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SCIENTIFIC OPINION

Scientific Opinion on Flavouring Group Evaluation 66, Revision 1 (FGE.66Rev1): Consideration of Furfuryl Alcohol and Related Flavouring Substances Evaluated by JECFA (55th meeting)[1]

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

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

The Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids of the European Food Safety Authority was requested to evaluate 14 flavouring substances in the Revision 1 of Flavouring Group Evaluation 66, using the Procedure in Commission Regulation (EC) No 1565/2000. None of the substances were considered to have genotoxic potential. 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. The Panel concluded that the 14 substances [FL-no: 13.001, 13.002, 13.003, 13.005, 13.018, 13.019, 13.025, 13.038, 13.057, 13.062, 13.067, 13.068, 13.073 and 13.128] do not give rise to safety concerns at their levels of dietary intake, estimated on the basis of the MSDI approach.

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1 On request from the Commission, Question No EFSA-Q-2011-00863, EFSA-Q-2011-00864, EFSA-Q-2011-00865, EFSA-Q-2011-00866 adopted on 6 July 2011.

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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 JECFA has evaluated 15 furfuryl alcohol derivatives at their 55th meeting. The Panel concluded that 14 substances in Flavouring Group Evaluation 66 (FGE.66) are structurally related to the subgroup of 10 furfuryl derivatives in the group of furfuryl and furan derivatives with and without additional side-chain substituents and heteroatoms evaluated by EFSA in FGE.13, Revision 2 (FGE.13Rev2). The 15th substance, 2-Benzofurancarboxaldehyde [FL-no: 13.031], is considered too structurally different from the other 14 to be included in the present FGE. This substance will be evaluated in FGE.67Rev1.

Eight of the candidate substances in this FGE are alpha,beta-unsaturated aldehydes or precursors for such which is a structural alert for genotoxicity. These eight substances [FL-no: 13.001, 13.018, 13.019, 13.057, 13.062, 13.067, 13.068 and 13.128] have initially been considered with respect to genotoxicity. The Panel concluded that for all eight substances the genotoxicity data available do not preclude their evaluation through the Procedure. No concern for genotoxicity was identified for the remaining six substances without the alpha,beta structural alert [FL-no: 13.002, 13.003, 13.005, 13.025, 13.038 and 13.073].

The Panel agrees with the application of the Procedure as performed by the JECFA for 13 substances considered in this FGE [FL-no: 13.002, 13.003, 13.005, 13.018, 13.019, 13.025, 13.038, 13.057, 13.062, 13.067, 13.068, 13.073 and 13.128]. In contrast to the JECFA, the Panel evaluated the fourteenth substance [FL-no: 13.001] by comparison with a Benchmark Dose, Lower Confidence Limit (BMDL) for a metabolite (5-hydroxymethylfurfural; [FL-no: 13.139]). For none of the candidate substances in this FGE a safety concern was identified.

For one substance, furfural [FL-no: 13.018], use levels have been provided by Industry and an mTAMDI figure of 9700 microgram/person/day could be calculated. For the remaining 13 substances [FL-no: 13.001, 13.002, 13.003, 13.005, 13.019, 13.025, 13.038, 13.057, 13.062, 13.067, 13.068, 13.073 and 13.128] 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.

Adequate specifications including purity criteria and identity test are available for all 14 JECFA evaluated substances considered in the present FGE.

Thus, for all 14 JECFA evaluated furfuryl derivatives [FL-no: 13.001, 13.002, 13.003, 13.005, 13.018, 13.019, 13.025, 13.038, 13.057, 13.062, 13.067, 13.068, 13.073 and 13.128] the Panel agrees with the JECFA conclusion “No safety concern at estimated levels of intake as flavouring substances” based on the MSDI approach.

KEY WORDS

Alpha,beta-unsaturated aldehydes, furfural, furfuryl derivatives, furfuryl alcohol, flavouring substances, safety evaluation, FGE.66, FGE.66Rev1.

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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.

After the finalisation of the evaluation programme 31 December 2009, in their letter of the 11 September 2009, the Commission requested EFSA to carry out a re-evaluation of 5-methylfurfural [FL-no: 13.001] in accordance with Commission Regulation (EC) No 1565/2000 (EC, 2000a).

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 if it 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 the 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 OF THE SUBSTANCES IN THE PRESENT FGE

The Flavouring Group Evaluation 66 concerned furfuryl alcohol and 13 related substances evaluated by the JECFA at their 55th meeting. Eight of the 14 substances are alpha,beta-unsaturated aldehydes or precursors for such, which the Panel considers to be a structural alert for genotoxicity. Accordingly, these eight substances were initially considered with respect to genotoxicity (see Section 1.1.2). The Panel concluded in FGE.218 (EFSA, 2009s) that the alpha,beta-unsaturated structure did not give rise to concern for genotoxicity in these eight substances, but for one of the substances, 5-methylfurfural [FL-no: 13.001], a genotoxicity concern could not be ruled out due to other structural alerts in the substance.

FGE	Opinion adopted by EFSA	Link	No. of candidate substances
FGE.66	9 July 2008	http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1211902338972.htm	14
FGE.66Rev1	6 July 2011		14

Additional genotoxicity data have become available for a structurally related substance to 5-methylfurfural [FL-no: 13.001], namely 5-hydroxymethylfurfural [FL-no: 13.139] for which additional genotoxicity data have been requested in FGE.13. It is anticipated that 5-methylfurfural can be oxidised to the primary alcohol 5-hydroxymethylfurfural (EFSA, 2010ak) and accordingly the new data submitted for 5-hydroxymethylfurfural was used in FGE.218Rev1 (EFSA, 2010ak) and will be used in this FGE to support the evaluation of 5-methylfurfural. Therefore the evaluation of these genotoxicity data submitted by the Industry are included in FGE.66Rev1.

Since the publication of FGE.66, the EU production volumes have been provided for three substances [FL-no: 13.003, 13.005 and 13.025], the evaluation of which could not be finalised in the previous version of this FGE, due to lack of these data. Based on the newly submitted EU production volumes these substances have already been evaluated in FGE.96, but for the sake of completion, the information has also been included here as well.

1. Presentation of the Substances in the JECFA Flavouring Group

1.1. Description

1.1.1. JECFA Status

The JECFA has evaluated a group of 14 furfuryl derivatives and one benzofurancarboxaldehyde at their 55th meeting (JECFA, 2001a).

1.1.2. EFSA Considerations

Nine of the substances in the group of furfuryl alcohol and related substances evaluated by the JECFA (JECFA, 2001a) are alpha,beta-unsaturated aldehydes or precursors for alpha,beta-unsaturated aldehydes. As the alpha,beta-unsaturated aldehyde and ketone structures are considered by the Panel to be structural alerts for genotoxicity, these nine substances, i.e. eight substances in FGE.218 (subgroup 4.2 of FGE.19 (EFSA, 2008b)) [FL-no: 13.001, 13.018, 13.019, 13.057, 13.062, 13.067, 13.068 and 13.128] (EFSA, 2009s) and one substance (2-benzofurancarboxaldehyde [FL-no: 13.031]) from subgroup 4.3 of FGE.19 (EFSA, 2008b), have initially been considered with respect to genotoxicity. The Panel concluded that although the eight substances in FGE.218 (EFSA, 2009s) and FGE.218Rev1 (EFSA, 2010ak) have a structural alert for genotoxicity, the data available do not preclude the evaluation of these eight substances through the Procedure (see Section 3.3 to 3.6). For the ninth substance, 2-benzofurancarboxaldehyde [FL-no: 13.031], the Panel concluded that the substance is structurally different from the eight substances in FGE.218 and should be considered separately in subgroup 4.3 from FGE.19 (EFSA, 2008b). This substance will be considered in Revision 1 of FGE.67, together with another structurally related flavouring substance. Accordingly the present FGE.66Rev1 deals with 14 of the 15 substances from the group of furfuryl alcohol and related substances evaluated by JECFA.

