EFSA ; Scientific Opinion on Flavouring Group Evaluation 67, Revision 1 (FGE.67Rev.1): Consideration of 40 furan-substituted aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids and related esters, sulfides, disulfides and ethers evaluated by JECFA at the 65th meeting (JECFA, 2006b) and re-evaluated at the 69th meeting (JECFA, 2009c)

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SCIENTIFIC OPINION

Scientific Opinion on Flavouring Group Evaluation 67, Revision 1 (FGE.67Rev.1):

Consideration of 40 furan-substituted aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids and related esters, sulfides, disulfides and ethers evaluated by JECFA at the 65th meeting (JECFA, 2006b) and re-evaluated at the 69th meeting (JECFA, 2009c)[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 consider evaluations of flavouring substances assessed since 2000 by the Joint FAO/WHO Expert Committee on Food Additives (the JECFA), and to decide whether further evaluation is necessary, as laid down in Commission Regulation (EC) No 1565/2000. The present consideration concerns a group of 33 furan-substituted aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids and related esters, sulfides, disulfides and ethers evaluated by the JECFA. In the present version of FGE.67 eight additional substances have been included. The 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. For twenty-two substances [FL-no: 13.029, 13.030, 13.045, 13.052, 13.054, 13.059, 13.061, 13.066, 13.069, 13.070, 13.083, 13.092, 13.101, 13.103, 13.105, 13.106, 13.107, 13.123, 13.138, 13.148, 13.163 and 13.191] a concern for genotoxicity was raised and therefore these were not evaluated using the Procedure. The Panel concluded that 8 substances [FL-no:

1 On request from the Commission, Question No EFSA-Q-2011-00867, EFSA-Q-2011-00868, EFSA-Q-2011-00869, EFSA-Q-2011-00870, EFSA-Q-2011-00871, EFSA-Q-2011-00872, EFSA-Q-2011-00873, EFSA-Q-2011-00874, EFSA-Q-2011-00875, EFSA-Q-2011-00876 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, Riccardo Crebelli, Karl-Heinz Engel, Henrik Frandsen, Rainer Gürtler, Frances Hill, Trine Husøy, John Christian Larsen, Catherine Leclercq, 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.

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13.006, 13.021, 13.022, 13.023, 13.024, 13.074, 13.116 and 13.190] do not give rise to safety concerns at the levels of dietary intake, estimated on the basis of the MSDI approach. For one substance [FL-no: 13.058] additional toxicity data are requested. Besides the safety assessment of these substances, the specifications for the materials of commerce have been considered. For three substances [FL-no: 13.031, 13.045 and 13.047] data on specifications / stereoisomerism are missing.

KEY WORDS

Furan-substituted; genotoxic; JECFA, 65th meeting, FGE.13Rev2; FGE.65; FGE.66Rev1.

SUMMARY

The European Food Safety Authority (EFSA) asked the Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (the Panel) to provide scientific advice to the Commission on the implications for human health of chemically defined flavouring substances used in or on foodstuffs in the Member States. In particular, the Panel was requested to consider the Joint FAO/WHO Expert Committee on Food Additives (the JECFA) evaluations of flavouring substances assessed since 2000, and to decide whether no further evaluation is necessary, as laid down in Commission Regulation (EC) No 1565/2000. These flavouring substances are listed in the Register, which was adopted by Commission Decision 1999/217/EC and its consecutive amendments.

The present Flavouring Group Evaluation deals with 39 substances, 38 substances of which were previously considered by the JECFA in a group of 40 furan-substituted aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids and related esters, sulfides, disulfides and ethers (JECFA, 2009a). One of these 40 substances [FL-no: 13.192] appeared to be a synonym of substance [FL-no: 13.178] which has already been evaluated in FGE.13Rev2. Therefore this substance [FL-no: 13.192] will not be further considered in this FGE and should be removed from the Register. Another substance [FL-no: 13.176] will be evaluated in FGE.75 rather than in FGE.67, because of better structural similarity with candidate substances in FGE.75. Furthermore, one candidate substance [FL-no: 13.031] from FGE.66Rev1 has been included in this revision of FGE.67, because this substance has better structural similarity to a candidate flavouring substance [FL-no: 13.074] in FGE.67 than to the other candidate flavouring substances in FGE.66Rev1. The flavouring substances considered in this FGE have been allocated to various subgroups, based on their chemical structures.

Thirteen of the 15 substances in subgroups VI-A and VI-B are alpha,beta-unsaturated carbonyls, which have been evaluated by EFSA in FGE.19 context with respect to a concern for a possible genotoxic potential. This concern for genotoxicity could not be alleviated for the six substances in subgroup VI-A [FL-no: 13.034, 13.043, 13.044, 13.046, 13.137 and 13.150], corresponding to FGE.19 subgroup 4.6 (EFSA, 2008b). These six substances were therefore not further considered in this FGE.

The thirty-three candidate substances considered in this FGE [FL-no: 13.006, 13.021, 13.022, 13.023, 13.024, 13.029, 13.030, 13.031, 13.045, 13.047, 13.052, 13.054, 13.058, 13.059, 13.061, 13.066, 13.069, 13.070, 13.074, 13.083, 13.092, 13.101, 13.103, 13.105, 13.106, 13.107, 13.116, 13.123, 13.138, 13.148, 13.163, 13.190 and 13.191] are structurally related to the group of 27 furfuryl and furan derivatives evaluated by EFSA in FGE.13Rev2. Part of the substances is also structurally related to a group of 33 sulphur-substituted furan derivatives used as flavouring agents, evaluated by EFSA in FGE.65 and another part is structurally related to 14 furfuryl derivatives evaluated in FGE.66Rev1 (EFSA, 2009an; EFSA, 2011ad).

The Panel agrees with the JECFA for 22 of the substances [FL-no: 13.029, 13.030, 13.045, 13.052, 13.054, 13.059, 13.061, 13.066, 13.069, 13.070, 13.083, 13.092, 13.101, 13.103, 13.105, 13.106, 13.107, 13.123, 13.138, 13.148, 13.163 and 13.191] that these substances cannot be evaluated through the Procedure, based on concerns with respect to genotoxicity.

In line with the approaches taken in previous FGEs (FGE.13Rev2, FGE.65 and FGE.66Rev1), the Panel considers that 11 substances [FL-no: 13.006, 13.021, 13.022, 13.023, 13.024, 13.031, 13.047, 13.058, 13.074, 13.116 and 13.190] can be evaluated using the Procedure.

It was concluded for ten substances [FL-no: 13.006, 13.021, 13.022, 13.023, 13.024, 13.031, 13.047, 13.074, 13.116 and 13.190], that they would be of no safety concern at their estimated intake levels based on the MSDI approach. For the remaining substance [FL-no: 13.058] this conclusion could not be drawn due to lack of an adequate NOAEL.

For all 33 substances use levels are needed to calculate the mTAMDIs in order to identify those flavouring substances that need more refined exposure assessment and to finalise the evaluation.

In order to determine whether the conclusion for the evaluated substances can be applied to the materials of commerce, it is necessary to consider the available specifications. Adequate specifications including complete purity criteria and identity are available for 30 of the 33 substances. Information on stereoisomeric composition has not been submitted for [FL-no: 13.047]. Information on solubility in water is missing for [FL-no: 13.045] and information on melting point is missing for [FL-no: 13.031].

Thus, for 25 of the 33 substances considered in this FGE the Panel has reservations. For three substances [FL-no: 13.031, 13.045 and 13.047] data on specifications / stereoisomerism are missing. For 23 substances [FL-no: 13.029, 13.030, 13.045, 13.052, 13.054, 13.058, 13.059, 13.061, 13.066, 13.069, 13.070, 13.083, 13.092, 13.101, 13.103, 13.105, 13.106, 13.107, 13.123, 13.138, 13.148, 13.163 and 13.191] the Panel concluded that additional toxicity / genotoxicity data are required.

For the remaining eight of these 33 furan derivatives [FL-no: 13.006, 13.021, 13.022, 13.023, 13.024, 13.074, 13.116 and 13.190] the Panel concluded that they would be of "no safety concern at estimated levels of intake as flavouring substances" based on the MSDI approach.

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

Substances which are listed in the Register are to be evaluated according to the evaluation programme laid down in Commission Regulation (EC) No 1565/2000 (EC, 2000a), which is broadly based on the Opinion of the Scientific Committee on Food (SCF, 1999a).

Commission Regulation (EC) No 1565/2000 lays down that substances that are contained in the Register and will be classified in the future by the Joint FAO/WHO Expert Committee on Food Additives (the JECFA) so as to present no safety concern at current levels of intake will be considered by the European Food Safety Authority (EFSA), who may then decide that no further evaluation is necessary.

In the period 2000 – 2008, during its 55th, 57th, 59th, 61st, 63rd, 65th, 68th and 69th meetings, the JECFA evaluated about 1000 substances, which are in the EU Register.

TERMS OF REFERENCE

EFSA is requested to consider the JECFA evaluations of flavouring substances assessed since 2000, and to decide whether no further evaluation is necessary, as laid down in Commission Regulation (EC) No 1565/2000 (EC, 2000a). These flavouring substances are listed in the Register which was adopted by Commission Decision 1999/217 EC (EC, 1999a) and its consecutive amendments.

ASSESSMENT

The approach used by EFSA for safety evaluation of flavouring substances is referred to in Commission Regulation (EC) No 1565/2000 (EC, 2000a), hereafter named the "EFSA Procedure". This Procedure is based on the Opinion of the Scientific Committee on Food (SCF, 1999a), which has been derived from the evaluation procedure developed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 1995; JECFA, 1996a; JECFA, 1997a; JECFA, 1999b), hereafter named the "JECFA Procedure". The Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (the Panel) compares the JECFA evaluation of structurally related substances with the result of a corresponding EFSA evaluation, focussing on specifications, intake estimations and toxicity data, especially genotoxicity data. The evaluations by EFSA will conclude whether the flavouring substances are of no safety concern at their estimated levels of intake, whether additional data are required or whether certain substances should not be put through the EFSA Procedure.

The following issues are of special importance.

Intake

In its evaluation, the Panel as a default uses the "Maximised Survey-derived Daily Intake" (MSDI) approach to estimate the *per capita* intakes of the flavouring substances in Europe.

In its evaluation, the JECFA includes intake estimates based on the MSDI approach derived from both European and USA production figures. The highest of the two MSDI figures is used in the evaluation

by the JECFA. It is noted that in several cases, only the MSDI figures from the USA were available, meaning that certain flavouring substances have been evaluated by the JECFA only on the basis of these figures. For Register substances for which this is the case the Panel will need EU production figures in order to finalise the evaluation.

When the Panel examined the information provided by the European Flavour Industry on the use levels in various foods, it appeared obvious that the MSDI approach in a number of cases would grossly underestimate the intake by regular consumers of products flavoured at the use level reported by the Industry, especially in those cases where the annual production values were reported to be small. In consequence, the Panel had reservations about the data on use and use levels provided and the intake estimates obtained by the MSDI approach. It is noted that the JECFA, at its 65th meeting considered "how to improve the identification and assessment of flavouring agents, for which the MSDI estimates may be substantially lower than the dietary exposures that would be estimated from the anticipated average use levels in foods" (JECFA, 2006c).

In the absence of more accurate 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.

As information on use levels for the flavouring substances has not been requested by the JECFA or has not otherwise been provided to the Panel, it is not possible to estimate the daily intakes using the mTAMDI approach for the substances evaluated by the JECFA. The Panel will need information on use levels in order to finalise the evaluation.

Threshold of 1.5 Microgram/Person/Day (Step B5) Used by the JECFA

The JECFA uses the threshold of concern of 1.5 microgram/person/day as part of the evaluation procedure:

"The Committee noted that this value was based on a risk analysis of known carcinogens which involved several conservative assumptions. The use of this value was supported by additional information on developmental toxicity, neurotoxicity and immunotoxicity. In the judgement of the Committee, flavouring substances for which insufficient data are available for them to be evaluated using earlier steps in the Procedure, but for which the intake would not exceed 1.5 microgram per person per day would not be expected to present a safety concern. The Committee recommended that the Procedure for the Safety Evaluation of Flavouring Agents used at the forty-sixth meeting be amended to include the last step on the right-hand side of the original procedure ("Do the condition of use result in an intake greater than 1.5 microgram per day?") (JECFA, 1999b).

In line with the Opinion expressed by the Scientific Committee on Food (SCF, 1999), the Panel does not make use of this threshold of 1.5 microgram per person per day.

Genotoxicity

As reflected in the Opinion of SCF (SCF, 1999a), the Panel has in its evaluation focussed on a possible genotoxic potential of the flavouring substances or of structurally related substances. Generally, substances for which the Panel has concluded that there is an indication of genotoxic potential *in vitro,* will not be evaluated using the EFSA Procedure until further genotoxicity data are provided. Substances for which a genotoxic potential *in vivo* has been concluded, will not be evaluated through the Procedure.

Specifications

Regarding specifications, the evaluation by the Panel could lead to a different opinion than that of JECFA, since the Panel requests information on e.g. isomerism.

Structural Relationship

In the consideration of the JECFA evaluated substances, the Panel will examine the structural relationship and metabolism features of the substances within the flavouring group and compare this with the corresponding FGE.

HISTORY OF THE EVALUATION

FGE	Opinion adopted by EFSA	Link	No. of candidate substances
FGE.67	26 November 2009	http://www.efsa.europa.eu/en/scdocs/scdoc/1404.htm	25
FGE.67Rev1	6 July 2011		33

The present revision of FGE.67, FGE.67Rev1 includes the consideration of additional eight substances. The sub-grouping of the two ketones [FL-no: 13.045 and 13.138] has been changed from subgroup III to subgroup VI-B. Due to a newly identified concern for genotoxicity, the evaluation of these two ketones has been revised since the previous version of FGE.67.

Seven of the eight additional substances [FL-no: 13.054, 13.066, 13.070, 13.083, 13.101, 13.105, 13.163] are alpha,beta-unsaturated ketones originally allocated to FGE.19 subgroup 4.5 (FGE.221) (EFSA 2008b). This structural characteristic is a known alert for genotoxicity, which may preclude the evaluation of substances through the Procedure. EFSA concluded in November 2008 that the alpha,beta-unsaturated structure in conjugation with an aromatic ring system, which is present in these seven substances, is comparable to acetophenone, i.e. no longer considered a structural alert for genotoxicity (EFSA, 2011ac). These seven substances are shown in Table 1.1.2, Subgroup VI-B.

The eighth substance, 2-benzofurancarboxaldehyde [FL-no: 13.031], is an alpha,beta-unsaturated aldehyde originally allocated to FGE.19 subgroup 4.3 (FGE.219) (EFSA 2008b). Also for this substance the alpha,beta-unsaturated structure is in conjugation with an aromatic ring system which is comparable to the situation in benzaldehyde for which no genotoxic concern is present (EFSA, 2011ac). Accordingly, this substance can also be evaluated using the Procedure. Although the substance originally was allocated by the JECFA to the group of furfuryl alcohol derivatives covered in FGE.66Rev1, the Panel considered that the substance belongs to the benzofurans in group V-B of the present FGE.67 (see Table 1.1.2).

1. Presentation of the Substances in the JECFA Flavouring Group

1.1. Description

The JECFA has evaluated a group of 40 diverse furan derivatives, first at their 65th meeting (JECFA, 2006b) where a request for additional data was expressed. The furan group was on the agenda again at the 69th JECFA meeting (JECFA, 2009c) where additional data had been provided.

1.1.1. JECFA Status

The JECFA expressed at its 69th meeting (JECFA, 2009a):

"At its sixty-fifth meeting (JECFA, 2006b), the JECFA reviewed a group of 40 furan-substituted aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids and related esters, sulfides, disulfides and ethers. The JECFA at that meeting took note of the extensive evidence for the genotoxicity of several members of this group of flavouring agents related to furan, including the clastogenicity of 2-furyl methyl ketone (JECFA-no: 1503) (2-acetylfuran, [FL-no: 13.054]) in mouse bone marrow. This substance accounts for 87 - 96 % of total exposure to this group of flavouring

agents. Noting also that furan is carcinogenic and is known to undergo epoxidation and ring opening to form a reactive 2-ene-1,4-dicarbonyl intermediate, the JECFA at its sixty-fifth meeting expressed concern that the observed genotoxicity might be due to formation of a reactive metabolite. Few data on genotoxicity *in vivo* were available, and specific assays to address potential carcinogenicity *in vivo* were lacking. The JECFA at its sixty-fifth meeting therefore concluded that the Procedure for the Safety Evaluation of Flavouring Agents could not be applied to this group because of the above concerns. It was also concluded that studies of metabolism and *in vivo* assays for deoxyribonucleic acid (DNA) reactivity, mutagenicity and carcinogenic potential of members of this group with representative structures would assist in resolving the concerns (JECFA, 2006b).

Additional studies of genotoxicity *in vitro* and *in vivo* with 2-furyl methyl ketone [FL-no: 13.054] were available to the Committee (the JECFA) at its present meeting (Durward, 2007a, 2007b; Sujatha, 2007). The Committee (JECFA, 2009c) included the new studies in its re-evaluation of the group of 40 furan-substituted aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids and related esters, sulfides, disulfides and ethers."

"The new data on 2-furyl methyl ketone (JECFA-no: 1503) (2-acetylfuran, [FL-no: 13.054]) available to the Committee at its present meeting were a study on UDS in cultured hepatocytes *in vitro*, a study on UDS in rat liver *in vivo*/*in vitro* and a test for SCEs in mouse bone marrow *in vivo*. 2-Furyl methyl ketone did not induce UDS either *in vitro* or *in vivo/in vitro*. However, it did induce SCEs, confirming the concern for clastogenicity as expressed by the Committee at its previous meeting. The Committee at its present meeting therefore considered that the new data available did not resolve the concerns expressed previously ".

"The Committee concluded that the Procedure for the Safety Evaluation of Flavouring Agents could not be applied to this group because of the unresolved toxicological concerns. Studies that would assist in the safety evaluation include investigations of the influence of the nature and position of ring substitution on metabolism and on covalent binding to macromolecules. Depending on the findings, additional studies might include assays related to the mutagenic and carcinogenic potential of representative members of this group"

At its 55th meeting (JECFA, 2001b) the JECFA has evaluated the substance 2-benzofuran carboxaldehyde [FL-no: 13.031] via the Procedure for the evaluation of flavouring substances. The JECFA conclude that the substance was of no safety concern.

