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Scientific Opinion on Flavouring Group Evaluation 67, Revision 3 (FGE.67Rev3): consideration of 23 furan-substituted compounds evaluated by JECFA at the 55th, 65th, 69th and 86th meetings

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Abstract

The Panel on Food Additives and Flavourings (FAF) was requested to consider the JECFA evaluations of 25 flavouring substances assigned to the Flavouring Group Evaluation 67 (FGE.67Rev3), using the Procedure as outlined in the Commission Regulation (EC) No 1565/2000. Eleven substances have already been considered in FGE.67 and its revisions (FGE.67Rev1 and FGE.67Rev2). During the current assessment, two substances were no longer supported by industry, therefore 12 candidate substances are evaluated in FGE.67Rev3. New genotoxicity and toxicity data are available for 2-pentylfuran [FL-no: 13.059] and 2-acetylfuran [FL-no: 13.054], which are representative substances of subgroup IV [FL-no: 13.069, 13.106, 13.148] and VI-B [FL-no: 13.045, 13.070, 13.083, 13.101, 13.105, 13.138, 13.163], respectively. Based on these data, the Panel concluded that the concern for genotoxicity is ruled out for both [FL-no: 13.054] and [FL-no: 13.059] and consequently for the substances that they represent. Since the candidate substances cannot be anticipated to be metabolised to innocuous products only, they were evaluated along the B-side of the Procedure. The Panel derived a NOAEL of 22.6 mg/kg bw per day and a BMDL of 8.51 mg/kg bw per day, for 2-acetylfuran and 2-pentylfuran, respectively. For all 12 substances sufficient margins of safety were calculated when based on the MSDI approach. Adequate specifications for the materials of commerce are available for all 23 flavouring substances. The Panel agrees with JECFA conclusions, for all 23 substances, 'No safety concern at estimated levels of intake as flavouring substances' based on the MSDI approach. For 18 substances [FL-no: 13.021, 13.022, 13.023, 13.024, 13.031, 13.045, 13.047, 13.054, 13.059, 13.074, 13.083, 13.101, 13.105, 13.106, 13.138, 13.148, 13.163 and 13.190], the mTAMDI intake estimates are above the threshold of toxicological concern (TTC) for their structural classes and more reliable data on uses and use levels are required to finalise their evaluation.

Keywords: Flavourings, FGE.67, FGE.13, furan-substituted

Requestor: European Commission

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1. Introduction

The European Food Safety Authority (EFSA) received mandates from the European Commission for evaluating new data on groups of substances evaluated in FGE.67Rev3 in three different occasions. Each mandate is reported below.

1.1. Background and Terms of Reference as provided by the requestor

1.1.1. Background of the mandate received on 25 October 2013

The use of flavourings in food is regulated under Regulation (EC) No 1334/2008 of the European Parliament and Council of 16 December 2008[1] on flavourings and certain food ingredients with flavouring properties for use in and on foods. On the basis of Article 9(a) of this Regulation, an evaluation and approval are required for flavouring substances.

The Union list of flavourings and source materials was established by Commission Implementing Regulation (EC) No 872/2012[2]. The list contains flavouring substances for which the scientific evaluation should be completed in accordance with Commission Regulation (EC) No 1565/2000[3].

On 6 July 2011, the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) adopted an opinion on Flavouring Group Evaluation 67, Revision 1: consideration of 40 furan-substituted aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids and related esters, sulfides, disulfides and ethers evaluated by JECFA at the 65th meeting and re-evaluated at the 69th meeting.[4]

For 23 substances [FL-no: 13.029, 13.030, 13.045, 13.052, 13.054, 13.058, 13.059, 13.061, 13.066, 13.069, 13.070, 13.083, 13.092, 13.101, 13.103, 13.105, 13.106, 13.107, 13.123, 13.138, 13.148, 13.163 and 13.191], the Panel concluded that additional toxicity/genotoxicity data are required.

Subsequently, these substances[5] were included in the Union List with a footnote 3 or 4.

As regards the 2 substances [FL-no: 13.029 and 13.030], the Commission was informed that these substances are no longer supported by the applicant and is proceeding with their removal from the Union List.

Additional data on 7 alkoyl-substituted furans [FL-no: 13.054, 13.066, 13.070, 13.083, 13.101, 13.105 and 13.163], represented by 2-acetylfuran [FL-no: 13.054], have now been submitted by the applicant.

In addition, according to the submitter, this group of alkoyl-substituted furans is closely related to the FGE.67 group of alkyl-substituted furans [FL-no: 13.059, 13.069, 13.103, 13.106 and 13.148] and furan-substituted aliphatic aldehydes, ketones and ethers [FL-no: 13.045, 13.052, 13.058, 13.061, 13.123 and 13.138]. For these substances, the submitter informed that data is going to be provided in December 2013.

Term of reference

The European Commission requests the European Food Safety Authority (EFSA) to finalise its safety assessment on this group of flavouring substances in accordance with Commission Regulation (EC) No 1565/2000.

1.1.2. Background of the mandate received on 19 May 2014

The use of flavourings in food is regulated under Regulation (EC) No 1334/2008 of the European Parliament and Council of 16 December 2008[1] on flavourings and certain food ingredients with flavouring properties for use in and on foods. On the basis of Article 9(a) of this Regulation, an evaluation and approval are required for flavouring substances.

[1] Regulation (EC) No 1334/2008 of the European Parliament and of the Council of 16 December 2008 on flavourings and certain food ingredients with flavouring properties for use in and on foods and amending Council Regulation (EEC) No 1601/91, Regulations (EC) No 2232/96 and (EC) No 110/2008 and Directive 2000/13/EC. OJ L 354, 31.12.2008, p. 34–50.

[2] Commission implementing Regulation (EU) No 872/2012 of 1 October 2012 adopting the list of flavouring substances provided for by Regulation (EC) No 2232/96 of the European Parliament and of the Council, introducing it in Annex I to Regulation (EC) No 1334/2008 of the European Parliament and of the Council and repealing Commission Regulation (EC) No 1565/2000 and Commission Decision 1999/217/EC. OJ L 267, 2.10.2012, p. 1–161.

[3] Commission Regulation (EC) No 1565/2000 of 18 July 2000 laying down the measures necessary for the adoption of an evaluation programme in application of Regulation (EC) No 2232/96. OJ L 180, 19.7.2000, p. 8–16.

[4] EFSA Journal 2011;9(10):2315.

[5] Except substances FL-no: 13.163 and 13.191, which were not included in the Union List when it was established.

The Union list of flavourings and source materials was established by Commission Implementing Regulation (EC) No 872/2012[2]. The list contains flavouring substances for which the scientific evaluation should be completed in accordance with Commission Regulation (EC) No 1565/2000[3].

On 23 October 2013, the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) was requested to re-evaluate 7 flavouring substances from FGE.67Rev1 (Alkoyl-substituted furans), represented by 2-acetylfuran [FL-no: 13.054] (Ares(2013)3318841).

On 10 December 2013, EFSA agreed to perform the safety assessment of these 7 substances by 25 July 2014: EFSA-Q-2013-00853 to 00859 (ref.: KL/cc (2013) out-8117458) (Ares (2013) 3762765).

On 1 April 2014, the European Flavour association (EFFA) submitted an updated dossier on this group of 7 alkoyl-substituted furans in order to cover two additional substances, namely 1-(2-furyl)-propan-2-one [FL-no: 13.045] and 1-(2-furyl)butan-3-one [FL-no: 13.138].

