] Industry has informed that the commercial substances are the racemates in all three cases. Two of the 27 substances can exist as geometrical isomers [FL-no: 13.011 and 13.129] and in both cases Industry has informed that the commercial substances are the (E)-isomers.

Eight of the candidate substances are classified into structural class II [FL-no: 13.122, 13.130, 13.136, 13.139, 13.145, 13.155, 13.125 and 13.162] and 19 candidate substances are classified into structural class III [FL-no: 13.011, 13.102, 13.108, 13.113, 13.114, 13.124, 13.127, 13.129, 13.132, 13.133, 13.135, 13.141, 13.143, 13.144, 13.146, 13.149, 13.178, 13.185 and 13.199].

Nineteen of the flavouring substances in the present group have been reported to occur naturally in a wide range of food items. For seven flavouring substances, no data on natural occurrence have been reported.

According to the default MSDI approach, the 27 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 volumes in Europe (MSDI approach), the total estimated combined intake of candidate and supporting substances would be 885 and 60 microgram/*capita*/day for structural class II and III, respectively. The total combined daily per capita intake of 885 microgram exceeds the threshold of concern of 540 microgram/person/day for structural class II substances. However, 50 % of the combined daily per capita intake of 885 microgram results from furfural for which, together with the furfural component of furfural diethyl acetal, an ADI of 0.5 mg/kg bw has been established by EFSA. The total combined intake for the class III substances of 60 microgram/capita/day does not exceed the threshold for the structural class of 90 microgram/person/day.

Genotoxicity studies were only available on some of the candidate substances included in main group I or on related supporting substances. For subgroup Ia, 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 provided for one candidate substance [FL-no: 13.139] and for two of the supporting substances. For the candidate substance in group Ib, *in vitro* and *in vivo* genotoxicity data were available for one supporting substance. For subgroup Ic, data on *in vitro* genotoxicity were provided for two supporting substances. For one of these, also data on *in vivo* genotoxicity were provided. In addition, data for a supporting substance for subgroup Ib contribute to the evaluation of the genotoxic potential of substances in subgroup Ic due to structural similarity of metabolites of the Ic candidates with the substances in subgroup Ib.

In subgroup Ia the data available do not indicate a concern for genotoxicity. For the one candidate substance in subgroup Ib [FL-no: 13.155] and the two candidate substances in subgroup Ic [FL-no: 13.125 and 13.162], metabolism studies on closely related substances indicate a potential for DNA-binding of metabolites. In addition, in several *in vitro* studies with related substances, indications for genotoxic activity were obtained. These data preclude the evaluation through the Procedure of the three candidate flavouring substances in subgroup Ib and Ic.

No genotoxicity data were available on candidate substances or on structurally related substances in main group II. The lack of data on the sulphur-containing candidate substances from main group II, or on the related supporting substances, does not allow concluding on their genotoxicity, but would not preclude the evaluation of these substances through the Procedure.

The metabolism of non-sulphur-containing furfuryl alcohol derivatives in FGE.13 [FL-no: 13.127, 13.129, 13.130, 13.132 and 13.133] includes the formation of furfural, a known reactive aldehyde, that may lead to hepatotoxicity. Furfural will be further metabolised to furoic acid [Fl no: 13.136] and furanacrylic acid, the hydrolysis product of [FL-no: 13.011]. 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 Ia cannot be predicted to be metabolised to innocuous compounds. In addition, 5HMF [FL-no: 13.139] has a free aldehyde function (*cf.* furfural*)* and can be anticipated to be reactive. In addition, this substance may be bioactivated to reactive intermediates by sulphotransferases for which genotoxicity has been reported. However, the available genotoxicity and carcinogenicity data for this substance do not indicate a concern for this substance. Extensive ring-opening with formation of intermediates reactive towards DNA has been reported for 2-alkyl-substituted furans in subgroup 1c [FL-no: 13.125 and 13.162]. In

addition, these compounds may be metabolised to ketones (subgroup 1b; [FL-no: 13.155]), for which genotoxicity may be anticipated.

Based on the metabolism of other sulphur-containing compounds, the candidate furfuryl and furan monosulphides are expected to be subject to oxidation mainly to sulphoxides and sulphones. Given the reactivity of thiol groups, whether free or from di- or trisulphides, 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 main group II can be predicted to be metabolised to innocuous substances.

Short-term and long-term toxicity studies are available for two candidate substances included in subgroup Ia, namely 2-furoic acid [FL-no: 13.136] and 5HMF [FL-no: 13.139] and for three related supporting substances. For the candidate substances structurally related to furfuryl alcohol or furfural, these studies indicate that the liver is the critical target for their toxicity. EFSA has established an ADI value of 0.5 mg/kg/d bw for furfural and the furfural component of furfural diethyl acetal. For 5-hydroxymethylfurfural [FL-no: 13.139], substance-specific data indicate that the kidney of the male mouse was the target organ, and for this substance a BMDL of 14.4 mg/kg bw/day could be derived, based on the results of a 13-weeks oral toxicity study.

No toxicity data are available on the sulphur-containing furan-derived candidate substances included in subgroups IIa-d. However, results from toxicity studies on 14 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 from 0.45 to 10 mg/kg bw/day.

It was considered on the basis of the default MSDI approach that for the 24 candidate substances [FL-no: 13.011, 13.102, 13.108, 13.113, 13.114, 13.122, 13.124, 13.127, 13.129, 13.130, 13.132, 13.133, 13.135, 13.136, 13.139, 13.141, 13.143, 13.144, 13.145, 13.146, 13.149, 13.178, 13.185 and 13.199], it could be concluded, at step B4 of the Procedure, that these 24 candidate substances do not pose a safety concern when used as flavouring substances at the estimated levels of intake based on the MSDI approach.

The Panel has reservations for three substances [FL-no: 13.125, 13.155 and 13.162] which could not be evaluated through the Procedure due to concern of genotoxicity *in vitro*. For these three substances additional date are required.

For three candidate substances [FL-no: 13.122, 13.136 and 13.139] in structural class II, evaluated through the Procedure, the mTAMDI range from 1400 to 3900 microgram/person/day, which are above the threshold of concern for this Cramer class. For one substance [FL-no: 13.145] the mTAMDI is 160 microgram/person/day, which is below the class threshold of 540 microgram/person/day. For substance [FL-no: 13.130, no use levels were provided, so no mTAMDI can be calculated.

The estimated intakes for 16 [FL-no: 13.011, 13.102, 13.108, 13.113, 13.114, 13.127, 13.129, 13.132, 13.133, 13.135, 13.141, 13.143, 13.146, 13.149, 13.178 and 13.185] of the 19 substances evaluated through the Procedure and assigned to structural class III, based on the mTAMDI, range from 160 to 3900 microgram/person/day, which are above the threshold of concern for structural class III substances of 90 microgram/person/day. For the remaining three substances from structural class III [FL-no: 13.124, 13.144 and 13.199] the mTAMDIs range from 49 to 78 microgram/person/day, which is below the threshold of concern.

Thus, for 20 of the candidate substances, evaluated using the Procedure, further information is required. This would include more reliable intake data and then, if required, additional toxicological data. 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 24 candidate substances evaluated through the Procedure can be applied to the material of commerce, it is necessary to consider the available specifications. Adequate specifications including complete purity criteria and identity for the materials of commerce have been provided for all 24 flavouring substances evaluated through the Procedure.

For these 24 candidate substances [FL-no: 13.011, 13.102, 13.108, 13.113, 13.114, 13.122, 13.124, 13.127, 13.129, 13.130, 13.132, 13.133, 13.135, 13.136, 13.139, 13.141, 13.143, 13.144, 13.145, 13.146, 13.149, 13.178, 13.185 and 13.199] the Panel concluded that they would be of "No safety concern at estimated levels of intake as flavouring substances" based on the MSDI approach.

TABLE 1: SPECIFICATION SUMMARY OF THE SUBSTANCES IN THE FGE.13REV2

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 13, Revision 2

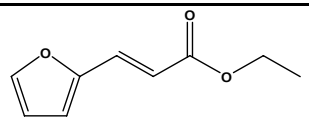
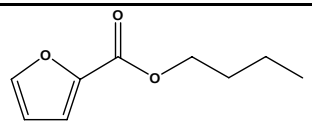
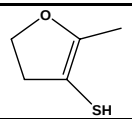
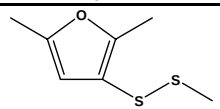
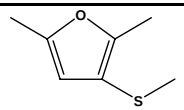
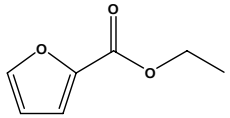
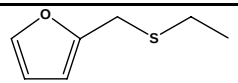
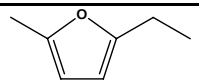
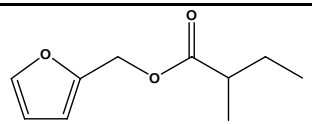
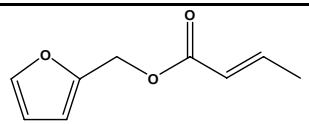
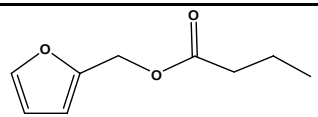
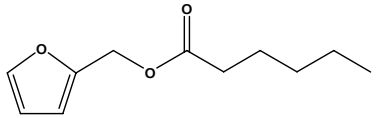
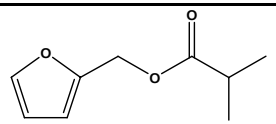
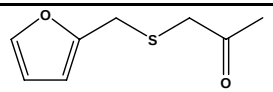
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
13.011	Ethyl furfuracrylate		545 623-20-1	Liquid $C_9H_{10}O_3$ 166.18	Practically insoluble or insoluble Freely soluble	229 14 MS 95 %	1.544-1.550 1.092-1.098	Register name to be changed to (E)-Ethyl furfuracrylate (EFFA). CASrn in Register does not specify (Z) or (E) isomer. CASrn in Register to be changed.
13.102	Butyl 2-furoate		583-33-5	Liquid $C_9H_{12}O_3$ 168.19	Practically insoluble or insoluble Freely soluble	233 MS 95 %	1.469-1.475 1.052-1.058	
13.108	4,5-Dihydro-3-mercapto-2-methylfuran		4683 26486-13-5	Liquid C_5H_8OS 116.18	Slightly soluble Freely soluble	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 Freely soluble	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 Freely soluble	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 Freely soluble	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 Freely soluble	73 (13 hPa) MS	1.509-1.515 1.047-1.053	

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 13, Revision 2

FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
						95 %		
13.125	2-Ethyl-5-methylfuran		10942 1703-52-2	Liquid $C_7H_{10}O$ 110.16	Practically insoluble or insoluble Freely soluble	118 MS 95 %	1.443-1.449 0.890-0.896	
13.127	Furfuryl 2-methylbutyrate		10643 13678-61-0	Liquid $C_{10}H_{14}O_3$ 182.22	Practically insoluble or insoluble Freely soluble	263 MS 95 %	1.455-1.461 1.009-1.015	Racemate, Register name to be changed to Furfuryl 2-methylbutyrate (EFFA).
13.129	Furfuryl but-2-enoate		59020-84-7	Liquid $C_9H_{10}O_3$ 166.17	Practically insoluble or insoluble Freely soluble	245 NMR 95 %	1.491-1.497 1.034-1.040	Register name to be changed to Furfuryl but-2(E)-enoate (EFFA). (Z) or (E) isomer not specified by CASrn in Register. CASrn in Register to be changed.
13.130 759	Furfuryl butyrate		638 623-21-2	Liquid $C_9H_{12}O_3$ 168.19	Insoluble Miscible	212 IR 99	1.457-1.462 1.051-1.057	
13.132	Furfuryl hexanoate		39252-02-3	Liquid $C_{11}H_{16}O_3$ 196.25	Practically insoluble or insoluble Freely soluble	224 MS 98 %	1.452-1.458 1.003-1.013	
13.133	Furfuryl isobutyrate		10641 6270-55-9	Liquid $C_9H_{12}O_3$ 168.19	Practically insoluble or insoluble Freely soluble	85 (20 hPa) MS 95 %	1.497-1.503 1.028-1.034	
13.135	1-(2-Furfurylthio)propanone		4676 58066-86-7	Liquid $C_8H_{10}O_2S$ 170.04	Insoluble Soluble	240.4 (1.2Torr) n.a. IR NMR 95 %	1.525-1.531 1.146-1.154	

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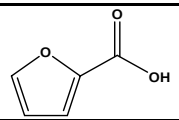
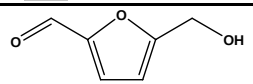
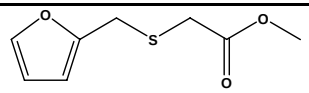
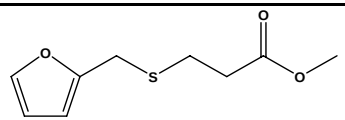
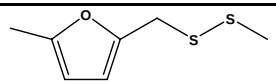
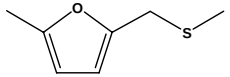
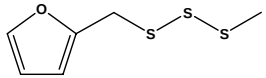
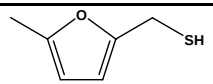
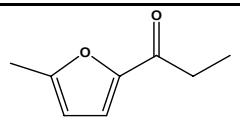
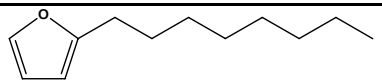
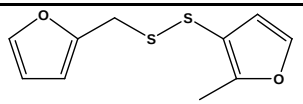
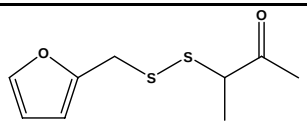
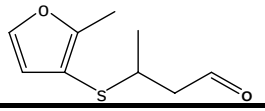
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
13.136	2-Furoic acid		10098 88-14-2	Solid $C_5H_4O_3$ 112.08	Slightly soluble Freely soluble	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 Freely soluble	154 (16 hPa) 34 MS 95 %	n.a. n.a.	
13.141	Methyl (2-furfurylthio)acetate		108499-33-8	Liquid $C_8H_{10}O_3S$ 186.23	Practically insoluble or insoluble Freely soluble	287 MS 95 %	1.510-1.520 1.195-1.205	
13.143	Methyl 3-(furfurylthio)propionate		94278-26-9	Liquid $C_9H_{12}O_3S$ 200.25	Practically insoluble or insoluble Freely soluble	310 MS 95 %	1.509-1.519 1.160-1.170	
13.144	Methyl 5-methylfurfuryl disulfide		78818-78-7	Solid $C_7H_{10}OS_2$ 174.28	Practically insoluble or insoluble Freely soluble	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 Freely soluble	84 (20 hPa) NMR 95 %	1.509-1.515 1.048-1.054	Register name to be changed to Methyl 5-methylfurfuryl sulphide.
13.146	Methyl furfuryl trisulfide		66169-00-4	Solid $C_6H_8OS_3$ 192.32	Practically insoluble or insoluble Freely soluble	320 43 NMR 95 %	n.a. n.a.	
13.149	5-Methyl-2-furanmethanethiol		59303-05-8	Liquid C_6H_8OS 128.19	Slightly soluble Freely soluble	62 (17 hPa) MS 95 %	1.523-1.529 1.041-1.047	
13.155	2-Methyl-5-propionylfuran		11158 10599-69-6	Liquid $C_8H_{10}O_2$ 138.17	Practically insoluble or insoluble Freely soluble	73 (13 hPa) MS 95 %	1.502-1.508 1.038-1.044	

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 13, Revision 2

FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
13.162	2-Octylfuran		10965 4179-38-8	Liquid $C_{12}H_{20}O$ 180.29	Practically insoluble or insoluble Freely soluble	103 (16 hPa) MS 95 %	1.313-1.319 0.892-0.898	
13.178	3-(Furfuryldithio)-2-methylfuran		109537-55-5	Solid $C_{10}H_{10}O_2S_2$ 226.32	Practically insoluble or insoluble Freely soluble	398 122 NMR 95 %	n.a. n.a.	Register name to be changed to Furan, 3-[(2-furanylmethyl)dithio]-2-methyl-.
13.185	2-Furfuryl 3-oxo-2-butyl disulphide			Solid $C_9H_{12}O_2S_2$ 216.32	Practically insoluble or insoluble Freely soluble	374 77 NMR 95 %	n.a. n.a.	Racemate (EFFA, 2010a). CASrn to be introduced in Register 159113-17-4. Register name to be changed to: 2-butanone, 3-[(2-furanylmethyl)dithio]- (EFFA).
13.199	3-[(2-Methyl-3-furyl)thio]-butanal		4501 915971-43-6	Liquid $C_9H_{12}O_2S$ 184.26	Practically insoluble Soluble	n.a. decomp at 198°C IR NMR MS 98%	1.5122-1.5222 1.101-1.121	Racemate (EFFA, 2010a).