Five [FL-no: 13.002, 13.003, 13.005, 13.025, 13.073] of the remaining six substances are esters of furoic acid and the sixth substance [FL-no: 13.038] is not structurally related to furfural. As for this substance, in other FGEs, no related compounds could be found, it will be evaluated in this FGE.

Thus, this FGE deals with in total 14 candidate substances. Thirteen substances in the JECFA flavouring group of furfuryl alcohol and related substances are structurally related to the subgroup of 10 furfuryl derivatives (without sulphur containing substituents) in the group of furfuryl and furan derivatives with and without additional side-chain substituents and heteroatoms evaluated by EFSA in FGE.13Rev2 subgroup Ia (EFSA, 2011h). The remaining substance [FL-no: 13.038] is to be evaluated on its own in this FGE.

1.2. Isomers

1.2.1. Status

None of the JECFA evaluated substances in the group of furfuryl alcohol and related substances has a chiral centre or can exist as geometrical isomers.

1.3. Specifications

1.3.1. Status

The JECFA specifications are available for all 14 substances (JECFA, 2000d). See Table 1.

1.3.2. EFSA Considerations

Adequate specifications are available for all 14 substances. .

2. Intake Estimations

2.1. Status

For all substances evaluated through the JECFA Procedure intake data are available for the EU, see Table 3.1.

2.2. EFSA Considerations

For one of the 14 substances, [FL-no: 13.018], the Industry has submitted use levels for normal and maximum use (EC, 2000a; EFFA, 2000e). Based on the normal use levels an mTAMDI figure of 9700 microgram/person/day can be calculated (EFSA, 2004d).

Table 2.2.1 Normal and Maximum use levels (mg/kg) available for JECFA evaluated substances in FGE.66Rev1

FL-no	Food Categories Normal use levels (mg/kg) Maximum use levels (mg/kg)																	
	01.0	02.0	03.0	04.1	04.2	05.0	06.0	07.0	08.0	09.0	10.0	11.0	12.0	13.0	14.1	14.2	15.0	16.0
13.018	25	-	25			25	5	5	-	-	-	-	25	25	15	17		
	60	-	50	0,25	0,25	700	25	125	-	-	-	-	63		28	30	25	25

Table 2.2.2 Estimated intakes based on the MSDI approach and the mTAMDI approach

FL-no	EU Register name	MSDI – EU (µg/*capita*/day)	MSDI – USA (µg/*capita*/day)	mTAMDI (µg/person/day)	Structural class	Threshold of concern (µg/person/day)
13.001	5-Methylfurfural	180	25		Class II	540
13.002	Methyl 2-furoate	30	37		Class II	540
13.003	Propyl 2-furoate	0.061	0.1		Class II	540
13.018	Furfural	440	460	9700	Class II	540
13.019	Furfuryl alcohol	180	24		Class II	540
13.057	Furfuryl isovalerate	0.024	1		Class II	540
13.062	Furfuryl propionate	1.7	5		Class II	540
13.068	Furfuryl valerate	0.24	14		Class II	540
13.128	Furfuryl acetate	16	21		Class II	540
13.005	Hexyl 2-furoate	0.061	0.1		Class III	90
13.025	Pentyl 2-furoate	0.36	0.1		Class III	90
13.038	2-Phenyl-3-carbethoxyfuran	0.012	2		Class III	90
13.067	Furfuryl octanoate	0.012	6		Class III	90
13.073	Octyl 2-furoate	2.2	0.1		Class III	90

3. Genotoxicity Data

3.1. Genotoxicity Studies – Text taken[4] from the JECFA (JECFA, 2001b)

No genotoxicity text was prepared by the JECFA on the group of furfuryl alcohol and related substances – the studies are only given in table format (see Table 2.1).

[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*.

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

One of the substances in the group of furfuryl alcohol and related substances is furfural [FL-no: 13.018], which also was considered separately at the 55th meeting (JECFA, 2001b) where the following was stated:

"Furfural was evaluated previously by the Committee at its thirty-ninth and fifty-first meetings (JECFA, 1992a; JECFA, 2000a). An ADI was not established at either meeting because of concern about the finding of tumours in male mice given furfural in corn oil by gavage and the fact that no NOEL was identified for hepatotoxicity in male rats. In a study in mice, the combined incidence of adenomas and carcinomas was increased in males at the highest dose (175 mg/kg bw per day). In order to address its concern with regard to the formation of liver tumours in mice, the Committee at its fifty-first meeting requested the results of studies of DNA binding or adduct formation *in vivo* to clarify whether furfural interacts with DNA in the liver of mice, and also requested the results of a 90-day toxicity study in rats to identify a NOEL for hepatotoxicity (JECFA, 2000a).

Since the last meeting, the results of a 14-day study to determine a dose range, a 90-day study of toxicity in rats, and an assay for unscheduled DNA synthesis in mice *in vivo* have become available. These data were reviewed and are summarized in the following monograph addendum."

"The ability of furfural to induce DNA repair in the hepatocytes of B6C3F1 mice was assessed in an assay for unscheduled DNA synthesis. The maximum tolerated dose for animals of each sex was determined in a preliminary study to be 320 mg/kg bw. In the study of unscheduled DNA synthesis, doses of 50, 175, and 320 mg/kg bw were given to groups of three animals of each sex, and expression of DNA repair was measured 2 - 4 and 12 - 16 hours after treatment. N-Nitrosodimethylamine (20 mg/kg bw) was used to measure expression within 2 - 4 hours and aminoazotoluene (200 mg/kg bw) for expression within 12 - 16 hours, as positive controls.

The animals treated with furfural did not show increased unscheduled DNA synthesis at either time after dosing, whereas the positive controls showed statistically significant increases in net nuclear grain counts. Little replicative DNA synthesis (0 - 0.4 %) was seen at either interval. The results provided no evidence that furfural damages DNA in mouse hepatocytes at doses up to 320 mg/kg bw (Edwards, 1999)."

"The results of an assay for unscheduled DNA synthesis in mice *in vivo* were reviewed by the Committee. This study, in which doses of up to 350 mg/kg bw were given, was particularly relevant since it addressed potential DNA repair in the cells in which tumours arose, namely hepatocytes. The negative results obtained in this assay were considered by the Committee to provide evidence that the liver tumours observed in the long-term study in mice were unlikely to have occurred through a genotoxic mechanism. The Committee considered that the concerns raised previously with respect to the liver tumours in mice were adequately addressed by this study and that a study of DNA binding was unnecessary."

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

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

Data on *in vitro* genotoxicity were provided for the two candidate substances, 5-hydroxymethylfurfural (5-HMF) [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. New genotoxicity data on the candidate substance 5-HMF have become available and will be considered in this revision of FGE.13.

[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.*

Candidate substances

5-hydroxymethyl furfural [FL-no: 13.139]

In the *in vitro* tests, 5-HMF 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 2.2 of this FGE). 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, 5-HMF 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, 5-HMF gave negative results in the HPRT and TK assay (Surh and Tannenbaum, 1994).

In an Ames test with TA104 strain upon inclusion of PAPS, a sulpho-group donor, and rat liver cytosol into the experimental model, 5-HMF 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 5-HMF 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 5-HMF (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 (> 40 microg/ml) reducing cell survival to > 63 %. No genotoxicity was observed with 5-HMF, 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 5-HMF in V79 and Caco-2 cells up to cytotoxic concentrations (80 mM). 5-HMF caused 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. 5-HMF 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 5-HMF *in vivo* (Janzowski et al., 2000).

To support the genotoxic potential of 5-HMF, some indications for tumorigenic activities of 5-HMF have been obtained with rats and mice. It has been reported that 5-HMF 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 5-HMF to mice (Surh et al., 1994).