EFFA stated that these 2 substances originally did not belong to the so-called "subgroup VI-B" which constitutes this "alkoyl-substituted furans"-group. They were added to this subgroup at a later stage and consequently EFFA failed to insert them as candidate chemicals at the time of drafting the initial Addendum of Additional Data to FGE.67Rev1.

EFFA has now updated the dossier by adding these two substances as candidate chemicals, supported by the data on the representative substance, 2-acetylfuran [FL-no: 13.054].

The present updated Footnote-10 Dossier on the "alkoyl-substituted furans"-group replaces the dossier submitted on 10 December 2013.

Term of reference

The European Commission requests the European Food Safety Authority (EFSA) to finalise its safety assessment on this group of flavouring substances in accordance with Commission Regulation (EC) No 1565/2000.

1.1.3. Background of the mandate received on 13 June 2014

The use of flavourings is regulated under Regulation (EC) No 1334/2008 of the European Parliament and Council of 16 December 2008[1] on flavourings and certain food ingredients with flavouring properties for use in and on foods. On the basis of Article 9(a) of this Regulation, an evaluation and approval are required for flavouring substances.

The Union list of flavourings and source materials was established by Commission Implementing Regulation (EC) No 872/2012[2]. The list contains flavouring substances for which the scientific evaluation should be completed in accordance with Commission Regulation (EC) No 1565/2000[3].

1. FGE.67Rev1

On 6 July 2011, the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) adopted an opinion on Flavouring Group Evaluation 67, Revision 1 (FGE.67Rev.1): Consideration of 40 furan-substituted aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids and related esters, sulfides, disulfides and ethers evaluated by JECFA at the 65th meeting (JECFA, 2006b) and re-evaluated at the 69th meeting (JECFA, 2009c)[4].

In its opinion, the Panel concluded that for the substances [FL-no: 13.059, 13.069, 13.103, 13.106 and 13.148] additional toxicity/genotoxicity data are required.

2. FGE.13Rev2

On 6 July 2011, the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) adopted an opinion on Flavouring Group Evaluation 13, Revision 2 (FGE.13Rev2): Furfuryl and furan derivatives with and without additional side-chain substituents and heteroatoms from chemical group 14.[6]

In its opinion the Panel stated that it has reservations for the substances [FL-no: 13.125 and 13.162] which could not be evaluated through the procedure due to concern of genotoxicity *in vitro*. For these two substances additional data are required.

On 29 April 2014, the European Flavour Association (EFFA) submitted additional data on 2-pentylfuran [FL-no: 13.059] from FGE.67, which is relevant to the safety assessment of this group of 7 alkylfurans.

[6] EFSA Journal 2011;9(8):2313.

Terms of Reference

The European Commission requests the European Food Safety Authority (EFSA) to evaluate this new information and, depending on the outcome, proceed to the full evaluation of these flavouring substances in accordance with Commission Regulation (EC) No 1565/2000.

1.2. Interpretation of the Terms of Reference

In FGE.67Rev1, the CEF Panel agreed with JECFA that the substances [FL-no: 13.045, 13.054, 13.059, 13.066, 13.069, 13.070, 13.083, 13.101, 13.103, 13.105, 13.106, 13.138, 13.148, 13.163] cannot be evaluated through the Procedure, based on concerns with respect to genotoxicity. These substances are structurally related to 2-ethyl-5-methylfuran [FL-no: 13.125] and 2-octylfuran [FL-no: 13.162] evaluated in FGE.13Rev2. In that scientific opinion, FGE.13Rev2, the CEF Panel had reservations for these substances [FL-no: 13.125 and 13.162] which could not be evaluated through the Procedure due to concern for genotoxicity *in vitro*, therefore additional data were required.

Industry has submitted data on the representative substances 2-pentylfuran [FL-no: 13.059] and 2-acetylfuran [FL-no: 13.054]. Data on 2-pentylfuran [FL-no: 13.059] are representative for [FL-no: 13.069, 13.103, 13.106, 13.148] in FGE.67Rev3 and supporting for [FL-no: 13.125 and 13.162] in FGE.13Rev3. The substances [FL-no: 13.125 and 13.162] will be considered in FGE.13Rev3. Data on 2-acetylfuran [FL-no: 13.054] are representative for [FL-no: 13.045, 13.066, 13.070, 13.083, 13.101, 13.105, 13.138 and 13.163] in FGE.67Rev3.

In addition, since the publication of FGE.67Rev2 (EFSA CEF Panel, 2015a), use levels data have been provided by industry for the following flavouring substances [FL-no: 13.006, 13.021, 13.022, 13.023, 13.024, 13.045, 13.054, 13.059, 13.069, 13.070, 13.083, 13.101, 13.105, 13.106, 13.116, 13.138, 13.148, 13.163, 13.190] for which the modified theoretical added maximum daily intake (mTAMDI) can be calculated.

1.3. History of the evaluation of the substances in Flavouring Group Evaluation 67Rev3

FGE.67

The Flavouring Group Evaluation 67 (FGE.67) dealt originally with 39 substances which were previously considered by the JECFA in a group of 40 furan-substituted aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids and related esters, sulfides, disulfides and ethers (JECFA, 2006a, 2009b).

One substance ([FL-no: 13.192], a synonym of substance [FL-no: 13.178]) of these 40 substances has already been evaluated in FGE.13Rev1 and will not be discussed further in this FGE.

In this group of 39 substances evaluated by the JECFA, 14 substances are α,β-unsaturated aldehydes or ketones which have been allocated to subgroups 4.4 ([FL-no: 13.176]), 4.5 ([FL-no: 13.054, 13.066, 13.070, 13.083, 13.101, 13.105 and 13.163]) and 4.6 ([FL-no: 13.034, 13.043, 13.044, 13.046, 13.137 and 13.150]) of FGE.19 in order to assess their genotoxic potential (EFSA, 2008a). Therefore, these 14 substances were not evaluated in FGE.67, which accordingly only deals with 25 flavouring substances.

The CEF Panel agreed with JECFA for 13 of the 25 substances [FL-no: 13.029, 13.030, 13.052, 13.059, 13.061, 13.069, 13.092, 13.103, 13.106, 13.107, 13.123, 13.148 and 13.191] that these substances cannot be evaluated through the procedure, based on concerns with respect to genotoxicity and carcinogenicity.

After application of the Procedure and consideration on information on stereochemistry, the CEF Panel concluded for nine substances [FL-no: 13.006, 13.021, 13.022, 13.023, 13.024, 13.047, 13.074, 13.116 and 13.190] that they would be of no safety concern at their estimated intake levels based on the maximised survey-derived daily intake (MSDI) approach.

Adequate specifications including complete purity criteria and identity were available for 22 of the 25 substances. For three substances [FL-no: 13.045, 13.058 and 13.190] data on specifications and stereoisomerism were missing. For 16 substances [FL-no: 13.029, 13.030, 13.045, 13.052, 13.058, 13.059, 13.061, 13.069, 13.092, 13.103, 13.106, 13.107, 13.123, 13.138, 13.148 and 13.191] the CEF Panel concluded that additional toxicity data are required.

For all 25 substances, use levels were needed to calculate the mTAMDIs in order to identify those flavouring substances that need more refined exposure assessment and to finalise the evaluation.

FGE.67Rev1

FGE.67Rev1 includes the consideration of additional eight substances resulting in a total of 33 substances (EFSA CEF Panel, 2011a).