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.*

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)

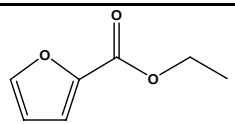
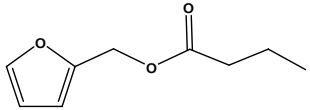
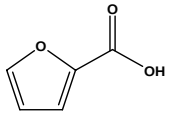
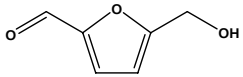
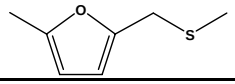
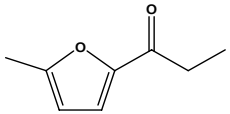
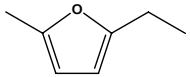
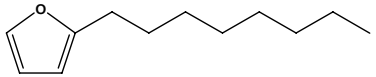
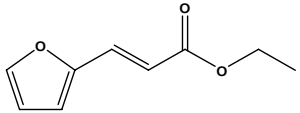
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
13.122	Ethyl 2-furoate		0.39	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
13.130 759	Furfuryl butyrate		0.24	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
13.136	2-Furoic acid		0.013	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
13.139	5-Hydroxymethylfurfuraldehyde		0.39	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
13.145	Methyl 5-methylfurfuryl sulfide		0.0024	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
13.155	2-Methyl-5-propionylfuran		0.011	Class II No evaluation			a)
13.125	2-Ethyl-5-methylfuran		0.011	Class II No evaluation			b)
13.162	2-Octylfuran		0.011	Class II No evaluation			b)
13.011	Ethyl furfuracrylate		0.12	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	

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

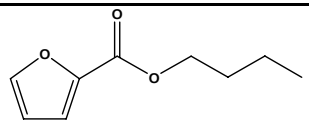
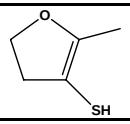
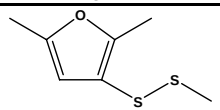
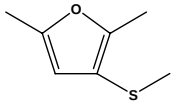
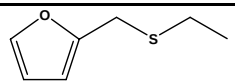
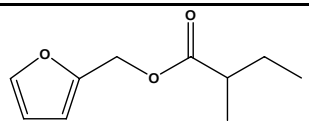
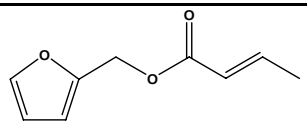
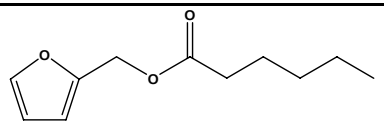
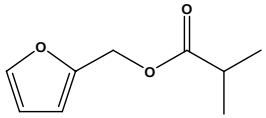
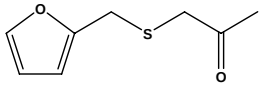
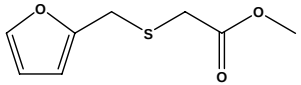
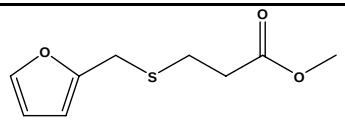
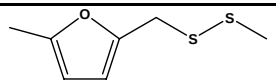
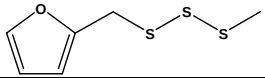
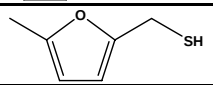
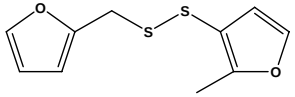
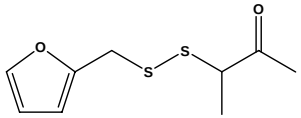
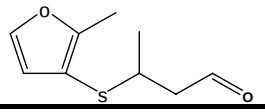
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
13.102	Butyl 2-furoate		0.12	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
13.108	4,5-Dihydro-3-mercapto-2-methylfuran		37	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
13.113	2,5-Dimethyl-3-(methyldithio)furan		0.0012	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
13.114	2,5-Dimethyl-3-(methylthio)furan		0.0024	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
13.124	Ethyl furfuryl sulfide		0.18	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
13.127	Furfuryl 2-methylbutyrate		0.73	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
13.129	Furfuryl but-2-enoate		0.11	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
13.132	Furfuryl hexanoate		0.58	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	

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

FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
13.133	Furfuryl isobutyrate		0.89	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
13.135	1-(2-Furfurylthio)propanone		0.61	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
13.141	Methyl (2-furfurylthio)acetate		0.011	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
13.143	Methyl 3-(furfurylthio)propionate		0.011	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
13.144	Methyl 5-methylfurfuryl disulfide		0.0024	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
13.146	Methyl furfuryl trisulfide		0.0024	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
13.149	5-Methyl-2-furanmethanethiol		0.37	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
13.178	3-(Furfuryldithio)-2-methylfuran		0.24	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
13.185	2-Furfuryl 3-oxo-2-butyl disulphide		0.011	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
13.199	3-[(2-Methyl-3-furyl)thio]-butanal		1.2	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	

1) *EU MSDI: Amount added to food as flavour in (kg / year) x 10E9 / (0.1 x population in Europe (= 375 x 10E6) x 0.6 x 365) = µg/capita/day.*
2) *Thresholds of concern: Class I = 1800 µg/person/day, Class II = 540 µg/person/day, Class III = 90 µg/person/day.*
3) *Procedure path A substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot.*
4) *No safety concern based on intake calculated by the MSDI approach of the named compound.*
5) *Data must be available on the substance or closely related substances to perform a safety evaluation.*
6) *No safety concern at estimated level of intake of the material of commerce meeting the specification of Table 1 (based on intake calculated by the MSDI approach).*
7) *Tentatively regarded as presenting no safety concern (based on intake calculated by the MSDI approach) pending further information on the purity of the material of commerce and/or information on stereoisomerism.*
8) *No conclusion can be drawn due to lack of information on the purity of the material of commerce.*

a) *Additional genotoxicity data required.*
b) *Genotoxic in vitro.*

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

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

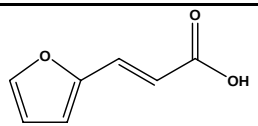
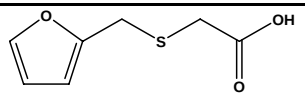
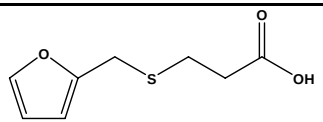
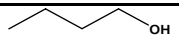
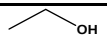
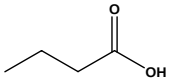
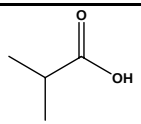
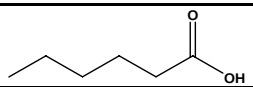
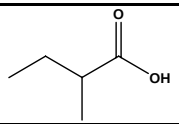
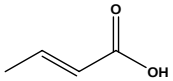
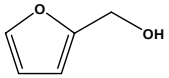
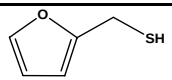
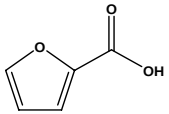
FL-no	EU Register name JECFA no	Structural formula	SCF status 1) JECFA status 2) CoE status 3) EFSA status	Structural class 4) Procedure path (JECFA) 5)	Comments
	Methanol		Not evaluated as flavour		Not in EU Register
	2-Furanacrylic acid		Not evaluated as flavour		Not in EU Register
	2-Furfurylthioacetic acid		Not evaluated as flavour		Not in EU Register
	3-Furfurylthiopropionic acid		Not evaluated as flavour		Not in EU Register
02.004	Butan-1-ol 85		Category 1 a) No safety concern b) Category A c)	Class I A3: Intake above threshold, A4: Endogenous	
02.078	Ethanol 41		Category 1 a) No safety concern d)	No evaluation	At the forty-sixth JECFA meeting (JECFA, 1997a), the Committee concluded that ethanol posed no safety concern at its current level of intake when ethyl esters are used as flavouring agents.
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	

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) EFSA status	Structural class 4) Procedure path (JECFA) 5)	Comments
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)	 (E)-isomer shown	FGE.05	Class I A3: Intake below threshold	
13.019	Furfuryl alcohol 451		No safety concern e) Category B c)	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	
13.026	2-Furanmethanethiol 1072		No safety concern f) Category A c)	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	
13.136	2-Furoic acid		FGE.13	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	

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

2) No safety concern at estimated levels of intake.

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

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

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

a) (SCF, 1995).

b) (JECFA, 1999b).

c) (CoE, 1992).

d) (JECFA, 1997a).

e) (JECFA, 2001a).

f) (JECFA, 2002c).

ND: Not detected.

TABLE 3: SUPPORTING SUBSTANCES SUMMARY

Table 3: Supporting Substances Summary

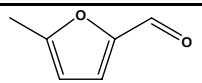
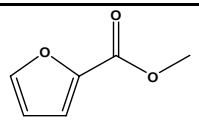
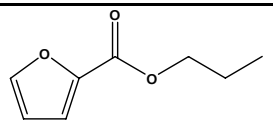
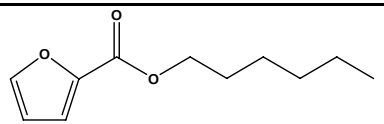
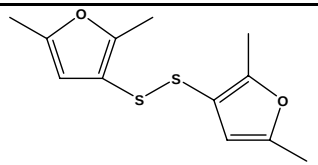
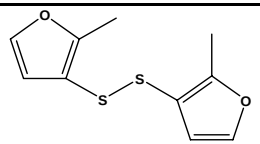
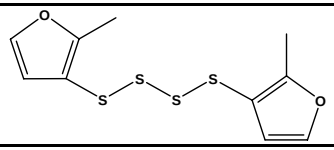
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (μg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
13.001	5-Methylfurfural		2702 119 620-02-0	745 JECFA specification (JECFA, 2002d)	180	No safety concern a) Category B b)	
13.002	Methyl 2-furoate		2703 358 611-13-2	746 JECFA specification (JECFA, 2000d)	30	No safety concern a) Deleted b)	GrADI: 0.5 (JECFA, 2001a). Deleted CoE (CoE, 1992)
13.003	Propyl 2-furoate		2946 359 615-10-1	747 JECFA specification (JECFA, 2000d)	0.061	No safety concern a) Deleted b)	GrADI: 0.5 (JECFA, 2001a). Deleted CoE (CoE, 1992)
13.005	Hexyl 2-furoate		2571 361 39251-86-0	749 JECFA specification (JECFA, 2001c)	0.061	No safety concern a) Deleted b)	GrADI: 0.5 (JECFA, 2001a). Deleted CoE (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)	0.97	No safety concern c) Category A b)	

Table 3: Supporting Substances Summary

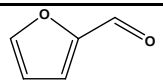
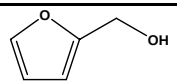
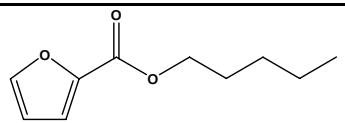
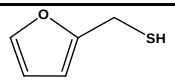
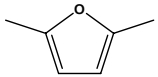
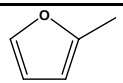
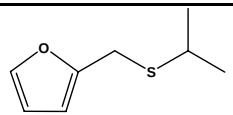
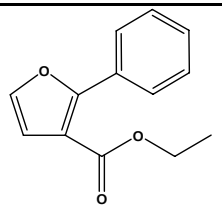
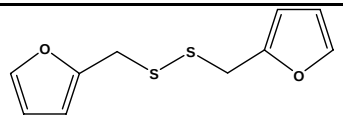
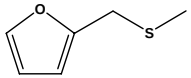
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (μg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
13.018	Furfural		2489 2014 98-01-1	450 JECFA specification (JECFA, 2000d)	440	Category 4 d) No safety concern a) Category B b)	GrADI: 0-0.5 (JECFA, 2001a). EFSA opinion, 2004.
13.019	Furfuryl alcohol		2491 2023 98-00-0	451 JECFA specification (JECFA, 2000d)	180	 No safety concern a) Category B b)	GrADI: 0.5 (JECFA, 2001a)
13.025	Pentyl 2-furoate		2072 2109 1334-82-3	748 JECFA specification (JECFA, 2001c)	0.36	 No safety concern a) Deleted b)	GrADI: 0.5 (JECFA, 2001a). Deleted CoE (CoE, 1992)
13.026	2-Furanmethanethiol		2493 2202 98-02-2	1072 JECFA specification (JECFA, 2002d)	29	 No safety concern c) Category A b)	
13.029	2,5-Dimethylfuran		 2208 625-86-5	1488 JECFA specification (JECFA, 2006b)	0.012	 No evaluation e) Deleted b)	f)
13.030	2-Methylfuran		4179 2209 534-22-5	1487 JECFA specification (JECFA, 2006b)	0.21	 No evaluation e) Category B b)	f)
13.032	Furfuryl isopropyl sulfide		3161 2248 1883-78-9	1077 JECFA specification (JECFA, 2002d)	0.0012	 No safety concern c) Category B b)	
13.038	2-Phenyl-3-carbethoxyfuran		3468 2309 50626-02-3	752 JECFA specification (JECFA, 2002d)	0.012	 No safety concern a) Category B b)	
13.050	Difurfuryl disulfide		3146 11480 4437-20-1	1081 JECFA specification (JECFA, 2002d)	3.3	 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)	

Table 3: Supporting Substances Summary

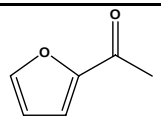
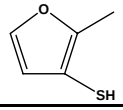
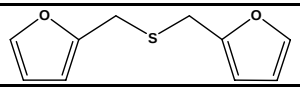
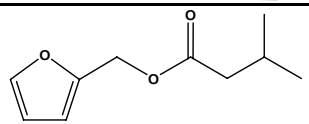
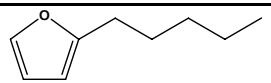
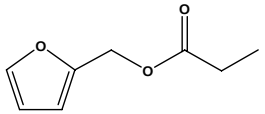
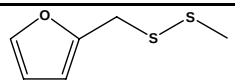
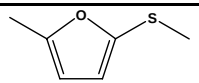
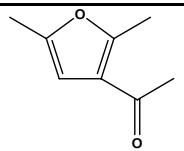
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (μg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
13.054	2-Acetylfuran		3163 11653 1192-62-7	1503 JECFA specification (JECFA, 2006b)	46	No evaluation e)	f)
13.055	2-Methylfuran-3-thiol		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 JECFA specification (JECFA, 2001c)	0.024	No safety concern a)	GrADI: 0.5 (JECFA, 2001a)
13.059	2-Pentylfuran		3317 10966 3777-69-3	1491 JECFA specification (JECFA, 2006b)	0.18	No evaluation e)	f)
13.062	Furfuryl propionate		3346 10646 623-19-8	740 JECFA specification (JECFA, 2001c)	1.7	No safety concern a)	GrADI: 0.5 (JECFA, 2001a)
13.064	Methyl furfuryl disulfide		3362 11513 57500-00-2	1078 JECFA specification (JECFA, 2002d)	0.85	No safety concern c)	
13.065	2-Methyl-5-(methylthio)furan		3366 11550 13678-59-6	1062 JECFA specification (JECFA, 2002d)	1.1	No safety concern c)	
13.066	3-Acetyl-2,5-dimethylfuran		3391 10921 10599-70-9	1506 Tentative JECFA specification (JECFA, 2006b)	ND	No evaluation e)	f)

Table 3: Supporting Substances Summary

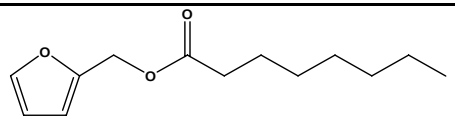
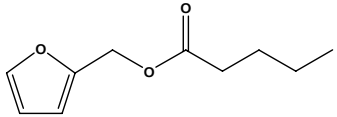
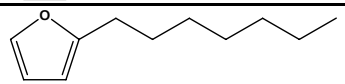
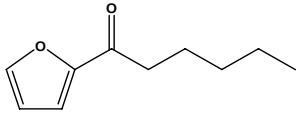
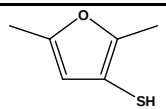
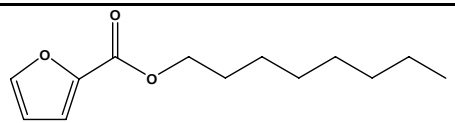
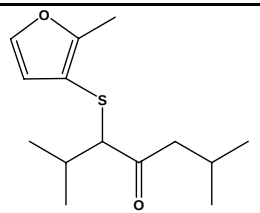
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (μg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
13.067	Furfuryl octanoate		3396 10645 39252-03-4	742 JECFA specification (JECFA, 2001c)	0.012	No safety concern a)	GrADI: 0.5 (JECFA, 2001a)
13.068	Furfuryl valerate		3397 10647 36701-01-6	741 JECFA specification (JECFA, 2001c)	0.24	No safety concern a)	GrADI: 0.5 (JECFA, 2001a)
13.069	2-Heptylfuran		3401 10952 3777-71-7	1492 JECFA specification (JECFA, 2006b)	0.012	No evaluation e)	f)
13.070	2-Hexanoylfuran		3418 11180 14360-50-0	1512 JECFA specification (JECFA, 2006b)	ND	No evaluation e)	f)
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 JECFA specification (JECFA, 2001c)	2.2	No safety concern a)	GrADI: 0.5 (JECFA, 2001a)
13.075	2,6-Dimethyl-3-((2-methyl-3-furyl)thio)heptan-4-one		3538 11915 61295-51-0	1086 JECFA specification (JECFA, 2002d)	1.8	No safety concern c)	