"Newly submitted data on 5-hydroxymethylfurfural[7]

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

At the end of a 3-month toxicity study, peripheral blood samples were obtained from male and female B6C3F1 mice receiving 0, 47, 188, 375 or 750 mg/kg bw/day of 5-HMF 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 (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 5-HMF 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 5-HMF 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 DNA damage induced in all cell lines was unrelated to the expression of SULT1A1 but they mentioned that the SULT1A1 activities in these three cell lines (Caco-2, HEK293 and L5178Y) were much lower than those that can be found in human gut and liver. The possibility was left open that SULT1A1 activity was too low to efficiently bioactivate 5-HMF 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 5-HMF.

These results are in conflict with the results of Glatt et al. (Glatt et al., 2005) who reported induction of SCE in 5-HMF-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 5-HMF 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

[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.

should be investigated, but that under the conditions of the test, 5-HMF is a rather weak DNA-damaging agent.

In a new publication by Severin et al. (2010), a dose dependent increase in DNA damage was observed in an COMET assay with HepG2 cells exposed to 5-HMF (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 5-HMF. 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 5-HMF was found in an *in vitro* micronucleous assay in the same cell line exposed to similar doses of HMF (20 hours). HMF was also tested in an Ames test performed according to the OECD guidelines 471. No increase in mutants was observed in *S. typhimurium* strains TA 98, TA 100, TA1535 and TA 1537 exposed to 5-HMF 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 5-HMF was unable to induce micronuclei *in vivo*, in the NTP 3-months study in mice by gavage, and *in vitro*, using the Hep-G2 human cell line expressing both CYP and SULT enzymes, its metabolite SMF has been reported to induce micronuclei in peripheral erythrocytes in mice ((Dahlberg, 2004) as cited by Glatt and Sommer, 2006 (no further data were available)).

According to Glatt and Sommer, incubation of DNA with SMF in a cell-free system led to the formation of DNA adducts that could be detected by the ^{32}P-postlabelling technique. No adducts were formed in incubations with 5-HMF 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 5-HMF. Although mutations were induced, adducts were not seen in these cells under the same conditions. 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 5-HMF, 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, 5-HMF 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 limit is the use of too high concentrations that can produce unpredictable effects, not related to the real genotoxic potential of 5-HMF, 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 5-HFM 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 5-HMF, intravenously. This indicates that there is a competition for the substrate 5-HMF between the oxidation pathway leading to the furoic acid derivative and the sulphonation pathway leading to the SMF metabolite. The Panel noted that SMF is very hydrophilic and therefore will have problems crossing the cell membrane and entering cells. Therefore SMF is more likely to induce mutation at the site of formation, mainly the liver. In addition, the half life was reported to be 4.2 minutes, and it is not likely that this metabolite will manage to reach the bone marrow and give any positive effect in an *in vivo* micronucleous test, taking into account that SMF will most likely be formed in the liver. However, 5-HMF has been found unable to

induce micronuclei also *in vitro*, using the HepG2 human cell line, expressing both CYP and SULT enzymes. In the rodents bioassays no carcinogenic response was observed and from this it may be concluded that the formation of the SMF metabolite is too low to result in a carcinogenic response. Assuming that in humans the ratio between the two competing pathways is not more favourable for the formation of SMF than in rodents, no gentoxicity or carcinogenicity is expected in humans either."

Furoic acid [FL-no: 13.136]

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

Supporting substances

In vitro genotoxicity data were available for five supporting substances (furfuryl acetate, furfuryl alcohol, furfural, 5-methylfurfural and methyl-2-furoate) and *in vivo* genotoxicity data for the two supporting substances furfuryl alcohol and furfural. Most studies were negative, although some positive results were reported. However, the genotoxicity of furfural has 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 the 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 5-HMF [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 of FGE.13Rev2, which could preclude their evaluation through the Procedure.

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

3.4. SCF Opinion on Furfural and Furfural Diethylacetal[8] (SCF, 2003a)

"Special studies on genotoxicity

Negative or weakly positive results have been obtained for most bacterial tests for genotoxicity. In particular, positive results were obtained in three out of several assays for reverse mutation in *S. typhimurium* at relatively high concentrations in the absence of metabolic activation. Furfural was found to be clearly genotoxic in cultured mammalian cells at the gene and chromosome level in the absence of metabolic activation. It induced SCE in cultured CHO cells and human lymphocytes. It was genotoxic in *Drosophila* in somatic cells (Wing spot test by inhalation) and germ cells (sex-chromosome loss by injection). It did not induce reciprocal translocations and sex-linked recessive lethal mutations, with only a doubtful increase in one study in *Drosophila*. Furfural was not genotoxic in any *in vivo* mammalian assays for chromosome aberrations, SCE or UDS."

Conclusion

"The Committee was of the opinion that the data were not totally convincing in demonstrating that the carcinogenicity of furfural was mediated via a thresholded mechanism and hence was unable to allocate an ADI to furfural at the present time. It was aware that a study in transgenic mice of the potential of furfural to induce gene mutations *in vivo* was in progress. The results were expected to be

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

available in the near future and the Committee would wish to re-evaluate furfural in the light of the results of this study."

The results of genotoxicity studies on furfural are summarised in Table 2.4.

3.5. EFSA Opinion on Furfural and Furfural Diethylacetal[9] (EFSA, 2004c)

"Special Studies on Genotoxicity

In a new study not previously evaluated by the SCF, furfural was examined for its potential to induce gene mutations of the λlacZ-gene *in vivo* in the liver of male transgenic mice (CD2F1(BALB/c x DBA/2) strain 40.6, with lacZ-genes as reporter genes). The study was carried out under GLP. As formal technical guidelines for this type of study are not available, the study protocol was designed in conformity with principles for transgenic studies identified by international expert groups (Gorelick and Mirsalis, 1996; Heddle et al., 2000). The study was conducted in five groups, three of which received furfural by gavage in corn oil, one negative control group received vehicle alone and one positive control group received ethylnitrosourea (ENU). The furfural and negative control groups each comprised 13 mice plus 2 back up animals; the positive control group comprised 8 mice plus 2 reserves. The furfural groups were given doses of 75, 150 or 300 mg furfural/kg bw in corn oil by gavage for 28 consecutive days; ENU was given to the positive control group by intraperitoneal injection in saline on days 5 - 9 of the study at a dose of 50 mg/kg bw/day. On day 28, three animals from each of the furfural and negative control groups were sacrificed for assessment of hepatotoxicity by clinical chemistry and histological examination. In addition, organ and body weights were monitored throughout. After a manifestation period of 34 - 35 days (days 62 - 63 of the study), the livers and samples of gastrointestinal tract tissues were fixed for mutation analysis. Mutation analysis was carried out on the livers of eight animals per group. At least 5000 (preferably 120,000) plaque-forming units (PFU) were examined (one PFU corresponding to one recovered copy of the λgt10lacZ shuttle vector).

There were three early decedents in the highest furfural dose group; two during treatment with no clinical signs, and one during the manifestation period. One animal from the low-dose group died during the manifestation period. The cause of death could not be ascertained.

Body weights in the furfural-treated groups showed a dose related increase compared to negative controls during the first week of treatment. In the post-treatment period the difference between control and two lower dose groups disappeared but the body weight of the group treated with 300 mg furfural/kg bw remained higher.

Evaluation of the clinical chemistry and gross and histopathology of the liver of the treated animals sacrificed at the end of the treatment period showed an increase in blood triglycerides, increased liver weight and centrilobular hypertrophy. This was interpreted by the authors as some evidence of hepatotoxicity. These changes did not persist until the end of the manifestation period, 34 - 35 days after the last dose.

The mutation frequency in DNA extracted from the livers of the negative control group was similar to historical data. There was no significant difference in mutation frequency between negative controls and the furfural-treated groups; the positive control group showed a significant increase in mutation frequency. It was concluded that oral administration of furfural in corn oil at levels of up to 300 mg/kg bw/day is not associated with an increase in the induction of mutations in liver cells of λlacZ transgenic mice (CIVO-TNO, 2003).

[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*.