All eight additional substances are in subgroup VI-B: seven of these [FL-no: 13.054, 13.066, 3.070, 13.083, 13.101, 13.105, and 13.163] were from the original JECFA group of 40 and one substance, 2-benzofurancarboxaldehyde [FL-no: 13.031], was moved over from FGE.66Rev1 because this substance is structurally more similar to the candidate flavouring substance [FL-no: 13.074] in subgroup V-B of FGE.67 than to the other substances in FGE.66Rev1 (EFSA CEF Panel, 2011b).

The substance [FL-no: 13.176] was moved over to FGE.99 and evaluated in that FGE because it is structurally more similar to candidate substances in FGE.99 (EFSA CEF Panel, 2012b).

Seven of the eight additional substances [FL-no: 13.054, 13.066, 13.070, 13.083, 13.101, 13.105 and 13.163] are α,β-unsaturated ketones allocated to FGE.19 subgroup 4.5 (EFSA, 2008a) and therefore not considered in FGE.67 (EFSA CEF Panel, 2010). In the course of the assessment, the CEF Panel concluded that the α,β-unsaturated structure in conjugation with an aromatic ring system, which is present in these seven substances as well as in acetophenone, is not considered a structural alert for genotoxicity, therefore subgroup 4.5 was not included in the updated list of FGE.19 substances (EFSA, 2008b). Nevertheless, the experimental genotoxicity data indicate that the substance [FL-no: 13.054] may give rise to DNA damage, which may result in chromosomal aberrations rather than gene mutations. The formation of DNA-reactive metabolites may be anticipated (EFSA CEF Panel, 2011c). The available genotoxicity data are sufficiently strong to raise a concern, which would preclude the evaluation of the substances in subgroup VI-B through the Procedure.

In FGE.67Rev1, the sub-grouping of the two ketones [FL-no: 13.045 and 13.138] has been changed from subgroup III to subgroup VI-B.

The CEF Panel considered that for the eight alkyl-substituted furans (subgroup IV) [FL-no: 13.029, 13.030, 13.059, 13.069, 13.092, 13.103, 13.106 and 13.148] the concern for formation of reactive metabolites could not be ruled out, because of insufficient data on genotoxicity.

For the substances in subgroups V-A [FL-no: 13.052, 13.061, 13.123] and V-C [FL-no: 13.107], a concern for genotoxicity was identified. In addition, the CEF Panel reiterated the concern for genotoxicity for one substance in subgroup I [FL-no: 13.191] that was already identified in FGE.67.

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

The CEF Panel agreed, in FGE67Rev1, with the conclusion reached by the JECFA at its 55th meeting (JECFA, 2001) that the substance [FL-no: 13.031] can be evaluated using the Procedure, and that this substance poses no safety concern when used as a flavouring substance.

Considering [FL-no: 13.031] evaluated in FGE.67Rev1 and the nine substances already evaluated through the Procedure in FGE.67, it was concluded for 10 substances [FL-no: 13.006, 13.021, 13.022, 13.023, 13.024, 13.031, 13.047, 13.074, 13.116 and 13.190], there would be no safety concern at their estimated intake levels based on the MSDI approach. For one substance [FL-no: 13.058], evaluated through the Procedure, this conclusion could not be drawn due to lack of an adequate NOAEL.

Poundage data for use as a flavouring substance in Europe were missing for [FL-no: 13.066 and 13.070].

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

Adequate specifications including complete purity criteria and identity are available for 30 of the 33 substances. For three substances [FL-no: 13.031, 13.045 and 13.047] data on specifications/ stereoisomerism are missing.

For 23 substances [FL-no: 13.029, 13.030, 13.045, 13.052, 13.054, 13.058, 13.059, 13.061, 13.066, 13.069, 13.070, 13.083, 13.092, 13.101, 13.103, 13.105, 13.106, 13.107, 13.123, 13.138, 13.148, 13.163 and 13.191], the CEF Panel concluded that additional toxicity/genotoxicity data are required.

FGE.67Rev2

The second revision of FGE.67 (FGE.67Rev2) was due to submission of a 90-day toxicity study for 3-(5-methyl-2-furyl)butanal [FL-no: 13.058] (IOFI, 2013). Furthermore, requested data on

specifications were submitted for three substances [FL-no: 13.031, 13.045 and 13.047] (EFFA, 2014). In addition, the industry informed the Commission that five substances [FL-no: 13.029, 13.030, 13.092, 13.107 and 13.191] are no longer supported for use in the EU as flavouring substances. These five substances will no longer be considered in this FGE (DG SANCO, 2012).

Accordingly, FGE.67Rev2 deals with 28 flavouring substances [FL-no: 13.006, 13.021, 13.022, 13.023, 13.024, 13.031, 13.045, 13.047, 13.052, 13.054, 13.058, 13.059, 13.061, 13.066, 13.069, 13.070, 13.074, 13.083, 13.101, 13.103, 13.105, 13.106, 13.116, 13.123, 13.138, 13.148, 13.163 and 13.190].

On the basis of the new data on toxicity for 3-(5-methyl-2-furyl)butanal [FL-no: 13.058] an appropriate no observed adverse effect level (NOAEL) of 5.9 mg/kg body weight (bw) per day was identified, supporting the evaluation of this candidate substance. Based on the MSDI approach, the CEF Panel concluded that [FL-no: 13.058] would be of no safety concern at the estimated intake level.

Adequate specifications including complete purity criteria and identity are available for all 28 substances. Thus, for the 11 furan derivatives evaluated through the Procedure in FGE.67 [FL-no: 13.006, 13.021, 13.022, 13.023, 13.024, 13.047, 13.074, 13.116 and 13.190], FGE.67Rev1 [FL-no: 13.031] and FGE.67Rev2 [FL-no: 13.058], the CEF Panel considered that the materials of commerce would not present a safety concern at their estimated levels of intake based on the MSDI approach. For the 17 remaining substances the evaluation could not be finalised due to lacking information on toxicity.

The mTAMDI could be calculated for 4 of the 11 substances that were evaluated through the Procedure. For three substances [FL-no: 13.031, 13.047 and 13.074], the mTAMDI exceeds the threshold for the corresponding structural class and therefore more reliable data on uses and use levels are required. For the remaining seven substances [FL-no: 13.006, 13.021, 13.022, 13.023, 13.024, 13.116 and 13.190], use levels are needed in order to calculate the mTAMDIs and to identify those flavouring substances that need more refined exposure assessment to finalise the evaluation.

In FGE.67Rev2, the CEF Panel concluded that for 17 substances [FL-no: 13.045, 13.052, 13.054, 13.059, 13.061, 13.066, 13.069, 13.070, 13.083, 13.101, 13.103, 13.105, 13.106, 13.123, 13.138, 13.148 and 13.163], additional toxicity/genotoxicity data are required. The CEF Panel noted further that for 7 of these 17 substances [FL-no: 13.045, 13.052, 13.054, 13.059, 13.061, 13.069 and 13.083] use levels have not been submitted.

FGE.67Rev3

The present revision is due to the submission of additional genotoxicity and toxicity data for 2-pentylfuran [FL-no: 13.059] and 2-acetylfuran [FL-no: 13.054], representative substances for subgroup IV [FL-no: 13.069, 13.103, 13.106 and 13.148] and subgroup VI-b [FL-no: 13.045, 13.066, 13.070, 13.083, 13.101, 13.105, 13.138 and 13.163], respectively.