Table 3: Supporting Substances Summary

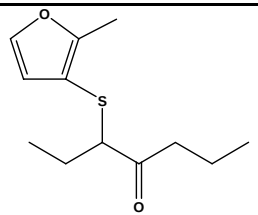
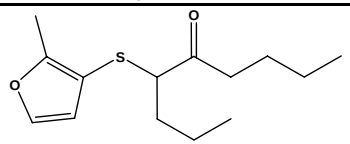
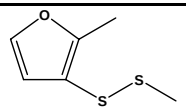
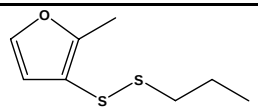
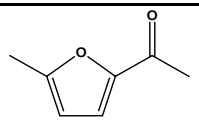
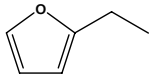
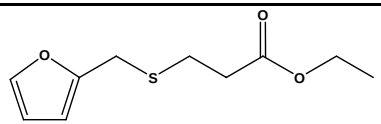
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (μg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
13.077	3-((2-Methyl-3-furyl)thio)heptan-4-one		3570 11922 61295-41-8	1085 JECFA specification (JECFA, 2002d)	2.9	No safety concern c)	
13.078	4-((2-Methyl-3-furyl)thio)nonan-5-one		3571 11923 61295-50-9	1087 JECFA specification (JECFA, 2002d)	0.73	No safety concern c)	
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)	0.12	No safety concern c)	
13.083	2-Acetyl-5-methylfuran		3609 11038 1193-79-9	1504 JECFA specification (JECFA, 2006b)	0.37	No evaluation e)	f)
13.092	2-Ethylfuran		3673 11706 3208-16-0	1489 JECFA specification (JECFA, 2006b)	0.061	No evaluation e)	f)
13.093	Ethyl 3-(2-furfurylthio)propionate		3674 94278-27-0	1088 JECFA specification (JECFA, 2002d)	0.012	No safety concern c)	

Table 3: Supporting Substances Summary

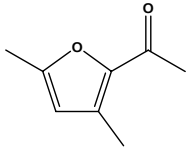
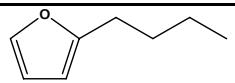
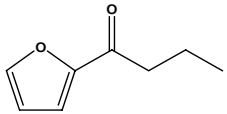
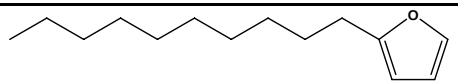
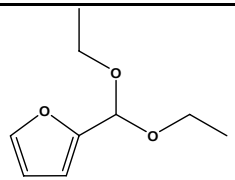
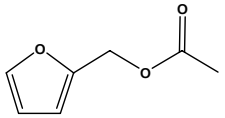
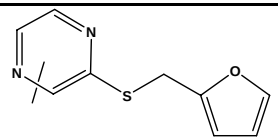
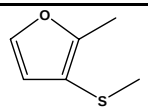
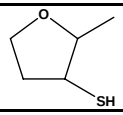
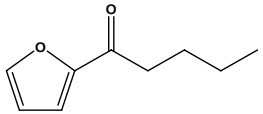
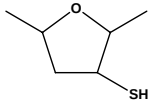
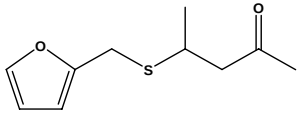
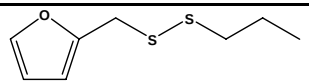
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (μg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
13.101	2-Acetyl-3,5-dimethylfuran		4071 22940-86-9	1505 JECFA specification (JECFA, 2006b)	0.0012	No evaluation e)	f)
13.103	2-Butylfuran		4081 10927 4466-24-4	1490 JECFA specification (JECFA, 2006b)	0.24	No evaluation e)	f)
13.105	2-Butyrylfuran		4083 11045 4208-57-5	1507 JECFA specification (JECFA, 2006b)	0.12	No evaluation e)	f)
13.106	2-Decylfuran		4090 83469-85-6	1493 JECFA specification (JECFA, 2006b)	0.0012	No evaluation e)	f)
13.126	Furfural diethyl acetal		 13529-27-6		0.085		Furfural diethyl acetal, structurally related to the substances in subgroup IA, has been evaluated by the AFC Panel (EFSA, 2004c)
13.128	Furfuryl acetate		2490 2065 623-17-6	739 JECFA specification (JECFA, 2000d)	16	No safety concern a) Category B b)	GrADI: 0.5 (JECFA, 2001a)
13.151	2-Methyl-3,5 and 6-(furfurylthio)pyrazine	 2 or 5 or 6 -Methyl-3-(furfurylthio)pyrazine	3189 2287 65530-53-2	1082 JECFA specification (JECFA, 2002d)	0.37	No safety concern c) Category B b)	
13.152	2-Methyl-3-(methylthio)furan		3949 63012-97-5	1061 JECFA specification (JECFA, 2002d)	1.2	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
13.160	2-Methyltetrahydrofuran-3-thiol		3787 57124-87-5	1090 JECFA specification (JECFA, 2002d)	3.5	No safety concern c)	
13.163	2-Pentanoylfuran		4192 3194-17-0	1509 JECFA specification (JECFA, 2006b)	0.061	No evaluation e)	f)
13.193	2,5-Dimethyltetrahydro-3-furanthiol		3971 26486-21-5	1091 JECFA specification (JECFA, 2002d)	0.024	No safety concern c)	
13.196	4-(Furfurylthio) pentan-2-one		3840 180031-78-1	1084 JECFA specification (JECFA, 2002d)	0.012	No safety concern c)	
13.197	Furyl propyldisulfide		3979 252736-36-0	1079 JECFA specification (JECFA, 2002d)	0.024	No safety concern c)	

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

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

3) No safety concern at estimated levels of intake.

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

a) (JECFA, 2001a).

b) (CoE, 1992).

c) (JECFA, 2002c).

d) (SCF, 1995).

e) (JECFA, 2009c.)

f) The procedure could not be applied to this furan-substituted substance due to concern with respect to available genotoxicity data, formation of possible toxic metabolites and structural similarity to furan which is carcinogenic (JECFA, 2006b).

ND) No intake data reported.

ANNEX I: PROCEDURE FOR THE SAFETY EVALUATION

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

The Procedure is a stepwise approach that integrates information on intake from current uses, structure-activity relationships, metabolism and, when needed, toxicity. One of the key elements in the Procedure is the subdivision of flavourings into three structural classes (I, II, III) for which thresholds of concern (human exposure thresholds) have been specified. Exposures below these thresholds are not considered to present a safety concern.

Class I contains flavourings that have simple chemical structures and efficient modes of metabolism, which would suggest a low order of oral toxicity. Class II contains flavourings that have structural features that are less innocuous, but are not suggestive of toxicity. Class III comprises flavourings that have structural features that permit no strong initial presumption of safety, or may even suggest significant toxicity (Cramer et al., 1978). The thresholds of concern for these structural classes of 1800, 540 or 90 microgram/person/day, respectively, are derived from a large database containing data on subchronic and chronic animal studies (JECFA, 1996a).

In Step 1 of the Procedure, the flavourings are assigned to one of the structural classes. The further steps address the following questions:

- can the flavourings be predicted to be metabolised to innocuous products[10] (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[11] (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.

[10] *"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).*

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

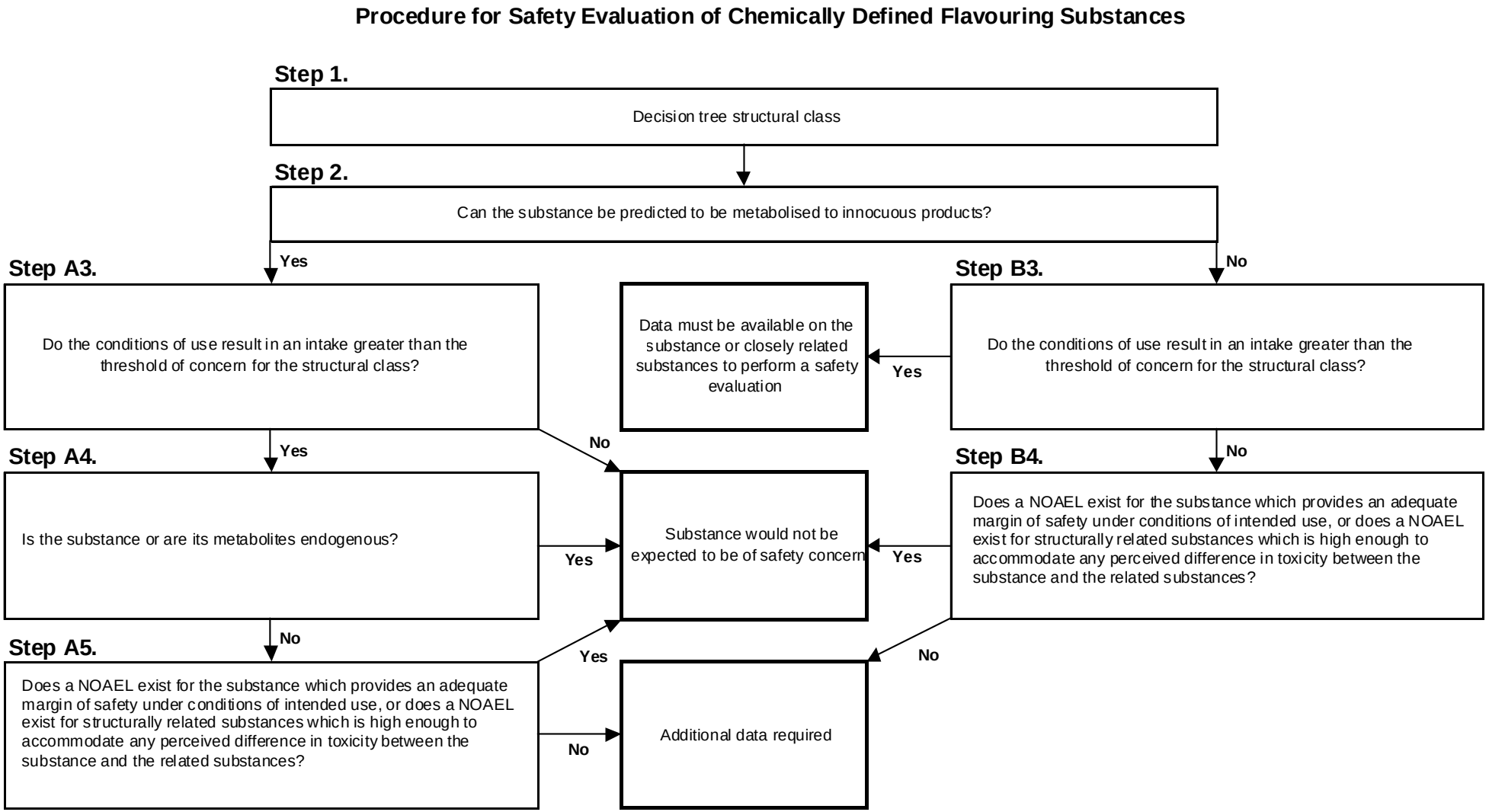


Figure I.1 *Procedure for Safety Evaluation of Chemically Defined Flavouring Substances*

ANNEX II: USE LEVELS / MTAMDI

II.1 Normal and Maximum Use Levels

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

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

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

The "normal and maximum use levels" are provided by Industry for 26 of the 27 candidate substances in the present flavouring group (EFFA, 2003g; EFFA, 2004y; EFFA, 2007a; Flavour Industry, 2009b) (Table II.1.2).

Table II.1.2 Normal and Maximum use levels (mg/kg) for candidate substances in FGE.13Rev1 (EFFA, 2003g; EFFA, 2004y; EFFA, 2007a; Flavour Industry, 2009b).

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	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
13.102	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
13.108	0,4	0,2	0,4	0,3	-	0,4	0,2	0,4	0,1	0,1	-	-	0,2	0,4	0,2	0,4	1	0,2
	2	1	2	1,5	-	2	1	2	0,4	0,4	-	-	1	2	1	2	5	1
13.113	0,4	0,2	0,4	0,3	-	0,4	0,2	0,4	0,1	0,1	-	-	0,2	0,4	0,2	0,4	1	0,2
	2	1	2	1,5	-	2	1	2	0,4	0,4	-	-	1	2	1	2	5	1
13.114	0,4	0,2	0,4	0,3	-	0,4	0,2	0,4	0,1	0,1	-	-	0,2	0,4	0,2	0,4	1	0,2
	2	1	2	1,5	-	2	1	2	0,4	0,4	-	-	1	2	1	2	5	1
13.122	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
13.124	0,2	0,1	0,2	0,2	-	0,2	0,1	0,2	0,1	0,1	-	-	0,1	0,2	0,1	0,2	0,4	0,1
	1	0,5	1	1	-	1	0,5	1	0,2	0,2	-	-	0,5	1	0,3	1	2	0,5
13.125	0,5	0,2	0,5	0,4	-	1	0,2	2	0,2	0,2	-	-	0,3	0,5	0,2	1	2	0,4
	2,5	1	2,5	2	-	5	1	10	1	1	-	-	1,5	2,5	1	5	10	2
13.127	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5

Table II.1.2 Normal and Maximum use levels (mg/kg) for candidate substances in FGE.13Rev1 (EFFA, 2003g; EFFA, 2004y; EFFA, 2007a; Flavour Industry, 2009b).

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**
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
13.129	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
13.130	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13.132	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
13.133	7	5	10	7	-	10	5	10	2	2	-	-	5	10	5	10	20	5
	35	25	50	35	-	50	25	50	10	10	-	-	25	50	25	50	100	25
13.135	0	0	0	0	-	2	0	1,5	1,1	0	-	-	0	0	1,5	0,5	1,5	0
	0	0	0	0	-	7	0	6	6	0	-	-	0	0	7	2	7	0
13.136	3	2	3	2	-	5	2	-	1	1	-	-	2	3	2	5	5	2
	15	10	15	10	-	25	10	-	5	5	-	-	10	15	10	25	25	10
13.139	3	2	3	2	-	4	2	5	1	1	-	-	2	3	2	4	5	2
	15	10	15	10	-	20	10	25	5	5	-	-	10	15	10	20	25	10
13.141	0,5	0,2	0,5	0,4	-	1	0,2	2	0,2	0,2	-	-	0,3	0,5	0,2	1	1	0,4
	2,5	1	2,5	2	-	5	1	10	1	1	-	-	1,5	2,5	1	5	5	2
13.143	0,5	0,2	0,5	0,4	-	1	0,2	2	0,2	0,2	-	-	0,3	0,5	0,2	1	2	0,4
	2,5	1	2,5	2	-	5	1	10	1	1	-	-	1,5	2,5	1	5	10	2
13.144	0,2	0,1	0,2	0,2	-	0,2	0,1	0,2	0,1	0,1	-	-	0,1	0,2	0,1	0,2	0,4	0,1
	1	0,5	1	1	-	1	0,5	1	0,2	0,2	-	-	0,5	1	0,3	1	2	0,5
13.145	0,4	0,2	0,4	0,3	-	0,4	0,2	0,4	0,1	0,1	-	-	0,2	0,4	0,2	0,4	1	0,2
	2	1	2	1,5	-	2	1	2	0,4	0,4	-	-	1	2	1	2	5	1
13.146	0,4	0,2	0,4	0,3	-	0,4	0,2	0,4	0,1	0,1	-	-	0,2	0,4	0,2	0,4	1	0,2
	2	1	2	1,5	-	2	1	2	0,4	0,4	-	-	1	2	1	2	5	1
13.149	0,4	0,2	0,4	0,3	-	0,4	0,2	0,4	0,1	0,1	-	-	0,2	0,4	0,2	0,4	1	0,2
	2	1	2	1,5	-	2	1	2	0,4	0,4	-	-	1	2	1	2	5	1
13.155	0,5	0,2	0,5	0,4	-	1	0,2	2	0,2	0,2	-	-	0,3	0,5	0,2	1	2	0,4
	2,5	1	2,5	2	-	5	1	10	1	1	-	-	1,5	2,5	1	5	10	2
13.162	0,5	0,2	0,5	0,4	-	1	0,2	2	0,2	0,2	-	-	0,3	0,5	0,2	1	2	0,4
	2,5	1	2,5	2	-	5	1	10	1	1	-	-	1,5	2,5	1	5	10	2
13.178	0,4	0,2	0,4	0,3	-	0,4	0,2	0,4	0,1	0,1	-	-	0,2	0,4	0,2	0,4	1	0,2
	2	1	2	1,5	-	2	1	2	0,4	0,4	-	-	1	2	1	2	5	1
13.185	0,5	0,2	0,5	0,4	-	1	0,2	2	0,2	0,2	-	-	0,3	0,5	0,2	1	2	0,4
	2,5	1	2,5	2	-	5	1	10	1	1	-	-	1,5	2,5	1	5	10	2
13.199	0,00	0,05	0,00	0,00	0,00	0,00	0,00	0,05	0,05	0,05	-	-	0,05	0,00	0,00	0,00	2	0,00
	5	0,1	1	1	1	5	5	0,1	0,2	0,1	-	-	0,1	5	2	5	10	5
	0,01		0,00	0,00	0,00	0,01	0,01							0,01	0,00	0,01		0,01
			3	3	3										5			

II.2 mTAMDI Calculations

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

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

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

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

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

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

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

The mTAMDI values are presented for 26 of the 27 flavouring substances in the present flavouring group, for which Industry has provided use and use levels (EFFA, 2003g; EFFA, 2004y; EFFA, 2007a; Flavour Industry, 2009b). The mTAMDI values are only given for highest reported use levels (see Table II.2.3).