Negative or weakly positive results have been obtained for most bacterial tests for genotoxicity. In particular, positive results were obtained in three out of several assays for reverse mutation in *Salmonella typhimurium* at relatively high concentrations in the absence of metabolic activation. Furfural was found to be clearly genotoxic in cultured mammalian cells at the gene and chromosome level in the absence of metabolic activation. It induced Sister Chromatid Exchange (SCE) in cultured Chinese Hamster Ovary (CHO) cells and human lymphocytes. It was genotoxic in *Drosophila* in somatic cells (Wing spot test by inhalation) and germ cells (sex-chromosome loss by injection). It did not induce reciprocal translocations and sex-linked recessive lethal mutations, with only a doubtful increase in one study in *Drosophila*. Furfural was not genotoxic in any *in vivo* mammalian assays for chromosome aberrations, SCE or Unscheduled DNA Synthesis (UDS) and the study in transgenic mice confirms that furfural does not induce gene mutations *in vivo*.

Discussion

The Panel noted the metabolic and toxicity data previously reviewed by the SCF together with the new results of the genotoxicity study in transgenic mice *in vivo*.

Furfural was negative in the *in vivo* genotoxicity assay and this corroborated earlier negative *in vivo* studies at the chromosome level and in a UDS assay.

In view of the absence of genotoxicity *in vivo*, the tumours observed in the long-term toxicity/carcinogenicity studies in male, but not female mice, are considered to arise as a consequence of chronic hepatotoxicity (hepatocellular necrosis) which was more marked in male animals. An increased tumour incidence was only observed at the highest dose level and at a dose higher than the minimal hepatotoxic dose.

It should be noted that no hepatocellular tumours were seen in the long-term rat study. However, liver toxicity was seen in this study (see SCF Opinion, Appendix 1) and the rat was considered more sensitive to liver toxicity.

The hepatotoxicity of furfural is dose-dependent but a NOEL was not established in the long-term studies. However, the short-term (90-day) study in rats was conducted to establish a NOEL for hepatotoxicity, which was determined to be 54 mg/kg bw. The Panel noted that because of possible formulation (corn oil) and dose regimen (bolus dose) effects observed in the gavage studies, the dietary administration studies were more appropriate for identifying a NOAEL. The Panel concluded that the NOAEL of 54 mg/kg bw/day for hepatic changes from the 90 day dietary study was appropriate and noted that the effects observed with doses up to threefold higher were of doubtful toxicological relevance. Therefore the Panel concluded that a safety factor of 100 would be sufficient in establishing an ADI from this subchronic study (see SCF Opinion).

Conclusion and Recommendation

The Panel concluded that furfural did not exhibit genotoxicity *in vivo* in male mice, the species and sex which displayed an increased tumour incidence in long-term studies and that the tumours arose by a secondary mechanism consequent on hepatotoxicity, which is dose dependent, displays a threshold and is seen in both rats and mice. It was therefore considered that the NOEL for hepatotoxicity in the rat could be used to derive an ADI for furfural.

An ADI for furfural was established at 0.5 mg/kg bw based on the NOEL of 54 mg/kg bw from the 90-day rat study to which a 100-fold safety factor was applied. Since furfural diethylacetal is rapidly converted to furfural at physiological pH, the ADI applies also to the furfural component of furfural diethylacetal since furfural is readily liberated from the acetal *in vivo*."

3.6. EFSA Considerations

In the evaluation of the genotoxic potential of the substances in FGE.218 (EFSA, 2009s), the Panel has taken into consideration the SCF Opinion expressed in December 2002 on furfural and furfural diethylacetal (SCF, 2003a) (See Section 3.4). This Opinion was later updated by EFSA in June 2004 (EFSA, 2004c) with consideration of additional data on the potential genotoxicity of furfural (See Section 3.5). Furthermore the evaluation by the JECFA on furfural and furfuryl alcohol and related flavouring agents at its 55th meeting (JECFA, 2001b) has been considered by the Panel (See Section 3.1 and 3.2).

The Panel concluded that the data available on seven of the eight substances in FGE.218 [FL-no: 13.018, 13.019, 13.057, 13.062, 13.067, 13.068 and 13.128] do not preclude the evaluation of these substances through the Procedure. Therefore, they can be evaluated together with the other six substances [FL-no: 13.002, 13.003, 13.005, 13.025, 13.038 and 13.073] from the JECFA group of furfuryl alcohol and related substances (JECFA, 2001b). 5-Methylfurfural [FL-no: 13.001] can be oxidised in the 5-methyl-group of the hetero-aromatic system to form 5-hydroxymethylfurfuraldehyde [FL-no: 13.139]. In FGE.13 (EFSA, 2005c), it was concluded that 5-hydroxymethylfurfuraldehyde may be metabolised to 5-[(sulphoxy)methyl]furfural, which shows genotoxic potential *in vitro* and that the Procedure could not be applied for this substance. However, after the publication of FGE.13 sufficient data have been provided to mitigate this concern with respect to genotoxic potential of [FL-no: 13.139] *in vivo* (EFSA, 2011h) and accordingly there is no concern for 5-methylfurfural [FL-no: 13.001], as concluded in FGE.218Rev1(EFSA, 2010ak). So, the Panel agrees with JECFA that [FL-no: 13.001] may also be evaluated using the Procedure.

4. Application of the Procedure

4.1. Application of the Procedure to Furfuryl Alcohol and Related Substances, Text taken from the JECFA (JECFA, 2001b)

Step 1

In applying the Procedure for the safety evaluation of flavouring substances, the JECFA assigned nine of the 14 substances in this consideration [FL-no: 13.001, 13.002, 13.003, 13.018, 13.019, 13.057, 13.062, 13.068 and 13.128] to structural class II (Cramer et al., 1978). Furfuryl propionate [FL-no: 13.062] was assigned to this class because it is structurally closely related to furfuryl acetate [FL-no: 13.128] and furfuryl pentanoate [FL-no: 13.068]. The remaining five substances [FL-no: 13.005, 13.025, 13.038, 13.067 and 13.073] were assigned to structural class III.

Step 2

The data on the metabolism of individual members of the group were sufficient to draw conclusions about their probable metabolic fate. Most substances ([FL-no: 13.002, 13.003, 13.005, 13.018, 13.019, 13.025, 13.057, 13.062, 13.067, 13.068, 13.073 and 13.128]) are predicted to be metabolised to 2-furoic acid or a 2-furoic acid derivative, which is either conjugated with glycine and excreted in the urine or condensed with acetyl CoA and conjugated with glycine before excretion in the urine. Because of concern about the results of the toxicological studies on furfural in rodents, these substances cannot be predicted to be metabolised to innocuous products. The evaluation of all substances in this group therefore proceeded via the B-side of the scheme.

Step 3

The estimated daily per capita intakes of all 14 substances in this group are below the threshold of concern for the respective structural classes (i.e. 540 µg/day for structural class II and 90 µg/day for structural class III). Accordingly, the evaluation of all 14 substances in the group proceeded to step B4.

Step 4

For furfural, the NOEL of 53 mg/kg bw/day in a 13-week feeding study in rats (Jonker, 2000b) provides an adequate margin of safety (> 1000 times) in relation to the known levels of intake of this substance. This NOEL is also appropriate for furfuryl alcohol [FL-no: 13.019] and the structurally related substances furfuryl acetate [FL-no: 13.128], furfuryl propionate [FL-no: 13.062], furfuryl pentanoate [FL-no: 13.068], furfuryl octanoate [FL-no: 13.067] and furfuryl 3-methylbutanoate [FL-no: 13.057], because all of these esters would be hydrolysed to furfuryl alcohol [FL-no: 13.019] and then oxidised to furfural [FL-no: 13.018]. The NOEL for furfural is also appropriate for the esters of furoic acid, methyl 2-furoate [FL-no: 13.002], propyl 2-furoate [FL-no: 13.003], amyl 2-furoate [FL-no: 13.025], hexyl 2-furoate [FL-no: 13.005] and octyl 2-furoate [FL-no: 13.073], which would be hydrolysed to furoic acid, the major metabolite of furfural. The NOEL for furfural is also appropriate for 5-methylfurfural [FL-no: 13.001], which would participate in the same metabolic pathways and also undergo alkyl oxidation. For 2-phenyl-3-carbethoxyfuran [FL-no: 13.038], the NOEL of 13 mg/kg bw/day in a 90-day feeding study in rats (Posternak et al., 1969) provides an adequate margin of safety (> 1000 times) in relation to the known levels of intake of this substance.