In FGE.67Rev2, 28 substances were considered. After publication of FGE.67Rev2, three substances [FL-no: 13.052, 13.061 and 13.123] were deleted from the Union List because of reasons mentioned in Regulation (EU) 2015/1102[7]. Therefore, these substances will not be considered further in FGE.67Rev3.

Industry communicated that the substances in subgroup VI-A, which were under evaluation in FGE.222 to clarify their potential genotoxicity, are no longer supported (DG SANTE, 2019). These six substances were part of the original JECFA group of 40 substances (see under FGE.67 above). Industry communicated also that the substances [FL-no: 13.066 and 13.103] are no longer supported (DG SANTE, 2020a,b). Therefore, these eight substances will not be further considered in this FGE.

The present revision of FGE.67, FGE.67Rev3, deals with 23 flavouring substances [FL-no: 13.006, 13.021, 13.022, 13.023, 13.024, 13.031, 13.045, 13.047, 13.054, 13.058, 13.059, 13.069, 13.070, 13.074, 13.083, 13.101, 13.105, 13.106, 13.116, 13.138, 13.148, 13.163 and 13.190].

Eleven of these substances have been evaluated through the Procedure in previous revisions of FGE.67: [FL-no: 13.006, 13.021, 13.022, 13.023, 13.024, 13.031, 13.047, 13.058, 13.074, 13.116 and 13.190].

Twelve substances will be evaluated based on new genotoxicity and toxicity data: subgroup IV [FL-no: 13.059, 13.069, 13.106, 13.148] and subgroup VI-b [FL-no: 13.045, 13.054, 13.070, 13.083, 13.101, 13.105, 13.138 and 13.163].

For the sake of completeness, the information for all the 40 substances is maintained in the various tables in this FGE.

[7] Commission Regulation (EU) 2015/1102 of 8 July 2015 amending Annex I to Regulation (EC) No 1334/2008 of the European Parliament and of the Council as regards removal from the Union list of certain flavouring substances. OJ L 181, 9.7.2015, p. 54–56.

FGE	Adopted by EFSA	Link	No. of substances
FGE.67	26 November 2009	https://www.efsa.europa.eu/en/efsajournal/pub/1404	25
FGE.67Rev1	6 July 2011	https://www.efsa.europa.eu/en/efsajournal/pub/2315	33
FGE.67Rev2	7 May 2015	https://www.efsa.europa.eu/en/efsajournal/pub/4115	28
FGE.67Rev3	25 November 2020	https://www.efsa.europa.eu/en/efsajournal/pub/6362	23

Several substances are structurally related to furfuryl- and furan derivatives evaluated by EFSA in FGE.13Rev1, FGE.65, FGE.66. The substances [FL-no: 13.116, 13.190] and [FL-no: 13.006] are considered structurally related to the substances in FGE.65 and in FGE.66, respectively. Since [FL-no: 13.006, 13.116, 13.190] have been already evaluated in previous revisions of FGE.67, no data from FGE.65 Rev1 (EFSA CEF Panel, 2015b) and FGE.66 Rev1 (EFSA CEF Panel, 2011b) are reported in the present opinion.

A summary of the history of the evaluation of the substances in FGE.67 is presented in Figure 1.

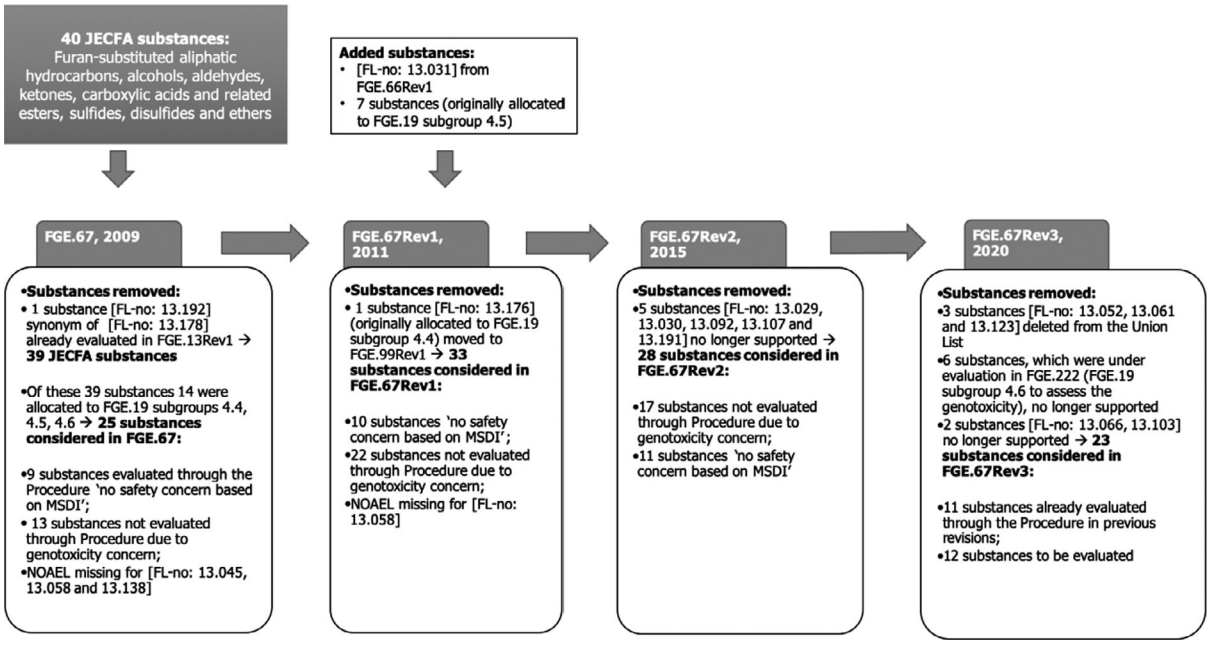


Figure 1: Summary of the history of evaluation of the substances in FGE.67

1.4. Presentation of the Substances in the JECFA Flavouring Group

The JECFA evaluated a group of 40 diverse furan derivatives, first at their 65th meeting (JECFA, 2006a) where a request for additional data was expressed. The furan group was on the agenda again at the 69th JECFA meeting (JECFA, 2009a,b), 76th JECFA meeting (JECFA, 2012) and at 86th JECFA meeting (JECFA, 2019) where additional data had been provided.

JECFA Status

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

At its 65th meeting, JECFA evaluated a group of 40 furan substituted substances (JECFA, 2006a). 'The Committee took note of the extensive evidence for the genotoxicity of several members of this group of flavouring agents related to furan, including the clastogenicity of 2-furyl methyl ketone (synonym: 2-acetyl furan; [FL-no: 13.054]) in mouse bone marrow. Furan, which is carcinogenic, is known to undergo oxidation and ring opening to form a reactive 2-ene-1,4- dicarbonyl intermediate. Accordingly, there is concern that the observed genotoxicity might be due to formation of a reactive metabolite. No data were available on the potential of members of this group of flavouring substances to form reactive metabolites, and no role of metabolism has been identified in the observed

genotoxicity with some of them. Moreover, there were few data on genotoxicity *in vivo*, and specific *in vivo* assays to address potential carcinogenicity were lacking'. JECFA concluded that the Procedure for the Safety Evaluation of Flavouring Agents could not be applied to this group and that additional data were needed.