1.1.2. EFSA Considerations

The group of furan derivatives evaluated by the JECFA is a very diverse group of 40 flavouring substances which can be subdivided into six major subgroups with further subdivision of subgroup V and VI, as depicted in the Table 1.1.2. The substance [FL-no: 13.031] has also been included in Table 1.1.2. [FL-no: 13.031] was evaluated by the JECFA at the 55th meeting (JECFA, 2001b) in the group of furfuryl derivatives evaluated by EFSA in FGE.66Rev1, but as substance [FL-no: 13.031] has structural similarity to [FL-no: 13.074] considered in FGE.67, it will be included in the current revision of FGE.67.

The structures of these 41 substances are given in Table 1.1.2.

Table 1.1.2 Sub-grouping of 40 furan-substituted substances considered by JECFA at the 55th, 65th and 69th meetings

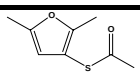
Sub-group*	FL-no JECFA-no	EU Register name	Structural formula	Supporting substances, represented in FGE and subgroup
I	13.116 1523	2,5-Dimethyl-3-thioacetoxyfuran		FGE.65 (Thioesters)

Table 1.1.2 Sub-grouping of 40 furan-substituted substances considered by JECFA at the 55th, 65th and 69th meetings

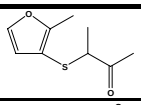
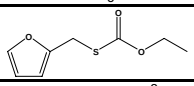
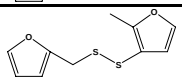
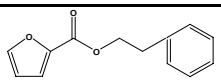
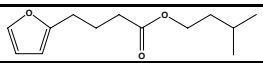
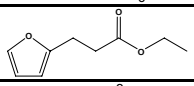
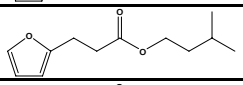
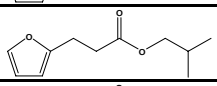
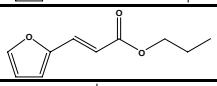
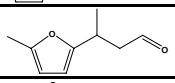
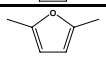
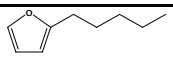
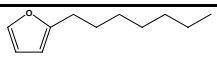
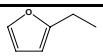
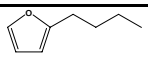
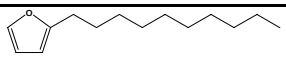
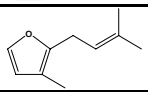
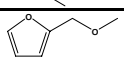
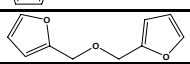
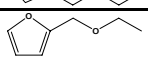
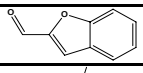
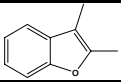
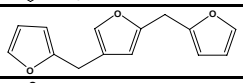
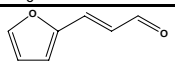
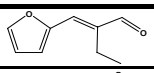
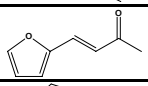
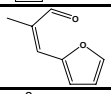
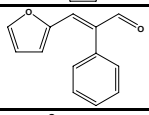
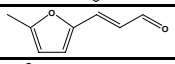
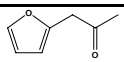
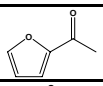
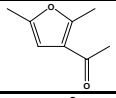
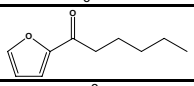
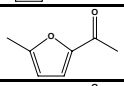
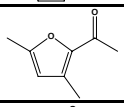
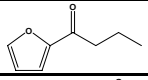
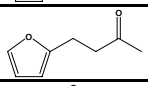
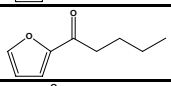
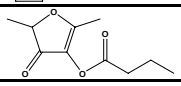
Sub-group*	FL-no JECFA-no	EU Register name	Structural formula	Supporting substances, represented in FGE and subgroup
I	13.190 1525	3-((2-Methyl3-furyl)thio)-2-butanone		FGE.13Rev2 Subgroup Iia (Sulphides)
I	13.191 1526	o-Ethyl S-(2-furylmethyl)thiocarbonate		None in FGE.13Rev2, FGE.65 or FGE.66Rev1
I	13.192* 1524	Furfuryl 2-methyl-3-furyldisulfide		FGE.13Rev2 Subgroup Iic (Disulphides)
II	13.006 1517	Phenethyl 2-furoate		FGE.13Rev2 Subgroup Ia (Related to furfuryl alcohol)
III	13.021 1516	Isopentyl 4-(2-furan)butyrate		FGE.13Rev2 Subgroup Ia
III	13.022 1513	Ethyl 3(2-furyl)propionate		FGE.13Rev2 Subgroup Ia
III	13.023 1515	Isopentyl 3-(2-furan)propionate		FGE.13Rev2 Subgroup Ia
III	13.024 1514	Isobutyl 3-(2-furyl)propionate		FGE.13Rev2 Subgroup Ia
III	13.047 1518	Propyl 3-(2-furyl)acrylate		FGE.13Rev2 Subgroup Ia
III	13.058 1500	3-(5-Methyl-2-furyl) butanal		FGE.13Rev2 Subgroup Ia
IV	13.029 1488	2,5-Dimethylfuran		FGE.13Rev2 Subgroup Ic (Alkyl-substituted furans)
IV	13.030 1487	2-Methylfuran		FGE.13Rev2 Subgroup Ic
IV	13.059 1491	2-Pentylfuran		FGE.13Rev2 Subgroup Ic
IV	13.069 1492	2-Heptylfuran		FGE.13rev2 Subgroup Ic
IV	13.092 1489	2-Ethylfuran		FGE.13rev2 Subgroup Ic
IV	13.103 1490	2-Butylfuran		FGE.13rev2 Subgroup Ic
IV	13.106 1493	2-Decylfuran		FGE.13rev2 Subgroup Ic
IV	13.148 1494	3-Methyl-2(3-methylbut-2-enyl)furan		FGE.13rev2 Subgroup Ic
V-A	13.052 1520	Furfuryl methyl ether		None in FGE.13Rev2, FGE.65 or FGE.66Rev1
V-A	13.061 1522	Difurfuryl ether		None in FGE.13Rev2, FGE.65 or FGE.66Rev1
V-A	13.123 1521	Ethyl furfuryl ether		None in FGE.13Rev2, FGE.65 or FGE.66Rev1
V-B	13.031 751	2-Benzofurancarboxaldehyde		None in FGE.13Rev2, FGE.65 or FGE.66Rev1
V-B	13.074 1495	2,3-Dimethylbenzofuran		None in FGE.13Rev2, FGE.65 or FGE.66Rev1
V-C	13.107 1496	2,4-Difurfurylfuran		None in FGE.13Rev2, FGE.65 or FGE.66Rev1
VI-A	13.034 1497	3-(2-Furyl)acrylaldehyde		Not yet considered due to concern for genotoxicity.

Table 1.1.2 Sub-grouping of 40 furan-substituted substances considered by JECFA at the 55th, 65th and 69th meetings

Sub-group*	FL-no JECFA-no	EU Register name	Structural formula	Supporting substances, represented in FGE and subgroup
VI-A	13.043 1501	Furfurylidene-2-butanal		Not yet considered due to concern for genotoxicity.
VI-A	13.044 1511	4-(2-Furyl)but-3-en-2-one		Not yet considered due to concern for genotoxicity.
VI-A	13.046 1498	3-(2-Furyl)-2-methylprop-2-enal		Not yet considered due to concern for genotoxicity.
VI-A	13.137 1502	3-(2-Furyl)-2-phenylprop-2-enal		Not yet considered due to concern for genotoxicity.
VI-A	13.150 1499	3-(5-Methyl-2-furyl)prop-2-enal		Not yet considered due to concern for genotoxicity.
VI-B	13.045 1508	1-(2-Furyl)-propan-2-one		FGE.13Rev2 Subgroup Ib (Alkoyl-substituted furans)
VI-B	13.054 1503	2-Acetylfuran		FGE.13Rev2 Subgroup Ib
VI-B	13.066 1506	3-Acetyl-2,5-dimethylfuran		FGE.13Rev2 Subgroup Ib
VI-B	13.070 1512	2-Hexanoylfuran		FGE.13Rev2 Subgroup Ib
VI-B	13.083 1504	2-Acetyl-5-methylfuran		FGE.13Rev2 Subgroup Ib
VI-B	13.101 1505	2-Acetyl-3,5-dimethylfuran		FGE.13Rev2 Subgroup Ib
VI-B	13.105 1507	2-Butyrylfuran		FGE.13Rev2 Subgroup Ib
VI-B	13.138 1510	1-(2-Furyl)butan-3-one		FGE.13Rev2 Subgroup Ib
VI-B	13.163 1509	2-Pentanoylfuran		FGE.13Rev2 Subgroup Ib
VI-C	13.176 1519	Furaneyl butyrate		None in FGE.13Rev2, FGE.65 or FGE.66Rev1.

***** [FL-no: 13.192] is a synonym of substance [FL-no: 13.178] which has already been evaluated in FGE.13Rev2.

Group I

Four substances [FL-no: 13.116, 13.190, 13.191 and 13.192] are structurally related to 14 sulphur-substituted furan derivatives evaluated by EFSA in FGE.13Rev2 (see Table 3.2) and 33 sulphur-substituted furan derivatives considered by EFSA in FGE.65 (see Table 3.3). Substance [FL-no: 13.192] appeared to be a synonym of substance [FL-no: 13.178] which has already been evaluated in FGE.13Rev2. Therefore this substance [FL-no: 13.192] will not be further considered in this FGE and should be removed from the Register.

Group II

One substance [FL-no: 13.006] is structurally related to five furoic acid esters considered by EFSA in FGE.66Rev1.

Group III

Six substances [FL-no: 13.021, 13.022, 13.023, 13.024, 13.047 and 13.058] are structurally related to a group of furfuryl and furoic acid substances evaluated by EFSA in FGE.13Rev2 in subgroup Ia.

Group IV

Eight substances [FL-no: 13.029, 13.030, 13.059, 13.069, 13.092, 13.103, 13.106 and 13.148] are alkyl-substituted furans and structurally related to two substances evaluated by EFSA in FGE.13Rev2 in subgroup Ic. In FGE.13Rev2 a concern for genotoxicity has been identified for structurally related substances.

Group V

The six substances in this group are not structurally related to any flavouring group evaluated or considered by EFSA.

Subgroup V-A

The three substances [FL-no: 13.052, 13.061 and 13.123] are furfuryl ethers.

Subgroup V-B

The two substances [FL-no: 13.031 and 13.074] are benzofuran derivatives. The substance [FL-no: 13.031] has been evaluated for genotoxicity in FGE.19 subgroup 4.3 (EFSA, 2008b), and thereafter concluded to be of no concern with respect to genotoxicity based on structural considerations (EFSA, 2011ac).

Subgroup V-C

The only substance [FL-no: 13.107] is a difurfurylfuran.

Group VI

Contains 16 alpha,beta-unsaturated carbonyls. These structures are considered by the Panel to be structural alerts for genotoxicity (EFSA, 2008b) and substances containing this structural characteristic have already been evaluated with respect to their genotoxic properties by EFSA in FGE.19 context (Subgroups 4.4b, 4.5 and 4.6 (EFSA, 2008b)).

Subgroup VI-A

Six substances (from FGE.19, subgroup 4.6, (EFSA, 2008b)) [FL-no: 13.034, 13.043, 13.044, 13.046, 13.137 and 13.150] for which a need for additional information on genotoxicity was identified (EFSA, 2008b).

Subgroup VI-B

This subgroup comprises nine substances. For seven substances in this subgroup (from FGE.19, subgroup 4.5, (EFSA, 2008b)) [FL-no: 13.054, 13.066, 13.070, 13.083, 13.101, 13.105 and 13.163] a need for additional information on genotoxicity was identified (EFSA, 2008b). After further considerations in the Panel it was concluded that these substances are comparable to acetophenone, an aromatic alpha,beta unsaturated ketone, which is not genotoxic. Therefore the alpha,beta-unsaturated ketone moiety in these seven furan derivatives is no longer considered to represent an alert for genotoxicity (EFSA, 2011ac); see also FGE.13Rev2).

Subgroup VI-C

One substance (from FGE.19, subgroup 4.4b, (EFSA, 2008b)) [FL-no: 13.176] for which a need for additional information on genotoxicity was identified (EFSA, 2009ae). After further evaluation of additional data submitted by the Industry, [FL-no: 13.176] has been cleared for genotoxicity in FGE.220Rev1 (EFSA, 2011a). Because this substance has closely related structural analogues in FGE.75, and no structural analogues in FGE.67, this substance will be further considered in a revision of FGE.75

Conclusion on subgroups

Of the 40 substances in the JECFA group of furan derivatives (JECFA, 2006b) the following will not be dealt with in the present revision of FGE.67; six substances from subgroup VI-A (corresponding to FGE.19 subgroup 4.6) for which there still is a genotoxicity concern, one substance from subgroup VI-C [FL-no: 13.176] which has been transferred to FGE.75Rev1 for further consideration based on structural similarities and one substance from subgroup I [FL-no: 13.192] which is a duplicate of the substance [FL-no: 13.178] which has already been evaluated in FGE.13Rev2. In conclusion, in the present FGE.67Rev1, 33 substances will be evaluated. These comprise 32 of the 40 substances in the JECFA group of furan derivatives and one additional benzofuran also evaluated by the JECFA in the group of furfuryl derivatives (JECFA, 2001b).

1.2. Isomers

1.2.1. Status

The following two substances [FL-no: 13.058 and 13.190] in the group of 33 JECFA evaluated furan-substituted substances have a chiral centre and [FL-no: 13.047] has geometrical isomerism.

1.2.2. EFSA Considerations

Information is lacking about the stereoisomeric composition for [FL-no: 13.047].

1.3. Specifications

1.3.1. Status

The JECFA specifications are available for all 33 substances (JECFA, 2005d). See Table 1.

1.3.2. EFSA Considerations

The available specifications are considered adequate for 30 of the 33 JECFA-evaluated substances. Information on stereoisomeric composition is lacking for [FL-no: 13.047]. Information on solubility in water is missing for [FL-no: 13.045] and information on melting point is missing for [FL-no: 13.031], see Table 1.

2. Intake Estimations

2.1. Status

For 31 of the 33 JECFA evaluated substances intake data are available for the EU, see Table 3.1.

2.2. EFSA Considerations

Tonnage data for use as a flavouring substance in Europe are missing for two [FL-no: 13.066 and 13.070] flavouring substances considered in this FGE.

3. Genotoxicity Data

3.1. Genotoxicity Studies – Text Taken[4] from the JECFA (JECFA, 2009a)

"Genotoxicity testing has been performed on eight [FL-no: 13.030, 13.029, 13.148, 13.034, 13.054 , 13.044, 13.022 and 13.191) representative furan-substituted aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids and related esters, sulfides, disulfides and ethers in this group. The results of these tests are summarised in Table 2.1 (of this FGE), see (JECFA, 2009a), and described below".

In vitro

"In standard Salmonella mutagenicity assays, 2,5-dimethylfuran [FL-no: 13.029], 3-methyl-2-(3-methylbut-2-enyl)-furan [FL-no: 13.148], 3-(2-furyl)acrolein [FL-no: 13.034], 4-(2-furyl)-3-buten-2-one [FL-no: 13.044], ethyl 3-(2-furyl)propanoate [FL-no: 13.022] and O-ethyl S-(2-furylmethyl)thiocarbonate [FL-no: 13.191] were not mutagenic in *Salmonella typhimurium* strains TA97, TA98, TA100, TA102, TA1535, TA1537 or TA1538 when tested at concentrations of up to 10 000 µg/plate, alone or in the presence of an exogenous rat liver metabolic activation system (S9) (Wild et al., 1983; Mortelmans et al., 1986; Shinohara et al., 1986; Asquith, 1989a; Eder et al., 1991a; Zeiger et al., 1992; Lee et al., 1994a; Verspeek-Rip, 2000). Likewise, with the exception of a single assay in which equivocal results of mutagenicity were reported in *S. typhimurium* strains TA97 and TA107 (Zeiger et al., 1992) 2-methylfuran [FL-no: 13.030] was consistently negative in several other strains of *S. typhimurium* (i.e. TA98, TA100, TA102 and TA1535) both alone and with an exogenous rat liver bioactivation system (S9) (Shinohara et al., 1986; Aeschbacher et al., 1989). Evaluated alone and with an exogenous bioactivation system in *S. typhimurium* at concentrations of up to 0.660 µmol/plate (54.2 µg/plate), 2-furyl methyl ketone [FL-no: 13.054] exhibited a significant positive mutagenic potential only in strain TA98 with bioactivation at the two lower concentrations (i.e. 0.165 and 0.330 µmol/plate) (Shinohara et al., 1986). At higher concentrations, significant cytotoxicity was observed, which was reflected by a concentration-dependent decrease in the number of revertants.

Bacterial mutagenicity testing of furans that can be metabolically oxidized to reactive alpha,beta-unsaturated dicarbonyl (2-ene-1,4-dicarbonyl) intermediates is problematic owing to their high bacterial toxicity. The cytotoxicity of these substances is believed to arise from their interactions with protein sulfhydryl and amino groups (Marnett et al., 1985a; Eder et al., 1992). Owing to the nature of the GSH conjugation pathway, genotoxicity studies in which high concentrations of alpha,beta-unsaturated carbonyl compounds are formed are likely to create oxidative stress. It is anticipated that cells exposed to high concentrations of these types of substances will rapidly deplete GSH levels, eventually leading to cellular damage and decreased cell viability, as indicated by the above study results.

O-Ethyl S-(2-furylmethyl)thiocarbonate [FL-no: 13.191] showed no mutagenic potential when tested in *Escherichia coli* WP2uvrA at concentrations of up to 3330 µg/plate, either alone or with a bioactivation system (Verspeek-Rip, 2000). Evaluated in *E. coli* PQ37 under the conditions of the SOS chromotest, 3-(2-furyl)acrolein [FL-no: 13.034] tested negative (Eder et al., 1991); however, in a subsequent evaluation, 3-(2-furyl)acrolein [FL-no: 13.034] as well as 2-furyl methyl ketone (2-acetylfuran) [FL-no: 13.054] were slightly positive in the SOS chromotest without metabolic activation, as evidenced by 1.72- and 1.75-fold increases, respectively, in the SOS induction factor

[4] *The text is taken verbatim from the indicated reference source, but text related to substances not included in the present FGE has been removed*.

over a background value of 1 (results were considered to be significant if the induction factor was at least 1.5) (Eder et al., 1993).