The evaluation of the 14 substances are summarised in Table 3.1: Summary of Safety Evaluation of Furfuryl Derivatives (JECFA, 2001b).

4.2. Application of the Procedure to a Group of Furfuryl and Furan Derivatives with and without Additional Side-Chain Substituents and Heteroatoms Evaluated by EFSA, Text taken from FGE.13Rev2 (EFSA, 2011h)

Only text relevant for substances in FGE.66Rev1 has been cited from the Procedure in FGE.13Rev2

One of the 10 candidate substances, 5-hydroxymethylfurfuraldehyde [FL-no: 13.139], may be metabolised to 5-[(sulphoxy)methyl]furfural, which shows genotoxic potential *in vitro*. Sufficient data have been provided to mitigate this concern with respect to genotoxic potential *in vivo*.

For the safety evaluation of the 10 candidate substances from subgroup Ia of FGE.13Rev2 the Procedure as outlined in Annex I was applied. The stepwise evaluations of the 10 substances are summarised in Table 3.2.

Step 1

According to the decision tree approach by Cramer et al. (Cramer et al., 1978) four candidate substances are classified into structural class II [FL-no: 13.122, 13.130, 13.136 and 13.139] and six substances are classified into structural class III [FL-no: 13.011, 13.102, 13.127, 13.129, 13.132 and 13.133].

Step 2

None of the candidate substances is predicted to be metabolised to innocuous products. Therefore, the evaluation of the 10 candidate substances proceeds via the B-side of the evaluation scheme.

Step B3

Four candidate substances, have been assigned to structural class II, and have estimated European daily *per capita* intakes (MSDI) ranging from 0.013 to 0.39 microgram (Table 3.2). These intakes are below the threshold of concern of 540 microgram/person/day for structural class II. Six candidate substances have been assigned to structural class III, and have estimated European daily *per capita* intakes (MSDI) ranging from 0.11 to 0.89 microgram (Table 3.2). These intakes are below the threshold of concern of 90 microgram/person/day for structural class III. Therefore, the safety evaluation proceeds to step B4 for all 10 candidate substances.

Step B4

Considering that the 10 candidate substances are metabolised to yield furfural and furoic acid or furanecrylic 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 bw has recently been 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 5-HMF [FL-no: 13.139] a substantial amount of substance-specific data are available, including 13-week subchronic studies and chronic studies in B6C3F1 mice and F344/N rats (NTP, 2010c). The carcinogenicity study in mice demonstrated that 5-HMF 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/day for 5 days/week and above with an intermittent dose regimen of five days per week. For this effects a Benchmark Dose, Lower Confidence Limit (BMDL) of 20.2 mg/kg bw for 5 days/week can be derived, which would be equivalent to 14.4 mg/kg bw/day, when corrected for continuous daily administration. 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 5-HMF [FL-no: 13.139] does not raise a safety concern as a flavouring substance, at its current level of use in foods.

In summary, it can be concluded at step B4 of the Procedure that the 10 candidate substances included in FGE.13Rev2 pose no safety concern when they are used as flavouring substances at the estimated levels of daily *per capita* intake based on the MSDI approach.

The stepwise evaluations of the 10 substances are summarised in Table 3.2: Summary of Safety Evaluation Applying the Procedure (EFSA / FGE.13Rev2)

4.3. EFSA Considerations

The Panel agrees with the application of the Procedure as performed by the JECFA for 13 of the 14 substances in the group of furfuryl alcohol and related substances considered in this FGE. For 5-methylfurfural [FL-no: 13.001] it is anticipated that the 5-methylgroup can be oxidised to the primary alcohol 5-hydroxymethylfurfural [FL-no: 13.139]. In FGE.13 it was concluded that 5-hydroxymethylfurfural may be metabolised to 5-[(sulphoxy)methyl]furfural, which shows genotoxic potential *in vitro* and could therefore not be evaluated through the Procedure (EFSA, 2005c). However, after the publication of FGE.13, sufficient data have been provided to mitigate this concern with respect to genotoxic potential *in vivo* (EFSA, 2011h), and the Panel can agree to evaluate [FL-no: 13.001] according to the Procedure. The Panel concluded it more appropriate to evaluate the exposure estimate of 180 microgram/*capita*/day (MSDI) for 5-methylfurfural [FL-no: 13.001] against the BMDL of 14.4 mg/kg bw/day derived in FGE.13Rev2 for 5-hydroxymethylfurfural [FL-no: 13.139]. For 5-methylfurfural an adequate Margin of Safety of 4800 can be calculated. So, the Panel can agree in the outcome of the Procedure as performed by the JECFA for [FL-no: 13.001] as well.

In the previous version of FGE.66, for three substances [FL-no: 13.003, 13.005 and 13.025] no European production figures were available and no MSDI could be calculated at the time where FGE.66 was published. Consequently the safety in use could not be assessed using the Procedure based on the MSDI approach for these three substances. Since the publication of FGE.66, the EU production volumes have been provided for these three substances. For all three substances [FL-no: 13.003, 13.005 and 13.025], which were evaluated via the B-side of the Procedure, an adequate

margin of safety could be calculated using the NOAEL for the structurally related furfural [FL-no: 13.018]. Therefore the Panel concluded at step B4 that these substances would be of no safety concern at their estimated levels of intake based on the MSDI approach (EFSA, 2010aj).

5. Conclusion

The JECFA has evaluated 15 furfuryl alcohol derivatives at their 55th meeting. The Panel concluded that 14 substances in FGE.66 are structurally related to the subgroup of 10 furfuryl derivatives in the group of furfuryl and furan derivatives with and without additional side-chain substituents and heteroatoms evaluated by EFSA in FGE.13Rev2. The 15th substance [FL-no: 13.031] is considered too structurally different from the other 14 to be included in the present FGE. This substance will be evaluated in FGE.67.

Eight of the candidate substances in this FGE are alpha,beta-unsaturated aldehydes or precursors for such which is a structural alert for genotoxicity. These eight substances [FL-no: 13.001, 13.018, 13.019, 13.057, 13.062, 13.067, 13.068 and 13.128] have initially been considered with respect to genotoxicity. The Panel concluded that for all eight substances, the genotoxicity data available do not preclude their evaluation through the Procedure. No concern for genotoxicity was identified for the remaining six substances without the alpha,beta structural alert [FL-no: 13.002, 13.003, 13.005, 13.025, 13.038 and 13.073].

The Panel agrees with the application of the Procedure as performed by the JECFA for 13 substances considered in this FGE [FL-no: 13.002, 13.003, 13.005, 13.018, 13.019, 13.025, 13.038, 13.057, 13.062, 13.067, 13.068, 13.073 and 13.128]. In contrast to the JECFA, the Panel evaluated the fourteenth substance [FL-no: 13.001] by comparison with a BMDL for a metabolite (5-hydroxymethylfurfural; [FL-no: 13.139]). For none of the candidate substances in this FGE a safety concern was identified.

For 13 substances [FL-no: 13.001, 13.002, 13.003, 13.005, 13.019, 13.025, 13.038, 13.057, 13.062, 13.067, 13.068, 13.073 and 13.128] 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. For one substance, furfural [FL-no: 13.018] use levels have been provided by Industry and an mTAMDI figure of 9700 microgram/person/day could be calculated.