At its 69th meeting, JECFA evaluated additional *in vitro* and *in vivo* genotoxicity studies on 2-acetylfuran [FL-no: 13.054] (JECFA, 2009a): *in vitro* and an *in vivo* unscheduled DNA Synthesis (UDS) studies and an *in vivo* sister chromatid exchange (SCE) study. JECFA considered that these studies did not resolve the concerns on genotoxicity and indicated 'Studies that would assist in the safety evaluation include investigations of the influence of the nature and position of ring substitution on metabolism and on covalent binding to macromolecules. Depending on the findings, additional studies might include assays related to the mutagenic and carcinogenic potential of representative members of this group'.

At its 76th meeting, JECFA re-evaluated these furans (JECFA, 2012) and evaluated a new *in vivo* comet assay comparing the potential genotoxicity of 2-pentylfuran and furan. Also this study was not considered sufficient to clarify the potential genotoxicity of this group of substances. The JECFA concluded that the additional information was not sufficient to alleviate the concerns raised during the 65th meeting.

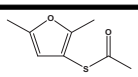
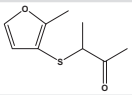
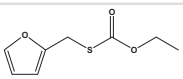
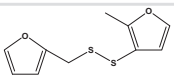
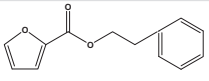
At its 86th meeting, JECFA considered additional *in vitro* genotoxicity studies for seven substances [FL-no: 13.024, 13.034, 13.044, 13.052, 13.054, 13.074 and, 13.083] and *in vivo* genotoxicity studies for four substances [FL-no: 13.034, 13.044, 13.054 and 13.059] (JECFA, 2019). Short–term toxicological studies were available for [FL-no: 13.058 and 13.059]. JECFA concluded that the new data were sufficient to rule out the previous concerns of the Committee. Therefore, the 39 substances were evaluated through the updated Procedure for the Safety evaluation of flavouring agents (JECFA, 2016).

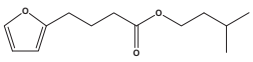
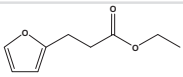
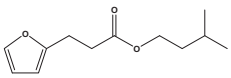
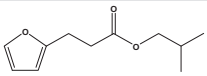
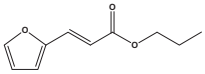
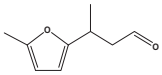
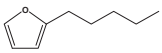
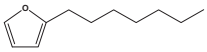
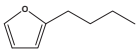
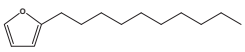
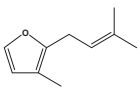
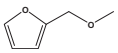
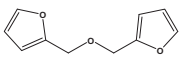
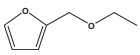
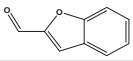
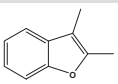
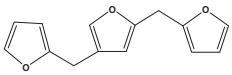
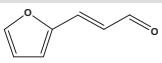
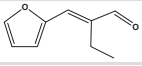
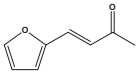
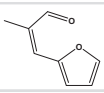
EFSA Considerations

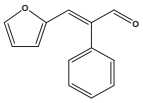
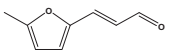
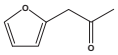
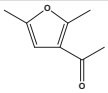
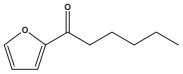
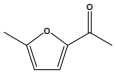
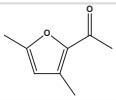
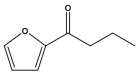
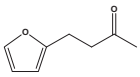
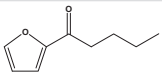
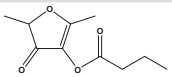
The group of furan derivatives evaluated by the JECFA is a very diverse group of flavouring substances which can be subdivided into six major subgroups with further subdivision of subgroup V and VI, as depicted in Table 1. The substance [FL-no: 13.031], also included in Table 1, was evaluated by the JECFA at the 55th meeting (JECFA, 2001) in the group of furfuryl derivatives evaluated by EFSA in FGE.66Rev1 (EFSA CEF Panel, 2011b), but as [FL-no: 13.031] has structural similarity to [FL-no: 13.074] considered in FGE.67, it has been included in FGE.67, rather than in FGE.66Rev1.

In the last column of Table 1, the status of the evaluation by EFSA of the individual members of FGE.67 is presented, based on the evaluation in FGE.67Rev2, i.e. before consideration of the information received by EFSA that leads to the present revision 3 of this FGE. In the meantime, several substances have been withdrawn by industry for use as flavourings substances. This is also taken into account in the table.

Table 1: Subgrouping of the furan-substituted substances considered by JECFA at the 55th, 65th, 69th and 86th meetings

Sub-group	FL-no JECFA-no	EU Register name	Structural formula	EFSA status following the publication of FGE67Rev2
I	13.116 1523	2,5-Dimethyl-3-thioacetoxyfuran		FGE.67 – no safety concern
I	13.190 1525	3-((2-Methyl-3-furyl)thio)-2-butanone		FGE.67 – no safety concern
I	13.191 1526	*o*-Ethyl S-(2-furylmethyl) thiocarbonate		Not supported by industry and not included in UL[(b)]
I	13.192 1524	Furfuryl 2-methyl-3-furyldisulfide		Duplicate of FL-no: 13.178 evaluated in FGE.13
II	13.006 1517	Phenethyl 2-furoate		FGE.67 – no safety concern

Sub-group	FL-no JECFA-no	EU Register name	Structural formula	EFSA status following the publication of FGE67Rev2
III	13.021 1516	Isopentyl 4-(2-furan)butyrate		FGE.67 – no safety concern
III	13.022 1513	Ethyl 3(2-furyl)propionate		FGE.67 – no safety concern
III	13.023 1515	Isopentyl 3-(2-furan) propionate		FGE.67 – no safety concern
III	13.024 1514	Isobutyl 3-(2-furyl) propionate		FGE.67 – no safety concern
III	13.047 1518	Propyl 3-(2-furyl)acrylate		FGE.67 – no safety concern
III	13.058 1500	3-(5-Methyl-2-furyl) butanal		FGE.67Rev2 – no safety concern
IV	13.029 1488	2,5-Dimethylfuran		Deleted from UL[a]
IV	13.030 1487	2-Methylfuran		Deleted from UL[a]
IV	13.059 1491	2-Pentylfuran		To be evaluated in FGE.67Rev3
IV	13.069 1492	2-Heptylfuran		To be evaluated in FGE.67Rev3
IV	13.092 1489	2-Ethylfuran		Deleted from UL[a]
IV	13.103 1490	2-Butylfuran		No longer supported[e]
IV	13.106 1493	2-Decylfuran		To be evaluated in FGE.67Rev3
IV	13.148 1494	3-Methyl-2(3-methylbut-2-enyl)furan		To be evaluated in FGE.67Rev3
V-A	13.052 1520	Furfuryl methyl ether		Deleted from UL[c]
V-A	13.061 1522	Difurfuryl ether		Deleted from UL[c]
V-A	13.123 1521	Ethyl furfuryl ether		Deleted from UL[c]
V-B	13.031 751	2-Benzofurancarboxaldehyde		FGE.67Rev1 – no safety concern
V-B	13.074 1495	2,3-Dimethylbenzofuran		FGE.67 – no safety concern
V-C	13.107 1496	2,4-Difurfurylfuran		Not supported by industry and not included in UL[b]
VI-A	13.034 1497	3-(2-Furyl)acrylaldehyde		No longer supported[d]
VI-A	13.043 1501	Furfurylidene-2-butanal		No longer supported[d]
VI-A	13.044 1511	4-(2-Furyl)but-3-en-2-one		No longer supported[d]
VI-A	13.046 1498	3-(2-Furyl)-2-methylprop-2-enal		No longer supported[d]