In the rec assay, which is based on differential inhibition of growth of repair-deficient strains as a measure of DNA-damaging activity, *Bacillus subtilis* strains H17 (rec+) and M45 (rec-) were incubated with 2-methylfuran (FL-no: 13.030), 2,5-dimethylfuran (FL-no: 13.029) and 2-furyl methyl ketone [FL-no: 13.054] at concentrations of up to 55 000 µg/disc, alone and with metabolic activation (Shinohara et al., 1986). 2-Furyl methyl ketone tested negative at a concentration of 550 µg/disc, but was reportedly positive at concentrations of 5500 µg/disc and greater alone and with metabolic activation. Likewise, 2,5-dimethylfuran was negative at the lowest concentration tested (i.e. 190 µg/disc) with metabolic activation, but tested positive at every concentration tested in the absence of metabolic activation. In contrast, 2-methylfuran was negative with metabolic activation and induced significant differences in the zones of inhibition only without metabolic activation. Additionally, 2-methylfuran and 2-acetylfuran were reported to cleave the double strand of *lambda*-phage DNA in the presence of Cu2+; however, a negative control was not included, and, therefore, the statistical significance of these results was not ascertained. Also, it should be noted that potential concomitant cytotoxicity was not monitored in this study.

The potential mammalian cell clastogenicities of 2-methylfuran (FL-no: 13.030), 2,5-dimethylfuran (FL-no: 13.029) and 2-furyl methyl ketone (FL-no: 13.054) were evaluated in Chinese hamster ovary (CHO) cells, in which induction of chromosomal aberrations was measured. Cells were exposed to substances from commercial sources (purity not given) for 3 hours, followed by 20 hours of maintenance. In the absence of exogenous metabolic activation, all three compounds produced increases in the number of chromosomal aberrations, mainly chromatid exchanges; however, in the presence of rat liver metabolic activation, only the clastogenicity of 2-furyl methyl ketone was increased, whereas the clastogenic activities of 2-methylfuran and 2,5-dimethylfuran were reduced in comparison with test systems without metabolic activation. Additionally, the authors noted that when NADP was eliminated from the activation system, the reduction in the chromosomal aberrations observed for 2-methylfuran and 2,5-dimethylfuran and the increase in the clastogenic activity observed with 2-furyl methyl ketone in the presence of the activation system were abolished. This suggests that mixed-function oxidases are integral in the metabolism of alkyl furan derivatives. It should be noted that the experiment with 2-furyl methyl ketone was performed at a limited number of concentrations (two), the active one of which far exceeded (112.6 mmol/l = 13 220 µg/ml) standard concentration limits for this assay and was toxic (Stich et al., 1981b).

Beginning in the late 1980s, researchers began studying test conditions (osmolality, ionic strength, low pH) that could cause an increase in clastogenic activity (increased chromosomal aberrations and micronuclei) in the absence of any chemical-induced effect on DNA (Zajac-Kaye and Ts'o, 1984; Brusick, 1986; Bradley et al., 1987; Galloway et al., 1987a; Seeberg et al., 1988; Morita et al., 1989; Scott et al., 1991). More recent research indicates that extreme culture conditions (hypo- and hyperosmolality and high pH) induce apoptosis and necrosis, leading to DNA fragmentation and producing false-positive responses in clastogenic assays (Meintieres and Marzin, 2004).

Apoptosis is a type of cell death that occurs under physiological conditions or in response to external stimuli (e.g. DNA-damaging agents, growth factor deprivation or receptor triggering). The mechanism of formation of apoptotic cells includes activation of cysteine proteases (caspases), leading to increased mitochondrial permeability, release of cytochrome c, DNA cleavage and redistribution of phosphatidylserine to the outer layers of the cell membrane, which enhances binding of cells to phagocytes. DNA cleavage, owing to irreversible activation of endonucleases, is followed by chromatin condensation and oligonucleosomal fragmentation due to double-strand cleavage of DNA in nucleosomal linker regions (Saraste and Pulkki, 2000). During chromatin condensation, the nucleus may split into a number of dense micronuclei. Fragmented DNA and chromatin condensation due to apoptotic events are not easily distinguished from direct action of a specific chemical.

In consideration of such knowledge, findings of chromosomal aberrations must be evaluated in the context of the potential for apoptosis to occur under test conditions. Relatively high concentrations (i.e. up to 1923 - 13 220 µg/ml or 20 - 150 mmol/l) were used in the study conducted by Stich et al. (Stich et al., 1981b). The Km for most enzyme kinetic processes is at or below 100 µmol/l (Bu, 2006; Wang and James, 2006), and thus the high concentrations used in this study may not be relevant to the human condition, especially with respect to the low levels of flavouring agents added to food. Furthermore, no information was available on culture conditions that may have promoted apoptosis. Results of chromosomal aberration and micronuclei assays are problematic to interpret in the absence of such information.

2-Furyl methyl ketone (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 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 a study examining the effect of oxygen scavengers on cadmium chloride–induced chromosomal aberrations in Chinese hamster V79 cells, 2,5-dimethylfuran (FL-no: 13.029) at 96.13 µg/ml (1 mmol/l) did not increase the frequency of chromosomal aberrations in comparison with control values. When 2,5-dimethylfuran at 96.13 µg/ml (1 mmol/l) was incubated with the V79 cells in the presence of cadmium chloride, no reduction in the clastogenic capacity of cadmium chloride was observed (Ochi and Ohsawa, 1985).

O-Ethyl-S-(2-furylmethyl)thiocarbonate [FL-no: 13.191] was evaluated for potential clastogenicity in a series of tests in human peripheral lymphocytes. Doses at which chromosomal aberrations were evaluated were based on a preliminary evaluation of effects on the mitotic index in the cells. Generally, O-ethyl-S-(2-furylmethyl)thiocarbonate exhibited marked mitogenicity and cytotoxicity, and accordingly only a relatively narrow range of concentrations was used. In the first set of tests in which an exposure time of 3 hours was utilized, the substance did not induce an increase at concentrations ranging between 150 and 350 µg/ml alone or in the presence of a bioactivation system; however, in another test employing a 3-hour exposure period with metabolic activation, significant and dose-dependent increases in the number of chromosomal aberrations were observed at concentrations of 325 and 375 µg/ml, but not at 150 µg/ml. Moreover, following a 24- or 48-hour exposure period, O-ethyl-S-(2-furylmethyl)thiocarbonate (up to 280 µg/ml) also induced dose-dependent and statistically significant increases in the number of chromosomal aberrations in the absence of metabolic activation in comparison with a negative control (Meerts, 2000).

In vivo

As reported in an abstract, 2-methylfuran [FL-no: 13.030] (purity not given) did not induce chromosomal aberrations in bone marrow cells or spermatocytes of Swiss albino mice evaluated at 24-hour intervals following administration in the diet at concentrations of 1000, 2000 or 4000 mg/kg (approximately 100, 200 and 400 mg/kg bw per day, respectively) for a period of 5 days. No positive control was included. Moreover, the authors noted that 2-methylfuran did not inhibit spindle protein synthesis or cell division in the somatic cells. In the germ cells, which were evaluated at weekly intervals for a period of 5 weeks following final dosing, in order to cover one full spermatogenesis cycle, no structural sperm-head abnormalities were reported (Subramanyam et al., 1989).

2-Furyl methyl ketone [FL-no: 13.054] was evaluated for clastogenic activity in bone marrow and germ cells of Swiss albino mice. Groups of two per dose per sampling time were administered the compound (99 % pure) orally at 0 (control), 1000, 2000 or 3000 mg/l in 0.5 ml of water (approximately 0, 20, 40 and 60 mg/kg bw, respectively) either as a single dose or once daily for 5 consecutive days. No positive control was included. Bone marrow cells were collected periodically for up to 72 hours following dosing, and meiotic and sperm preparations from testes and epididymis,

respectively, were assessed at 24 hours and weekly for a total of 5 weeks post-dosing. In bone marrow cells, the substance at the high dose level was observed to inhibit mitosis beginning at 18 hours following single- or multiple-dose treatment. At 24 hours, mitodepression was also observed at the high dose level in the single-dose experiment, as well as at the middle and high dose levels in mice administered multiple doses. In the repeat-dose test protocol, the effect remained significant for up to 36 hours post-treatment. Mitodepression was accompanied by increases in the frequency of structural chromosomal aberrations, mainly gaps and breaks, in the bone marrow cells. Specifically, at the high dose level (i.e. 3000 mg/l), between 18 and 24 hours following single-dose administration and 12 and 48 hours following final treatment of multiple-dose groups, the frequency of aberrations was elevated. Additionally, in animals receiving multiple doses of 2-furyl methyl ketone, significant increases in the number of chromosomal aberrations were also observed at the middle dose level (i.e. 2000 mg/l) between 24 and 36 hours post-treatment. In contrast to the dose- and time-dependent increase in chromosomal aberrations in the somatic cells, only a single isolated increase in structural chromosomal aberrations was observed in mouse spermatocytes 3 weeks following single-dose administrations of the substance, and only at the highest dose level. Following multiple-dose administration, abnormalities in germ cells were limited to significant increases in polyploidy and XY univalents occurring at weeks 3 and 4 at the highest dose level. Furthermore, no sperm-head abnormalities were observed at any dose level, irrespective of the treatment protocol. The absence of sperm-head abnormalities at all dose levels was indicative of a lack of sperm toxicity of the substance. The authors concluded that 2-furyl methyl ketone exhibits only mild clastogenic activity in mouse bone marrow and is not clastogenic in germ cells (Sujatha et al., 1993).

2-Furyl methyl ketone was 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).

2-Furyl methyl ketone was evaluated for induction of UDS in hepatocytes isolated from livers of dosed male Sprague-Dawley rats. The assay was conducted according to Good Laboratory Practices 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, in contrast to the positive controls 2-acetylaminofluorene and N,N'-dimethylhydrazine (Durward, 2007b).

O-Ethyl S-(2-furylmethyl)thiocarbonate (FL-no: 13.191) was evaluated for induction of micronuclei in bone marrow erythrocytes of NMRI BR mice. Groups of five per sex per dose per sampling time were administered single doses of compound (99 % pure) at 0 (vehicle control), 100, 250 or 500 mg/kg bw in corn oil via gavage. Dosed animals at every dose level and controls were killed at 24 hours postdosing. Additionally, a second group of high-dose mice (i.e. 500 mg/kg bw) and the positive control (cyclophosphamide) group were terminated at 48 hours post-dosing. Bone marrow smears were prepared from the femurs. No increase in the incidence of micronucleated polychromatic erythrocytes was observed in dosed mice compared with controls, in contrast to the positive control, which induced a 20-fold increase. However, the authors also noted that cells obtained from dosed animals did not exhibit a reduction in the ratio of polychromatic to normochromatic erythrocytes, indicating an absence of toxicity, which could be due to lack of adequate exposure of bone marrow (Verspeek-Rip, 2001)".

Conclusions on genotoxicity

"With few exceptions, eight representative substances of this group were consistently negative in mutation assays conducted in various strains of *S. typhimurium* and *E. coli* under appropriate testing conditions. Negative and positive results were obtained in the rec assay in *B. subtilis* for 2-methylfuran and 2,5-dimethylfuran. In mammalian genotoxicity assays conducted in CHO and V79 cells and human peripheral lymphocytes, study results were inconsistent, with both negative (2,5-dimethylfuran, O-ethyl-S-(2-furylmethyl)thiocarbonate) and positive (2-methylfuran, 2,5-dimethylfuran) results reported. Although positive results were reported in the chromosomal aberration assay in CHO cells with 2-methylfuran and 2,5-dimethylfuran, relatively high concentrations were utilized (i.e. up to 13220 and 1923 µg/ml, respectively); the statistical significance of the results was not specified, and the potential cytotoxicity was not monitored in the assay. Moreover, as previously discussed, positive *in vitro* results of chromosomal aberrations are difficult to interpret in the presence of concomitant cytotoxicity and cell cycle delay, which, based on the results of the studies, are a feature of the furan derivatives. Therefore, it may be expected that mammalian cells in culture might not have adequate metabolic capacities to counter this toxicity. In fact, with the exception of one assay in which clastogenic activity was reported for a single compound (i.e. 2-furyl methyl ketone) with a metabolic activation system, results obtained with other representative furan derivatives demonstrated a reduction in the frequency of chromosomal aberrations in the presence of metabolic activation. Furthermore, unlike the positive results reported for 2,5-dimethylfuran among several other compounds evaluated in CHO cells at the high concentrations used in the study of Stich et al. (Stich et al., 1981b) 2,5-dimethylfuran, tested at lower concentrations in V79 cells, did not exhibit clastogenic activity (Ochi and Ohsawa, 1985). The negative findings in the human hepatocyte DNA damage assay provide evidence that the chromosomal aberration findings are not due to a DNA-reactive mechanism.

Three representative compounds were studied in *in vivo* assays. With 2-methylfuran, no increase in chromosomal aberrations was found in either mouse bone marrow cells or spermatocytes. In a study in which mild clastogenic activity was reported in mouse bone marrow cells at the middle and high doses of 2-furyl methyl ketone (i.e. 40 and 60 mg/kg bw, respectively), at which the authors also reported significant mitodepression following single- and multiple dose administrations, no increase in chromosomal aberrations was observed in the spermatocytes obtained from the same mice, and the weak clastogenic effects achieved statistical significance only after repeated daily exposure to near-lethal doses. A study from the same laboratory reported induction of SCEs in mouse bone marrow cells by 2-furyl methyl ketone. However, 2-furyl methyl ketone did not elicit UDS in hepatocytes isolated from rat liver, suggesting that any possible *in vivo* genotoxicity is not attributable to DNA reactivity. The frequency of micronucleus formation in bone marrow cells of mice administered single doses of O-ethyl-S-(2-furylmethyl)thiocarbonate was comparable with control values (Verspeek-Rip, 2001), although adequacy of exposure was not demonstrated.

In conclusion, results of the *in vitro* genotoxicity/mutagenicity tests revealed mixed results, with positive results reported less frequently in the presence of an activation system. This could indicate metabolic detoxication of these substances. The *in vivo* single-dose studies with 2-furyl methyl ketone did not indicate evidence for genotoxicity, whereas two repeat-dose studies showed weak effects for induction of chromosomal aberrations and SCEs. However, evidence indicates that 2-furyl methyl ketone does not exhibit DNA reactivity. The basis for the positive clastogenicity findings remains unclear.

For a summary of *in vitro / in vivo* genotoxicity data considered by the JECFA, see Table 2.1.

3.2. Genotoxicity Studies – Text taken[5] from the JECFA (JECFA, 2001b)

The JECFA did not provide a text on the genotoxicity in their evaluation of the group of furfuryl alcohol and related substances including flavouring substance [FL-no: 13.031]. The JECFA only presented the study results in table format.

For a summary of *in vitro / in vivo* genotoxicity data considered by the JECFA see Table 2.5.

3.3. Genotoxicity Studies - Text taken[6] from EFSA FGE.13Rev2 (EFSA, 2011h)

In the following text, which is taken from FGE.13Rev2, the FGE.13-(sub)grouping is maintained.

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] min subgroup Ib no genotoxicity data are available, but *in vitro* and *in vivo* genotoxicity data are available for the supporting substance 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 (i.e. furans with sulphur-containing ring substituents).

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 TA98 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 and Tannenbaum, 1994)

[5] *The text is taken verbatim from the indicated reference source, but text related to substances not included in the present FGE has been removed.*

[6] *The text is taken verbatim from the indicated reference source, but text related to substances not included in the present FGE has been removed.*

In an Ames test with TA 104 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 and Tannenbaum, 1994).

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

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

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

Newly submitted data on 5-hydroxymethylfurfural[7] (Included in Table 2.2 and 2.3 of this FGE)

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 B6C3F1 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 1,000 normochromatic erythrocytes (NCEs) in 10 animals per sex per treatment group. In addition, the percentage of polychromatic erythrocytes

[7] 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.

(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 authors (Durling et al., 2009) concluded that 5HMF DNA damage 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 a 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 TA98, TA100, TA1535 and TA1537 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 (2006), incubation of DNA with SMF in a cell-free system led to the formation of DNA adducts that could be detected by the ^{32}P-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 B6C3F1 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 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* micronucleus 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 genotoxicity 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 TA 98 and TA 100. Furoic acid was also negative in DNA repair test in *E.coli* and in a UDS assay using primary rat hepatocytes.

Supporting substances

In vitro genotoxicity data were available for five supporting substances: furfuryl acetate, furfuryl alcohol, furfural, 5-methylfurfural and methyl-2-furoate [FL-no: 13.128, 13.019, 13.018, 13.001 and 13.002] and *in vivo* genotoxicity data for the two supporting substances furfuryl alcohol and furfural [FL-no: 13.019 and 13.018]. Most studies were negative, although some positive results were reported.

However, the genotoxicity of furfural has recently been re-evaluated by the AFC panel, which concluded that furfural did not induce gene mutations *in vivo*, on the basis of new studies with transgenic mice (EFSA, 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 aberrations 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 (gender not given) hepatocytes from two batches purchased from a commercial provider were incubated with concentrations of the compound (purity not given) 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-hour 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 considered 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 test 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 structural 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 (EFSA, 2011ac). 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 (FGE.13Rev2 (EFSA, 2011h), 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. With 2-methylfuran an equivocal result was obtained in a bacterial reverse gene mutation assay with *S. typhimurium* in strain TA97. This test was considered valid (Zeiger et al., 1992).

For a 2-alkyl- and 2,5-dialkyl-substituted furans, formation of reactive intermediates cannot be excluded (see Chapter 4 and Annex III, FGE.13Rev2 (EFSA, 2011h). 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.

For a summary of *in vitro / in vivo* genotoxicity data considered by EFSA, see Table 2.2 and 2.3.