TableII.2.3 Estimated intakes based on the mTAMDI approach

FL-no	**EU Register name**	**mTAMDI (µg/person/day)**	**Structural class**	**Threshold of concern (µg/person/day)**

13.122	Ethyl 2-furoate	3900	Class II	540
13.130	Furfuryl butyrate		Class II	540
13.136	2-Furoic acid	1400	Class II	540
13.139	5-Hydroxymethylfurfuraldehyde	1600	Class II	540
13.145	Methyl 5-methylfurfuryl sulfide	160	Class II	540
13.155	2-Methyl-5-propionylfuran	420	Class II	540
13.125	2-Ethyl-5-methylfuran	420	Class II	540
13.162	2-Octylfuran	420	Class II	540
13.011	Ethyl furfuracrylate	3900	Class III	90
13.102	Butyl 2-furoate	3900	Class III	90
13.108	4,5-Dihydro-3-mercapto-2-methylfuran	160	Class III	90
13.113	2,5-Dimethyl-3-(methyldithio)furan	160	Class III	90
13.114	2,5-Dimethyl-3-(methylthio)furan	160	Class III	90
13.124	Ethyl furfuryl sulfide	78	Class III	90
13.127	Furfuryl 2-methylbutyrate	3900	Class III	90
13.129	Furfuryl but-2-enoate	3900	Class III	90
13.132	Furfuryl hexanoate	3900	Class III	90
13.133	Furfuryl isobutyrate	3900	Class III	90
13.135	1-(2-Furfurylthio)propanone	780	Class III	90
13.141	Methyl (2-furfurylthio)acetate	400	Class III	90
13.143	Methyl 3-(furfurylthio)propionate	420	Class III	90
13.144	Methyl 5-methylfurfuryl disulfide	78	Class III	90
13.146	Methyl furfuryl trisulfide	160	Class III	90
13.149	5-Methyl-2-furanmethanethiol	160	Class III	90
13.178	3-(Furfuryldithio)-2-methylfuran	160	Class III	90
13.185	2-Furfuryl 3-oxo-2-butyl disulphide	420	Class III	90
13.199	3-[(2-Methyl-3-furyl)thio]-butanal	49	Class III	90

ANNEX III: METABOLISM

III.1. Introduction

The candidate substances in FGE.13Rev2 are furan derivatives which can be divided into two main groups (I and II). Ten candidate substances in subgroup Ia are furfuryl alcohol derivatives. In subgroup Ib, there is only one candidate substance, an alkoyl-substituted furan. The two candidate substances in subgroup Ic are alkyl-substituted furans, without functional groups in the side chains

The 14 candidate substances in main group II are furan derivatives, containing sulphur substituents as mono-, di- and tri-sulphides (subgroups IIa, IIc and IId) or free thiol groups (subgroup IIb). The candidate substance 4,5-dihydro-3-mercapto-2-methylfuran [FL-no: 13.108] in subgroup IIb is a non-aromatic furan derivative.

The subgrouping has been presented in Table 4.1 (Section 4). Further details on the structural properties of the substances in these two subgroups are presented in the respective sections of this Annex.

III.2. Main Group I – Non-sulphur-containing Furan Derivatives

Furfuryl alcohol derivatives (Ia), alkoyl-substituted furans (Ib) and alkyl-substituted furans (Ic)

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, 13,130, 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

Five of the candidate substances in subgroup I [FL-no: 13.127, 13.129, 13.130, 13.132 and 13.133] are esters of furfuryl alcohol [FL-no: 13.019] and two [FL-no: 13.122 and 13.102] are esters of 2-furoic acid [FL-no: 13.136]. One candidate substance is an ester of furanacrylic acid [FL-no: 13.011].

The furfuryl alcohol esters (i.e. candidate substances furfuryl butyrate [FL-no: 13.130], 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 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 µl/L isoamyl furylpropion-1-ate or 40 µl/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 in humans the eight candidate esters in subgroup Ia will be hydrolysed to their corresponding acids and alcohols within a relatively short time, possibly prior to gastrointestinal absorption or otherwise during their passage through the liver. The expected hydrolysis products for these esters and their evaluation status, when used as flavouring substances, are shown in table 2b. Ester hydrolysis is not an issue for the substances in subgroups Ib an Ic.

III.2.2. Absorption, distribution and elimination

Subgroup Ia

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-hours 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 et al., 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 hours (Nomeir et al., 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 hours 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 hours. 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 hours 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).

Subgroup Ic

Alkylfuran derivatives also exhibit rapid uptake, metabolism and excretion. Male Sprague-Dawley rats administered 100 mg [^{14}C]-2-methylfuran/kg bw in sesame oil via intraperitoneal injection showed radiolabelled 2-methylfuran [FL-no: 13.030] metabolites in the 12-hours urine (Ravindranath & Boyd, 1991). Maximal hepatic radioactivity was detected at 4 hours post-administration.

Tissue distribution of 50 - 200 mg [^{14}C]-2-methylfuran/kg bw over 24 hours showed the presence of radiolabel from greatest to least as follows: liver > kidney > lung > blood. The maximal amount of radiolabel was detected in the liver at 8 hours post administration, followed by a steady decline up to 24 hours (Ravindranath et al., 1986).

Based on these data, the members of this group of furan-substituted aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids and related esters are anticipated to be rapidly absorbed, distributed through key organs involved in metabolic processes and then eliminated, primarily in the urine.

III.2.3. Biotransformation

Subgroup Ia:

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 et al., 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 sulphate 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 et al., 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; Friedmann, 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.

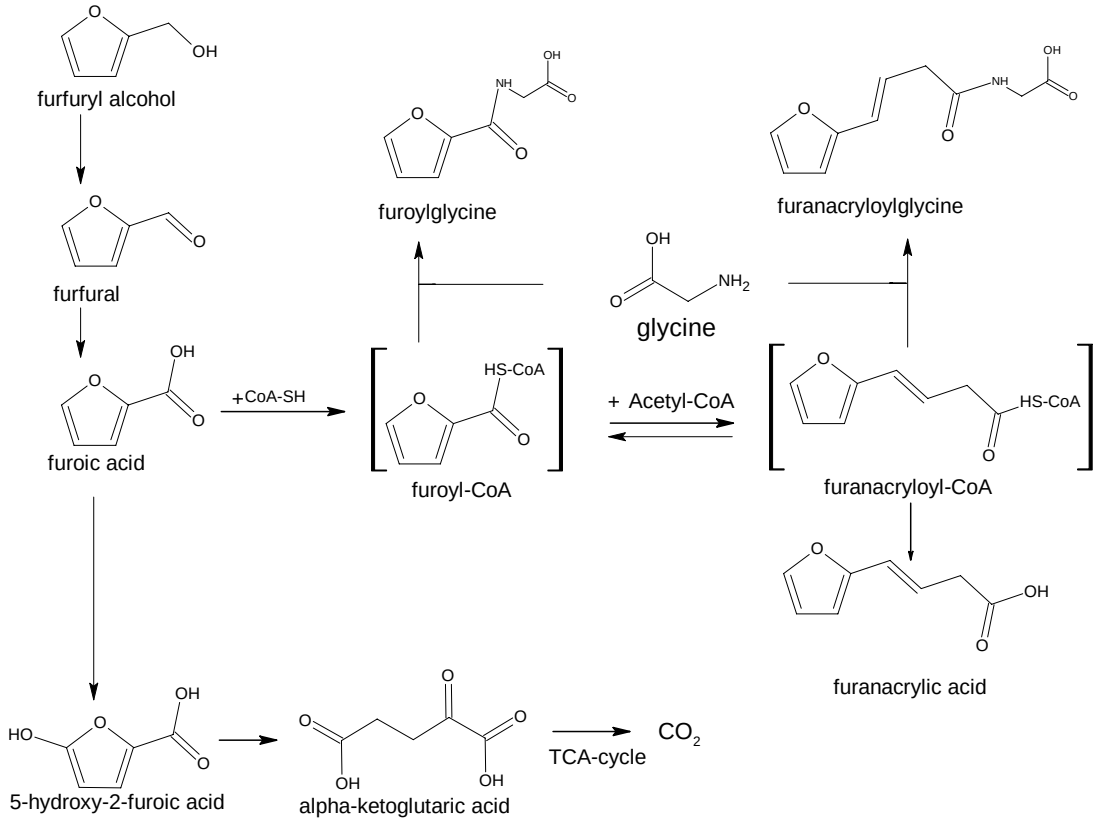


Figure III.1. Biotransformation of furfuryl alcohol and furfural. Formation of glycine conjugates are major pathways in mammals. Formation of carbon dioxide also occurs in mammals. The pathway via alpha-ketoglutaric acid has been described for micro-organisms, and might occur in mammals, as well.

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 (5HMFA) and its glycine conjugate [*N*-(5-hydroxymethyl-2-furoyl)glycine, 5HMFG]. A third, minor polar metabolite was not further identified.

The ratio of 5HMFA to 5HMFG 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 5HMFA/5HMFG ratio 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 B6C3F$_1$ mice following oral gavage administration of 5, 100 or 500 mg (rats) and 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 hours) than in rats.

5-Hydroxymethyl-2-furoic acid (5HMFA) 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 (5HMFG) 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

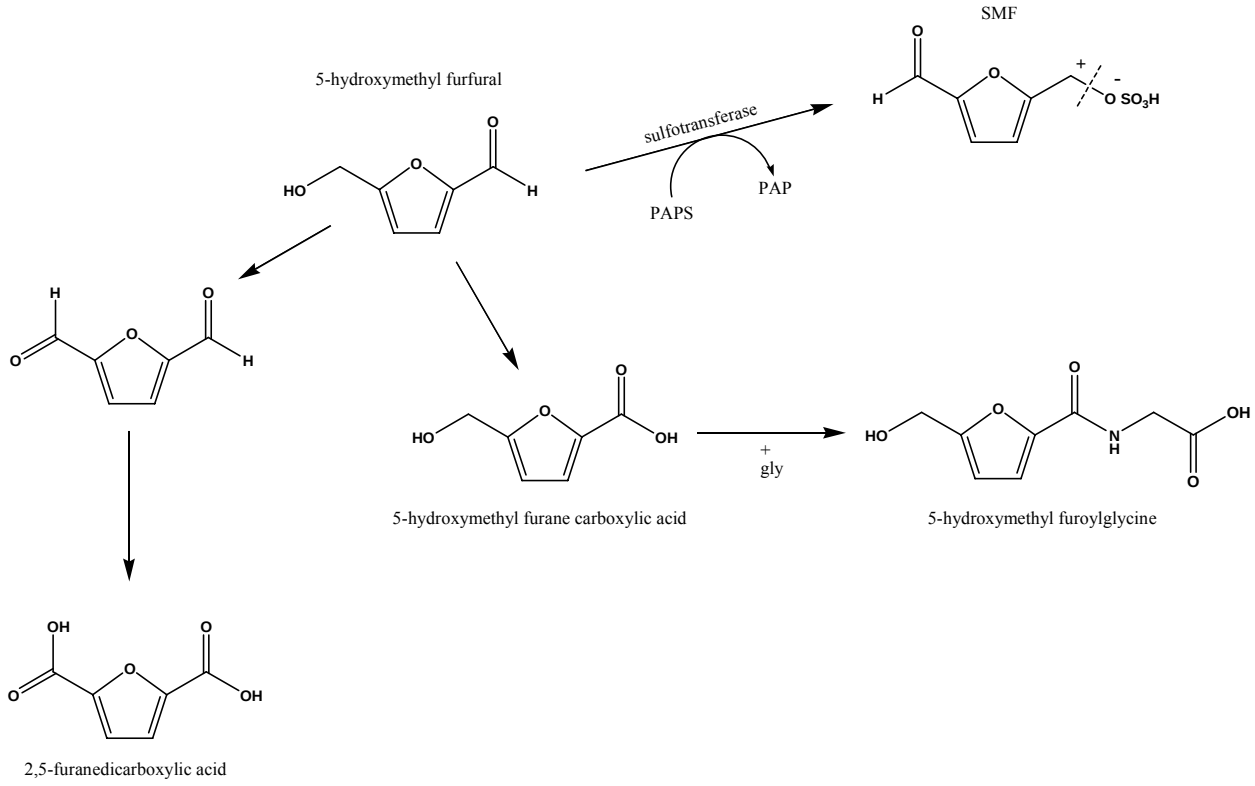


Figure III.2 Metabolic pathways for 5-hydroxymethylfurfuraldehyde. Glycine conjugation will require transient coupling to CoA (see Figure III.1)

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).

In addition to the above mentioned pathways, 5HMF has been shown to be bioactivated *in vitro* to 5-[(sulphoxy)methyl] furfural (SMF), through sulphonation of its allylic hydroxyl functional group, catalyzed by sulphotransferases. In the resulting ester, the sulphate is a good leaving group, thus producing a highly electrophilic allyl carbocation (Glatt, 1997), 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) maybe 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 sulpho-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 sulpho-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).

Twelve weeks old FVB/N mice (n = 28) were given an intravenous injection of 100 mg/kg bw 5HMF in isotonic saline. Two blood samples were taken from each animal, and blood samples from different animals were combined to give information on 5HMF and SMF pharmacokinetics. SMF was detected in plasma from animals given 5HMF, and the half life of SMF was calculated to be 4.2 minutes (Monien et al., 2009).

As part of the NTP program 3-weeks and 3-months sub-chronic toxicity studies, male and female F344/N rats and B6C3F$_1$ mice were administered 5HMF at different dose levels up to 1,500 mg/kg bw per day (see section 8.2). At various time points during the studies, urine of the animals was collected for 24 hours and analyzed for the presence of 5-(hydroxymethyl)-2-furoic acid and the corresponding glycine conjugate 5-(hydroxymethyl)-furoyl glycine. In male mice and in rats in both sexes, the percentages of the dose recovered following the last exposure increased during the exposure period. In female mice no such induction was observed. Also in male mice the percentage recovered as the glycine conjugate amounted to 10 - 20 % of the urinary metabolites, while in the females the proportion of the glycine conjugate was only *ca.* 5 %. The proportion of the glycine conjugate was not dependent on the dose level. In male rats, the fraction of the dose excreted as glycine conjugate was ~ 10 % at the lower end of the dose range and this percentage fell to below 2 % at the high end of the dose range, due to saturation of the glycine conjugation pathway. In female rats a similar phenomenon was observed, but in the females the proportion of the glycine conjugate remained at about 3 - 5 % of the total amount in urine. Excretion of SMF was not studied (NTP, 2010c).

Subgroups Ib and Ic:

No data were available on the candidate substances in these groups. However, some data on supporting substances have been summarised by the JECFA (JECFA, 2009a). The following text is based on this JECFA evaluation in which some modifications have been included after consultation of the original publications.

The candidate substance in subgroup Ib [FL-no: 13.155] is an alkoyl-substituted furan which has a keto group in the C1-position of the furan ring substituent. It is considered that the alpha,beta-unsaturated ketone structure in this substance is structurally related and comparable to the structure in acetophenone (EFSA, 2008am). It is anticipated that the ketone in the C_1 position of the sidechain will undergo reduction to the corresponding secondary alcohol which subsequently is excreted after conjugation (JECFA, 2006d). For this

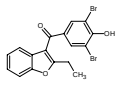
substance also ring oxidation followed by ring opening may be anticipated (see subgroup Ic) (JECFA, 2009a).

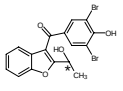
For the candidate substances in subgroup Ic it is of relevance that alkyl-substituted furan and benzofuran derivatives may undergo cytochrome P450–mediated side-chain oxidation to yield an alcohol functional group located at the position bonded directly to the furan ring. The resulting secondary alcohol may be excreted in the urine primarily as the glucuronic acid or sulphate conjugate, or it may be converted to the corresponding ketone, which may also be excreted in the urine. This kind of side-chain oxidation, preferably at the C_1' position of furan, is similar to that observed with other alkyl-substituted heterocyclic derivatives (e.g. pyridine derivatives and indoles) (Hawksworth & Scheline, 1975; Thornton-Manning et al., 1993). It is noted that the resulting secondary furyl alcohol forms an α,β-unsaturated carbonyl system with the double bonds in the furan ring. In addition to side-chain oxidation, the furan ring can undergo cytochrome P450-induced oxidation followed by opening of the ring to yield reactive 2-enal or 2-enedial intermediates. It is not entire clear if in these reactions also epoxide intermediates are involved, but if so, there have to be very unstable. The 2-en(edi)al intermediates have been shown to form protein and DNA adducts. They also may conjugate readily with GSH, but as their GSH conjugates are unstable, this conjugation offers no protection. However, conjugation with cysteine results in a stable non-reactive product (Ravindranath et al., 1983; Ravindranath et al., 1984; Ravindranath & Boyd, 1985; Ravindranath et al., 1986; Ravindranath & Boyd, 1991).