In order to determine whether the conclusion for the substances considered in this FGE can be applied to the materials of commerce, it is necessary to consider the available specifications. Adequate specifications including purity criteria and identity test are available for all 14 JECFA evaluated substances considered in the present FGE.

Thus, for all 14 JECFA evaluated furfuryl derivatives [FL-no: 13.001, 13.002, 13.003, 13.005, 13.018, 13.019, 13.025, 13.038, 13.057, 13.062, 13.067, 13.068, 13.073 and 13.128] the Panel agrees with the JECFA conclusion “No safety concern at estimated levels of intake as flavouring substances” based on the MSDI approach.

TABLE 1: SPECIFICATION SUMMARY

Table 1: Specification Summary of the Substances in the Group of furfuryl alcohol and related flavouring substances evaluated by the JECFA (JECFA, 2000d)

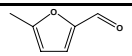
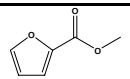
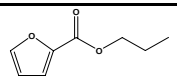
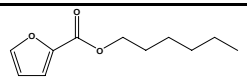
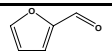
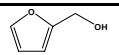
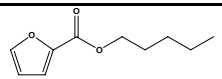
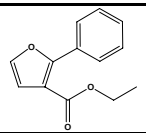
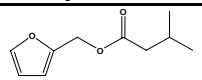
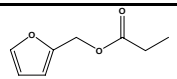
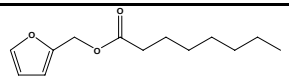
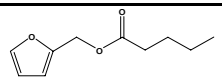
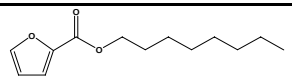
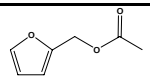
FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)
13.001 745	5-Methylfurfural		2702 119 620-02-0	Liquid $C_6H_6O_2$ 110.11	Slightly soluble Miscible	187 IR 97 %	1.525-1.532 1.098-1.108
13.002 746	Methyl 2-furoate		2703 358 611-13-2	Liquid $C_6H_6O_3$ 126.11	Insoluble to slightly soluble Miscible	181 IR 98 %	1.485-1.490 1.176-1.181
13.003 747	Propyl 2-furoate		2946 359 615-10-1	Liquid $C_8H_{10}O_3$ 154.17	Slightly soluble Miscible	211 MS 97 %	1.471-1.475 1.067-1.075
13.005 749	Hexyl 2-furoate		2571 361 39251-86-0	Liquid $C_{11}H_{16}O_3$ 196.25	Insoluble Miscible	252 IR 97 %	1.468-1.473 1.015-1.020
13.018 450	Furfural		2489 2014 98-01-1	Liquid $C_5H_4O_2$ 96.10	soluble miscible	161-162 IR 95 %	1.521-1.529 1.153-1.162
13.019 451	Furfuryl alcohol		2491 2023 98-00-0	Liquid $C_5H_6O_2$ 98.1	miscible miscible	169-171 IR 97 %	1.481-1.489 1.126-1.136
13.025 748	Pentyl 2-furoate		2072 2109 1334-82-3	Liquid $C_{10}H_{14}O_3$ 182.22	Insoluble Miscible	95-97 (1.3 hPa) IR 98 %	1.469-1.475 1.031-1.038
13.038 752	2-Phenyl-3-carbethoxyfuran		3468 2309 50626-02-3	Liquid $C_{13}H_{12}O_3$ 216.24	Slightly soluble Miscible	148-154 (8 hPa) IR 99 %	1.515-1.527 1.120-1.132
13.057 743	Furfuryl isovalerate		3283 10642 13678-60-9	Liquid $C_{10}H_{14}O_3$ 182.22	Insoluble Miscible	97-98 (14 hPa) IR 98 %	1.456-1.464 1.014-1.023
13.062 740	Furfuryl propionate		3346 10646 623-19-8	Liquid $C_8H_{10}O_3$ 154.17	Slightly soluble Miscible	195-196 IR 98 %	1.457-1.464 1.076-1.086

Table 1: Specification Summary of the Substances in the Group of furfuryl alcohol and related flavouring substances evaluated by the JECFA (JECFA, 2000d)

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)
13.067 742	Furfuryl octanoate		3396 10645 39252-03-4	Liquid $C_{13}H_{20}O_3$ 224.30	Insoluble Miscible	139 (13 hPa) IR 98 %	1.456-1.464 0.980-0.989
13.068 741	Furfuryl valerate		3397 10647 36701-01-6	Liquid $C_{10}H_{14}O_3$ 182.22	Insoluble Miscible	228-229 IR 98 %	1.457-1.462 1.024-1.031
13.073 750	Octyl 2-furoate		3518 10864 39251-88-2	Liquid $C_{13}H_{20}O_3$ 224.30	Insoluble Miscible	126-127 (1 hPa) IR 98 %	1.466-1.472 0.984-0.990
13.128 739	Furfuryl acetate		2490 2065 623-17-6	Liquid $C_7H_8O_3$ 140.14	Insoluble Miscible	175-177 IR 97 %	1.457-1.466 1.110-1.119

1) Solubility in water, if not otherwise stated.
2) Solubility in 95 % ethanol, if not otherwise stated.
3) At 1013.25 hPa, if not otherwise stated.
4) At 20°C, if not otherwise stated.
5) At 25°C, if not otherwise stated.

TABLE 2: GENOTOXICITY DATA

Table 2.1: Summary of Genotoxicity Data of Furfuryl Derivatives Evaluated by the JECFA (JECFA, 2001b)

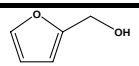
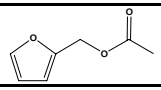
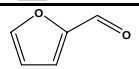
FL-no JECFA-no	EU Register name JECFA name	Structural formula	End-point	Test system	Concentration	Results	Reference
13.019 451	Furfuryl alcohol		Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	294 µg/plate	Negative[a,b]	(Florin et al., 1980)
			Reverse mutation	*S. typhimurium* TA98, TA100, 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	*S. typhimurium* TA1535, TA100, TA1537 (modified assay)	200 000 µg/ml	Positive[a,b]	(McGregor et al., 1981)
			DNA repair and H17 (rec+)	*B. subtilis* M45 (rec–)	2000 - 20 000 µg/disc	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* TA1535, 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 2.1: Summary of Genotoxicity Data of Furfuryl Derivatives Evaluated by the JECFA (JECFA, 2001b)

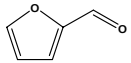
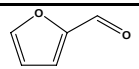
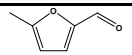
FL-no JECFA-no	EU Register name JECFA name	Structural formula	End-point	Test system	Concentration	Results	Reference
			Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537	33 - 6666 µg/plate	Negative[a,b] Equivocal in TA100[a]	(NTP, 1990a)
	Furfural (cont.)		Reverse mutation	*S. typhimurium* 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	100 - 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	100 - 10 000 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/L[a] 0.07 - 0.14 mmol/L[c]	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	Negative	(NTP, 1990a)

Table 2.1: Summary of Genotoxicity Data of Furfuryl Derivatives Evaluated by the JECFA (JECFA, 2001b)

FL-no JECFA-no	EU Register name JECFA name	Structural formula	End-point	Test system	Concentration	Results	Reference
					injection		
			Spermhead abnormalities	Mice	4000 mg/kg of diet daily for 5 weeks	Negative	(Subramanyam et al., 1989)
	Furfural (cont.)		Somatic chromo-somal mutation	Swiss albino mouse bone- marrow cells	1000 - 2000 mg/kg of diet 4000 mg/kg bw for 5 days	Negative Positive	(Subramanyam et al., 1989)
			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.

C Concentration added to culture.