3.4. EFSA Considerations

The Panel considered that the entire group of furans used as chemically defined flavouring substances (i.e. all flavouring substances discussed in FGE.13Rev2, FGE.65, FGE.66Rev1 and FGE.67Rev1) is a very diverse group. Based on this diversity, the Panel considers it justified to differentiate between the various subgroups with respect to the way the substances are metabolised and therefore also with respect to their possible genotoxic activity. Information on furan ring oxidation and opening, which results in the formation of reactive intermediates, was already considered in FGE.13Rev2. In this FGE, for the substances containing oxygenated ring substituents ring-opening was not considered a major issue with respect to genotoxicity. This was also supported by the fact that for the supporting substance furfural, for which this ring opening also has been reported, data show that furfural is not genotoxic *in vivo*. However for the eight alkyl-substituted furans [FL-no: 13.029, 13.030, 13.059, 13.069, 13.092, 13.103, 13.106 and 13.148] in this FGE the concern for formation of reactive metabolites could not be taken away, because of insufficient data on genotoxicity. It may be considered that ring oxidation and opening would be more relevant for these alkyl-substituted furans because they lack other simple options for metabolism like hydrolysis and / or immediate conjugation. In addition, oxidation of the C_1'-carbon of the alkyl substituent results in the formation of a ketone and for one such ketone [FL-no: 13.054], data are available to indicate a genotoxic potential (see section on genotoxicity on substances in FGE13.Rev2, above and Chapter 4 and Annex III in FGE.13Rev2). Therefore, the two candidate alkyl-substituted furans in subgroup Ic of FGE.13Rev2 were not evaluated via the Procedure. The same would apply to the eight alkyl-substituted furans in group IV in FGE.67Rev1.

Apart from the alkylfurans, simple hydrolysis / conjugation reactions are also not possible for the substances in subgroups V-A and V-C, either. As ethers (group V-A [FL-no: 13.052, 13.061 and 13.123]) are more resistant to hydrolysis than the corresponding esters, it may be anticipated that these ethers can also be more prone to ring oxidations and opening than the substances in subgroup Ia in FGE.13Rev2 or group III in FGE.67. The substance in group V-C [FL-no: 13.107] is anticipated to be metabolised even more similar to the alkylfurans than the substances in group V-A. Also for the substances in these two groups a concern for genotoxicity cannot be excluded. The concern is not identified for the one substance in group V-B, 2,3-dimethylbenzofuran [FL-no: 13.074], because for this substance furan ring opening is considered unlikely due to the two methyl substituents at the double bond in the furan ring. Another substance in subgroup V-B [FL-no: 13.031] has been considered for genotoxicity in FGE.219, because this substance is an alpha,beta-unsaturated aldehyde. Afterwards, the Panel considered that since the double bond in alpha-position to the carbonyl group is part of an aromatic system, the reactivity of this double bond is less than in non-aromatic alpha,beta-unsaturated carbonyls, and for that reason the concern for genotoxicity of this candidate substance [FL-no: 13.031] has been waived (EFSA, 2011ac).

Seven of the nine substances in subgroup VI-B are alpha,beta-unsaturated ketones [FL-no: 13.054, 13.066, 13.070, 13.083, 13.101, 13.105 and 13.163]. This structural 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 (EFSA, 2011ac). 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. The formation of DNA-reactive metabolites may be anticipated (EFSA, 2011h). 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 VI-B through the Procedure. Based on the concern raised by the genotoxicity data on [FL-no: 13.054] and the anticipation that keto-reduction is less favourable for biotransformation than e.g. alcohol or aldehyde oxidation and conjugation, the Panel considered it necessary to re-evaluate the two remaining alkoyl-substituted furans in subgroup VI-B [FL-no: 13.045 and 13.138]. In similarity with the other ketones in this subgroup VI-B in FGE.67Rev1

(supported by subgroup Ib in FGE.13Rev2), for these two substances now also a concern for genotoxicity is identified.

No data on genotoxicity are available for the previously evaluated (FGE.13Rev2) furans with sulphur-containing ring substituents. As it is anticipated that the predominant metabolic attack for these substances will be on the sulphur atom(s), for these substances ring opening is also not considered to be a major metabolic route. In absence of further data on genotoxicity the Panel decided that these substances could be evaluated through the Procedure. In the group of substances evaluated by the JECFA (JECFA, 2009a) additional data, both *in vitro* and *in vivo* are available on an additional furan with a sulphur-containing ring substituent (O-ethyl-S-(2-furylmethyl)thio-carbonate [FL-no: 13.191]). This substance is not supporting for the flavouring substances in FGE.13Rev2, FGE.65 and FGE.67Rev1. In several good quality *in vitro* studies (reports also available to EFSA) genotoxicity has been observed with this substance, but in a valid micronucleus test in mice *in vivo,* no genotoxic effects were seen. The reports of these studies (Verspeek-Rip, 2000; Verspeek-Rip, 2001); and (Meerts, 2000)) were also available to EFSA. In the micronucleus test (Verspeek-Rip, 2001) no indications for bone-marrow toxicity were obtained. Although from the clinical signs[8] it could be anticipated that the substance may have reached the systemic circulation and subsequently the bone-marrow, given the clearly positive response *in vitro*, this evidence was considered not strong enough and therefore the Panel concluded that this substance should also not be evaluated through the Procedure. Thus for one [FL-no: 13.191] of the three flavouring substances in subgroup I [FL-no: 13.116, 13.190 and 13.191], a concern for genotoxicity was identified, precluding the evaluation of this substance through the Procedure.

Thus, the Panel concluded that 11 of the 33 substances evaluated in this FGE can be evaluated through the Procedure [FL-no: 13.006, 13.021, 13.022, 13.023, 13.024, 13.031, 13.047, 13.058, 13.074, 13.116 and 13.190]. For one of the substances in group I [FL-no: 13.191], for all (21) substances in groups IV, V-A, V-C and VI-B ([FL-no: 13.029, 13.030, 13.045, 13.052, 13.054, 13.059, 13.061, 13.066, 13.069, 13.070, 13.083, 13.092, 13.101, 13.103, 13.105, 13.106, 13.107, 13.123, 13.138, 13.148 and 13.163] a concern for genotoxicity was identified, precluding these substances to be evaluated through the Procedure.

4. Application of the Procedure

4.1. JECFA Statement on the Application of the Procedure to 40 furan-substituted substances evaluated by JECFA[9] (JECFA, 2009a)

As stated above, the main concern with this group arises primarily from the carcinogenicity of furan itself, which is believed to involve a reactive genotoxic metabolite formed by epoxidation and opening of the furan ring. Furan is not a member of this group of flavouring agents, but all the members of the group contain a furan ring with either one or two substituents of varying complexity. In some flavouring agents, a substituent is present on one side of the furan ring only, whereas in others, substituents are present on both sides. The presence of an extended side-chain attached to the furan ring would reduce the potential for epoxidation of the double bond and provide a site for detoxication via metabolism and elimination. The flavouring agent that has the simplest structure and would be predicted to have the greatest potential for ring oxidation is 2-methylfuran [FL-no: 13.030]; there is evidence from studies *in vitro* and *in vivo* that this compound undergoes bioactivation to a reactive ring-opened metabolite that binds covalently to both protein and DNA. Data are not available on the influence of the nature and position of the ring substitution on potential for metabolic activation and

[8] Clinical signs were: uncoordinated movements, lethargy, rough coat, slow breathing and hunched posture.

[9] The text is taken verbatim from the indicated reference source, but text related to substances not included in the present FGE has been removed.

adduct formation. After administration of a single dose, 2-methylfuran produced liver toxicity in rats from 50 mg/kg bw, but hepatotoxicity has not been reported for other members of this group in more extensive studies.

Testing for genotoxicity has been performed on eight members of this group of flavouring agents. The results of the studies of genotoxicity/mutagenicity *in vitro* that were already available to the Committee (the JECFA) at its previous meeting were both positive and negative, with most positive results reported for chromosomal aberration. These, however, were less frequent in the presence of metabolic activation, indicating possible metabolic detoxication rather than bioactivation. 2-Methylfuran (FL-no: 13.030), for example, produced chromosomal aberrations *in vitro*, but the clastogenic activity was lower in the presence of a metabolizing system. The limited data available on genotoxicity *in vivo* showed no evidence of chromosomal aberration in mouse bone marrow or spermatocytes for 2-methylfuran. 2-Furyl methyl ketone [FL-no: 13.054] also induced no chromosomal aberrations in mouse spermatocytes, but a weak, transient increase in chromosomal aberrations was observed in mouse bone marrow, associated with mitodepression. O-Ethyl-S-(2-furylmethyl)thiocarbonate [FL-no: 13.191] appeared not to induce micronucleus formation in mouse bone marrow.

The new data on 2-furyl methyl ketone [FL-no: 13.054] available to the Committee (the JECFA) at its present meeting were a study on UDS in cultured hepatocytes *in vitro*, a study on UDS in rat liver *in vivo/in vitro* and a test for SCEs in mouse bone marrow *in vivo*. 2-Furyl methyl ketone did not induce UDS either *in vitro* or *in vivo*/*in vitro*. However, it did induce SCEs, confirming the concern for clastogenicity as expressed by the Committee (the JECFA) at its previous meeting. The Committee (the JECFA) at its present meeting therefore considered that the new data available did not resolve the concerns expressed previously.

The Committee (the JECFA) concluded that the Procedure for the Safety Evaluation of Flavouring Agents could not be applied to this group because of the unresolved toxicological concerns. Studies that would assist in the safety evaluation include investigations of the influence of the nature and position of ring substitution on metabolism and on covalent binding to macromolecules. Depending on the findings, additional studies might include assays related to the mutagenic and carcinogenic potential of representative members of this group."

4.2. JECFA Statement on the Application of the Procedure to one furfuryl alcohol related substance [FL-no: 13.031] evaluated by the JECFA[10] (JECFA, 2001b)

Step 1.

In applying the Procedure for the Safety Evaluation of Flavouring Agents to the above-mentioned substances, the Committee assigned substance 2-benzofurancarboxaldehyde (JECFA No. 751) [FL-no: 13.031] to structural class III.

Step 2.

The JECFA provided no statement on the possible metabolism of substance [FL-no: 13.031]. The JECFA concluded that the evaluation of all substances (thus including substance [FL- no: 13.031] in this group proceeded via the right-hand (the B-side) side of the scheme (i.e. they cannot be predicted to be readily metabolised to innocuous products).

Step B3.

[10] The text is taken verbatim from the indicated reference source, but text related to substances not included in the present FGE has been removed.

The estimated daily per capita intakes of substance [FL-no: 13.031] is below the threshold of concern for its structural class (i.e. 90 µg/day for structural class III). Accordingly, the evaluation of this substance proceeded to step B4.

Step B4.

For 2-benzofurancarboxaldehyde [FL-no: 13.031], the NOEL of 25 mg/kg bw per day in a 90-day feeding study in rats (Posternak et al., 1969) provides an adequate margin of safety (> 1,000,000) in relation to the known levels of intake of this substance.

4.3. Application of the Procedure to 27 Furfuryl and furan derivatives with and without additional side-chain substituents and heteroatoms by EFSA[11] in FGE.13Rev2 (EFSA, 2011h)

In FGE.13Rev2 data have been presented, which indicate 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 (FGE.13Rev2).

Thus, the Procedure for the safety evaluation of flavouring substances as outlined in Annex I (FGE.13Rev2) 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 (FGE.13Rev2), 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

[11] The text is taken verbatim from the indicated reference source, but text related to substances not included in the present FGE has been removed.

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 3.2). 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 (FGE.13Rev2)). 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 observed 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 and 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.

4.4. EFSA Considerations

For eight of the 33 flavouring substances [FL-no: 13.006, 13.021, 13.022, 13.023, 13.024, 13.116, 13.190 and 13.191], the classification according to Cramer et al., 1977 was revised from structural class II to III. These revisions are due to the question of natural occurrence for the substances involved and were consistent with FGE.13Rev2 and FGE.65. The Panel notes that for the substances involved, this will not affect the final conclusions.

The Panel agrees with the JECFA (JECFA, 2009a) for 22 of the 33 substances [FL-no: 13.029, 13.030, 13.045, 13.052, 13.054, 13.059, 13.061, 13.066, 13.069, 13.070, 13.083, 13.092, 13.101, 13.103, 13.105, 13.106, 13.107, 13.123, 13.138, 13.148, 13.163 and 13.191] in the group of furan-substituted aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids and related esters, sulfides, disulfides and ethers that these substances cannot be evaluated through the Procedure, based on concerns with respect to genotoxicity and carcinogenicity.

The Panel also agrees with the conclusion reached by the JECFA at its 55th meeting (JECFA, 2001b) that substance [FL-no: 13.031] can be evaluated using the Procedure, and that this substances poses no safety concern when used as a flavouring substance.

Contrary to the JECFA and in line with the decisions taken in previous FGEs (FGE.13.Rev2, FGE.66Rev1, FGE.67 (EFSA, 2011h; EFSA, 2011ad; EFSA, 2009ao)), the Panel considers that the remaining 10 substances [FL-no: 13.006, 13.021, 13.022, 13.023, 13.024, 13.047, 13.058, 13.074, 13.116 and 13.190] can also be evaluated using the Procedure.

Step 1

These 10 substances which can be evaluated through the Procedure [FL-no: 13.006, 13.021, 13.022, 13.023, 13.024, 13.047, 13.058, 13.074, 13.116, and 13.190] have been allocated to structural class III according to the Cramer et al. decision tree.

Step 2

In line with the previous evaluations (FGE.13.Rev2, FGE.66Rev1 and FGE.65 (EFSA, 2011h; EFSA, 2011ad; EFSA, 2009ao)), none of the 10 substances can be anticipated to be metabolised to innocuous products. Therefore all 10 substances should be evaluated through the B-side of the Procedure.

Step B3

At step B3 of the Procedure, all 10 substances have exposure estimates less than the thresholds for their respective classes, and therefore these substances should proceed to step B4 of the Procedure.

Step B4

Phenethyl 2-furoate [FL-no: 13.006]

After hydrolysis this substance will yield 2-furoic acid [FL no: 13.019] and phenethyl alcohol [FL no: 02.019]. At an exposure at the level of the MSDI (0.012 μg *per capita* per day), the respective amounts of 2-furoic acid and phenethyl alcohol would amount to 0.006 μg per person per day and 0.007 μg per person per day, respectively. In FGE.13Rev1 2-furoic acid has been evaluated by comparison with the ADI of 0.5 mg/kg bw for the related substance 2-furfural (EFSA, 2004c). Phenethyl alcohol was considered of no safety concern at step A3 of the Procedure by the Panel in FGE.53. Based on these considerations, it is concluded that at the estimated level of exposure, based on the MSDI approach, phenethyl 2-furoate [FL-no: 13.006] is of no safety concern.

2,5-Dimethyl-3-thioacetoxyfuran [FL-no: 13.116]

At step B4 of the Procedure the exposure estimate of 3 microgram *per capita* per day for [FL-no: 13.116] can be compared to the NOAEL of 0.73 mg/kg bw/day for the supporting substance 2,5-dimethyl-3-(isopentylthio)furan [FL-no: 13.041] (FGE.65 (EFSA, 2009an)), as determined in a 90-day study reported by Morgareidge et al. (Morgareidge et al., 1974a) and Cox et al., (Cox et al., 1974a). An adequate margin of safety of 14.6×10^3 can be calculated.

3-((2-Methyl-3-furyl)thio)-2-butanone [FL-no: 13.190]

At step B4 of the Procedure the exposure estimate of 0.012 microgram *per capita* per day for [FL-no: 13.190] can be compared to the NOAEL of 3.76 mg/kg bw/day for the supporting substance 3-((2-methyl-3-furyl)thio)heptan-4-one [FL-no: 13.077] (FGE.65 (EFSA, 2009an)), as determined in a 90-day study reported by Gallo et al. (Gallo et al., 1976b). An adequate margin of safety of 1.9×10^7 can be calculated.

Isopentyl 4-(2-furan)butyrate [FL-no: 13.021], Ethyl 3(2-furyl)propionate [FL-no: 13.022], Isopentyl 3-(2-furan)propionate [FL-no: 13.023] and Isobutyl 3-(2-furyl)propionate [FL-no: 13.024]

At step B4 of the Procedure for substance [FL-no: 13.024] a NOAEL of 35 mg/kg bw/day, determined in a 90-day study, has been reported (Lough et al., 1985). When at step B4 the NOAEL for this substance is compared to its exposure estimate of 0.12 microgram *per capita* per day based on the MSDI approach, an adequate margin of safety of 18×10^6 can be calculated. The same NOAEL can also be used to evaluate [FL-no: 13.021, 13.022 and 13.023], for which exposure estimates of 0.24, 0.012 and 0.24 microgram *per capita* per day were calculated. Comparison of these MSDI exposure estimates with the NOAEL for [FL-no: 13.024] provides adequate margins of safety of 8.9×10^6, 1.8×10^8 and 8.9×10^6 for [FL-no: 13.021, 13.022 and 13.023], respectively.

2,3-Dimethylbenzofuran [FL-no: 13.074]

At step B4 of the Procedure for substance [FL-no: 13.074] a NOAEL of 0.6 mg/kg bw/day, determined in a 90-day study has been reported (Long et al. (Long, 1977a). When at step B4 the NOAEL for this substance is compared to its exposure estimate of 0.52 microgram *per capita* per day based on the MSDI approach, an adequate margin of safety of 69×10^3 can be calculated.

Propyl 3-(2-furyl)acrylate [FL-no: 13.047]

This substance can be anticipated to be hydrolysed into propanol and 2-furanacrylic acid. The latter fragment should be further considered. At an exposure at the level of the MSDI (2.2 µg *per capita* per day) for [FL no: 13.047], the amount of 2-furanacrylic acid released would amount to 1.7 µg per person per day. In FGE.13Rev1 2-furanacrylic acid has been evaluated by comparison with the ADI of 0.5 mg/kg bw for the related substance 2-furfural (EFSA, 2004c). It may be concluded that at the estimated level of exposure, based on the MSDI approach, propyl 3-(2-furyl)acrylate [FL-no: 13.047] is of no safety concern.

3-(5-Methyl-2-furyl) butanal [FL-no: 13.058]

No NOAEL could be identified to support the evaluation of substance [FL-no: 13.058] at step B4. Therefore for this substance no conclusion as to its safety when used as a chemically defined flavouring substance can be reached.