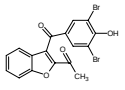
The metabolic fate of a 2-ethylbenzofuran derivative has been investigated in humans, rats and dogs. Two healthy male subjects were each given an oral 100 mg dose of [3-^{14}C]-benzarone [3-(4-hydroxybenzoyl) 2-ethylbenzofuran] in two gelatine capsules. Approximately 73 % of the radioactivity was excreted in the urine over 5 days, with more than 59 % being excreted in the first 24 hours. Approximately 19 % of the radioactivity was excreted in the faeces over 5 days. The principal metabolites included benzarone hydroxylated at the C_1' position of the furan ring and a glucuronic acid conjugate either of the C_1' hydroxyl or the phenolic hydroxyl group. In the dog or rat, more than 80 % of a 0.5 or 2 mg/kg bw dose of [3-^{14}C]-benzarone was excreted in the faeces during the first 48 hours. In the rat or dog, most (> 70 %) of the absorbed dose was eliminated by direct conjugation of the administered substance, whereas in humans, >70 % was hydroxylated before conjugation. The authors speculated that the benzarone glucuronic acid conjugate was excreted directly into the bile more readily in rats and dogs than in humans, thereby minimizing further hydroxylation in the liver (Wood et al., 1987).

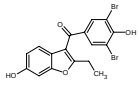
Two healthy male volunteers were administered a single oral daily dose of 100 mg benzbromarone [(3,5-dibromo-4-hydroxyphenyl) (2-ethyl-3-benzofuranyl)methanone][12] for 8 consecutive days (De Vries et al., 1993). The major metabolites were formed by C_1'-hydroxylation to yield the corresponding 1'-hydroxybenzbromarone and by hydroxylation of the fused benzene ring to yield 6-hydroxybenzbromarone. The corresponding C_1' ketone (= 1'-oxobenzbromanone) formed by oxidation of the 1'-hydroxy group was also identified in the urine. The ratio of C_1' enantiomers of 1'-hydroxybenzbromarone was 2.12 in the plasma and 7.32 in the urine. These metabolic data support the conclusion that alkyl-substituted furan and benzofuran derivatives undergo side-chain oxidation to yield the corresponding alcohol metabolite, followed by excretion in the urine. As no comparison was made between urinary metabolites before and after treatment with glucuronidase/sulphatase the study does not provide information on the extent of conjugation of benzbromanone metabolites.

Initial *in vitro* experiments in rat microsomal preparations suggested that high concentrations of alkyl-substituted furans are partly metabolized to reactive acetylacrolein$_1$-type intermediates (Ravindranath et al.,


12 Benzbromarone


1'-hydroxybenzbromarone
asterix indicates chiral centre


1'-Oxobenzbromarone


6-Hydroxybenzbromarone

1983, 1984). Acetylacrolein is a potent microsomal mixed-function oxidase inhibitor that has been reported to bond covalently and irreversibly to the oxidizing enzyme, thus inactivating it (Ravindranath & Boyd, 1985).

Acetylacrolein (= 4-oxo-pent-2-enal) is a potent microsomal mixed-function oxidase inhibitor which has been reported to bind covalently and irreversibly to the oxidizing enzyme, thus deactivating it (Ravindranath and Boyd, 1985). Significant protein binding (> 55 nmol/mg protein) was reported when 10 mmol/l of [2-^{14}C]-methylfuran were incubated with rat hepatic microsomes in the presence of NADPH and oxygen. In the absence of oxygen or NADPH, little binding was observed (< 2 nmol/mg protein). These findings suggest that NADPH-dependent oxidation of 2-methylfuran is a prerequisite for protein binding. Increased protein binding (> 80 nmol/mg protein) was also reported when Sprague-Dawley rats were pre-treated with phenobarbital, a cytochrome P450 inducer, while decreased or no protein binding was observed in the presence of piperonyl butoxide or N-octyl imidazole, both of which inhibit cytochrome P450. The V_{max} and K_m for 2-methylfuran metabolism in phenobarbital pre-treated rats were 0.81 µmol/2 mg microsomal protein per min and 0.463 mmol/l, respectively, and those in rats without phenobarbital pre-treatment were 0.53 µmol/2 mg microsomal protein per min and 1.417 mmol/l, respectively. These values suggest that 2-methylfuran undergoes cytochrome P450-mediated oxidation to yield a reactive metabolite (i.e. acetylacrolein) which binds covalently to protein (Ravindranath & Boyd, 1985). With 3-methylfuran a similar ring opening product (3-methyl-but-2-enedial) has been found (Ravindranath et al., 1983).

In the same study, when acetylacrolein at 0.25 mmol/l (24.5 µg/ml) was added to the incubation mixture, microsomal metabolism of 2-methylfuran was almost completely inhibited (covalent binding was 1.5 % of the control incubation). At a concentration of 0.5 mmol acetylacrolein/l (49.1 µg/ml), no metabolism of 2-methylfuran was detectable, suggesting that acetylacrolein inhibits cytochrome P450-mediated oxidation, probably through direct covalent bonding with the enzyme. Thus, 2-methylfuran is a suicide substrate for this enzyme. Conjugation of the reactive metabolite with sulphydryl trapping agents, including cysteine (10 mmol/l) and GSH (10 mmol/l), showed a marked decrease in microsomal protein binding, suggesting that sulphydryl conjugation plays a role in the detoxication of acetylacrolein. Cysteine was the better trapping agent for the prevention of microsomal protein binding when compared with GSH, semicarbazide, lysine or *N*-acetylcysteine. The authors postulated that cysteine forms a stable cyclic conjugate with alpha,beta-unsaturated aldehydes, whereas the ability of GSH to form stable conjugates with alpha,beta-unsaturated aldehydes varies (Esterbauer et al., 1975; Esterbauer et al., 1976; Ravindranath & Boyd, 1985).

Other *in vitro* experiments support the conclusion that cytochrome P450-mediated oxidation of 2-methyl-furan is directly related to its toxicity. This was studied in hepatocytes isolated from adult male Wistar rats that were untreated or treated with phenobarbitone (0.1 % in drinking-water for 5 days) or beta-naphthoflavone (80 mg/kg bw by intraperitoneal injection daily for 3 days). The cultured hepatocytes were incubated with 2-methylfuran at 0, 100, 300, 600 or 1000 µmol/l (0, 8.2, 24.6, 49.3 and 82.1 µg/ml, respectively) for 24 hours. The median lethal concentrations (LC_{50} values) for untreated, phenobarbitone-treated or β-naphthoflavone-treated hepatocytes were 794, 34 and 57 µmol/l (65.2, 2.8 and 4.7 µg/ml), respectively, indicating that enzyme induction increased the toxicity of 2-methylfuran (Hammond and Fry, 1991).

Male Sprague-Dawley rats (150 - 200 g) were administered a single dose of 50, 100, 200 or 400 mg 2-methylfuran/kg bw in sesame oil by intraperitoneal injection and were sacrificed 24 hours later. The 50 mg/kg bw group did not show any evidence of liver necrosis, but they exhibited endothelial injury, with blebbing of the endothelium into the vascular lumen of the central veins. Animals given 100, 200 or 400 mg 2-methylfuran/kg bw showed a dose-related increase in the severity of hepatocellular injury (e.g. eosinophilic cytoplasm, vacuolation), centrilobular necrosis, and necrosis and sloughing of the bronchiolar epithelium, which, at the high dose, resulted in complete obliteration of numerous respiratory and terminal bronchioles. Dose-related increases in serum glutamic pyruvic transaminase (GPT) were observed up to 200 mg 2-methylfuran/kg bw; however, the levels of serum GPT in the animals given 50 mg 2-methylfuran/kg

bw were not significantly higher than those of the control rats. Free GSH levels in the liver, lungs and kidneys, investigated over a period of 0.5 - 36 hours after administration of 100 mg 2-methylfuran/kg bw, were initially decreased (67.5 % of control in the liver and 87 % of control in the kidneys at 0.5 hours), but then reached or exceeded control levels within 8 - 24 hours (137 % of control in the kidneys and 130 % of control in the lungs at 12 hours). Tissue distribution and covalent binding studies were conducted over a period of 0.5 - 24 hours after an intraperitoneal dose of 100 mg [^{14}C]-2-methylfuran/kg bw. The radiolabelled [^{14}C]-2-methylfuran covalently bound to protein was detected at the highest concentration in the liver, followed by the kidney, lung and blood. Liver and kidney DNA also showed covalent binding of ^{14}C label. Maximal covalent binding to DNA was observed in the liver at 1 hour and in the kidney at 4 hours. With phenobarbital pretreatment a 2-fold increase in binding in the liver was observed. Conversely, *N*-octylimidazole pretreatment decreased the level of covalent binding of the ^{14}C label to proteins and DNA in the liver, lung and kidney. Increased and decreased protein binding and hepatotoxicity measured as serum GPT levels were observed in rats pretreated with phenobarbital and *N*-octylimidazole, respectively. 3-Methylcholanthrene or piperonyl butoxide pretreatment did not affect either covalent binding or hepatotoxicity. These results provide evidence that bioactivation of 2-methylfuran by a CYP system is a prerequisite for tissue necrosis in rats (Ravindranath et al., 1986).

In a study examining GSH and cysteine conjugation on the toxic potential of 2-methylfuran, male Sprague-Dawley rats were treated subcutaneously with a 900 mg/kg bw dose of buthionine sulphoximine 1.5 hours prior to intraperitoneal administration of 100 mg [^{14}C]2-methylfuran/kg bw prepared in sesame oil. Marked decreases in covalent DNA and protein binding in the liver and reduced hepatotoxicity, as indicated by lower serum GPT levels, were observed. Buthionine sulphoximine treatment revealed a transient increase in plasma cysteine levels, concurrent with a decrease in GSH levels. However, administration of 100 mg 2-methylfuran/kg bw 1.5 hours after buthionine sulphoximine administration significantly reduced plasma cysteine levels and increased (20 %) urinary elimination of 2-methylfuran-labelled metabolites compared with the control group ([^{14}C]-2-methylfuran only). Subcutaneous pretreatment with diethylmaleate, a depletor of liver GSH, at 0.4 ml/kg bw increased binding to liver proteins and increased hepatotoxicity, as indicated by a rise in serum GPT levels compared with rats that received only 2-methylfuran. Subcutaneous pretreatment of rats with GSH synthesis promoter L-2-oxothiazolidine-4-carboxylate at a dose of 1000 mg/kg bw resulted in a marked increase of covalent protein binding in the liver and potentiated hepatotoxicity (increased serum GPT levels compared with rats that received only 2-methylfuran). When rats were pretreated with both buthionine sulphoximine and L-2-oxothiazolidine-4-carboxylate, a marked decrease in covalent protein binding in the liver and hepatotoxicity, as indicated by a reduction in serum GPT levels, was observed. No unchanged 2-methylfuran was observed in the urine, indicating that pretreatment did not inhibit metabolic processes (Ravindranath & Boyd, 1991). The authors proposed that buthionine sulphoximine pretreatment indirectly aids in the detoxication of 2-methylfuran through a reduction of GSH supply and an increase in the availability of cysteine, which forms a more stable conjugate with acetylacrolein than GSH.

Adult male Swiss albino mice (10 - 15 per group) were administered 2-ethylfuran (commercial grade, FL-no: 13.092) at 200 mg/kg bw in sesame oil via intraperitoneal injection with or without phenobarbital, piperonyl butoxide or cobalt(II) chloride pretreatment. The mortality rates were 1/10, 2/10, 3/15 and 2/11 for the untreated, phenobarbital pretreatment, piperonyl butoxide pretreatment and cobalt(II) chloride pretreatment groups, respectively. 2-Ethylfuran produced a moderate necrosis of the liver and mild to moderate necrosis of the kidneys. The kidney necrosis was described as a coagulative lesion of the proximal convoluted tubules of the outer cortex, without damage to the glomerular or medullary cells. Piperonyl butoxide and cobalt(II) chloride decreased the severity of necrosis in the liver and kidney (McMurtry et al., 1977).

In the same study, mice were injected intraperitoneally with 70 mg 2-acetylfuran [FL-no: 13.054] (commercial grade)/kg bw in 0.9 % sodium chloride, with and without phenobarbital pretreatment, and 80 mg 2-acetylfuran/kg bw, with and without cobalt(II) chloride pretreatment. The mortality rates were 1/12, 0/12, 0/12 and 0/12 for the 70 mg 2-acetylfuran/kg bw, 70 mg 2-acetylfuran/kg bw plus phenobarbital, 80

mg 2-acetylfuran/kg bw, and 80 mg 2-acetylfuran/kg bw plus cobalt(II) chloride treatment groups, respectively. Mice treated with 2-acetylfuran showed no evidence of toxicity in the kidneys. Hepatic necrosis, described as midzonal-centrilobular necrosis of the parenchymal hepatocytes, was mild in severity with cobalt(II) chloride pretreatment, showing a marked decrease in the incidence and severity of necrosis (McMurtry et al., 1977).

Ten male ICR mice were injected intraperitoneally with 2-ethylfuran (analytical reagent grade) at 2.6 mmol/kg bw (250 mg/kg bw) in sesame oil. Histopathology of tissues collected 24 hours later revealed extensive proximal tubular necrosis of the kidneys and focal hydroptic degeneration of the liver. Significant increases in the plasma urea nitrogen level (approximately 5 times control level) and GPT level were reported (Wiley et al., 1984).

Severe bronchiolar necrosis was reported when 2-ethylfuran (2.6 mmol/kg bw or 250 mg/kg bw) in sesame oil was administered by intraperitoneal injection to male ICR mice. Administration of 1.56 mmol 2-ethylfuran/kg bw (150 mg/kg bw) via intraperitoneal injection to five male ICR mice showed approximately a doubling, compared with control values, of the amount of [^{14}C]-thymidine incorporation into pulmonary DNA measured at 3 days after dosing, which indicates increased cell replication and lung repair (Gammal et al., 1984).

In a study of the tumour-inhibiting properties of 2-heptylfuran [FL-no: 13.069], increased cytosolic glutathione *S*-transferase activity was observed in tissue preparations of the liver, forestomach and small bowel mucosa isolated from 7-weeks old female A/J mice (five mice per group) that received doses of 12, 25, 50 or 80 µmol of 2-heptylfuran dissolved in cottonseed oil via gavage every other day for a total of three doses. A 50 µmol dose of 2-heptylfuran showed a significant increase in acid-soluble sulphydryl levels, which is a good measure of GSH content in tissues, in all four tissue types (liver, small bowel mucosa, forestomach and lung) when compared with controls. At lower dose levels the increases became inconsistent. At the highest dose level the increase was lower than at 50 µmol probably because of the toxicity of the substance (Lam & Zheng, 1992).

In summary, 2-alkyl-substituted furans can be metabolized by side-chain oxidation to initially yield the 1'-alcohol derivative, which can be either conjugated and excreted or oxidized to the corresponding (α,β,-unsaturated) ketone. The conversion to the ketone is anticipated to be reversible, in which case the ketones are reduced to the corresponding alcohols and excreted mainly in the urine. In a second pathway, the furan ring can be oxidized and may undergo rapid ring opening to yield reactive 2-ene-1,4-dicarbonyls (e.g. acetylacrolein) possibly through an unstable epoxide intermediate. These reactive 2-ene-1,4-dicarbonyls can be conjugated with available sulphydryl trapping agents, such as GSH and cysteine, or can be covalently bound to proteins and DNA.

III.2.4. Summary

Subgroup Ia:

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. 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.

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.

Metabolism data confirm that for 5HMF the principal route of metabolism in mice and rats is oxidation to the furoic acid derivative followed by glycine conjugation and rapid elimination in the urine. 5HMF has been shown to be bioactivated *in vitro* to 5-(sulphoxy)-methyl furfural (SMF), through sulphonation of its allylic hydroxyl functional group, catalyzed by sulphotransferases. Recent data have also shown that SMF can be formed *in vivo* upon intravenous administration of 5HMF. The half-life was reported to be 4.2 minutes indicating that this sulphate conjugate is very unstable. Nevertheless, the formation of SMF raises a concern for genotoxicity, which needs specific data on genotoxicity to be overcomed.

Subgroups Ib and Ic:

The candidate substance in subgroup Ib [FL-no: 13.155] is an alkoyl furan which has a keto group in the 1-position of the furan ring substituent. It is considered that the alpha,beta-unsaturated structure in this substance is structurally related and comparable to the structure in acetophenone (EFSA, 2008am). It is anticipated that the ketone in the C_1 position of the sidechain will undergo reduction to the corresponding secondary alcohol which subsequently is excreted after conjugation (JECFA, 2006d). For this substance also ring oxidation followed by ring opening may be anticipated (see subgroup Ic).

The mono-alkyl furans from subgroup Ic, such as the candidate substance 2-octylfuran [FL-no: 13.162], may undergo oxidation (possibly epoxidation of the unsubstituted double bond) and rearrangement to an alkyl substituted dialdehyde. For several 2-alkylsubstituted furans reactivity of these dialdehydes towards proteins and DNA has been demonstrated, resulting in toxicity to liver and kidneys. In addition, oxidation of the C_1'-carbon of the alkyl substituent may result in the formation of an α,β-unsaturated ketone, for which genotoxicity might be anticipated. No data on metabolism of 2,5-dialkyfurans were available, but for this substance also oxidation of the side chain may be anticipated. It is not clear if this substance would be subject to ring-opening.

III.3. Main Group II – Furan derivatives with sulphur substitution

The candidate substances in main group II are sulphur-substituted furan derivatives. These substances are further divided in four subgroups, based on the structural properties of the sulphur-containing functional group. The sulphur is present, either:

- as sulphides [FL-no: 13.114, 13.124, 13.135, 13.141, 13.143, 13.145 and 13.199] (subgroup IIa),
- as free thiol group: [FL-no: 13.108 and 13.149] (subgroup IIb),
- as disulfides [FL-no: 13.113, 13.144, 13.178 and 13.185] (subgroup IIc),
- as trisulfide [FL-no: 13.146] (subgroup IId).