Sub-group	FL-no JECFA-no	EU Register name	Structural formula	EFSA status following the publication of FGE67Rev2
VI-A	13.137 1502	3-(2-Furyl)-2-phenylprop-2-enal		No longer supported(d)
VI-A	13.150 1499	3-(5-Methyl-2-furyl)prop-2-enal		No longer supported(d)
VI-B	13.045 1508	1-(2-Furyl)-propan-2-one		To be evaluated in FGE.67Rev3
VI-B	13.054 1503	2-Acetylfuran		To be evaluated in FGE.67Rev3
VI-B	13.066 1506	3-Acetyl-2,5-dimethylfuran		No longer supported(f)
VI-B	13.070 1512	2-Hexanoylfuran		To be evaluated in FGE.67Rev3
VI-B	13.083 1504	2-Acetyl-5-methylfuran		To be evaluated in FGE.67Rev3
VI-B	13.101 1505	2-Acetyl-3,5-dimethylfuran		To be evaluated in FGE.67Rev3
VI-B	13.105 1507	2-Butyrylfuran		To be evaluated in FGE.67Rev3
VI-B	13.138 1510	1-(2-Furyl)butan-3-one		To be evaluated in FGE.67Rev3
VI-B	13.163 1509	2-Pentanoylfuran		To be evaluated in FGE.67Rev3
VI-C	13.176 1519	Furaneyl butyrate		FGE.99 – no safety concern

FL-No: FLAVIS number; JECFA: The Joint FAO/WHO Expert Committee on Food Additives; FGE: Flavouring Group Evaluation.
(a): Commission Regulation (EU) No 246/2014 of 13 March 2014 amending Annex I to Regulation (EC) No 1334/2008 of the European Parliament and of the Council as regards removal from the Union list of certain flavouring substances. OJ L74, 14.3.2014, p. 58–60.
(b): Commission Implementing Regulation (EU) No 872/2012 of 1 October 2012 adopting the list of flavouring substances provided for by Regulation (EC) No 2232/96 of the European Parliament and of the Council, introducing it in Annex I to Regulation (EC) No 1334/2008 of the European Parliament and of the Council and repealing Commission Regulation (EC) No 1565/2000 and Commission Decision 1999/217/EC. OJ L 267, 2.10.2012, p. 1–161.
(c): Commission Regulation (EU) 2015/1102 amending annex I to Commission Regulation (EC) no 1334/2008 of the European Parliament and of the Council as regards removal from the Union list of certain flavouring substances, OJ L181, 9.7.2015, p. 54–56.
(d): Letter from DG-SANTE to EFSA (DG SANTE, 2019).
(e): Letter from DG-SANTE to EFSA (DG SANTE, 2020a).
(f): Letter from DG-SANTE to EFSA (DG SANTE, 2020b).

Only groups of substances evaluated in FGE.67Rev3 are described. No descriptions are given for groups I, II III, V and VI-C, which can be found in FGE.67Rev2 (EFSA CEF Panel, 2015a).

Group IV

This subgroup comprises eight substances [FL-no: 13.029, 13.030, 13.059, 13.069, 13.092, 13.103, 13.106 and 13.148]. All are alkyl-substituted furans and structurally related to two substances evaluated by EFSA in FGE.13Rev2 in subgroup Ic [FL-no: 13.125, 13.162]. In FGE.13Rev2 (EFSA CEF Panel, 2011c), a concern for genotoxicity has been identified for these two structurally related substances. However, four of the substances in this subgroup (IV) are no longer supported by industry

[FL-no: 13.029, 13.030 and 13.092][8] and [FL-no: 13.103] (DG SANTE, 2020a). Consequently, only four substances [FL-no: 13.059, 13.069, 13.106 and 13.148] will be considered in FGE.67Rev3.

Group VI

This subgroup consists of 16 α,β-unsaturated carbonyls initially allocated to FGE.19 for evaluating the potential genotoxicity (EFSA, 2008a,b). The substances in this group have been allocated to three subgroups. Subgroups relevant for the current assessment are described below; information on subgroup VI-C is reported in FGE.67Rev2 (EFSA CEF Panel, 2015a).

Subgroup VI-A

For six substances (from FGE.19, subgroup 4.6, (EFSA, 2008b)) [FL-no: 13.034, 13.043, 13.044, 13.046, 13.137 and 13.150] a need for additional information on genotoxicity was identified in FGE.222 (EFSA CEF Panel, 2012a). However, the evaluation of these substances is not supported by industry (DG SANTE, 2019). Therefore, these six substances will not be further evaluated.

Subgroup VI-B

This subgroup comprises nine substances. For seven substances in this subgroup (from FGE.19, subgroup 4.5, (EFSA, 2008b)) [FL-no: 13.054, 13.066, 13.070, 13.083, 13.101, 13.105 and 13.163] a need for additional information on genotoxicity was identified (EFSA, 2008a). After further considerations in the course of the assessment, the CEF Panel concluded that these substances are comparable to acetophenone, an aromatic α,β-unsaturated ketone, which is not genotoxic. Therefore, the α,β-unsaturated ketone moiety in these seven furan derivatives is no longer considered to represent an alert for genotoxicity (EFSA, 2008b, see also FGE.13Rev2). However, based on other considerations with respect to metabolism and possible genotoxic properties of substance [FL-no: 13.054], for all substances in this subgroup additional data on genotoxicity were requested in FGE.67Rev1 and in FGE.67Rev2. Meanwhile industry has communicated that [FL-no: 13.066] is no longer supported for use as a flavouring substance in the EU (DG SANTE, 2020b). Therefore, this substance will no longer be considered in this FGE.

In conclusion, revision 3 of FGE.67 will include 23 substances:

- 11 flavouring substances [FL-no: 13.006, 13.021, 13.022, 13.023, 13.024, 13.031, 13.047, 13.058, 13.074, 13.116, 13.190] which have been considered in previous versions of FGE.67. Data on these substances will not be further discussed with the exception of the considerations on exposure based on the new data available. As requested by the CEF Panel in FGE.67Rev2, use levels data have been submitted for the substances [FL-no: 13.006, 13.021, 13.022, 13.023, 13.024, 13.116, 13.190] which will be considered in the present revision.
- 12 additional substances: four substances from subgroup IV [FL-no: 13.059, 13.069, 13.106 and 13.148] and eight substances from subgroup VI-B [FL-no: 13.045, 13.054, 13.070, 13.083, 13.101, 13.105, 13.138 and 13.163]. In subgroup IV, 2-pentylfuran [FL-no: 13.059] is considered the representative substance, while in subgroup VI-B, 2-acetylfuran [FL-no: 13.054] is the representative substance.

For the sake of completeness, the information on identity of all substances is maintained in various tables of this FGE. Information on specifications is only maintained for the substances which are currently in the Union List (see Appendix B). For substances that are no longer in the Union List, FGE.67Rev2 can be consulted.