5. Conclusion

The present Flavouring Group Evaluation deals with 39 substances, 38 substances of which were previously considered by the JECFA in a group of 40 furan-substituted aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids and related esters, sulfides, disulfides and ethers (JECFA, 2009a). One of these 40 substances [FL-no: 13.192] appeared to be a synonym of substance

[FL-no: 13.178] which has already been evaluated in FGE.13Rev2. Therefore this substance [FL-no: 13.192] will not be further considered in this FGE and should be removed from the Register. Another substance [FL-no: 13.176] will be evaluated in FGE.75 rather than FGE.67, because of better structural similarity with candidate substances in FGE.75. Furthermore, one candidate substance [FL-no: 13.031] from FGE.66Rev1 has been included in this revision of FGE.67, because this substance has better structural similarity to a candidate flavouring substance [FL-no: 13.074] in FGE.67 than to the other candidate flavouring substances in FGE.66Rev1. The flavouring substances considered in this FGE have been allocated to various subgroups, based on their chemical structures.

Thirteen of the 15 substances in subgroups VI-A and VI-B are alpha,beta-unsaturated carbonyls, which have been evaluated by EFSA in FGE.19 context with respect to a concern for a possible genotoxic potential. This concern for genotoxicity could not be alleviated for the six substances in subgroup VI-A [FL-no: 13.034, 13.043, 13.044, 13.046, 13.137 and 13.150], corresponding to FGE.19 subgroup 4.6 (EFSA, 2008b). These six substances were therefore not further considered in this FGE.

The thirty-three candidate substances considered in this FGE [FL-no: 13.006, 13.021, 13.022, 13.023, 13.024, 13.029, 13.030, 13.031, 13.045, 13.047, 13.052, 13.054, 13.058, 13.059, 13.061, 13.066, 13.069, 13.070, 13.074, 13.083, 13.092, 13.101, 13.103, 13.105, 13.106, 13.107, 13.116, 13.123, 13.138, 13.148, 13.163, 13.190 and 13.191] are structurally related to the group of 27 furfuryl and furan derivatives evaluated by EFSA in FGE.13Rev2. Part of the substances is also structurally related to a group of 33 sulphur-substituted furan derivatives used as flavouring agents, evaluated by EFSA in FGE.65 and another part is structurally related to 14 furfuryl derivatives evaluated in FGE.66Rev1 (EFSA, 2009an; EFSA, 2011ad).

For eight alkyl-substituted furans in group IV [FL-no: 13.029, 13.030, 13.059, 13.069, 13.092, 13.103, 13.106 and 13.148] for three furfuryl ethers in subgroup V-A [FL-no: 13.052, 13.061 and 13.123] and a difurfuryl furan [FL-no: 13.107] in subgroup V-C and a sulphur-substituted furan from group I [Fl no: FL-no: 13.191] a concern for genotoxicity was identified.

For the seven alpha,beta-unsaturated substances in subgroup VI-B [FL-no: 13.054, 13.066, 13.070, 13.083, 13.101, 13.105 and 13.163], corresponding to FGE.19 subgroup 4.5 (EFSA, 2008b) no concern for genotoxicity with respect to this alpha,beta-unsaturation was identified afterwards, based on additional evaluation of their chemical structures. However, based on available genotoxicity data, a concern for genotoxicity was still identified, which was also relevant for the two remaining substances [FL-no: 13.045 and 13.138] in this subgroup.

Thus, the Panel agrees with JECFA for 22 of the 33 substances [FL-no: 13.029, 13.030, 13.045, 13.052, 13.054, 13.059, 13.061, 13.066, 13.069, 13.070, 13.083, 13.092, 13.101, 13.103, 13.105, 13.106, 13.107, 13.123, 13.138, 13.148, 13.163 and 13.191] that these substances cannot be evaluated through the procedure, based on concerns with respect to genotoxicity.

In line with the decisions taken in previous FGEs (FGE.13Rev2, FGE.65 and FGE.66Rev1), the Panel considers that 11 substances [FL-no: 13.006, 13.021, 13.022, 13.023, 13.024, 13.031, 13.047, 13.058, 13.074, 13.116 and 13.190] can be evaluated using the Procedure.

It was concluded for ten substances [FL-no: 13.006, 13.021, 13.022, 13.023, 13.024, 13.031, 13.047, 13.074, 13.116 and 13.190], that they would be of no safety concern at their estimated intake levels based on the MSDI approach. For the remaining substance [FL-no: 13.058] this conclusion could not be drawn due to lack of an adequate NOAEL.

For all 33 substances use levels are needed to calculate the mTAMDIs in order to identify those flavouring substances that need more refined exposure assessment and to finalise the evaluation.

In order to determine whether the conclusion for the evaluated substances can be applied to the materials of commerce, it is necessary to consider the available specifications. Adequate specifications including complete purity criteria and identity are available for 30 of the 33 substances. Information

on stereoisomeric composition has not been submitted for [FL-no: 13.047]. Information on solubility in water is missing for [FL-no: 13.045] and information on melting point is missing for [FL-no: 13.031].

Thus, for 25 of the 33 substances considered in this FGE the Panel has reservations. For three substances [FL-no: 13.031, 13.045 and 13.047] data on specifications / stereoisomerism are missing. For 23 substances [FL-no: 13.029, 13.030, 13.045, 13.052, 13.054, 13.058, 13.059, 13.061, 13.066, 13.069, 13.070, 13.083, 13.092, 13.101, 13.103, 13.105, 13.106, 13.107, 13.123, 13.138, 13.148, 13.163 and 13.191] the Panel concluded that additional toxicity / genotoxicity data are required.

For the remaining eight of these 33 furan derivatives [FL-no: 13.006, 13.021, 13.022, 13.023, 13.024, 13.074, 13.116 and 13.190] 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

Table 1: Specification Summary for 33 furan derivatives evaluated by JECFA (JECFA, 2005d)

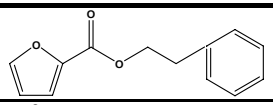
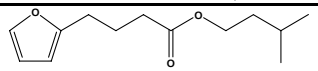
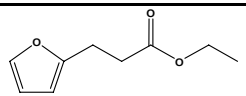
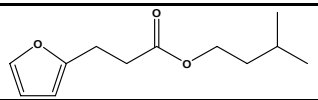
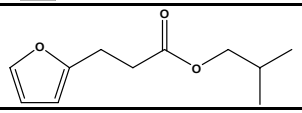
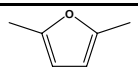
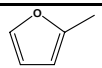
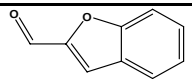
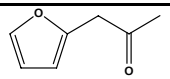
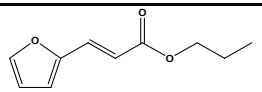
FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	EFSA comments
13.006 1517	Phenethyl 2-furoate		2865 362 7149-32-8	Liquid $C_{13}H_{12}O_3$ 216.24	Insoluble Soluble	275 NMR 96 %	1.585-1.593 1.136-1.142	
13.021 1516	Isopentyl 4-(2-furan)butyrate		2070 2080 7779-66-0	Liquid $C_{13}H_{20}O_3$ 224.30	Insoluble Insoluble	263-265 NMR 95 %	1.551-1.555 0.975-0.981	
13.022 1513	Ethyl 3(2-furyl)propionate		2435 2091 10031-90-0	Solid $C_9H_{12}O_3$ 168.19	Very slightly soluble Soluble	n.a. 24-25 NMR 95 %	n.a. n.a.	Register name to be changed to: Ethyl 3-(2-furyl)propionate.
13.023 1515	Isopentyl 3-(2-furan)propionate		2071 2092 7779-67-1	Liquid $C_{12}H_{18}O_3$ 210.27	Insoluble Soluble	258 NMR 96 %	1.549-1.557 0.987-0.993	
13.024 1514	Isobutyl 3-(2-furyl)propionate		2198 2093 105-01-1	Liquid $C_{11}H_{16}O_3$ 196.25	Very slightly soluble Soluble	105 (4 hPa) NMR 96 %	1.531-1.537 1.007-1.013	
13.029 1488	2,5-Dimethylfuran		2208 625-86-5	Liquid C_6H_8O 96.13	Slightly soluble Soluble	93 IR NMR MS 95 %	1.437-1.443 0.892-0.898	
13.030 1487	2-Methylfuran		4179 2209 534-22-5	Liquid C_5H_6O 82.10	Slightly soluble Soluble	64 IR NMR MS 97 %	1.431-1.437 0.908-0.917	
13.031 751	2-Benzofurancarboxaldehyde		3128 2247 4265-16-1	Solid $C_9H_6O_2$ 146.15	Insoluble Slightly soluble	130-131 (17hPa) MS 96 %	n.a. n.a.	MP 7).
13.045 1508	1-(2-Furyl)-propan-2-one		2496 11837 6975-60-6	Liquid $C_7H_8O_2$ 124.14	Soluble	179-180 NMR 97 %	1.499-1.505 1.074-1.080	Missing data on solubility in water.
13.047 1518	Propyl 3-(2-furyl)acrylate 6)		2945 11842 623-22-3	Liquid $C_{10}H_{12}O_3$ 180.20	Insoluble Soluble	119 (9 hPa) NMR 97 %	1.520-1.526 1.071-1.077 (20°)	Stereoisomeric composition to be specified.

Table 1: Specification Summary for 33 furan derivatives evaluated by JECFA (JECFA, 2005d)

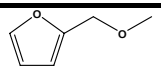
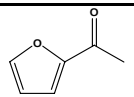
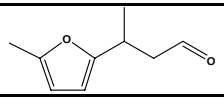
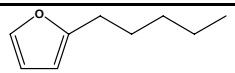
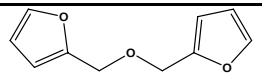
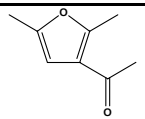
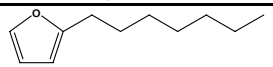
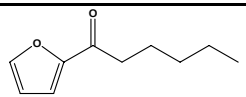
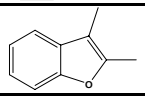
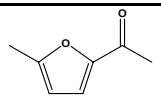
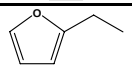
FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	EFSA comments
13.052 1520	Furfuryl methyl ether		3159 10944 13679-46-4	Liquid $C_6H_8O_2$ 112.13	Insoluble Soluble	134-135 NMR 99 %	1.454-1.460 1.013-1.019	
13.054 1503	2-Acetylfuran		3163 11653 1192-62-7	Liquid $C_6H_6O_2$ 110.11	Very slightly soluble Soluble	67 (13 hPa) IR 97 %	1.505-1.510 1.102-1.107	
13.058 1500	3-(5-Methyl-2-furyl) butanal		3307 10355 31704-80-0	Liquid $C_9H_{12}O_2$ 152.19	Insoluble Soluble	88-91 (16 hPa) NMR 98 %	1.575-1.581 1.006-1.012	Racemate.
13.059 1491	2-Pentylfuran		3317 10966 3777-69-3	Liquid $C_9H_{14}O$ 138.21	Slightly soluble Soluble	58-60 (13 hPa) NMR 99 %	1.443-1.449 0.886-0.893	
13.061 1522	Difurfuryl ether		3337 10930 4437-22-3	Liquid $C_{10}H_{10}O_3$ 178.19	Insoluble Soluble	88-89 (1 hPa) NMR 97 %	1.138-1.144 1.506-1.512	
13.066 1506	3-Acetyl-2,5-dimethylfuran		3391 10921 10599-70-9	Liquid $C_8H_{10}O_2$ 138.17	Slightly soluble Soluble	83 (14 hPa) NMR 99 %	1.484-1.492 1.027-1.048	
13.069 1492	2-Heptylfuran		3401 10952 3777-71-7	Liquid $C_{11}H_{18}O$ 166.26	Insoluble Soluble	209-210 NMR 99 %	1.446-1.452 0.860-0.866	
13.070 1512	2-Hexanoylfuran		3418 11180 14360-50-0	Liquid $C_{10}H_{14}O_2$ 166.22	Slightly soluble Soluble	65-67 (0.7 hPa) NMR 99 %	1.490-1.496 0.992-0.998	
13.074 1495	2,3-Dimethylbenzofuran		3535 11913 3782-00-1	Liquid $C_{10}H_{10}O$ 146.19	Insoluble Soluble	96-98 (20 hPa) NMR 97 %	1.554-1.563 1.031-1.037	
13.083 1504	2-Acetyl-5-methylfuran		3609 11038 1193-79-9	Liquid $C_7H_8O_2$ 124.14	Slightly soluble Soluble	71-72 (10 hPa) IR NMR 99 %	1.511-1.517 1.066-1.072 (20°)	
13.092 1489	2-Ethylfuran		3673 11706 3208-16-0	Liquid C_6H_8O 96.13	Insoluble Soluble	92-93 NMR 95 %	1.444-1.450 0.909-0.915	

Table 1: Specification Summary for 33 furan derivatives evaluated by JECFA (JECFA, 2005d)

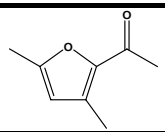
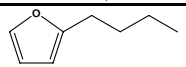
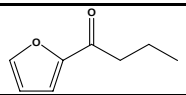
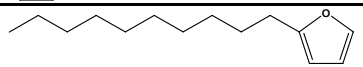
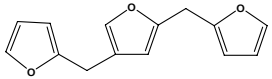
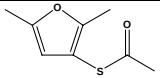
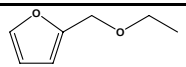
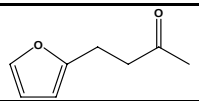
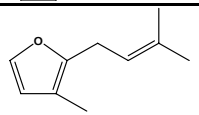
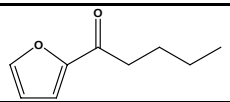
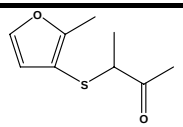
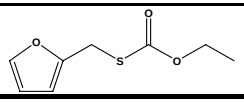
FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	EFSA comments
13.101 1505	2-Acetyl-3,5-dimethylfuran		4071 22940-86-9	Liquid $C_8H_{10}O_2$ 138.17	Insoluble Soluble	195 18 MS 95 %	1.494-1.500 1.041-1.047	
13.103 1490	2-Butylfuran		4081 10927 4466-24-4	Liquid $C_8H_{12}O$ 124.18	Insoluble Soluble	139 MS 95 %	1.444-1.450 0.884-0.890	
13.105 1507	2-Butyrylfuran		4083 11045 4208-57-5	Liquid $C_8H_{10}O_2$ 138.17	Insoluble Soluble	195 NMR MS 95 %	1.489-1.495 1.050-1.056	
13.106 1493	2-Decylfuran		4090 83469-85-6	Solid $C_{14}H_{24}O$ 208.34	Insoluble Soluble	 30 NMR 95 %	n.a. n.a.	
13.107 1496	2,4-Difurfurylfuran		4095 64280-32-6	Solid $C_{14}H_{12}O_3$ 228.24	Insoluble Soluble	 153 NMR 95 %	n.a. n.a.	
13.116 1523	2,5-Dimethyl-3-thioacetoxyfuran		4034 55764-22-2	Liquid $C_8H_{10}O_2S$ 170.23	Practically insoluble Soluble	230 IR NMR MS 98 %	1.527-1.533 1.137-1.143	
13.123 1521	Ethyl furfuryl ether		4114 10940 6270-56-0	Liquid $C_7H_{10}O_2$ 126.15	Slightly soluble Soluble	150 MS 95 %	1.449-1.455 0.982-0.988	
13.138 1510	1-(2-Furyl)butan-3-one		4120 11084 699-17-2	Solid $C_8H_{10}O_2$ 138.17	Slightly soluble Soluble	n.a. 37 MS 95 %	n.a. n.a.	
13.148 1494	3-Methyl-2(3-methylbut-2-enyl)furan		4174 15186-51-3	Liquid $C_{10}H_{14}O$ 150.22	Slightly soluble Soluble	70 (15 hPa) MS 98 %	1.473-1.479 0.998-1.004	Register name to be changed to: 3-Methyl-2/(3-methylbut-2-enyl)furan.
13.163 1509	2-Pentanoylfuran		4192 3194-17-0	Liquid $C_9H_{12}O_2$ 152.19	Slightly soluble Soluble	101 (13 hPa) MS 95 %	1.486-1.492 1.009-1.015	

Table 1: Specification Summary for 33 furan derivatives evaluated by JECFA (JECFA, 2005d)

FL-no JECFA-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)	EFSA comments
13.190 1525	3-((2-Methyl3-furyl)thio)-2-butanone		4056 61296-44-1	Liquid $C_9H_{12}O_2S$ 184.25	Practically insoluble Soluble	70 (1 hPa) IR NMR MS 99 %	1.510-1.516 1.104-1.110	CASrn in Register is not valid; CASrn to be changed to: 61295-44-1 (The Good Scents Company, 2011). Reister name to be changed to 3-((2-Methyl-3-furyl)thio)-2-butanone. Racemate.
13.191 1526	o-Ethyl S-(2-furylmethyl)thiocarbonate		4043 376595-42-5	Liquid $C_8H_{10}O_3S$ 186.23	Practically insoluble Soluble	130-135 IR NMR MS 99 %	1.504-1.510 1.167-1.173	

1) Solubility in water, if not otherwise stated.

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

3) At 1013.25 hPa, if not otherwise stated.

4) At 20°C, if not otherwise stated.

5) At 25°C, if not otherwise stated.

6) Stereoisomeric composition not specified.

7) MP: Missing melting point.