In candidate substance [FL-no: 13.178] the disulfide bridge forms a link between two furan rings. In the other members of this main group II, only one furan ring is present. One of the candidate substances in

subgroup IIb is 4,5-dihydro-3-mercapto-2-methylfuran [FL-no: 13.108]; the only compound containing a non-aromatic ring.

For none of the candidate substances, absorption, distribution, metabolism or elimination studies were found in the published or (submitted) unpublished literature, but it is anticipated that these substances will be absorbed from the GI tract at least to some extent. 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. This supporting substance is a normal ester and thus the ester hydrolysis is relevant for the two candidate substances [FL-no: 13.141, 13.143] in subgroup IIa.

When the supporting substance 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)disulphide was formed (Salzer, 1991). This observation is relevant for the candidate substances in subgroup IIb.

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 sulphides is given in Figure III.3

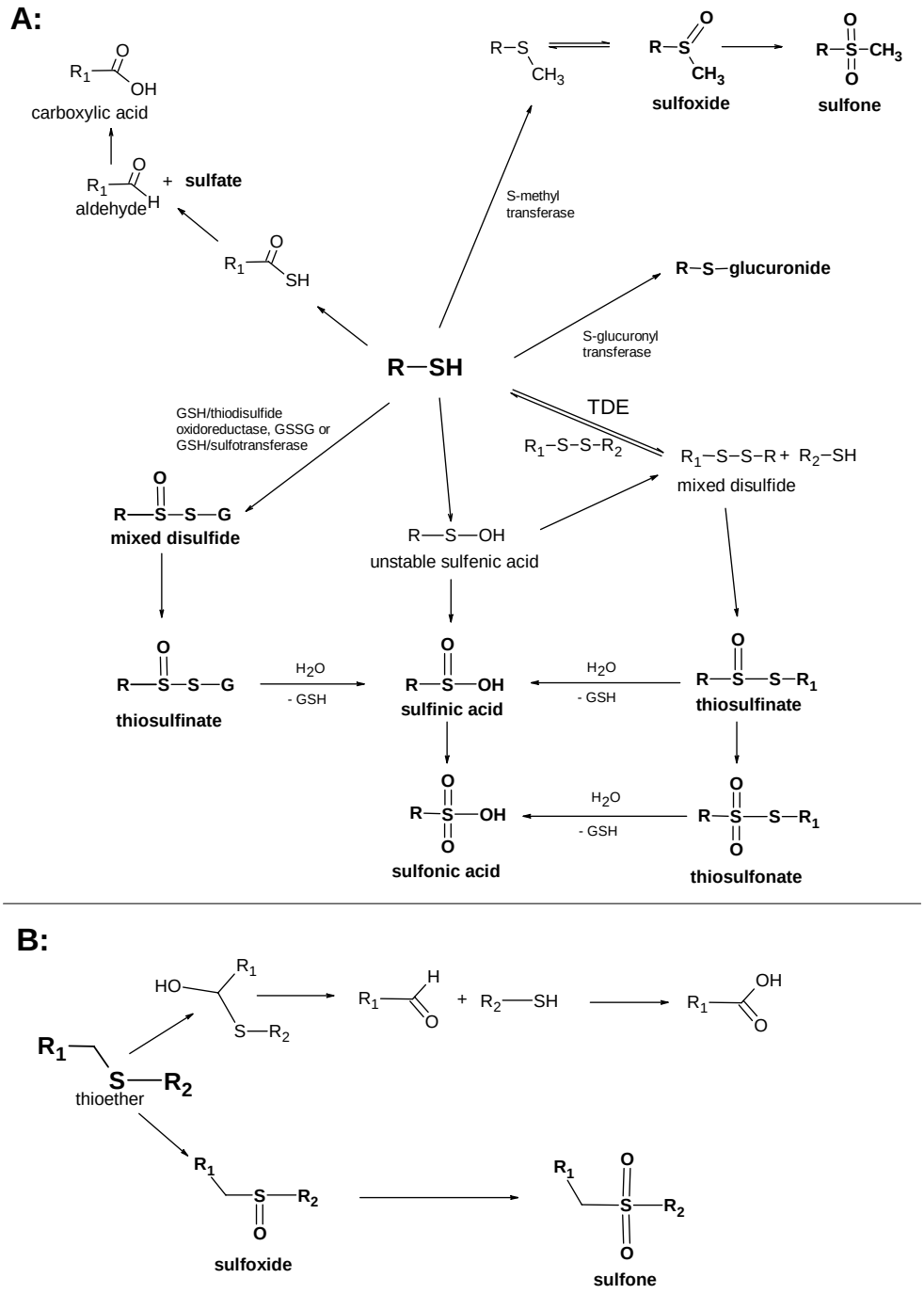


Figure III.3 Metabolism of organic sulphur compounds. A panel: metabolism of thiols and disulphides. B panel: metabolism of thioethers. Structures in bold are major excretion products. TDE = Thiol-Disulphide Exchange

Metabolism of sulphides (subgroup IIa):

The major reactions by which simple sulphides can be metabolised involve oxidation of the sulphor (S) to give sulphoxides, which can be further converted to sulphones. Alternatively, sulphides 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 sulphate (see Figure III.3, Panel A) (Damani, 1987; Richardson et al., 1991). For dipropyl sulphide it was

demonstrated that the sulphoxide was the most important metabolite. Formation of dipropyl sulphone was much less important and complete oxidation, resulting in excretion of free sulphate accounted for less than 3 % of the dose (Nickson & Mitchell, 1994). These authors also indicated that with diethyl and dimethyl sulphides, sulphur oxidation to the sulphones was more important than for dipropylsulphide. No metabolism occurred in other parts of the molecule.

Metabolism of free thiol groups (subgroup IIb):

Thiols are highly reactive *in vivo* mainly because most thiols exist in the ionised form at physiologic pH. Thiols are oxidised to unstable sulphenic acids, which are further oxidised to the corresponding sulphinic and sulphonic acids (Damani, 1987). Methylation of thiols primarily by methyl-S-transferases, which require S-adenosyl methionine as a co-factor, yields methyl sulphides, which then are readily oxidised to sulphoxides and sulphones. Substances which contain free thiol groups may also react with endogenous thiol-containing compounds such as proteins or glutathione to form mixed disulphides 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). Sulphoxides and sulphones are physiologically stable and are excreted unchanged in the urine (Nickson & Mitchell, 1994; Nickson et al., 1995; McBain & Menn, 1969).

The oxidation to sulphoxides is mainly catalysed by two enzyme systems, cytochromes P450 and Flavine-containing Mono Oxygenase (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 P450. This is the case for simple aliphatic (e.g. diethyl sulphide), alicyclic (e.g. thiolane) and aromatic (e.g., ethyl p-tolyl sulphide) sulphides (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 disulphides (subgroup IIc):

The labile nature of the S-S bond also presents a variety of metabolic options for detoxication. Disulphides 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 disulphide 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 disulphides.

Thiol-disulphide exchange (TDE) reactions occur *in vivo* and result from nucleophilic substitution by sulphur. TDE reactions with endogenous cellular thiols (GSH) and disulphides (GSSG) produce mixed disulphides 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).

2-Furfuryl 3-oxo-2-butyl disulphide [FL-no: 13.185], which also carries a keto group in the side chain, might be subject to keto-reduction after which the resulting hydroxyl group could be conjugated. However, there is no data to support this observation.

Metabolism of tri-sulphides (subgroup IId)

Trisulphides may react with endogenous thiols such as reduced glutathione (GSH) or cysteine forming a thiol and a hydropersulphide or perthiol (RSH + R'SSH or R'SSSH or R'SxH, respectively). (Münchberg et al.,

2007) Compared to thiols, perthiols may be strong reducing agents, reacting rapidly with oxidants to form reactive products.

In the literature it is discussed that trisulphides are readily cleaved by GSH to form an equimolar mixture of thiol and perthiol, while tetrasulphides are symmetrically cleaved forming two molecules of perthiol. Redox cycling and production of "active oxygen" may be expected with tri-, tetra- and polysulphides. In this context the chain of reduction is proposed to start with GSH, which reduces the polysulphide and continues via the perthiol and haemoglobin to O_2 which is reduced to H_2O_2. Experiments with a synthetic persulphide, benzyl hydrodisulphide (benzyl-SSH), gave evidence that persulphides may produce reactive oxygen species (O_2*-, H_2O_2 and HO*) under physiologically relevant conditions. This was proposed to be the mechanism behind the cytotoxicity of some naturally occurring products (Chatterji et al., 2005). These interactions have been discussed to greater extent in FGE.08Rev1 (EFSA, 2009z).

Summary

The fourteen representatives of main group II are all furan derivatives containing sulphur substituents as free thiol groups, mono-, di- and tri-sulphides. Substances bearing a free thiol group (subgroup IIb), 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 sulphoxides or sulphones, which can be readily excreted. Alternatively, conjugation with glutathione may occur resulting in a mixed disulphide, which can be reduced to give the free thiols. These can be oxidised to give thiosulphinates or thiosulphones or can undergo thiol-disulphide 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 sulphides (subgroup IIa) may undergo sulphur oxidation reactions, similar to the methylether conjugates of free thiol groups; in additions thiols may be formed. Disulphides (subgroup IIc) can be reduced to give the free thiols, or can be oxidised to give thiosulphinates or thiosulphones. For the one trisulphide candidate substance (in subgroup IId) no direct information on biotransformation was available. However, it has been described that tri and higher sulphides may be cleaved upon reaction with GSH, resulting in the formation of reactive perthiols.

III.4. Summary

The candidate substances in FGE.13Rev2 are all furan derivatives which can be divided into two main groups and several subgroups.

Main group I - Non-sulphur Containing Furan Derivatives

The 13 candidate substances in main group I are ten furfuryl alcohol derivatives such as esters of furfuryl alcohol, furoic acid, or furanacrylic acid, in addition to furoic acid and 5-hydroxymethylfurfuraldehyde (5MHF), a furfural with an additional hydroxymethyl side chain at C5 of the furan ring. These ten substances have been allocated to subgroup Ia. One candidate substance is an alkoyl-substituted furan, allocated to subgroup Ib, and two substances in subgroup Ic are alkylsubstituted furans.

Subgroup Ia

The esters of furfuryl alcohol [FL-no: 13.127, 13.129, 13.130, 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 both subgroups or there hydrolysis products are rapidly absorbed after oral exposure. 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] has been shown to be bioactivated *in vitro* by sulphotransferases to SMF, a reactive intermediate. The same metabolism has been demonstrated *in vivo* after intravenous administration, but the SMF has a very short half-life. This metabolite raises a concern for genotoxicity and therefore additional data on genotoxicity of 5HMF need to be present to overcome this concern.

Subgroups Ib and Ic

The alkyl furans from subgroup Ic, such as the candidate substance 2-octylfuran [FL-no: 13.162], may undergo oxidation (possibly epoxidation of the unsubstituted double bond) and rearrangement to an alkyl substituted dialdehyde (a ring-opening product). For several 2-alkylsubstituted furans reactivity of these dialdehydes towards proteins and DNA has been demonstrated, resulting in toxicity to liver and kidneys. In addition, oxidation of the C_1'-carbon of the alkyl substituent may result in the formation of a ketone, with a structure similar to the candidate and supporting substances in subgroup Ib. Although such ketons may undergo keto-reduction and subsequent conjugation and excretion, it cannot be excluded that a significant part of the dose may also be subject to ring oxidation and opening, similar to the substances in subgroup Ic. No data on metabolism of 2,5-dialkylfurans were available, but for these substances also oxidation of the side chain may be anticipated. It is not clear if these substances would be subject to ring-opening.

Main group II - Sulphur-containing Furan Derivatives

The fourteen representatives of main group II are all sulphur-substituted furan derivatives, containing sulphur substituents as free thiol groups, mono-, di- and tri-sulphides. The substance 4,5-dihydro-3-mercapto-2-methylfuran [FL-no: 13.108] is the only candidate containing a non aromatic ring, in contrast to the other 13 candidate substances in this group.

Substances bearing a free thiol group (subgroup IIb), 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 sulphoxides or sulphones, which can be readily excreted. Alternatively, conjugation with glutathione may occur resulting in a mixed disulphide, which can be reduced to give the free thiols. These can be oxidised to give thiosulphinates or thiosulphones or can undergo thiol-disulphide 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 sulphides (subgroup IIa) may undergo sulphur oxidation reactions, similar to the methylether conjugates of free thiol groups; in additions thiols may be formed. Disulphides (subgroup IIc) can be reduced to give the free thiols, or can be oxidised to give thiosulphinates or thiosulphones. For the one trisulphide candidate substance (in subgroup IId), no direct information on biotransformation was available. However, it has been described that tri and higher sulphides may be cleaved upon reaction with GSH, resulting in the formation of reactive perthiols.

From the above text it is anticipated that the predominant metabolic attack for these substances in main group II will be on the sulphur atom(s) and ring opening is not considered to be a major metabolic route.

III.5. Conclusion

Based on the available information, it is concluded that the candidate substances included in subgroup Ia, as far as their metabolism includes formation of furfural [FL-no: 13.127, 13.129, 13.130, 13.132 and 13.133], a known hepatotoxic reactive aldehyde, cannot be predicted to be metabolised to innocuous or endogenous compounds. The two furoate esters in this subgroup [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 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 candidate substances are metabolised to innocuous products. The same would apply to the candidate substance [FL-no: 13.011]. In addition, to the above mentioned pathways, 5HMF [FL-no: 13.139], the remaining substance in subgroup Ia, has been shown to be bioactivated *in vitro* to a reactive intermediate, SMF, by sulphotransferases. The same metabolism has been demonstrated *in vivo* after intravenous administration, but the SMF has a short half-life. This metabolite raises a concern for genotoxicity and therefore additional data on genotoxicity of 5HMF need to be present to overcome this concern.

In contrast to the substances in subgroup Ia, the candidate substances in subgroups Ib and Ic do not have options for rapid oxidation and subsequent conjugation to innocuous glycine conjugates. For these substances, oxidation and opening of the furan ring, which results in the formation of very reactive metabolites, can be anticipated to be much more important that for the furan derivatives in subgroup Ia.

Given the reactivity of thiols groups, whether free or as obtained from the metabolism of did- or trisulphides, and the importance of thiols groups in cell physiology, it cannot be excluded that these substances interfere with normal cell function. In addition, for the trisulphide candidate substance formation of very reactive partial-metabolites has to be anticipated. Therefore, it is concluded that the following candidate substances cannot be predicted to be metabolised to innocuous products:

[FL-no: 13.114, 13.124, 13.135, 13.141, 13.143, 13.145 and 13.199,] included in subgroup IIa;

[FL-no: 13.108 and 13.149,] included in subgroup IIb;

[FL-no: 13.113, 13.144, 13.178 and 13.185] included in subgroup IIc; and

[FL-no: 13.146] included in subgroup IId.

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 the JECFA at the 55th and 59th meetings. The supporting substances are listed in brackets.