2. Data and methodologies

2.1. Data

Following the concern for genotoxicity, expressed by the CEF Panel in FGE.67Rev2, for the substances in subgroup IV and VI-B, industry provided information from literature. However, most of the submitted publications had already been considered previously. The new studies submitted were a combined 28-day and 90-day toxicity study for 2-acetylfuran [FL-no: 13.054] (Bio-Research

[8] Commission Implementing Regulation (EU) No 246/2014 of 13 March 2014 amending Annex I to Regulation (EC) No 1334/2008 of the European Parliament and of the Council as regards removal from the Union list of certain flavouring substances. OJ L 74, 14.3.2014, p. 58–60.

Laboratories, 1985) and an *in vivo* combined bone marrow micronucleus test and comet assay for 2-pentylfuran [FL-no: 13.059] (Covance, 2014); see documentation provided to EFSA n. 2, 5, 15.

Additional information was provided by the applicant during the assessment process in response to requests from EFSA sent on 1/4/2015, 18/12/2017, 29/11/2018, 29/4/2020, 15/7/2020 (see Documentation provided to EFSA n. 6, 19, 20, 22, 24, 25, 3, 4, 17). Moreover, industry provided updated poundage and use levels data (Documentation provided to EFSA n.16,18).

The new available data considered in the present revision of FGE.67 are summarised in Table 2.

Table 2: Data evaluated in FGE.67Rev3

Subgroup	FL-no	Chemical name	Data provided for the current revision 3 of FGE.67	Appendix (Table nr) and relevant section of the opinion	Documentation provided to EFSA/Reference
II	13.006	Phenethyl 2-furoate	Use levels	Appendix C (Tables C.1 and C.4); Section 3.2	EFFA (2018)
III	13.021	Isopentyl 4-(2-furan) butyrate	Use levels		EFFA (2018)
III	13.022	Ethyl 3-(2-furyl) propionate	Use levels		EFFA (2018)
III	13.023	Isopentyl 3-(2-furan) propionate	Use levels		EFFA (2018)
III	13.024	Isobutyl 3-(2-furan) propionate	Use levels		EFFA (2018)
VI-B	13.045	1-(2-Furyl)-propan-2-one	Use levels, poundage data		EFFA (2020b)
VI-B	13.054	2-Acetylfuran	Genotoxicity and toxicity data Use levels poundage data	Appendix E (Tables E.1 and E.2); Section 3.4.5 Appendix F (Table F.1); Section 3.4.6 Appendix C (Tables C.1 and C.4); Section 3.2	Covance (2016) Charles River (2020a) Bio-Research Laboratories (1985) EFFA (2020a,b)
IV	13.059	2-Pentylfuran	Genotoxicity and toxicity data Use levels, poundage data		New York Medical College (2012) Covance (2014) Charles River (2020b) Gulf South Research Institute (1971a,b) Product Safety Labs (2016, 2017) EFFA (2020a,b)
IV	13.069	2-Heptylfuran	Use levels, poundage data	Appendix C (Tables C.1 and C.4); Section 3.2	EFFA (2020b)
VI-B	13.070	2-Hexanoylfuran	Use levels, poundage data		EFFA (2020b)
VI-B	13.083	2-Acetyl-5-methylfuran	Use levels, poundage data		EFFA (2020b)
VI-B	13.101	2-Acetyl-3,5-dimethylfuran	Use levels, poundage data		EFFA, 2020b
VI-B	13.105	2-Butyrylfuran	Use levels, poundage data		EFFA (2020b)
IV	13.106	2-Decylfuran	Use levels, poundage data		EFFA (2020b)
I	13.116	2,5-Dimethyl-3-thioacetoxyfuran	Use levels		EFFA (2018)
VI-B	13.138	1-(2-Furyl)butan-3-one	Use levels, poundage data		EFFA (2020b)

Subgroup	FL-no	Chemical name	Data provided for the current revision 3 of FGE.67	Appendix (Table nr) and relevant section of the opinion	Documentation provided to EFSA/Reference
IV	13.148	3-Methyl-2(3-methylbut-2-enyl)furan	Use levels, poundage data		EFFA (2020b)
VI-B	13.163	2-Pentanoylfuran	Use levels, poundage data		EFFA (2020b)
I	13.190	3-((2-Methyl-3-furyl) thio)-2-butanone	Use levels		EFFA (2018)

FL-No: FLAVIS number; FGE: Flavouring Group Evaluation.

In addition, the following references were used:

- JECFA specifications for the 12 candidate flavouring substances [FL-no: 13.045, 13.054, 13.059, 13.069, 13.070, 13.083, 13.101, 13.105, 13.106, 13.138 13.148 and 13.163] (JECFA, 2005, 2018).
- 65th JECFA report (JECFA, 2006a,b), JECFA monograph and report of the 69th meeting (JECFA, 2009a,b), 76th JECFA report (JECFA, 2012) and 86th JECFA report (JECFA, 2019).
- EFSA scientific opinion on FGE.67Rev2 (EFSA CEF Panel, 2015a).
- EFSA scientific opinion on FGE.13Rev2 (EFSA CEF Panel, 2011c).

2.2. Methodologies

This opinion was prepared based on the principles described in the EFSA Guidance on transparency with regard to scientific aspects of risk assessment (EFSA Scientific Committee, 2009) and following the relevant existing guidance documents from the EFSA Scientific Committee. The assessment strategy applied for the evaluation programme of flavouring substances, as laid down in Commission Regulation (EC) No 1565/2000, is based on the Opinion on a Programme for the Evaluation of Flavouring substances of the Scientific Committee on Food (SCF, 1999).

2.2.1. Procedure for the safety evaluation of flavouring substances

The approach for safety evaluation of chemically defined flavouring substances as referred to in Commission Regulation (EC) No 1565/2000, named the 'Procedure', is described in Appendix A.

2.2.2. Approach used for the calculation of exposure

The approach used for calculation of the intake of the flavouring substances is described in Appendix A (point 'a) *Intake*') and in Appendix C (Section C.2 'mTAMDI calculation').

3. Assessment

3.1. Specifications

JECFA status

The JECFA specifications are available for the all 23 flavouring substances in FGE.67Rev3 (JECFA, 2005, 2018).

Two substances [FL-no: 13.058 and 13.190] in the group of JECFA evaluated furan-substituted substances have a chiral centre and [FL-no: 13.047] has geometrical isomerism. None of the remaining flavouring substances has possibility for stereoisomerism.

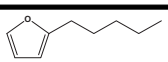
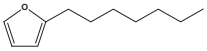
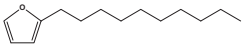
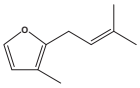
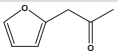
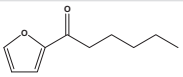
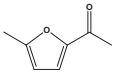
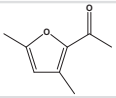
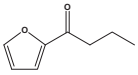
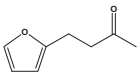
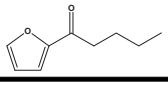
EFSA considerations

The available specifications are considered adequate for all 23 flavouring substances (see Appendix B) and information on stereoisomerism is adequate for all substances.

The Panel noted that the name in the Union List of the substance isopentyl 3-(2-furan)propionate [FL-no: 13.023] should be changed to isopentyl 3-(2-furyl)propionate.

Table 3 shows the chemical structures of the 12 substances under evaluation in this revision of FGE.67 (FGE.67Rev3).