TABLE 2: GENOTOXICITY DATA

TABLE 2.1: GENOTOXICITY DATA FOR 40 FURAN-SUBSTITUTED SUBSTANCES EVALUTED BY JECFA (JECFA, 2009A)

FL-no: JECFA-no:	Flavouring agent	End-point	Test system	Concentration/ dose	Results	References
			In vitro			
13.030 1487	2-Methylfuran	Reverse mutation	*Salmonella 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)[a]	Negative[b]	(Shinohara et al., 1986)
		Reverse mutation	*S. typhimurium* TA98, TA100, TA102 and TA1535	Up to 10 000 µg/plate	Negative[b,c,d]	(Zeiger et al., 1992)
		Reverse mutation	*S. typhimurium* TA97 and TA104	Up to 10 000 µg/plate	Equivocal[b,c,d]	(Zeiger et al., 1992)
		Reverse mutation	*S. typhimurium* TA98, TA100 and TA102	11 nmol/plate to 1,1 mmol/ plate (0,9-90 310 µg/plate)[a]	Negative[b]	(Aeschbacher et al., 1989)
		DNA damage	*Bacillus subtilis* H17 (rec+) and M45 (rec˜)	0.16, 16 or 1600 µg/disc	Negative Positive[b,e]	(Shinohara et al., 1986)
		Chromosomal aberration	CHO cells	0-150 mmol/l (0-12315 µg/ml)[a]	Positive[b,f]	(Stich et al., 1981b)
13.029 1488	2,5-Dimethylfuran	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)[g]	Negative[b]	(Shinohara et al., 1986)
		Reverse mutation	*S. typhimurium* TA98 and TA100	Not specified	Negative[b]	(Lee et al., 1994a)
		Reverse mutation	*S. typhimurium* TA97, TA98, TA100 and TA1535	Up to 3333 µg/plate	Negative[b,c,d]	(Zeiger et al., 1992)
		DNA damage	*B. subtilis* H17 (rec+) and M45 (rec-)	190, 1900 or 9500 µg/disc	Negative Positive[b,h]	(Shinohara et al., 1986)
		Chromosomal aberration	Chinese hamster V79 cells	1 mmol/l (96.13 µg/ml)[g]	Negative	(Ochi and Ohsawa, 1985)
		Chromosomal aberration	CHO cells	0-20 mmol/l (0-1923 µg/ml)[g]	Positive[b,f]	(Stich et al., 1981b)
13.148 1494	3 -Methyl-2-(3-methylbut-2-enyl)-furan	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535 and TA1537	3.2, 16, 80, 400 or 2000 µg/plate	Negative[b]	(Asquith, 1989a)
13.034	3-(2-Furyl)acrolein	Reverse mutation	*S. typhimurium* TA100	Not specified	Negative[b,c]	(Eder et al., 1991b)
		DNA damage (SOS chromotest)	*E. coli* PQ37	Not specified	Negative[i]	(Eder et al., 1991b)
		DNA damage (SOS chromotest)	*E. coli* PQ37	Not specified	Weakly positive[j]	(Eder et al., 1993)
13.054 1503	2-Furyl methyl ketone	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)[j]	Negative Positive[b,k]	(Shinohara et al., 1986)

TABLE 2.1: GENOTOXICITY DATA FOR 40 FURAN-SUBSTITUTED SUBSTANCES EVALUTED BY JECFA (JECFA, 2009A)

FL-no: JECFA-no:	Flavouring agent	End-point	Test system	Concentration/ dose	Results	References
	2-Furyl methyl ketone (cont.)	DNA damage	*E. coli* PQ37 (SOS chromotest)	Not specified	Slightly positive[j]	(Eder et al., 1993)
		DNA damage	*B. subtilis* H17 (rec+) and M45 (rec-)	550, 5500 or 55000 µg/disc	Negative Positive[b,l]	(Shinohara et al., 1986)
		Chromosomal aberration	CHO cells	0-112.6 mmol/l (0-13220 µg/ml)[j]	Positive[b,m,n]	(Stich et al., 1981b)
		UDS	Human hepatocytes	2.19, 4.38, 8.75, 17.5, 35, 70, 140 or 280 µg/ml	Negative	(Durward, 2007a)
13.044 1511	4-(2-Furyl)-3-buten-2-one	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535 and TA1537	33, 100, 333, 1000, 2166 or 3333 µg/ plate	Negative[b,c,o]	(Mortelmans et al., 1986)
13.022 1513	Ethyl 3-(2-furyl)propanoate	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537 and TA1538	Up to 3600 µg/plate	Negative[b]	(Wild et al., 1983)
13.191 1526	O-Ethyl-S-(2-furylmethyl)thio-carbonate	Reverse mutation	*S. typhimurium* TA98, TA100, TA1535 and TA1537	33, 100, 333, 1000 or 3330 µg/plate	Negative[b,p]	(Verspeek-Rip, 2000)
		Reverse mutation	*E. coli* WP2uvrA	33, 100, 333, 1000 or 3330 µg/plate	Negative[b,q]	(Verspeek-Rip, 2000)
		Chromosomal aberration	Human peripheral lymphocytes	150, 300 or 350 µg/ml	Negative[b,r]	(Meerts, 2000)
		Chromosomal aberration	Human peripheral lymphocytes	130, 240 or 280 µg/ml	Positive[i,s]	(Meerts, 2000)
		Chromosomal aberration	Human peripheral lymphocytes	100, 130 or 240 µg/ml	Positive[i,t]	(Meerts, 2000)
		Chromosomal aberration	Human peripheral lymphocytes	150, 325 or 375 µg/ml	Negative Positive[r,u,v]	(Meerts, 2000)
			In vivo			
13.030 1487	2-Methylfuran	Chromosomal aberration	Mouse bone marrow cells and spermatocytes	1000, 2000 or 4000 mg/kg (100, 200 or 400 mg/kg bw per day)[w]	Negative	(Subramanyam et al., 1989)
13.054 1503	2-Furyl methyl ketone	Chromosomal aberration	Mouse bone marrow	1000, 2000 or 3000 mg/l (20, 40 or 60 mg/ kg bw)[x]	Positive[y,z]	(Sujatha et al., 1993)
		Chromosomal aberration	Mouse spermatocytes	1000, 2000 or 3000 mg/l (20, 40 or 60 mg/ kg bw)[x]	Negative[aa]	(Sujatha et al., 1993)
		SCE	Mouse bone marrow	1000, 2000 or 3000 mg/l (20, 40 or 60 mg/ kg bw)[x]	Positive	(Sujatha, 2007)
		UDS	Rat liver	7 or 21 mg/kg bw	Negative	(Durward, 2007b)
13.191 1526	O-Ethyl-S-(2-furylmethyl)thio-carbonate	Micronucleus induction	Mouse bone marrow	100, 250 or 500 mg/kg bw[bb]	Negative	(Verspeek-Rip, 2001)

CHO, Chinese hamster ovary; SCE, sister chromatid exchange; UDS, unscheduled DNA synthesis.

a Calculated using relative molecular mass of 2-methylfuran = 82.1.

b With and without metabolic activation.

c Preincubation method.

d Occasional incidences of slight to complete clearing of the background lawn at the higher concentrations.

e Negative at all concentrations with metabolic activation; positive without metabolic activation.

f Clastogenic activity decreased with metabolic activation (statistical significance of results was not specified).

g Calculated using relative molecular mass of 2,5-dimethylfuran = 96.13.

h Positive at every concentration without metabolic activation; with metabolic activation, negative at 190 µg/disc, but positive at higher concentrations.

i Without metabolic activation.

j Calculated using relative molecular mass of 2-furyl methyl ketone = 110.11.

k Positive only in strain TA98 with an increase in the presence of metabolic activation.

l Negative at 550 µg/disc; positive at 5500 and 55 000 µg/disc (with and without metabolic activation).

m Cytotoxicity was observed at 12 398 µg/ml (112.6 mmol/l) in the presence of metabolic activation.

n Clastogenic activity increased with metabolic activation (statistical significance of results was not specified).

o Cytotoxicity was observed at 3333 µg/plate in all S. typhimurium strains and at 2166 µg/plate inS. typhimurium strains TA100 and TA1537.

p Cytotoxicity was observed at the 3330 µg/plate level in all S. typhimurium strains and at 1000 µg/plate in S. typhimurium strains TA100 and TA1535.

q Cytotoxicity was observed at 3330 µg/plate in the absence of metabolic activation.

r 3-h continuous exposure time.

s 24-h continuous exposure time.

t 48-h continuous exposure time.

u With metabolic activation.

v Statistically significant dose-dependent increases in chromosomal aberrations were seen at the two highest concentrations only, 325 and 375 µg/ml.

w Mice received 2-methylfuran in the diet for 5 consecutive days at 24-h intervals.

x 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.

y 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.

z Chromosomal aberrations were observed in the presence of significant mitodepression.

aa 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.

bb Single dose administered by gavage.

Substances listed in brackets are the JECFA evaluated supporting substances in FGE.13Rev2

TABLE 2.2: GENOTOXICITY (*IN VITRO*) EVALUATED BY EFSA IN FGE.13REV2 (EFSA, 2011H)

Chemical Name [FL-no:]	Test system	Test Object	Concentration	Result	Reference	Comments
(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 to 196 µg/ml	Negative	(Jansson et al., 1986)	
	Sister chromatid exchange	Human Lymphocytes	Up to 970 µg/ml	Negative	(Gomez-Arroyo and 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)	
	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 and 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)	
	Ames test	*S. typhimurium* TA102, TA104	100 - 10000 µg/plate	Negative[3]	(Dillon et al., 1998)	

TABLE 2.2: GENOTOXICITY (*IN VITRO*) EVALUATED BY EFSA IN FGE.13REV2 (EFSA, 2011H)

Chemical Name [FL-no:]	Test system	Test Object	Concentration	Result	Reference	Comments
	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 and 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 and 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 and Schadly, 1996)	
	Chromosomal aberration	CHO cells	Up to 1,230 µg/ml	Positive[1]	(NTP, 1990a)	
	Unscheduled DNA Synthesis	Human liver slices	0.005 - 10 mM	Negative	(Adams et al., 1998b)	
	DNA-protein cross-links	EBV- human Burkitt's lymphoma cells	25 mM	Positive[5]	(Costa et al., 1997)	
5-Hydroxymethyl-furfuraldehyde [13.139]	Ames test	*S. typhimurium* TA98; TA100	0.2 - 1 µmol/plate	Negative	(Surh et al., 1994)	The study is considered valid. Purity 99 %.
	Ames test	*S. typhimurium* TA98; TA100	0.2 - 2.0 µg/plate	Positive[3]	(Omura et al., 1983)	Positive dose related respons 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[3]	(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 the 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

TABLE 2.2: GENOTOXICITY (*IN VITRO*) EVALUATED BY EFSA IN FGE.13REV2 (EFSA, 2011H)

Chemical Name [FL-no:]	Test system	Test Object	Concentration	Result	Reference	Comments
						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 and McGregor, 1992)	The study is considered valid.
	Ames test	*S. typhimurium* TA97, TA98, TA102, TA1535	100 - 10,000 µg/plate	Negative[1]	(NTP, 2010c)	
	Ames test	*S. typhimurium* TA100	100 - 10,000 µg/plate	Weakly positive[2]	(NTP, 2010c)	
	Ames test	*S. typhimurium* TA100 and TA98	1,500 - 10,000 µg/plate	Negative[1]	(NTP, 2010c)	
	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537	At 0.5 µg/mL up to 5000 µg/mL	Negative[1]	(Severin et al., 2010)	
	Reverse mutation assay	*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[28]	(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[29]	(Glatt et al., 2005)	
	Umu assay	*S. typhimurium* TA1535	20 mM	Positive[9]	(Janzowski et al., 2000)	Positive results were only obtained at high concentrations resulting in reduced cell viability and growth. The study is considered valid but interpretation of data is questionable.
	Rec assay	*B. subtilis* H 17 rec+; M 45 rec-	0.25 - 12.5 mg/disk	Positive[1]	(Shinohara et al., 1986)	Experimental details are lacking. The validity of the study can not be evaluated.
	Chromosomal aberration	Chinese hamster V79 cells	Up to 2000 µg/ml	Positive[10]	(Nishi et al., 1989)	Weak positive response were only obtained at high concentrations. The study is considered valid.
	Comet assay	V79, Caco-2, primary human colon cells and primary rat hepatocytes	Up to 80 mM	Negative[2]	(Janzowski et al., 2000)	The study is considered valid but interpretation of data is questionable.
	Comet assay	HepG2 cells	0, 5.35, 7.87, 11.57, 17, 25, 36.6 mM	Positive[27, 28]	(Severin et al., 2010)	
	Comet assay	Human Caco-2 cells	3,153 - 12,611 µg/mL (25 - 100 mM)	Positive[25]	(Durling et al., 2009)	
	Comet assay	Human HEK293 cells	3,153 - 12,611 µg/mL (25 - 100 mM)	Positive[25]	(Durling et al., 2009)	
	Comet assay	Mouse lymphoma L5178Y cells	3,153 - 12,611 µg/mL (25 - 100 mM)	Positive[25]	(Durling et al., 2009)	
	Comet assay	Chinese hamster V-79 cells	315 - 12,611 µg/mL (2.5 - 100 mM)	Positive[26]	(Durling et al., 2009)	
	Comet assay	Chinese hamster V-79-hP-PST cells	315 - 12,611 µg/mL (2.5 - 100 mM)	Positive[26]	(Durling et al., 2009)	
	HPRT assay	V79 cells	Up to 140 mM	Positive[1, 11]	(Janzowski et al., 2000)	Positive response were only obtained at high concentrations resulting in reduced cell viability and growth. The study is considered valid but interpretation of data is

TABLE 2.2: GENOTOXICITY (*IN VITRO*) EVALUATED BY EFSA IN FGE.13REV2 (EFSA, 2011H)

Chemical Name [FL-no:]	Test system	Test Object	Concentration	Result	Reference	Comments
						questionable.
	HPRT and tk assay	TK6 human lymphoblast cells	20 - 75 µg/ml	Negative	(Surh and Tannenbaum, 1994)	The study is considered valid.
(5-Methylfurfural [13.001])	Ames test	*S. typhimurium* TA1537, TA100 and TA1535	288 µg/plate	Negative[1]	(Florin et al., 1980)	
	Ames test	*S. typhimurium* TA98, TA100 and TA102	96100 µg/plate	Negative[1]	(Aeschbacher et al., 1989)	
	Ames test	*S. typhimurium* TA98 and TA100	79 - 316 µg/plate	Negative[1]	(Shinohara et al., 1986)	
	Rec-assay	*B. subtilis* strains H17 & M45	0.55 - 5500 µg/disk	Positive[1]	(Shinohara et al., 1986)	
	Sister chromatid exchange	CHO cells	2200 - 4070 µg/ml	Positive[1]	(Stich et al., 1981a)	
2-Furoic acid [13.136]	Ames test	*S. typhimurium* TA98; TA100	25 - 100 µg/plate	Negative[2]	(Ichikawa et al., 1986b)	The study is considered valid.
	Ames test	*S. typhimurium* TA100		Negative	(Soska et al., 1981)	Dose not reported. The validity of the study can not be evaluated.
	Ames test	*S. typhimurium* TA100	1000 µg/plate	Negative	(Kitamura et al., 1978)	The study is considered valid.
	DNA repair test	*E. coli* WP2l WP2 uvrA; WP67; WP100; CM 561; CM 571; CM 611	1000 µg/disk	Negative	(Soska et al., 1981)	The study is considered valid.
	Unscheduled DNA synthesis	Primary rat hepatocytes	1000 µg/ml	Negative[10, 12]	(Aaron et al., 1989)	Study performed in accordance with GLP. The study is considered valid.
(Methyl-2-furoate [13.002])	Ames test	*S. typhimurium* TA98; TA100	100 µg/plate	Negative[10]	(Ichikawa et al., 1986b)	
(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 aberration	CHO cells	0 - 112.6 mmol/l (0 - 13220 µg/ml)[20]	Positive[22,23,24]	(Stich et al., 1981b)	Study reported by 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

TABLE 2.2: GENOTOXICITY (*IN VITRO*) EVALUATED BY EFSA IN FGE.13REV2 (EFSA, 2011H)

Chemical Name [FL-no:]	Test system	Test Object	Concentration	Result	Reference	Comments
						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.
	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

1With 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-furyl methyl ketone = 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).

25 Positive only at the highest concentration tested with significant decrease in cell viability.

26 Positive at high concentration with significantly reduced cell viability.

27Cytotoxic at the two highest doses.

28 20 hours of exposure.

29Weakly positive but statistically significant at each concentration.

Substances listed in brackets are the JECFA evaluated supporting substances in FGE.13Rev2

TABLE 2.3: GENOTOXICITY (*IN VIVO*) EVALUATED BY EFSA IN FGE.13REV2 (EFSA, 2011H)

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 and Souza, 1985)	
	Chromosomal aberration assay	Adult Human Lymphocytes	Inhalation (occupational atmosphere)	32300 mg/m^3	Negative	(Gomez-Arroyo and Souza, 1985)	
	Chromosomal aberration assay	Mouse bone marrow cells	Drinking water	0.5 mg/kg 1 - 2 mg/kg	Negative Positive	(Sujatha and 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 day/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)	
	Nondisjunction assay	*Drosophila melanogaster* (females)	Inhalation	1.5 %	Negative[1]	(Muñoz and 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 and Souza, 1985)	
	Chromosomal aberration assay	Adult Human Lymphocytes	Inhalation (occupational	9454 mg/m^3	Negative	(Gomez-Arroyo and Souza, 1985)	

TABLE 2.3: GENOTOXICITY (*IN VIVO*) EVALUATED BY EFSA IN FGE.13REV2 (EFSA, 2011H)

Chemical Name [FL-no:]	Test system	Test Object	Route	Dose	Result	Reference	Comments
			atmosphere)				
	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-Furyl methyl ketone [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 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.
5-Hydroxymethylfurfural [13.139]	Micronucleus assay	Mouse peripheral blood cells		47, 94, 188, 375 or 750 mg/kg bw/day	Negative	(NTP, 2010c)	

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 2.4: GENOTOXICITY FGE.65

No genotoxicity data available for the sulphur containing furan-derivatives

TABLE 2.5: SUMMARY OF GENOTOXICITY DATA OF FURFURYL DERIVATIVES EVALUATED BY JECFA (JECFA, 2001B)