TABLE IV.1: ACUTE TOXICITY

Chemical Name [FL-no:]	Species	Sex	Route	LD50 (mg/kg bw)	Reference	Comments
Main group I – Non-sulphur-containing Furan Derivatives						
(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	IP injection	100	(Phatak & Emerson, 1936)	
Ethyl 2-furoate [13.122]	Rat	NR	IP injection	75 – 100	(Phatak & Emerson, 1936)	Questionable route of exposure.
(Propyl 2-furoate [13.003])	Rat	NR	IP injection	75 – 100	(Phatak & Emerson, 1936)	
Butyl 2-furoate [13.102]	Mouse	NR	Oral	1500	(Stasenkova & Shchirskaya, 1967)	
	Rat	NR	IP injection	100 – 150	(Phatak & Emerson, 1936)	Questionable route of exposure.
(Amyl 2-furoate [13.025])	Rat	NR	IP injection	250 – 500	(Phatak & Emerson, 1936)	
Main group II – Sulphur-containing 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	IP injection	100 – 200	(Doull et al., 1962)	

TABLE IV.1: ACUTE TOXICITY

Chemical Name [FL-no:]	Species	Sex	Route	LD50 (mg/kg bw)	Reference	Comments
(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)	
	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 17 supporting substances and two structurally related substances evaluated by the JECFA at the 55th and 59th meetings. The supporting and related substances are listed in brackets

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

Chemical Name [FL-no:]	Species; Sex No./Group	Route	Dose levels mg/kg bw/day	Duration	NOAEL (mg/kg /day)	Reference	Comments
			Main group I – Non-sulphur-containing 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, F 100	Gavage	0, 30, 60	2 years	> 60	(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-Hydroxymethylfurfur-aldehyde [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

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
							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.
	Mouse; M, F 10	Gavage	0, 94, 188, 375, 750, 1500;	22 days	750	(NTP, 2010c)	Dosing 5 day/week.
	Mouse; M, F 20	Gavage	0, 47, 94, 188, 375, 750	13 weeks	20.2	(NTP, 2010c)	Dosing 5 day/week; the reported value under NOAEL is actually a BMDL (see section 8.2).
	Mouse; M, F 100	Gavage	0, 188, 375, 750	2 years	< 188	(NTP, 2010c)	Dosing 5 day/week.
	Rat; M, F 10	Gavage	0, 94, 188, 375, 750, 1500;	22 days	750	(NTP, 2010c)	Dosing 5 day/week.
	Rat; M, F 20	Gavage	0, 94, 188, 375, 750, 1500; 5d/w	13 weeks	< 188	(NTP, 2010c)	Dosing 5 day/week.
	Rat; M, F 100	Gavage	0, 188, 375, 750	2 years	< 188	(NTP, 2010c)	Dosing 5 day/week.
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.
	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)	Summary publication of study using appropriate methodology. Nu numerical data presented to enable

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
							verification of NOAEL. Otherwise considered valid.
(2-Phenyl-3-carbethoxy furan [13.038])	Rat; M, F 30	Diet	0, 13	90 days	13[2]	(Posternak et al., 1969)	Summary publication of study using appropriate methodology. Nu numerical data presented to enable verification of NOAEL. Otherwise considered valid.
(2-Pentylfuran [FL-no: 13.059])	Rat; M, F 23	Diet	0, 25.6 (26)	90 days	25.6[2]	(Shellenberger, 1971e)	
			Main group II – Sulphur-containing Furan Derivatives				
(2-Methyl-3-furanthiol [13.055])	Rat; M, F 30	Diet	0, 5	90 days	5[2]	(Oser, 1970b)	The target dose was 3.96 mg/kg bw/day; the actual dose was 5 mg/kg bw/d.
(Bis(2-methyl-3-furyl) disulfide [13.016])	Rat; M, F 30	Diet	0, 0.45	90 days	0.45[6]	(Morgareidge and Oser, 1970e)	Good quality study.
	Rat; M, F 30	Diet	0, 3.96	90 days	Not established	(Oser, 1970a)	Good quality study The target dose was 3.96 mg/kg bw/day; the actual dose was 5 mg/kg bw/day.
(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)	Summary publication of study using appropriate methodology. Nu numerical data presented to enable verification of NOAEL. Otherwise considered valid.
(4-[(2-Furanmethyl)thio]-2-pentanone [13.196])	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'-	Rat; M, F	Diet	0, 10	14 days	10	(Gill and Van Miller,	

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
(Thiodimethylene)difuran [13.056])	10					1987b)	
(Bis(2-methyl-3-furyl) tetrasulfide [13.017])	Rat; M, F 30	Diet	0, 0.56	90 days	0.56[2]	(Morgareidge and Oser, 1970f)	Taken as a valid study, but test report did not contain results of carried out histopathology.
(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)	Summary publication of study using appropriate methodology. Nu numerical data presented to enable verification of NOAEL. Otherwise considered valid.
(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)	Summary publication of study using appropriate methodology. Nu numerical data presented to enable verification of NOAEL. Otherwise considered valid.
(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)	Valid study.
(Ethyl 3-(furfurylthio)propionate [13.093])	Rat; M, F 20	Diet	0, 5.78, 17.24	90 days	5.78[2]	(Bio-Research Laboratory, 1980)	Limitedly reported, but considered valid.
(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)	
	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

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 – Non-sulphur-containing Furan Derivatives							
(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 the JECFA at the 55th meeting and three supporting substances evaluated at the 65th and 69th (re-evaluation) meeting. Supporting substances are listed in brackets.

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

Chemical Name [FL-no:]	Test system	Test Object	Concentration	Result	Reference	Comments
Main group I – Non-sulphur-containing Furan Derivatives						
(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, 1999a)	
	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, 1999a)	
	Sister chromatid exchange	Human Lymphocytes	Up to196 µg/ml	Negative	(Jansson et al., 1986)	
	Sister chromatid exchange	Human Lymphocytes	Up to 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, 1999a)	

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

Chemical Name [FL-no:]	Test system	Test Object	Concentration	Result	Reference	Comments
	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)	
	Ames test	S.*typhimurium* TA98, TA100	Up to 1000 µg	Negative[2]	(Osawa & Namiki, 1982)	
	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)	

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

Chemical Name [FL-no:]	Test system	Test Object	Concentration	Result	Reference	Comments
	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., 1987b)	
	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-	EBV- human Burkitt's lymphoma cells	25 mM	Positive[5]	(Costa, 1997)	

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

Chemical Name [FL-no:]	Test system	Test Object	Concentration	Result	Reference	Comments
	links					
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[1]	(Omura et al., 1983)	Positive dose related response inTA100 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[1]	(Shinohara et al., 1986)	Positive results only obtained in TA100 with S9. Reverse dose-respons relationship. Experimental details are lacking.
	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/plate[3]	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., 1987b)	Single dose only.
	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

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

Chemical Name [FL-no:]	Test system	Test Object	Concentration	Result	Reference	Comments
						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 cannot be evaluated.
	Chromosomal aberration	Chinese hamster V79 cells	Up to 2000 µg/ml	Positive[10]	(Nishi et al., 1989)	Weak positive responses 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 responses 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.,	

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

Chemical Name [FL-no:]	Test system	Test Object	Concentration	Result	Reference	Comments
					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)	
(2-acetylfuran [13.054])	UDS	Human hepatocytes	2.19, 4.38, 8.75, 17.5, 35, 70, 140 or 280 µg/ml	Negative	(Durward, 2007a)	New study submitted to JECFA for the 69th meeting.
	Reverse mutation	*S. typhimurium* TA98 and TA100	0.165, 0.330, 0.495 or 0.660 µmol/plate (13.5, 27.1, 40.6 or 54.2 µg/plate)[20]	Negative/positive [1,21]	(Shinohara et al., 1986)	Study reported by JECFA at the 65th meeting.
	DNA damage	*E. coli* PQ37 (SOS chromotest)	Not specified	Slightly positive	(Eder et al., 1993)	Study reported by JECFA at the 65th meeting.
	DNA damage	*B. subtilis* H17 (rec+) and M45 (rec-)	550, 5500 or 55000 µg/disc	Negative/ positive[1,22]	(Shinohara et al., 1986)	Study reported by JECFA at the 65th meeting.
	Chromosomal	CHO cells	0-112.6 mmol/l	Positive[22,23,24]	(Stich et al., 1981b)	Study reported by

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

Chemical Name [FL-no:]	Test system	Test Object	Concentration	Result	Reference	Comments
	aberration		(0-13220 µg/ml)[20]			JECFA at the 65th meeting.
(2-Methylfuran [13.030])	Reverse mutation	*S .typhimurium* TA98 and TA100	0.165, 0.330, 0.495 or 0.660 µmol/plate (13.5, 27.1, 40.6 or 54.2 µg/plate)[13]	Negative[1]	(Shinohara et al., 1986)	Study reported by JECFA at the 65th meeting.
	Reverse mutation	*S. typhimurium* TA98, TA100, TA102 and TA1535	Up to 10 000 µg/plate	Negative[1,14,15]	(Zeiger et al., 1992)	Study reported by JECFA at the 65th meeting.
	Reverse mutation	*S. typhimurium* TA97 and TA104	Up to 10 000 µg/plate	Equivocal[1,14,15]	(Zeiger et al., 1992)	Study reported by JECFA at the 65th meeting.
	Reverse mutation	*S. typhimurium* TA98, TA100 and TA102	11 nmol/plate to 1,1 mmol/ plate (0,9-90 310 µg/plate)[13]	Negative[1]	(Aeschbacher et al., 1989)	Study reported by JECFA at the 65th meeting.
	DNA damage	*Bacillus subtilis* H17 (rec+) and M45 (rec˜Ñ)	0.16, 16 or 1600 µg/disc	Negative/positive [1,16]	(Shinohara et al., 1986)	Study reported by JECFA at the 65th meeting.
	Chromosomal aberration	CHO cells	0-150 mmol/l (0-12315 µg/ml)[13]	Positive[1,17]	(Stich et al., 1981b)	Study reported by JECFA at the 65th meeting.
(2,5-Dimethylfuran [13.029])	Reverse mutation	*S. typhimurium* TA98 and TA100	0.165, 0.330, 0.495 or 0.660 µmol/plate (13.5, 27.1, 40.6 or 54.2 µg/plate)[18]	Negative[1]	(Shinohara et al., 1986)	Study reported by JECFA at the 65th meeting.
	Reverse mutation	*S. typhimurium* TA98 and TA100	Not specified	Negative[1]	(Lee et al., 1994a)	Study reported by JECFA at the 65th meeting.
	Reverse mutation	*S. typhimurium* TA97, TA98, TA100 and TA1535	Up to 3333 µg/plate	Negative[1,14,15]	(Zeiger et al., 1992)	Study reported by JECFA at the 65th meeting.
	DNA damage	*B. subtilis* H17 (rec+) and M45 (rec-)	190, 1900 or 9500 µg/disc	Negative/positive [1,19]	(Shinohara et al., 1986)	Study reported by JECFA at the 65th meeting.
	Chromosomal aberration	Chinese hamster V79 cells	1 mmol/l (96.13 µg/ml)[18]	Negative	(Ochi and Ohsawa, 1985)	Study reported by JECFA at the 65th meeting.

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

Chemical Name [FL-no:]	Test system	Test Object	Concentration	Result	Reference	Comments
	Chromosomal aberration	CHO cells	0-20 mmol/l (0-1923 µg/ml)[18]	Positive[1,17]	(Stich et al., 1981b)	Study reported by JECFA at the 65th meeting.

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.
[13] Calculated using relative molecular mass of 2-methylfuran = 82.1.
[14] Preincubation method.
[15] Occasional incidences of slight to complete clearing of the background lawn at the higher concentrations.
[16] Negative at all concentrations with metabolic activation; positive without metabolic activation.
[17] Clastogenic activity decreased with metabolic activation (statistical significance of results was not specified).
[18] Calculated using relative molecular mass of 2,5-dimethylfuran = 96.13.
[19] Positive at every concentration without metabolic activation; with metabolic activation, negative at 190 µg/disc, but positive at higher concentrations.
[20] Calculated using relative molecular mass of 2-acetylfuran = 110.11.
[21] Positive only in strain TA98 with an increase in the presence of metabolic activation.
[22] Negative at 550 µg/disc; positive at 5500 and 55 000 µg/disc (with and without metabolic activation).
[23] Cytotoxicity was observed at 12 398 µg/ml (112.6 mmol/l) in the presence of metabolic activation.
[24] Clastogenic activity increased with metabolic activation (statistical significance of results was not specified).

TABLE IV.5: SUMMARY OF ADDITIONAL GENOTOXICITY DATA ON 5-HMF (*IN VITRO*)

TABLE IV.5: SUMMARY OF ADDITIONAL GENOTOXICITY DATA ON 5-HYDROXYMETHYLFURFURAL (*IN VITRO*)

Chemical name [FL-no:]	Test system	Test object	Concentration	Result	Reference
5-Hydroxymethylfurfural [13.139]	Ames	*S. typhimurium* TA97, TA98, TA102, TA1535	100-10,000 µg/plate	Negative[1]	(NTP, 2010c)
	Ames	*S. typhimurium* TA100	100-10,000 µg/plate	Weakly positive[2]	(NTP, 2010c)
	Ames	*S. typhimurium* TA100 and TA98	1,500-10,000 µg/plate	Negative[1]	(NTP, 2010c)
	Ames	*S. typhimurium* TA98, TA100, TA1535, TA1537	at 0.5 µg/mL up to 5000 µg/mL	Negative[1]	(Severin et al., 2010)
	Reverse mutation	*E. coli* WP2 uvrA/pKM101	1,500-10,000 µg/plate	Negative[1]	(NTP, 2010c)
	Micronucleus assay	HepG2 cells	0, 5.35, 7.87, 11.57, 17, 25, 36.6 mM	Negative[6]	(Severin et al., 2010)
	SCE induction	V79-hCYP2E1-hSULT1A1 cells	19.8-3808 µM	Positive	(Glatt et al., 2005)
	SCE induction	V79-Mz cells	238-3808 µM,	Positive[7]	(Glatt et al., 2005)
	Comet Assay	HepG2 cells	0, 5.35, 7.87, 11.57, 17, 25, 36.6 mM	Positive[5, 6]	(Severin et al., 2010)
	Comet Assay	Human Caco-2 cells	3,153-12,611 µg/mL (25-100 mM)	Positive[3]	(Durling et al., 2009)
	Comet Assay	Human HEK293 cells	3,153-12,611 µg/mL (25-100 mM)	Positive[3]	(Durling et al., 2009)
	Comet Assay	Mouse lymphoma L5178Y cells	3,153-12,611 µg/mL (25-100 mM)	Positive[3]	(Durling et al., 2009)
	Comet Assay	Chinese hamster V-79 cells	315-12,611 µg/mL (2.5-100 mM)	Positive[4]	(Durling et al., 2009)
	Comet Assay	Chinese hamster V-79-hP-PST cells	315-12,611 µg/mL (2.5-100 mM)	Positive[4]	(Durling et al., 2009)
	Micronucleus assay	Mouse peripheral blood cells	47, 94, 188, 375 or 750 mg/kg bw/d	Negative	(Durling et al., 2009)

[1] With and without S9 metabolic activation.
[2] Without S9 metabolic activation.
[3] Positive only at the highest concentration tested with significant decrease in cell viability.
[4] Positive at high concentration with significantly reduced cell viability.
[5] Cytotoxic at the two highest doses.
[6] 20 hours of exposure.
[7] Weakly positive but statistically significant at each concentration.

In vivo mutagenicity/genotoxicity data are not available for any of the candidate substances of the present flavouring group evaluation from chemical group 14 but for two supporting substances evaluated by the JECFA at the 55th meeting and two supporting substances evaluated at the 65th and 69th (re-evaluation) meeting.

TABLE IV.6: GENOTOXICITY (*IN VIVO*)

Chemical Name [FL-no:]	Test system	Test Object	Route	Dose	Result	Reference	Comments
(Furfuryl alcohol [13.019])	Sex-linked recessive lethal test	*Drosophila melanogaster*	Injection	Up to 6500 ppm	Negative	(Rodriquez-Arnaiz et al., 1989)	
	Sister chromatid exchange	Adult Human Lymphocytes	Inhalation (occupational atmosphere)	32300 mg/m^3	Negative	(Gomez-Arroyo & Souza, 1985)	
	Chromosomal aberration assay	Adult Human Lymphocytes	Inhalation (occupational atmosphere)	32300 mg/m^3	Negative	(Gomez-Arroyo & Souza, 1985)	
	Chromosomal aberration assay	Mouse bone marrow cells	Drinking water	0.5 mg/kg 1 - 2 mg/kg	Negative Positive	(Sujatha & Subramanyam, 1994)	
	Sister chromatid exchange	Mouse bone marrow cells	IP injection	300 mg/kg	Negative	(NTP, 1999a)	
	Chromosomal aberration assay	Mouse bone marrow cells	IP injection	300 mg/kg	Negative	(NTP, 1999a)	
	Micronucleus assay	Mouse bone marrow cells	IP injection	250 mg/kg	Negative	(NTP, 1999a)	
	Mouse bioassay	Tg·AC transgenic mice	Dermal exposure	1.5 mg; 5 d/week for 20 weeks	Negative	(Spalding et al., 2000)	
(Furfural [13.018])	Sex-linked recessive lethal test	*Drosophila melanogaster*	Diet	1000 ppm	Negative	(Woodruff et al., 1985)	
	Sex-linked recessive lethal test	*Drosophila melanogaster*	Injection	100 ppm	Positive	(Woodruff et al., 1985)	
	Sex-linked recessive lethal test	*Drosophila melanogaster*	Injection	Up to 6500 ppm	Negative	(Rodriquez-Arnaiz et al., 1989)	
	Chromosome Loss	*Drosophila melanogaster*	Oral or injected	3750 –5000 ppm. Mated with repair-proficient females	Negative	(Rodriquez-Arnaiz et al., 1992)	
	Chromosome Loss	*Drosophila melanogaster*	Oral or injected	3750 –5000 ppm. Mated with repair-deficient females	Positive	(Rodriquez-Arnaiz et al., 1992)	
	Reciprocal translocations	*Drosophila melanogaster*	Injection	100 ppm	Negative	(Woodruff et al., 1985)	

TABLE IV.6: GENOTOXICITY (*IN VIVO*)

Chemical Name [FL-no:]	Test system	Test Object	Route	Dose	Result	Reference	Comments
	Nondisjunction assay	*Drosophila melanogaster* (females)	Inhalation	1.5 %	Negative[1]	(Muñoz & Barnett, 1999)	
	Sister chromatid exchange	Mouse bone marrow cells	Injection	50 - 200 mg/kg	Negative	(NTP, 1990a)	
	Sperm head abnormalities	Mouse	Oral	4000 ppm daily for 5 weeks	Negative	(Subramanyam et al., 1989)	
	Somatic chromosome mutations	Swiss albino mouse (bone marrow cells)		1000 – 2000 ppm 4000 ppm for 5 days	Negative Positive	(Subramanyam et al., 1989)	
	Sister chromatid exchange	Adult Human Lymphocytes	Inhalation (occupational atmosphere)	9454 mg/m^3	Negative	(Gomez-Arroyo & Souza, 1985)	
	Chromosomal aberration assay	Adult Human Lymphocytes	Inhalation (occupational atmosphere)	9454 mg/m^3	Negative	(Gomez-Arroyo & Souza, 1985)	
	Unscheduled DNA synthesis	Mouse	Oral	50 - 320 mg/kg	Negative	(Edwards, 1999)	
	Unscheduled DNA synthesis	F344 Rat	Oral	5 - 50 mg/kg	Negative	(Phillips et al., 1997)	
	Gene mutation in the λ*lacZ-gene* in liver	Transgenic mouse $CD2F_1$ (BALB/c x DBA/2)	Oral	75 – 300 mg/kg	Negative	(CIVO-TNO, 2003)	
(2-acetylfuran [13.054])	SCE	Mouse bone marrow		1000, 2000 or 3000 mg/l (20, 40 or 60 mg/ kg bw)[2]	Positive	(Sujatha, 2007)	New study submitted to JECFA for the 69th meeting.
	UDS	Rat liver		7 or 21 mg/kg bw	Negative	(Durward, 2007b)	New study submitted to JECFA for the 69th meeting.
	Chromosomal aberration	Mouse bone marrow		1000, 2000 or 3000 mg/l (20, 40 or 60 mg/ kg bw)[2]	Positive[3,4]	(Sujatha et al., 1993)	Study reported by JECFA at the 65th meeting.
	Chromosomal aberration	Mouse spermatocytes		1000, 2000 or 3000 mg/l (20, 40 or 60 mg/ kg bw)[2]	Negative[5]	(Sujatha et al., 1993)	Study reported by JECFA at the 65th

TABLE IV.6: GENOTOXICITY (*IN VIVO*)

Chemical Name [FL-no:]	Test system	Test Object	Route	Dose	Result	Reference	Comments
							meeting.
(2-Methylfuran [13.030])	Chromosomal aberration	Mouse bone marrow cells and spermatocytes		1000, 2000 or 4000 mg/kg (100, 200 or 400 mg/kg bw per day)[6]	Negative	(Subramanyam et al., 1989)	Study reported by JECFA at the 65th meeting.