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
Main group I – Non-sulphur-containing Furan Derivatives						
(Furfuryl alcohol [13.019])	Ames test	*S. typhimurium* TA98, TA100, TA1535 and TA1537	294 µg/plate	Negative[1]	(Florin et al., 1980)	
	Ames test	*S. typhimurium* TA98, TA100, TA1535 and TA1537	10000 µg/plate	Negative[1]	(Mortelmans et al., 1986) (NTP, 1999a)	
	Ames test	*S. typhimurium* TA100	2500 - 12500 µg/ml	Negative[1]	(Stich et al., 1981a)	
	Ames test	*S. typhimurium* TA98, TA100 and TA102	198000 µg/plate	Negative[1]	(Aeschbacher et al., 1989)	
	Ames test	*S. typhimurium* TA98 and TA100	81 - 323 µg/plate	Negative[1]	(Shinohara et al., 1986)	
	Modified Ames test	*S. typhimurium* TA1535, TA100 and TA1537	200000 µg/ml	Positive[1]	(McGregor et al., 1981)	
	Rec assay	*B. subtilis*	2000 - 20000 µg/disk	Positive[1]	(Shinohara et al., 1986)	
	Sister chromatid exchange	CHO cells	245 µg/ml	Positive[1]	(Stich et al., 1981a)	
	Sister chromatid exchange	CHO cells	500 µg/ml	Positive/Weakly positive[2] Negative[3]	(NTP, 1999a)	
	Sister chromatid exchange	Human Lymphocytes	Up 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* TA1535, 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)	

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	8000 µg/plate	Positive[1]	(Zdzienicka et al., 1978)	
	Ames test	*S. typhimurium* TA98	8000 µg/plate	Negative[1]	(Zdzienicka et al., 1978)	
	Ames test	*S. typhimurium* TA100, TA102	100 - 10000 µg/plate	Negative[2]	(Dillon et al., 1998)	
	Ames test	*S. typhimurium* TA104	100 - 10000 µg/plate	Equivocal[2]	(Dillon et al., 1998)	
	Ames test	*S. typhimurium* TA102, TA104	100 - 10000 µg/plate	Negative[3]	(Dillon et al., 1998)	
	Ames test	*S. typhimurium* TA100	100 - 10000 µg/plate	Equivocal[3]	(Dillon et al., 1998)	
	Modified Ames test	*S. typhimurium* TA100	426 µg/plate	Negative	(Kim et al., 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[1]	(Omura et al., 1983)	Posisitive dose related respons inTA100 only, most potent without S9. Purity and other experimental details not reported. The validity of the study cannot be evaluated.
	Ames test	*S. typhimurium* TA98; TA100	0.17 - 0.66 µmol/plate	Positive[1]	(Shinohara et al., 1986)	Positive results only obtained in TA100 with S9. Reverse dose-respons relationship. Experimental details are lacking.
	Ames test	*S. typhimurium* TA104	0.1 - 0.8 mM	Negative[2]	(Lee et al., 1995b)	Positive result was obtained

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
				Positive		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 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)	The study is considered valid.
	Ames test	*S. typhimurium* TA100	100 - 10,000 µg/plate	Weakly positive[2]	(NTP, 2010c)	The study is considered valid.
	Ames test	*S. typhimurium* TA100 and TA98	1,500 - 10,000 µg/plate	Negative[1]	(NTP, 2010c)	The study is considered valid.
	Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537	at 0.5 µg/mL up to 5000 µg/mL	Negative[1]	(Severin et al., 2010)	For discussion and interpretation see text.
	Reverse mutation assay	*E. coli* WP2 uvrA/pKM101	1,500 - 10,000 µg/plate	Negative[1]	(NTP, 2010c)	The study is considered valid.
	Micronucleus assay	HepG2 cells	0, 5.35, 7.87, 11.57, 17, 25, 36.6 mM	Negative[15]	(Severin et al., 2010)	For discussion and interpretation see text.
	SCE induction	V79-hCYP2E1-hSULT1A1 cells	19.8 - 3808 µM	Positive	(Glatt et al., 2005)	For discussion and interpretation see text.
	SCE induction	V79-Mz cells	238 - 3808 µM,	Positive[16]	(Glatt et al., 2005)	For discussion and interpretation see text.
	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 cannot 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,	Positive[14, 15]	(Severin et al., 2010)	For discussion and

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
			25, 36.6 mM			interpretation see text.
	Comet assay	Human Caco-2 cells	3,153 - 12,611 µg/mL (25 - 100 mM)	Positive[12]	(Durling et al., 2009)	For discussion and interpretation see text.
	Comet assay	Human HEK293 cells	3,153 - 12,611 µg/mL (25 - 100 mM)	Positive[12]	(Durling et al., 2009)	For discussion and interpretation see text.
	Comet assay	Mouse lymphoma L5178Y cells	3,153 - 12,611 µg/mL (25 - 100 mM)	Positive[12]	(Durling et al., 2009)	For discussion and interpretation see text.
	Comet assay	Chinese hamster V-79 cells	315 - 12,611 µg/mL (2.5 - 100 mM)	Positive[13]	(Durling et al., 2009)	For discussion and interpretation see text.
	Comet assay	Chinese hamster V-79-hP-PST cells	315 - 12,611 µg/mL (2.5 - 100 mM)	Positive[13]	(Durling et al., 2009)	For discussion and interpretation see text.
	HPRT assay	V79 cells	Up to 140 mM	Positive[1, 11]	(Janzowski et al., 2000)	Positive response were only obtained at high .concentrations resulting in reduced cell viability and growth. The study is considered valid but interpretation of data is questionable.
	HPRT and tk assay	TK6 human lymphoblast cells	20 - 75 µg/ml	Negative	(Surh 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, 17]	(Aaron et al., 1989)	Study performed in accordance with GLP. The study is considered valid.
(Methyl-2-furoate [13.002])	Ames test	*S. typhimurium* TA98; TA100	100 µg/plate	Negative[10]	(Ichikawa et al., 1986b)	

NR=Not Reported.
[1]*With and without S9 metabolic activation.*
[2] *Without S9 metabolic activation.*
[3] *With S9 metabolic activation.*
[4] *Concentration 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] *Positive only at the highest concentration tested with significant decrease in cell viability.*
[13] *Positive at high concentration with significantly reduced cell viability.*
[14] *Cytotoxic at the two highest doses.*
[15] *20 hours of exposure.*
[16] *Weakly positive but statistically significant at each concentration.*
[17] *Dose levels above 300 microgram/ml were cytotoxic.*

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	*D. 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 days/week for 20 weeks	Negative	(Spalding et al., 2000)	
(Furfural [13.018])	Sex-linked recessive lethal test	*D. melanogaster*	Diet	1000 ppm	Negative	(Woodruff et al., 1985)	
	Sex-linked recessive lethal test	*D. melanogaster*	Injection	100 ppm	Positive	(Woodruff et al., 1985)	
	Sex-linked recessive lethal test	*D. melanogaster*	Injection	Up to 6500 ppm	Negative	(Rodriquez-Arnaiz et al., 1989)	
	Chromosome Loss	*D. melanogaster*	Oral or injected	3750 - 5000 ppm. Mated with repair-proficient females	Negative	(Rodriquez-Arnaiz et al., 1992)	
	Chromosome Loss	*D. melanogaster*	Oral or injected	3750 - 5000 ppm. Mated with repair-deficient females	Positive	(Rodriquez-Arnaiz et al., 1992)	
	Reciprocal translocations	*D. melanogaster*	Injection	100 ppm	Negative	(Woodruff et al., 1985)	
	Nondisjunction assay	*D. 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 atmosphere)	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
	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)	
5-Hydroxymethylfurfural [13.139]	Micronucleus assay	Mouse peripheral blood cells		47, 94, 188, 375 or 750 mg/kg bw/day	Negative	(NTP, 2010c)	3-months micronucleus assay. Limited validity in absence of bone-marrow toxicity.