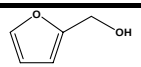
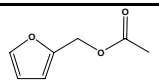
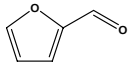
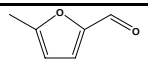
FL-no JECFA-no	EU Register name JECFA name	Structural formula	End-point	Test system	Concentration	Results	Reference
13.019 451	Furfuryl alcohol		Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	294 µg/plate	Negative[a,b]	(Florin et al., 1980)
			Reverse mutation	*S. typhimurium* TA98, TA100, TA1535	Up to10 000 µg/plate	Negative[a,b]	(Mortelmans et al., 1986)
			Reverse mutation	*S. typhimurium* TA100	2500 - 12 500µg/ml	Negative[a,b]	(Stich et al., 1981a)
			Reverse mutation	*S. typhimurium* TA98, TA100, TA102	Up to 198 000 µg/plate	Negative[a,b]	(Aeschbacher et al., 1989)
			Reverse mutation	*S. typhimurium* TA98, TA100	81 - 323 µg/plate	Negative[a,b]	(Shinohara et al., 1986)
			Reverse mutation	k TA1535, TA100, TA1537 (modified assay)	200 000 µg/ml	Positive[a,b]	(McGregor et al., 1981)
			DNA repair and H17 (rec+)	k M45 (rec–) µg/disc	2000–20 000	Positive[a,b]	(Shinohara et al., 1986)
			Sister chromatid exchange	Chinese hamster ovary cells	245 µg/ml	Positive[a,b]	(Stich et al., 1981b)
			Sister chomatid exchange	Human lymphocytes	Up to 196 µg/ml	Negative	(Jansson et al., 1986)
			Siser chromatid exchange	Human lymphocytes	Up to 970 µg/ml	Negative	(Gomez-Arroyo and Souza, 1985)
			Chromosomal aberration	Chinese hamster ovary cells	2000 µg/ml	Positive	(Stich et al., 1981b)
			Gene conversion	*S. cerevisiae* strain D7	13 500 - 16 000 µg/ml	Positive[a]	(Stich et al., 1981a)
			Sex-linked recessive l ethal mutation	*D. melanogaster*	Up to 6500 ppm by injection	Negative	(Rodriquez-Arnaiz et al., 1989)
			Sister chromatid exchange	Adult human lymphocytes	32 300 mg/m^3 in occupational atmosphere	Negative	(Gomez-Arroyo and Souza, 1985)
			Sister chomatid exchange	Adult human lymphocytes	32 300 mg/m^3 in occupational atmosphere	Negative	(Gomez-Arroyo and Souza, 1985)
			Chromosomal aberration	Mouse bone-marrow cells	0.5 mg/kg bw in drinking-water 1 - 2 mg/kg bw in drinking-water	Negative Positive	(Sujatha and Subramanyam, 1994)
13.128 739	Furfuryl acetate		Reverse mutation	*S. typhimurium* TA1535, TA98, TA100	33 - 666 µg/plate	Positive[a,b]	(Mortelmans et al., 1986)
13.018 450	Furfural		Reverse mutation	*S. typhimurium* TA 1535, TA100, TA1537, TA1538, TA98	0.1 - 1000 µg/ml	Negative[a,b]	(McMahon et al., 1979)
			Reverse mutation	*S. typhimurium* TA100, TA98, TA1535	Up to 3460 µg/plate 5766 µg/plate	Negative[a,b] Positive[a] (weakly)	(Loquet et al., 1981)
			Reverse mutation	*S. typhimurium* TA100, TA98, TA102	Up to 115 320 µg/plate	Negative[a,b]	(Aeschbacher et al., 1989)
			Reverse mutation	*S. typhimurium* TA100, TA98	15 - 63 µg/plate	Negative[a,b]	(Shinohara et al., 1986)
			Reverse mutation	*S. typhimurium* TA104	5 - 500 µg/plate	Positive[b]	(Shane et al., 1988)
			Reverse mutation	*S. typhimurium* TA100, TA102	5 - 500 µg/plate	Negative[b]	(Shane et al., 1988)
			Reverse mutation	*S. typhimurium* TA104, TA102	96 µg/plate	Negative	(Marnett et al., 1985a)
			Reverse mutation	*S. typhimurium* TA98, TA100, TA1535	Up to 6667 µg/plate	Negative[a,b]	(Mortelmans et al., 1986)
			Reverse mutation	*S. typhimurium* TA98, TA100	Up to 1000 µg	Negative[a]	(Osawa and Namiki, 1982)

TABLE 2.5: SUMMARY OF GENOTOXICITY DATA OF FURFURYL DERIVATIVES EVALUATED BY JECFA (JECFA, 2001B)

FL-no JECFA-no	EU Register name JECFA name	Structural formula	End-point	Test system	Concentration	Results	Reference
			Reverse mutation	S. typhimurium TA98, TA100, TA1535, TA1537	33– 6666 µg/plate	Negative[a,b] Equivocal in TA100[a]	(NTP, 1990a)
			Reverse mutation	*S. typhimurium* TA100	8000 µg/plate	Positive[a,b]	(Zdzienicka et al., 1978)
			Reverse mutation	*S. typhimurium* TA98	8000 µg/plate	Negative[a,b]	(Zdzienicka et al., 1978)
			Reverse mutation	*S. typhimurium* TA100, TA102	1 00 - 10 000 µg/plate	Negative[a]	(Dillon et al., 1998)
			Reverse mutation	*S. typhimurium* TA104	100 - 10 000 µg/plate	Equivocal[a]	(Dillon et al., 1998)
			Reverse mutation	*S. typhimurium* TA102, TA104	100 - 10 000 µg/plate	Negative[b]	(Dillon et al., 1998)
			Reverse mutation	*S. typhimurium* TA100	10010 000 µg/plate	Equivocal[b]	(Dillon et al., 1998)
			Reverse mutation	*S. typhimurium* TA100 (modified assay)	426 µg/plate	Negative[a,b]	(Kim et al., 1987b)
			Reverse mutation	*S. typhimurium* TA100, TA1535, TA1537 (modified assay)	200 000 µg/ml	Negative	(McGregor et al., 1981)
			Reverse mutation	*E. coli* WP2, WP2 uvrA (modified assay)	0.1- 1000 µg/ml	Negative[a,b]	(McMahon et al., 1979)
			SOS induction	*S. typhimurium* TA1535/ pSK1002	1932 µg/ml	Negative[a,b]	(Nakamura et al., 1987)
			DNA repair	*B. subtilis* H17 (rec+) and M45 (rec–)	Up to 1000 µg	Negative[a]	(Osawa and Namiki, 1982)
			DNA repair	*B. subtilis* H17 (rec+) and M45 (rec–)	0.6 ml	Negative[a,b]	(Matsui et al., 1989)
			DNA repair	*B. subtilis* H17 (rec+) and M45 (rec–)	1700 - 17 000 µg/disc	Positive[a,b]	(Shinohara et al., 1986)
			Forward mutation	L5178Y mouse lymphoma cells, Tk+/– locus	25 - 100 µg/ml 200 µg/ml	Negative[a] Positive[a]	(McGregor et al., 1988b)
			Sister chromatid exchange	Chinese hamster ovary cells	2500 - 4000 µg/ml	Positive[a,b]	(Stich et al., 1981b)
			Sister chromatid exchange	Chinese hamster ovary cells	Up to 1170 µg/ml	Positive[a,b]	(NTP, 1990a)
			Sister chromatid exchange	Human lymphocytes	Up to 0.035 mmol/La 0.07 - 0.14 mmol/Lc	Negative[a,b] Positive[a,b]	(Gomez-Arroyo and Souza, 1985)
			Chromosomal aberration	Chinese hamster ovary cells	500 µg/ml 1000 - 2000 µg/ml	Negative Positive	(Nishi et al., 1989)
			Chromosomal aberration	Chinese hamster ovary cells	Up to 40 mmol/L (3840 mg)	Positive[a,b]	(Stich et al., 1981b)
			Chromosomal aberration	Chinese hamster ovary cells	3000 µg/ml	Positive	(Stich et al., 1981a)
			Chromosomal aberration	Chinese hamster ovary cells	Up to 1230 µg/ml	Positive[a,b]	(NTP, 1990a)
			Unscheduled DNA synthesis	Human liver slices	0.005 - 10 mmol/L	Negative	(Adams et al., 1998b)
			Sex-linked recessive lethal mutation	*D. melanogaster*	1000 mg/kg of diet	Negative	(Woodruff et al., 1985)
			Sex-linked recessive lethal mutation	*D. melanogaster*	100 mg/kg by injection	Positive	(Woodruff et al., 1985)
			Sex-linked recessive lethal mutation	*D. melanogaster*	Up to 6500 mg/kg by injection	Negative	(Rodriquez-Arnaiz et al., 1989)
			Chromosomal loss	*D. melanogaster*	Oral or injected dose of 3750 - 5000 mg/kg of diet. Mated with repair-proficient females	Negative	(Rodriquez-Arnaiz et al., 1992)
			Chromosomal loss	*D. melanogaster*	Oral or injected dose of 3750 - 5000 mg/kg of diet. Mated with repair-deficient females	Positive	(Rodriquez-Arnaiz et al., 1992)
			Reciprocal trans- location	*D. melanogaster*	100 mg/kg by injection	Negative	(Woodruff et al., 1985)
			Sister chromatid exchange	Mouse bone-marrow cells	50 - 200 mg/kg bw by injection	Negative	(NTP, 1990a)
			Spermhead abnormalities	Mice	4000 mg/kg of diet daily for 5	Negative	(Subramanyam et al., 1989)

TABLE 2.5: SUMMARY OF GENOTOXICITY DATA OF FURFURYL DERIVATIVES EVALUATED BY JECFA (JECFA, 2001B)

FL-no JECFA-no	EU Register name JECFA name	Structural formula	End-point	Test system	Concentration	Results	Reference
					weeks		
			Somatic chromo-somal mutation	Swiss albino mouse bone- marrow cells	1000 - 2000 mg/kg of diet	Negative	(Subramanyam et al., 1989)
					4000 mg/kg bw for 5 days	Positive	
			Sister chromatid exchange	Adult human lymphocytes	9454 mg/m^3 in occupational atmosphere	Negative	(Gomez-Arroyo and Souza, 1985)
			Chromosomal aberration	Adult human lymphocytes	9454 mg/m^{3}in occupational atmosphere	Negative	(Gomez-Arroyo and Souza, 1985)
			Unscheduled DNA synthesis	B6C3F1 mice	50 - 320 mg/kg bw orally	Negative	(Edwards, 1999)
			Unscheduled DNA synthesis	Fischer 344 rats	5 - 50 mg/kg bw orally	Negative	(Phillips et al., 1997)
13.001 745	5-Methylfurfural		Reverse mutation	S. typhimurium TA1537, TA100, TA1535	288 µg/plate	Negative[a,b]	(Florin et al., 1980)
			Reverse mutation	*S. typhimurium* TA98, TA100, TA102	96,100 µg/plate	Negative[a,b]	(Aeschbacher et al., 1989)
			Reverse mutation	*S. typhimurium* TA98, TA100	79 - 316 µg/plate	Negative[a,b]	(Shinohara et al., 1986)
			DNA repair	*B. subtilis* H17 (rec+) and M45 (rec–)	0.55 - 5500 µg/disk	Positive[a,b]	(Shinohara et al., 1986)
			Sister chromatid exchange	Chinese hamster ovary cells	2200 - 4070 µg/ml	Positive[a,b]	(Stich et al., 1981b)

a Without metabolic activation from a 9000 g supernatant of rat liver.

b With metabolic activation.

TABLE 3: SUMMARY OF SAFETY EVALUATIONS

Table 3.1: Summary of safety evaluation of 33 furan derivatives evaluated by JECFA (JECFA, 2001b) and considered in FGE.67Rev1

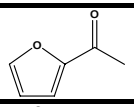
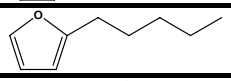
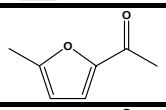
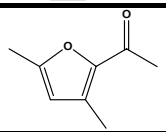
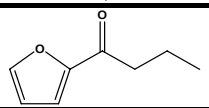
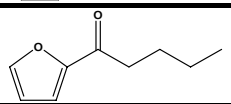
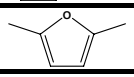
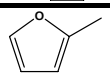
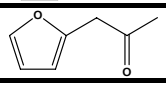
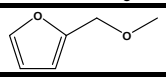
FL-no JECFA-no	EU Register name	Structural formula	EU MSDI 1) US MSDI (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
13.054 1503	2-Acetylfuran		46 13	Class II No evaluation		Additional genotoxicity data required	
13.059 1491	2-Pentylfuran		0.18 0.03	Class II No evaluation		Additional genotoxicity data required	
13.083 1504	2-Acetyl-5-methylfuran		0.37 0.1	Class II No evaluation		Additional genotoxicity data required	
13.101 1505	2-Acetyl-3,5-dimethylfuran		0.0012 0.002	Class II No evaluation		Additional genotoxicity data required	
13.105 1507	2-Butyrylfuran		0.12 0.2	Class II No evaluation		Additional genotoxicity data required	
13.163 1509	2-Pentanoylfuran		0.061 0.09	Class II No evaluation		Additional genotoxicity data required	
13.029 1488	2,5-Dimethylfuran		0.012 0.02	Class II No evaluation		Additional genotoxicity data required	
13.030 1487	2-Methylfuran		0.21 0.3	Class II No evaluation		Additional genotoxicity data required	
13.045 1508	1-(2-Furyl)-propan-2-one		0.037 0.02	Class II No evaluation		Additional genotoxicity data required	
13.052 1520	Furfuryl methyl ether		0.024 0.09	Class II No evaluation		Additional genotoxicity data required	

Table 3.1: Summary of safety evaluation of 33 furan derivatives evaluated by JECFA (JECFA, 2001b) and considered in FGE.67Rev1

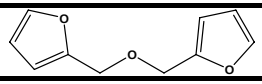
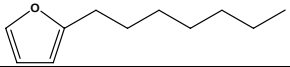
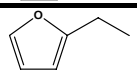
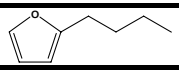
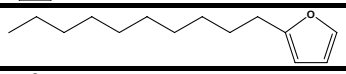
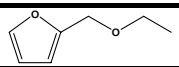
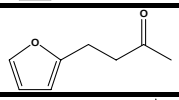
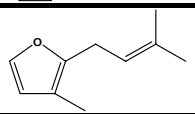
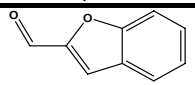
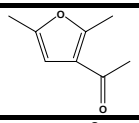
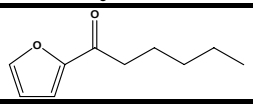
FL-no JECFA-no	EU Register name	Structural formula	EU MSDI 1) US MSDI (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
13.061 1522	Difurfuryl ether		0.12 0.09	Class II No evaluation		Additional genotoxicity data required	
13.069 1492	2-Heptylfuran		0.012 0.9	Class II No evaluation		Additional genotoxicity data required	
13.092 1489	2-Ethylfuran		0.061 0.5	Class II No evaluation		Additional genotoxicity data required	
13.103 1490	2-Butylfuran		0.24 0.5	Class II No evaluation		Additional genotoxicity data required	
13.106 1493	2-Decylfuran		0.0012 0.002	Class II No evaluation		Additional genotoxicity data required	
13.123 1521	Ethyl furfuryl ether		0.0012 0.002	Class II No evaluation		Additional genotoxicity data required	
13.138 1510	1-(2-Furyl)butan-3-one		2.2 3	Class II No evaluation		Additional genotoxicity data required	
13.148 1494	3-Methyl-2(3-methylbut-2-enyl)furan		0.12 0.2	Class II No evaluation		Additional genotoxicity data required	
13.031 751	2-Benzofurancarboxaldehyde		0.012 0.01	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	Evaluted in FGE.219, No genotoxicity concern (EFSA, Nov 2007). No safety concern at the estimated level of intake based on the MSDI approach	Melting point to be provided.
13.066 1506	3-Acetyl-2,5-dimethylfuran		ND 2	Class III No evaluation		Additional genotoxicity data required	
13.070 1512	2-Hexanoylfuran		ND 0.9	Class III No evaluation		Additional genotoxicity data required	

Table 3.1: Summary of safety evaluation of 33 furan derivatives evaluated by JECFA (JECFA, 2001b) and considered in FGE.67Rev1

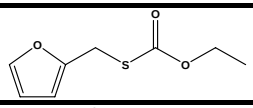
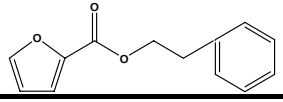
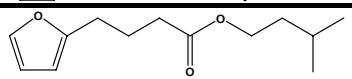
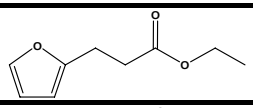
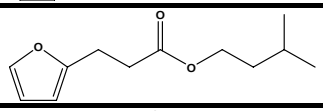
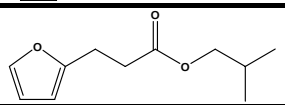
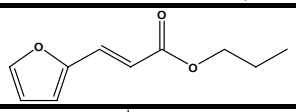
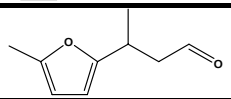
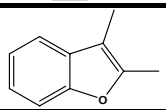
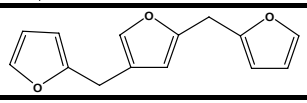
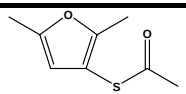
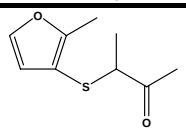
FL-no JECFA-no	EU Register name	Structural formula	EU MSDI 1) US MSDI (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
13.191 1526	o-Ethyl S-(2-furylmethyl)thiocarbonate		0.61 0.9	Class III No evaluation		Additional genotoxicity data required	
13.006 1517	Phenethyl 2-furoate		0.012 0.2	Class III No evaluation		No safety concern at the estimated level of intake based on the MSDI approach	No safety concern at the estimated level of intake based on the MSDI approach.
13.021 1516	Isopentyl 4-(2-furan)butyrate		0.24 0.09	Class III No evaluation		No safety concern at the estimated level of intake based on the MSDI approach	No safety concern at the estimated level of intake based on the MSDI approach.
13.022 1513	Ethyl 3(2-furyl)propionate		0.012 0.07	Class III No evaluation		No safety concern at the estimated level of intake based on the MSDI approach	No safety concern at the estimated level of intake based on the MSDI approach.
13.023 1515	Isopentyl 3-(2-furan)propionate		0.24 0.09	Class III No evaluation		No safety concern at the estimated level of intake based on the MSDI approach	No safety concern at the estimated level of intake based on the MSDI approach.
13.024 1514	Isobutyl 3-(2-furyl)propionate		0.12 24	Class III No evaluation		No safety concern at the estimated level of intake based on the MSDI approach	No safety concern at the estimated level of intake based on the MSDI approach.
13.047 1518	Propyl 3-(2-furyl)acrylate		2.2 1	Class III No evaluation		No safety concern at the estimated level of intake based on the MSDI approach	Stereoisomeric composition to be specified.
13.058 1500	3-(5-Methyl-2-furyl) butanal		0.0012 0.5	Class III No evaluation		Additional data required. No adequate NOAEL exists	
13.074 1495	2,3-Dimethylbenzofuran		0.52 0.01	Class III No evaluation		No safety concern at the estimated level of intake based on the MSDI approach	No safety concern at the estimated level of intake based on the MSDI approach.
13.107 1496	2,4-Difurfurylfuran		0.0012 0.002	Class III No evaluation		Additional genotoxicity data required	

Table 3.1: Summary of safety evaluation of 33 furan derivatives evaluated by JECFA (JECFA, 2001b) and considered in FGE.67Rev1

FL-no JECFA-no	EU Register name	Structural formula	EU MSDI 1) US MSDI (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
13.116 1523	2,5-Dimethyl-3-thioacetoxyfuran		3.0 4	Class III No evaluation		No safety concern at the estimated level of intake based on the MSDI approach	No safety concern at the estimated level of intake based on the MSDI approach.
13.190 1525	3-((2-Methyl3-furyl)thio)-2-butanone		0.012 0.02	Class III No evaluation		No safety concern at the estimated level of intake based on the MSDI approach	No safety concern at the estimated level of intake based on the MSDI approach. Racemate.