[1] Exposure to 1 % solutions did not affect the flies' behaviour and they had a 95 % survival rate. At dose concentrations of 1.3 and 1.5 % the results indicate a threshold for the induction of nondisjunction.
[2] Two experimental protocols were utilized. In one experiment, animals received single oral dose administrations of the test compound. In the other experiment, the test compound was orally administered once per day at the same concentrations as in the single-dose study for 5 consecutive days with 24-h intervals between doses.
[3] No effects observed at 20 mg/kg bw dose level and only mild, but significant ($P < 0.05$) effects seen at higher concentrations in bone marrow cells.
[4] Chromosomal aberrations were observed in the presence of significant mitodepression.
[5] A single statistically significant occurrence of increased chromosomal aberrations observed 3 weeks following a single dose administration in the 60 mg/kg bw test group; statistically significant increases in polyploidy and XY univalents observed at weeks 3 and 4 at 60 mg/kg bw in multipledose-treated rats.
[6] Mice received 2-methylfuran in the diet for 5 consecutive days at 24 hours intervals.

TABLE IV.7: SUMMARY OF ADDITIONAL GENOTOXICITY DATA ON 5-HMF (*IN VIVO*)

TABLE IV.7: SUMMARY OF ADDITIONAL GENOTOXICITY DATA ON 5-HYDROXYMETHYLFURFURAL (*IN VIVO*)

Chemical name [FL-no:]	Test system	Test object	Route	Concentration	Result	Reference	EFSA Comments
5-Hydroxymethylfurfural [13.139]	Micronucleus assay	Mouse peripheral blood cells	Gavage	47, 94, 188, 375 or 750 mg/kg bw/d	Negative	(NTP, 2010c)	3-months micronucleous assay.

ANNEX V: DOSE RESPONSE MODELLING FOR 5HMF

In compliance with the Opinion of the EFSA Scientific Committeee on the use of the Bench Mark Dose (BMD) approach in Risk Assessment (EFSA, 2011g), the results obtained in the (NTP, 2010c) subchronic and chronic bioassays with 5HMF [FL-no: 13.139] have been submitted to statistical dose response modelling. The studies performed with rats did not indicate adverse effects at dose levels sufficiently low to drive the determination of a point-of-departure. In addition, the effects reported in rats appeared to be much more of questionable relevance than the effects reported in the mice (see section 8.2 of the main text). Therfore, only the data from the mouse study have been included in this analysis.

For all modelling the statistical package PROAST (version 27.4) has been used. This package is available via: http://www.rivm.nl/en/foodnutritionandwater/foodsafety/proast.jsp. Using this statistical package, 95 % lower confidence limit (single sided) of the Benchmark doses (BMDLs) were calculated (see (EFSA, 2011g)) for the various effects. For each evaluation, all statistical models available in PROAST (i.e. the "EPA-models" plus the Exponential and Hill families of models) were used.

All evaluations were carried out with the following settings:

° Benchmark respons 10 % extra risk (default for quantal data)

° No restrictions for model parameters to limit e.g. steepness of the fitted dose-response curves

° Sex was used as a covariate unless effects were only observed in one sex.

° For all evaluations the following criteria were used to decide on acceptability of modelling output:

° P-value for goodness of fit: 0.05.

° The ratio BMDL/BMDU < 10, otherwise the uncertainty in the data and model results is too high

Dose-response analysis for renal changes in male mice in the NTP (NTP, 2010c) subchronic gavage study with 5HMF.

For the subchronic study in the mouse, the critical effect was the occurrence of cytoplasmic alterations in the renal proximal tubule epithelium at 188 mg/kg or above in male mice.

The following data were extracted from the study report (NTP, 2010c)

TABLE V.1

dose (mg/kg bw/d)	No of animals affected	No of animals per group
0	1	10
47	0	10
94	1	10
188	6	10
375	8	10
750	10	10

The results of this analysis are presented in Table V.2.

TABLE V.2 Results of a BMD analysis of the data from NTP (2010c) on cytoplasmic alterations in the renal proximal tubule epithelium in the male mouse subchronic bioassay with 5HMF.

model	covar	npar	loglik	acc	BMD*	BMDL*	BMDU*
null	NA	1	-41.05	--	NA	NA	NA
full	NA	6	-18.24	--	NA	NA	NA
one-stage	--	2	-22.87	yes	29.5	20.2	45
two-stage	--	3	-20.24	yes	87.4	68.9	116
log-logist	--	3	-19.83	yes	95.9	55.5	145
Weibull	--	3	-20.24	yes	84.3	40.7	145
log-prob	--	3	-19.64	yes	96	57.4	146
gamma	--	3	-19.92	yes	92.6	47.1	147
logistic	--	2	-20.68	yes	84.5	57.1	122
probit	--	2	-20.73	yes	74.2	62	116
LVM: E2-	--	2	-20.67	yes	79.6	54.9	115
LVM: H3-	--	3	-20	yes	90	48.9	143

*: BMD, BMDL and BMDU are in mg/kg bw/d; 5d/w
BMR: 0.1
constraint: no
P-value GoF: 0.05

From the table it can be concluded that all models gave an acceptable fit. Since none of the models is a priori preferable above one of the others, the BMDL, which can be selected from this table, is 20.2 mg/kg bw/d from the EPA one-stage model. This BMDL is the lowest BMDL calculated. A graphical representation of the modelled dose response curve is given in figure V.1. Note that in this plot the maximal observed incidence of the effect is normalised to 1.

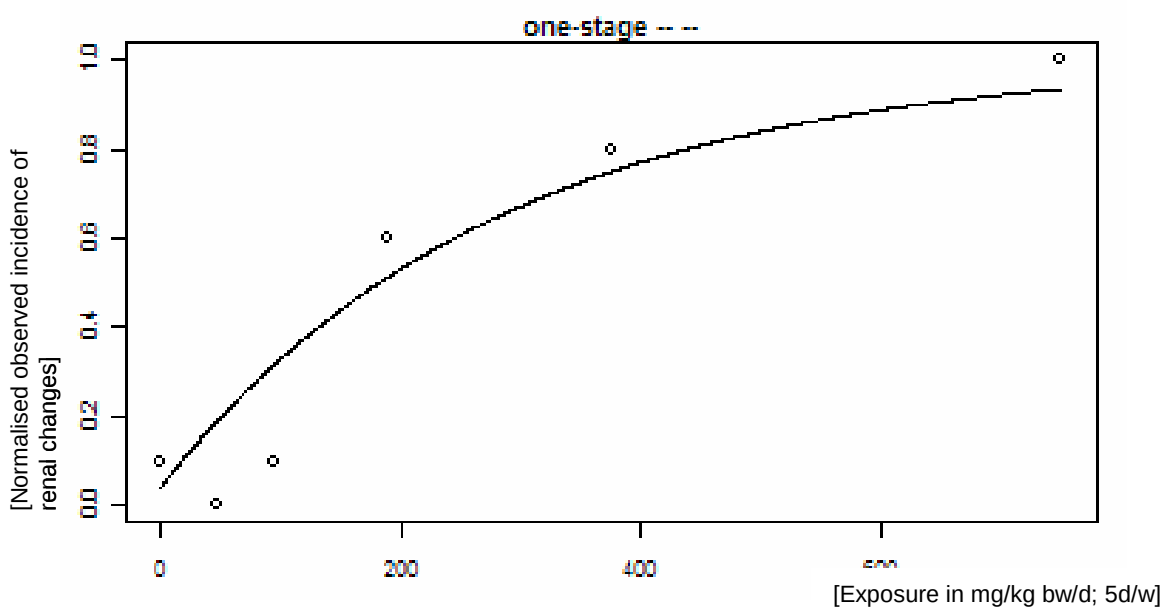


Figure V.1. Plot of EPA one-stage model fitted to the data from NTP (2010c) on cytoplasmic alterations in the renal proximal tubule epithelium in the male mouse subchronic bioassay with 5HMF.

Dose-response analysis for nasal changes in male and femal mice in the NTP (2010c) chronic gavage study with 5HMF.

A spectrum of non-neoplastic nasal lesions considered to be related to administration of 5HMF was observed. The following effects extended into the low dose group in either or both sexes:

- Olfactory epithelial metaplasia,
- Chronic active olfactory epithelium inflammation,
- Bowman gland hyperplasia
- Bowman gland dilatation
- Chronic active inflamation of Bowman glands

The following data were extracted from the study report:

TABLE V.3

		No of animals / group which showed effect*					No of animals / group
dose	**sex**	**OEM**	**ICA**	**GlH**	**GlD**	**GlCAI**	
0	m	1	0	3	16	4	50
188	m	7	6	7	22	12	50
375	m	38	18	45	47	34	50
750	m	43	45	45	45	43	47
0	f	1	0	0	12	1	49
188	f	5	1	7	36	6	49
375	f	30	14	42	48	21	50
750	f	40	41	43	47	38	50

* OEM: Olfactory epithelial metaplasia; ICA: Chronic active olfactory epithelium inflammation; GlH: Bowman gland hyperplasia; GlD: Bowman gland dilatation; GlICAI: Chronic active inflammation of Bowman glands.

For all five effects for which the data were analysed, at least two of the 10 models provided an acceptable fit. The data of the statistical analysis are only given for the analysis of the data on chronic active inflammation of Bowman glands (GlCAI) for which the lowest BMDL of 62.5 mg/kg for the males could be calculated (see Table V.4). The BMDLs for the other effects were 65.6, 142, 152 and 155 mg/kg for GlD, ICA, OEM and GlH, respectively.

TABLE V.4 Results of a BMD analysis of the data from NTP (2010c) on chronic active inflammation of Bowman glands in the mouse chronic bioassay with 5HMF.

model	**covar**	**npar**	**loglik**	**acc**	**BMD***	**BMDL***	**BMDU***
null	NA	1	-266.24	--	NA	NA	NA
full	NA	8	-171.18	--	NA	NA	NA
one-stage	b	3	-180.3	no	41.3	NA	NA
two-stage	b	4	-174.34	yes	101	62.5	147
log-logist	b	4	-172.5	yes	129	97.2	164
Weibull	b	4	-173.81	yes	105	72.7	140
log-prob	b	4	-172.51	yes	132	101	166
gamma	b	4	-173.14	yes	119	83.2	156
logistic	b	3	-175.67	yes	121	102	142
probit	--	2	-186	no	168	NA	NA

LVM: E4-	a	4	-172.04	yes	94.9	77.2	116
LVM: H3-	a	4	-172.07	yes	114	81.6	149

*: BMD, BMDL and BMDU are in mg/kg bw/d; 5d/w
covariate: sex (no covariate is implemented for the probit model)
BMR: 0.1
constraint: no
P-value GoF: 0.05

From Table V.4 it can be concluded that no acceptable fit could be found for the EPA one-stage model and the probit model. All other models gave an acceptable fit. Since none of the accepted models is a priori preferable above one of the others, the BMDL, which can be selected from this table is 62.5 mg/kg bw/d from the EPA two-stage model. This BMDL is the lowest BMDL calculated. A graphical representation of the modelled dose response curve is given in figure V.2. Note that in this plot the maximal observed incidence of the effects is normalised to 1.

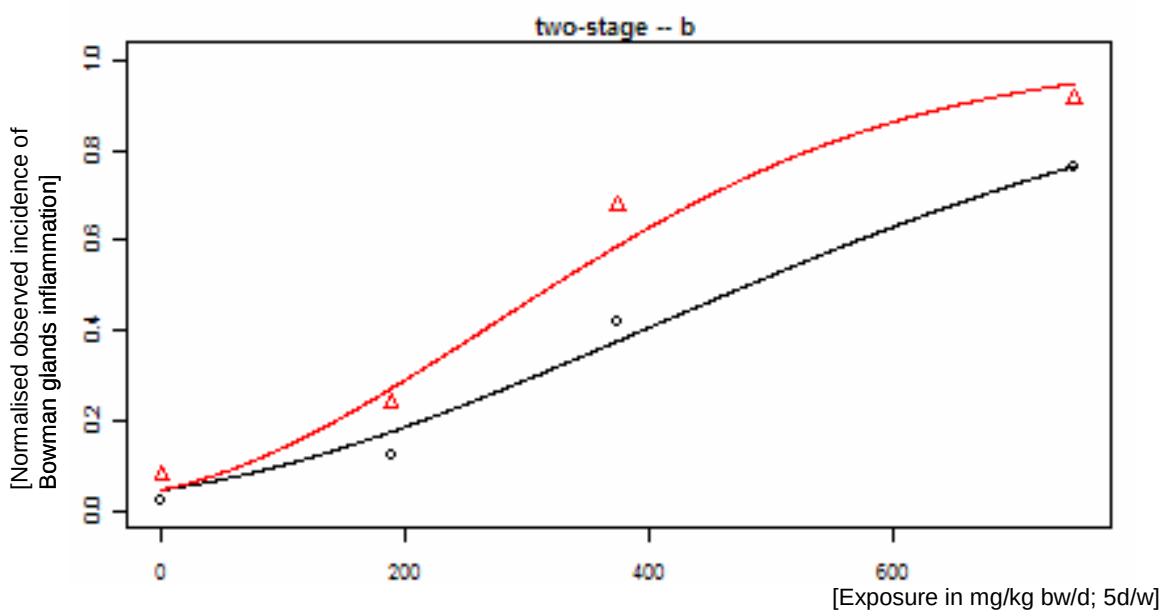


Figure V.2. Plot of EPA two-stage model fitted to the data from NTP (2010c) chronic active inflammation of Bowman glands in the mouse chronic bioassay with 5HMF. (upper curve and triangles males; lower curve and circles: females)

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ABBREVIATIONS

ADI	Acceptable Daily Intake
AFC	Food Additives, Flavourings, Processing Aids and Materials in Contact with Food
AST	Aspartate aminotransferase
BUN	Blood Urea Nitrogen
BW	Body weight
CAS	Chemical Abstract Service
CEF	Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CHO	Chinese hamster ovary (cells)
CoE	Council of Europe
DNA	Deoxyribonucleic acid
EC	European Commission
EFSA	The European Food Safety Authority
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FEMA	Flavor and Extract Manufacturers Association
FGE	Flavouring Group Evaluation
FLAVIS (FL)	Flavour Information System (database)
FMO	Flavine-containing Mono Oxygenase
GI	Gastrointestinal
GLP	Good Laboratory Practice
GPT	Glutamic Pyruvic Transaminase
GSH	Glutathione
HMF	Hydroxymethylfurfualdehyde
HMFA	Hydroxymethyl-2-furoic acid
ID	Identity
IP	Intra Peritoneal
IOFI	International Organization of the Flavour Industry
IR	Infrared spectroscopy
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
LD_{50}	Lethal Dose, 50 %; Median lethal dose
LH	Luteinizing hormone
MS	Mass spectrometry
MSDI	Maximised Survey-derived Daily Intake
mTAMDI	Modified Theoretical Added Maximum Daily Intake
NAD	Nicotinamide Adenine Dinucleotide

NADP	Nicotinamide Adenine Dinucleotide Phosphate
NADPH	Nicotinamide Adenine Dinucleotide Phosphate, reduced form
NCE	Normochromatic erythrocytes
No	Number
NOAEL	No Observed Adverse Effect Level
NOEL	No Observed Effect Level
NTP	National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
PCE	Polychromatic erythrocytes
SCE	Sister Chromatid Exchange
SCF	Scientific Committee on Food
SMART	Somatic Mutation and Recombination Test
TAMDI	Theoretical Added Maximum Daily Intake
TDE	Thiol-disulphide exchange
UDS	Unscheduled DNA Synthesis
WHO	World Health Organisation