[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.*

Table 2.4: Genotoxicity on Furfural, SCF Opinion December 2002 (SCF, 2003a)

Substance	End-point	Test object	Concentration	Result	Reference
In vitro					
Furfural [13.018]	Reverse mutation	*S. typhimurium* TA100, TA98, TA1535	0.05 - 60 µmol/plate	Weakly positive (TA100)[b]	(Loquet et al., 1981)
		S. typhimurium TA100, TA98, TA102	≤ 1.2 mmol/plate	Negative[a]	(Aeschbacher et al., 1989)
		S. typhimurium TA100, TA98	0.165 - 0.660 µmol/plate	Negative[a]	(Shinohara et al., 1986)
		S. typhimurium TA102, TA104	5 - 500 µg/plate	Positive (TA104)	(Shane et al., 1988)
		S. typhimurium TA98, TA100,TA1535, TA1537	33.3 - 6666 µmol/plate	Negative[a]	(Mortelmans et al., 1986)
		S. typhimurium TA98, TA100	1 - 15 µL/plate	Positive[a] (TA100)	(Zdzienicka et al., 1978)
		S. typhimurium TA98, TA100	7 µL/plate	Negative[a]	(Sasaki and Endo, 1978)
		S. typhimurium TA100	4.44 µmol/plate	Negative[a]	(Osawa and Namiki, 1982)
		S. typhimurium TA98, TA100, TA104, E.coliWP2uvrA/PKM101	20 µL/plate	Negative[a]	(McMahon et al., 1979)
		S. typhimurium TA104	1 µmol (max. non-toxic dose)	Negative[b]	(Marnett et al., 1985a)
	Umu gene expression	*S. typhimurium* TA1535/pSK/1002	1932 µg/mL	Negative[a]	(Nakamura et al., 1987)
	Rec assay	*B.subtilis* H17, M45	1.7 - 17 mg/disk	Positive[a]	(Shinohara et al., 1986)
		B.subtilis H17, M45	1 mg/disk	Negative[a]	(Matsui et al., 1989)
	Forward mutation	L5178Ytk+/- mouse lymphoma cells	25 - 800 µg/mL	Positive[b]	(McGregor et al., 1988b)
	Chromosomal aberration	Chinese hamster ovary cells	10-40 mM	Positive[a]	(Stich et al., 1981a; Stich et al., 1981b)
		Chinese hamster ovary cells	200 - 1230 µg/mL	Positive[a]	(Galloway et al., 1985)
		Chinese hamster ovary cells	1.5 - 5000 µg/mL	Positive[a]	(Gudi and Schadly, 1996)
		Chinese hamster V79 cells	500 - 2000 µg/mL	Positive[a]	(Nishi et al., 1989)
	Sister chromatid exchange	Chinese hamster ovary cells	11.7 - 3890 µg/mL	Positive[a]	(Galloway et al., 1985)
		Human peripheral lymphocytes	3.5 - 14x10^{-5} M	Positive[b]	(Gomez-Arroyo and Souza, 1985)
	Unscheduled DNA synthesis	Human liver slices	0.14 mmol/L 0-25 mmol/L	Negative	(Lake, 1998)
In vivo					
Furfural [13.018]	Sex-linked recessive lethal mutation	*D. melanogaster*	1000 ppm, in diet	Negative	(Woodruff et al., 1985)
		D. melanogaster	100 ppm, by injection	Positive	(Woodruff et al., 1985)
	Wing spot test	*D. melanogaster*	3750 - 7500 ppm by aerial exposure	Positive	(Rodriquez-Arnaiz et al., 1989)
	Sex-chromosome loss	*D. melanogaster*	3750 - 5000 ppm, in diet and by injection	Positive on injection	(Rodriquez-Arnaiz et al., 1989; Rodriquez-Arnaiz et al., 1992)
	Reciprocal translocation	*D. melanogaster*	1000 ppm, in diet	Negative	(Woodruff et al., 1985)
	Sister chromatid exchange/ chromosomal aberration	B6C3F1 mouse bone marrow cells	50 - 200 mg/kg bw, once i.p.	Negative	(NTP, 1990a)
	Somatic chromosomal aberration	Swiss albino mouse bone marrow cells	4000 ppm for 5 days, in diet	Negative	(Subramanyam et al., 1989)+
	Sperm head abnormalities	Swiss albino mouse	4000 ppm for 5 weeks, in diet	Negative	(Subramanyam et al., 1989)+
	Unscheduled DNA synthesis	Fischer 344 rat hepatocytes	5.0, 16.7 or 50 mg/kg bw, orally	Negative	(Phillips et al., 1997)
		B6C3F1 mouse hepatocytes	50, 175 or 320 mg/kg bw, orally	Negative	(Edwards, 1999)

a With and without metabolic activation.
b Without metabolic activation.

+ Aabstract only; no details availabe.

TABLE 3: SUMMARY OF SAFETY EVALUATIONS

Table 3.1: Summary of Safety Evaluation of 14 JECFA-Evaluated Furfuryl Derivatives (JECFA, 2001b)

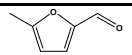
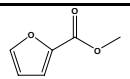
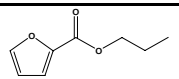
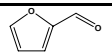
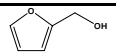
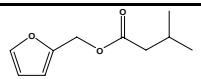
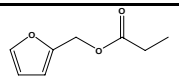
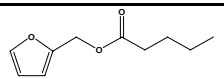
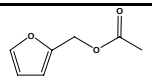
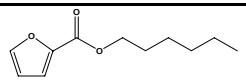
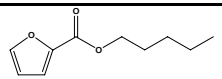
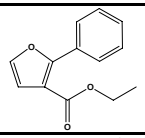
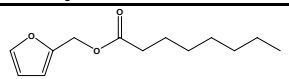
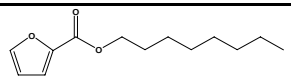
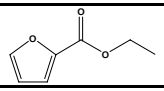
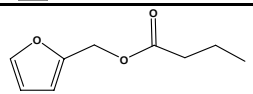
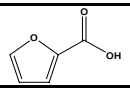
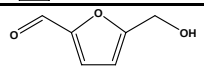
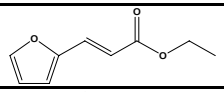
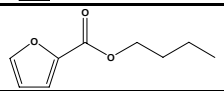
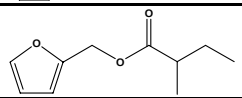
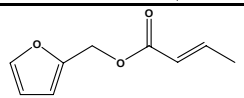
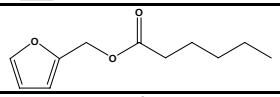
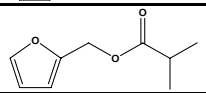
FL-no JECFA-no	EU Register name	Structural formula	EU MSDI 1) US MSDI (µg/*capita*/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
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. Separate EFSA opinion available.	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 3.1: Summary of Safety Evaluation of 14 JECFA-Evaluated Furfuryl Derivatives (JECFA, 2001b)

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.

Table 3.2: Summary of Safety Evaluation Applying the Procedure for 10 Furfuryl Derivatives of FGE.13Rev2 (REF:6915)

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)	
13.011	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)	
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)	

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.

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ABBREVIATIONS

ADI	Acceptable daily intake
BMDL,	Benchmark Dose (Lower Confidence Limit).
BW	Body weight
CAS	Chemical Abstract Service
CEF	Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CHO	Chinese hamster ovary (cells)
CoE	Council of Europe
DNA	Deoxyribonucleic acid
EFSA	The European Food Safety Authority
ENU	Ethylnitrosourea
EPA	United States Environmental Protection Agency
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 practice
GSH	Glutathione
HMF	Hydroxymethylfurfualdehyde
HPRT	Hypoxanthine phosphoribosyltransferase
ID	Identity
Ip	Intraperitoneal
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 erythrocyte

No	Number
NOAEL	No observed adverse effect level
NOEL	No observed effect level
NTP	National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
PAPS	3'-Phosphoadenosine-5'-phosphosulfate
PCE	Polychromatic erythrocyte
PFU	Plaque-forming units
SCE	Sister chromatic exchange
SCF	Scientific Committee on Food
UDS	Unsheduled DNA Synthesis
WHO	World Health Organisation