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.

ND: not determined.

Table 3.2: Summary of Safety Evaluation Applying the Procedure for substances in FGE.13Rev2 (EFSA, 2011h) (based on intakes calculated by the MSDI approach) for FGE.13Rev2 (EFSA, 2011h)

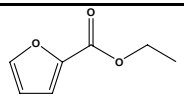
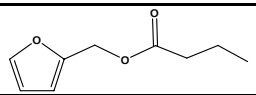
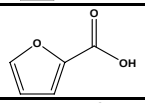
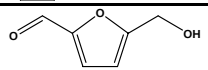
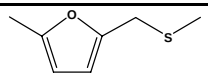
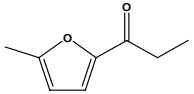
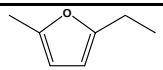
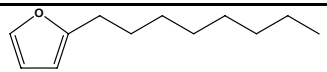
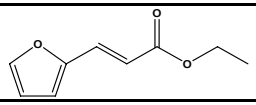
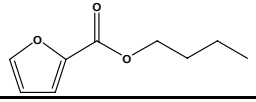
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
13.122	Ethyl 2-furoate		0.39	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	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)	
13.102	Butyl 2-furoate		0.12	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	

Table 3.2: Summary of Safety Evaluation Applying the Procedure for substances in FGE.13Rev2 (EFSA, 2011h) (based on intakes calculated by the MSDI approach) for FGE.13Rev2 (EFSA, 2011h)

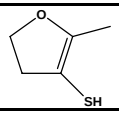
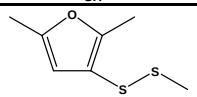
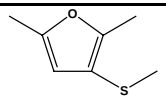
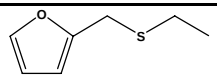
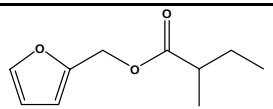
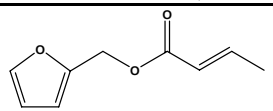
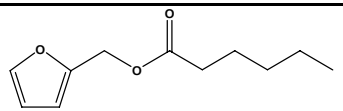
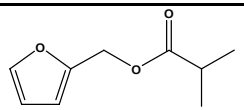
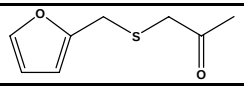
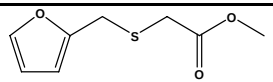
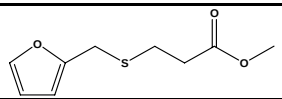
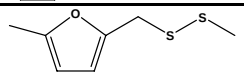
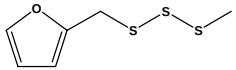
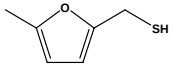
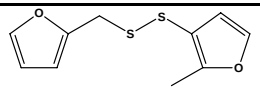
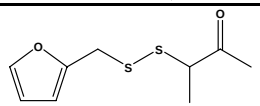
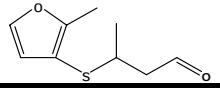
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
13.108	4,5-Dihydro-3-mercapto-2-methylfuran		37	Class III B3: Intake below threshold,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

Table 3.2: Summary of Safety Evaluation Applying the Procedure for substances in FGE.13Rev2 (EFSA, 2011h) (based on intakes calculated by the MSDI approach) for FGE.13Rev2 (EFSA, 2011h)

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
13.143	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 3.3: Summary of Safety Evaluation of 33 sulphur substituted furan derivatives (JECFA, 2002d) considered in FGE.65 (EFSA, 2009an)

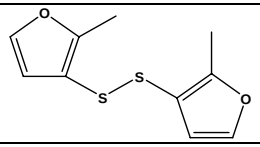
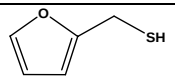
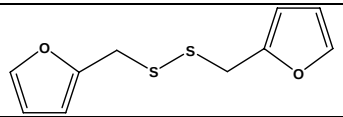
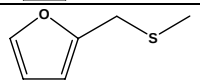
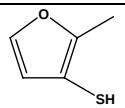
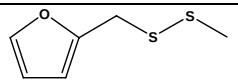
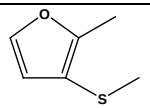
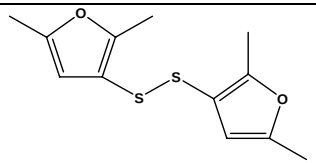
FL-no JECFA-no	EU Register name	Structural formula	EU MSDI 1) US MSDI (μg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
13.016 1066	bis-(2-Methyl-3-furyl) disulfide		0.27 0.7	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach	
13.026 1072	2-Furanmethanethiol		29 11	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach	
13.050 1081	Difurfuryl disulfide		3.3 0.7	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach	
13.053 1076	Methyl furfuryl sulfide		0.97 0.1	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach	
13.055 1060	2-Methylfuran-3-thiol		0.52 0.9	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach	According to JECFA: Min. assay value is "95" and secondary components "Bis(2-methyl-3-furyl)disulfide".
13.064 1078	Methyl furfuryl disulfide		0.85 0.04	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach	
13.152 1061	2-Methyl-3-(methylthio)furan		1.2 0.1	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach	
13.015 1067	bis-(2,5-Dimethyl-3-furyl) disulfide		0.012 0.7	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach	

Table 3.3: Summary of Safety Evaluation of 33 sulphur substituted furan derivatives (JECFA, 2002d) considered in FGE.65 (EFSA, 2009an)

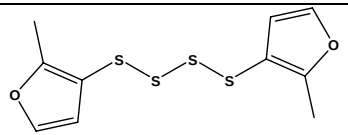
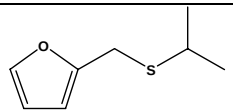
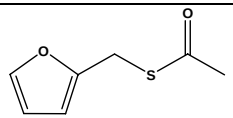
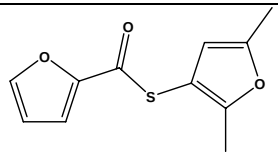
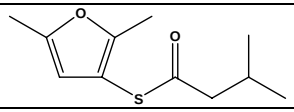
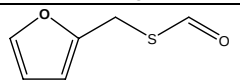
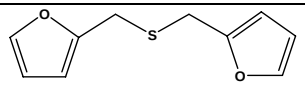
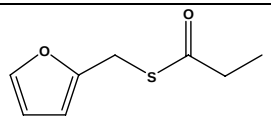
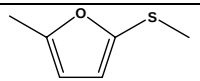
FL-no JECFA-no	EU Register name	Structural formula	EU MSDI 1) US MSDI (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
13.017 1068	bis-(2-Methyl-3-furyl) tetrasulfide		0.97 0.7	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach	
13.032 1077	Furfuryl isopropyl sulfide		0.0012 0.1	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach	
13.033 1074	S-Furfuryl acetothioate		0.43 0.05	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach	
13.040 1071	2,5-Dimethyl-3-thiofuroylfuran		0.012 0.01	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach	JECFA evaluated (S)-2,5-dimethyl-3-thiofuroylfuran (CASrn as in Register). Register CASrn refers to the (S)-enantiomer.
13.041 1070	2,5-Dimethyl-3-(isopentylthio)furan		0.49 0.7	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach	Registername to be changed to 2,5-dimethyl-3-(isovalerylthio)furan.
13.051 1073	2-Furfuryl thioformate		1.3 0.02	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach	
13.056 1080	Difurfuryl sulfide		0.73 0.005	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	No adequate NOAEL exists, additional data required	
13.063 1075	S-Furfuryl propanethioate		0.012 0.005	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach	
13.065 1062	2-Methyl-5-(methylthio)furan		1.1 0.02	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach	

Table 3.3: Summary of Safety Evaluation of 33 sulphur substituted furan derivatives (JECFA, 2002d) considered in FGE.65 (EFSA, 2009an)

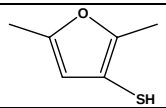
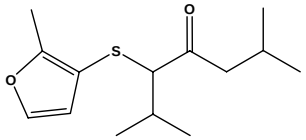
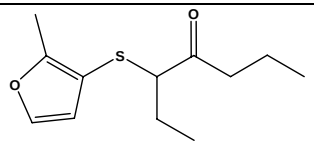
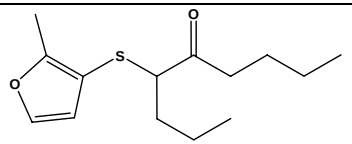
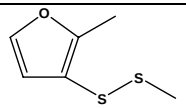
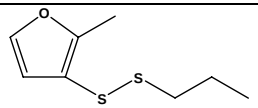
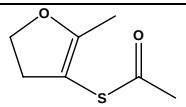
FL-no JECFA-no	EU Register name	Structural formula	EU MSDI 1) US MSDI (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
13.071 1063	2,5-Dimethylfuran-3-thiol		0.024 0.7	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach	
13.075 1086	2,6-Dimethyl-3-((2-methyl-3-furyl)thio)heptan-4-one		1.8 0.7	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach	According to JECFA: Min. assay value is "94" and secondary components "2,6-Dimethyl-2-[(2-methyl-3-furyl)thio]-4-heptanone" Composition of mixture to be specified.
13.077 1085	3-((2-Methyl-3-furyl)thio)heptan-4-one		2.9 0.7	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach	
13.078 1087	4-((2-Methyl-3-furyl)thio)nonan-5-one		0.73 0.7	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach	
13.079 1064	Methyl 2-methyl-3-furyl disulfide		0.73 0.05	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach	According to JECFA: Min. assay value is "97" and secondary components "up to 3% bis(2-methyl-3-furyl)disulfide".
13.082 1065	Propyl 2-methyl-3-furyl disulfide		0.12 0.7	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach	According to JECFA: Min. assay value is "97" and secondary components "up to 2% bis(2-methyl-3-furyl)disulfide and propyl disulfide".
13.086 1089	4,5-Dihydro-2-methyl-3-thioacetoxyfuran		0.49 0.7	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach	

Table 3.3: Summary of Safety Evaluation of 33 sulphur substituted furan derivatives (JECFA, 2002d) considered in FGE.65 (EFSA, 2009an)

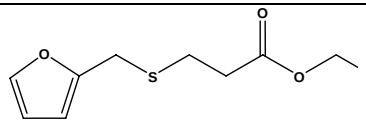
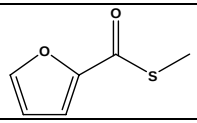
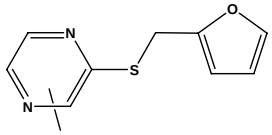
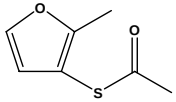
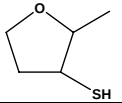
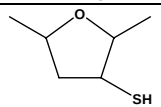
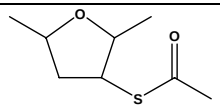
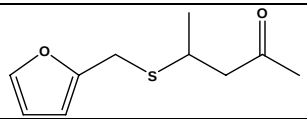
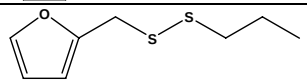
FL-no JECFA-no	EU Register name	Structural formula	EU MSDI 1) US MSDI (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
13.093 1088	Ethyl 3-(2-furfurylthio)propionate		0.012 0.2	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach	
13.142 1083	S-Methyl 2-furanthiocarboxylate		0.37 0.1	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach	
13.151 1082	2-Methyl-3,5 and 6-(furfurylthio)pyrazine 2 or 5 or 6 -Methyl-3-(furfurylthio)pyrazine		0.37 0.7	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach	According to JECFA: Min. assay value is "99" and "Mixture of isomers: 70% 2,3-; 29% 2,6-; trace 2,5-".
13.153 1069	2-Methyl-3-furyl thioacetate		0.012 0.07	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach	According to JECFA: Min. assay value is "92" and secondary components "cis- and trans-2-Methyl-3-tetrahydrofuranthiol acetate" Composition of mixture to be specified.
13.160 1090	2-Methyltetrahydrofuran-3-thiol		3.5 0.7	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	No adequate NOAEL exists, additional data required	According to JECFA: Min. assay value is "97" and "71% trans and 26% cis isomer".
13.193 1091	2,5-Dimethyltetrahydro-3-furanthiol		0.024 0.9	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	No adequate NOAEL exists, additional data required	According to JECFA: Min. assay value is "96 (mixture of 4 stereoisomers)" Composition of mixture to be specified.
13.194 1092	2,5-Dimethyltetrahydro-3-furyl thio acetate		0.012 2	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	No adequate NOAEL exists, additional data required	According to JECFA: Min. assay value is "90 (mixture of 4 stereoisomers)" and secondary components "2,5-Dimethyltetrahydrofuran-3-thiol, Dimethyltetrahydro-3-furyl dithioacetate" Composition of mixture to

Table 3.3: Summary of Safety Evaluation of 33 sulphur substituted furan derivatives (JECFA, 2002d) considered in FGE.65 (EFSA, 2009an)

FL-no JECFA-no	EU Register name	Structural formula	EU MSDI 1) US MSDI (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
							be specified.
13.196 1084	4-(Furfurylthio) pentan-2-one		0.012 0.6	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach	Register name to be changed to 4-[(2-furanylmethyl)thio]-2-pentanone.
13.197 1079	Furyl propyldisulfide		0.024 3	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	No safety concern at estimated level of intake as flavouring substance based on the MSDI approach	Register name to be changed to Furfuryl propyl disulfide.

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.

ND: not determined

Table 3.4: Summary of Safety Evaluation of 15 JECFA-Evaluated Furfuryl Derivatives (JECFA, 2001b) considered in FGE.66Rev1 (EFSA, 2011ad)

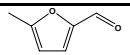
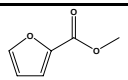
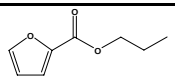
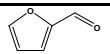
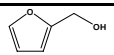
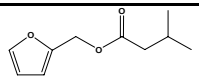
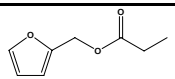
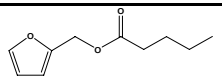
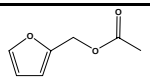
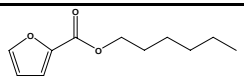
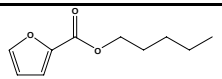
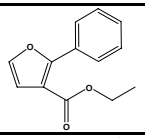
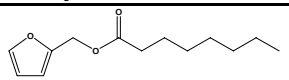
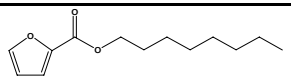
FL-no JECFA-no	EU Register name	Structural formula	EU MSDI 1) US MSDI (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
13.001 745	5-Methylfurfural		180 25	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
13.002 746	Methyl 2-furoate		30 37	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	No safety concern at the estimated level of intake based on the MSDI approach	No safety concern at the estimated level of intake based on the MSDI approach.
13.003 747	Propyl 2-furoate		0.061 0.1	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	No safety concern at the estimated level of intake based on the MSDI approach	No safety concern at the estimated level of intake based on the MSDI approach.
13.018 450	Furfural		440 460	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	No safety concern at the estimated level of intake based on the MSDI approach	No safety concern at the estimated level of intake based on the MSDI approach.
13.019 451	Furfuryl alcohol		180 24	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	No safety concern at the estimated level of intake based on the MSDI approach. Evaluated by JECFA before 2000 - No EFSA consideration required	No safety concern at the estimated level of intake based on the MSDI approach.
13.057 743	Furfuryl isovalerate		0.024 1	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	No safety concern at the estimated level of intake based on the MSDI approach	No safety concern at the estimated level of intake based on the MSDI approach.
13.062 740	Furfuryl propionate		1.7 5	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	No safety concern at the estimated level of intake based on the MSDI approach	No safety concern at the estimated level of intake based on the MSDI approach.
13.068 741	Furfuryl valerate		0.24 14	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	No safety concern at the estimated level of intake based on the MSDI approach	No safety concern at the estimated level of intake based on the MSDI approach.
13.128 739	Furfuryl acetate		16 21	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	No safety concern at the estimated level of intake based on the MSDI approach	No safety concern at the estimated level of intake based on the MSDI approach.
13.005 749	Hexyl 2-furoate		0.061 0.1	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	No safety concern at the estimated level of intake based on the MSDI approach	No safety concern at the estimated level of intake based on the MSDI approach.

Table 3.4: Summary of Safety Evaluation of 15 JECFA-Evaluated Furfuryl Derivatives (JECFA, 2001b) considered in FGE.66Rev1 (EFSA, 2011ad)

FL-no JECFA-no	EU Register name	Structural formula	EU MSDI 1) US MSDI (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
13.025 748	Pentyl 2-furoate		0.36 0.1	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	No safety concern at the estimated level of intake based on the MSDI approach	No safety concern at the estimated level of intake based on the MSDI approach.
13.038 752	2-Phenyl-3-carbethoxyfuran		0.012 2	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	No safety concern at the estimated level of intake based on the MSDI approach	No safety concern at the estimated level of intake based on the MSDI approach.
13.067 742	Furfuryl octanoate		0.012 6	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	No safety concern at the estimated level of intake based on the MSDI approach	No safety concern at the estimated level of intake based on the MSDI approach.
13.073 750	Octyl 2-furoate		2.2 0.1	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	No safety concern at the estimated level of intake based on the MSDI approach	No safety concern at the estimated level of intake based on the MSDI approach.

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.

ND: not determined.

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ABBREVIATIONS

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
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)
GLP	Good laboratory practise
GSH	Glutathione
ID	Identity
IR	Infrared spectroscopy
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
MSDI	Maximised Survey-derived Daily Intake
mTAMDI	Modified Theoretical Added Maximum Daily Intake
NCE	Normochromatic erythrocytes
No	Number
NOAEL	No observed adverse effect level
NTP	National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
PCE	Polychromatic erythrocytes
SCE	Sister chromatic exchange
SCF	Scientific Committee on Food
UDS	Unscheduled DNA synthesis
WHO	World Health Organisation