Table 3: Flavouring substances under evaluation in FGE.67Rev3

Sub-group	FL-no JECFA-no	EU Register name	Structural formula	Structural class[(a)]
IV	13.059 1491	2-Pentylfuran		III
IV	13.069 1492	2-Heptylfuran		III
IV	13.106 1493	2-Decylfuran		III
IV	13.148 1494	3-Methyl-2(3-methylbut-2-enyl)furan		III
VI-B	13.045 1508	1-(2-Furyl)-propan-2-one		III
VI-B	13.054 1503	2-Acetylfuran		III
VI-B	13.070 1512	2-Hexanoylfuran		III
VI-B	13.083 1504	2-Acetyl-5-methylfuran		III
VI-B	13.101 1505	2-Acetyl-3,5-dimethylfuran		III
VI-B	13.105 1507	2-Butyrylfuran		III
VI-B	13.138 1510	1-(2-Furyl)butan-3-one		III
VI-B	13.163 1509	2-Pentanoylfuran		III

FL-No: FLAVIS number; FGE: Flavouring Group Evaluation; JECFA: The Joint FAO/WHO Expert Committee on Food Additives.
(a): Determined with OECD Toolbox (version 4.3.1 available online https://www.oecd.org/chemicalsafety/risk-assessment/oecd-qsar-toolbox.htm).

3.2. Estimation of intake

JECFA status

For the 23 JECFA evaluated substances, production volumes are available for the EU and accordingly MSDI figures for the EU can be calculated, see Appendix C, Table C.4.

JECFA (2019) calculated both MSDI and SPET (single-portion exposure technique) according to the updated JECFA Procedure described in the 82nd JECFA report (JECFA, 2016).

For all substances in FGE.67Rev3, the SPET value is higher than the MSDI except for one substance [FL-no: 13.006].

EFSA considerations

Updated poundage data for use as flavouring substances in the EU have been submitted by industry for 12 substances (EFFA, 2018, 2020b) allowing for the calculation of MSDIs for all candidate substances in this group [FL-no: 13.045, 13.054, 13.059, 13.069, 13.070, 13.083, 13.101, 13.105, 13.106, 13.138, 13.148 and 13.163].

Although included in Table C.4 (Appendix C), the SPET exposure estimates as calculated by JECFA are not part of the consideration of the candidate substances in this FGE.

Updated use levels have been submitted for [FL-no: 13.006, 13.021, 13.022, 13.023, 13.024, 13.116 and 13.190] (EFFA, 2018) and for [FL-no: 13.045, 13.054, 13.059, 13.069, 13.070, 13.083, 13.101, 13.105, 13.106, 13.138, 13.148 and 13.163] (EFFA, 2020b).

Based on the normal use levels, mTAMDI values can be calculated for all the 23 substances.

For 18 substances [FL-no: 13.021, 13.022, 13.023, 13.024, 13.031, 13.045, 13.047, 13.054, 13.059, 13.074, 13.083, 13.101, 13.105, 13.106, 13.138, 13.148, 13.163 and 13.190], the mTAMDI intake estimates are above the threshold of toxicological concern (TTC) for their structural class and more reliable data on uses and use levels are required to finalise their evaluation. For five substances [FL-no: 13.006, 13.058, 13.069, 13.070 and 13.116], the mTAMDI intake estimates is below the TTC for structural class (III).

Use levels and mTAMDI values are presented in Appendix C, Tables C.1 and C.4.

3.3. Natural occurrence

Data on natural occurrence were provided (EFFA, 2020a), but are not considered for the evaluation. However, the information provided by industry is included in Appendix C.

3.4. Biological and toxicological data

3.4.1. ADME data

JECFA status

JECFA reported that alkylfuran derivatives exhibit rapid uptake, metabolism and excretion (JECFA, 2009a).

According to JECFA monograph (JECFA 2009a), 'Alkyl-substituted furan and benzofuran derivatives undergo cytochrome P450 (CYP)–induced side-chain oxidation to yield an alcohol functional group located at the position bonded directly to the furan ring. The resulting alcohol may be excreted in the urine primarily as the glucuronic acid or sulfate conjugate, or it may be converted to the corresponding ketone, which may also be excreted in the urine. CYP-induced side-chain oxidation, preferably at the $C_{1'}$ position of furan, is similar to that observed with other alkyl-substituted heterocyclic derivatives (e.g. pyridine derivatives) (Hawksworth and Scheline, 1975; Ruangyuttikarn et al., 1992; Thornton-Manning et al., 1993; Gillam et al., 2000). In addition to side-chain oxidation, the furan ring may undergo CYP-mediated oxidation (epoxidation) to yield unstable epoxides that may ring-open to yield reactive 2-enedial intermediates. These types of intermediates have been shown to readily conjugate with GSH, depleting free GSH and subsequently, at high levels, forming protein and DNA adducts (Ravindranath et al., 1983, 1984, 1986; Ravindranath and Boyd, 1985)'.

'Unsubstituted and short-chain alkyl-substituted furans have also been shown to undergo ring epoxidation in the liver by mixed-function oxidases. Epoxy-substituted furans have been reported to undergo ring opening to yield reactive 2-ene-1,4-dicarbonyl intermediates (. . .) that can be conjugated with GSH and readily eliminated in the urine or, at relatively high concentrations, react with proteins and DNA to form adducts. Initial *in vitro* experiments in rat microsomal preparations suggested that high concentrations of alkyl-substituted furans are partly metabolized to reactive acetylacrolein1-type intermediates (Ravindranath et al., 1983, 1984). Acetylacrolein is a potent microsomal mixed-function oxidase inhibitor that has been reported to bind covalently and irreversibly to the oxidizing enzyme, thus inactivating it (Ravindranath and Boyd, 1985)'.

JECFA (2009a) concluded that 'alkyl-substituted furans can be metabolized by side-chain oxidation to initially yield the 1'-alcohol derivative, which can be either conjugated and excreted or oxidized to the corresponding ketone. The conversion to the ketone is anticipated to be reversible, in which case the ketones are reduced to the corresponding alcohols and excreted mainly in the urine. In a second pathway, the furan ring can be oxidized to form an unstable epoxide that may undergo rapid ring opening to yield reactive 2-ene-1,4-dicarbonyl intermediates (e.g. acetylacrolein). The reactive intermediate can be conjugated with available sulfhydryl trapping agents, such as GSH and cysteine, or, at high *in vivo* concentrations, can be covalently bound to proteins and DNA'.

In 2019, JECFA confirmed the same conclusions on the metabolism of alkyl-substituted furans reached in the previous evaluation (JECFA, 2009a): 'At that meeting, the Committee noted that the biotransformation processes applicable to members of this group of furan-substituted flavouring agents are, in large part, dependent on the presence or absence of specific functional groups attached to the furan ring. The Committee also noted that at higher dose levels, low relative molecular mass alkyl furans (e.g. 2-methylfuran) can undergo ring oxidation to yield reactive 2-ene-1,4-dicarbonyl intermediates that can react with protein and DNA. For example, furan has limited metabolic options and is biotransformed via ring oxidation to an enedialdehyde species that is a potent hepatotoxin. The

Committee further noted that the presence of an extended side-chain attached to the furan ring would reduce the potential for epoxidation of the double bond and provide a site for detoxication via metabolism and elimination'.

EFSA considerations

The Panel considered data on ADME reported by JECFA. Although JECFA evaluated this group of substances through the new Procedure (JECFA, 2016), where the question 'can the substance be predicted to be metabolised to innocuous products' is not foreseen, the Panel considerations on these substances are based on the former Procedure (Appendix A).

Based on the available data as described by JECFA, the Panel considered that these substances could not be anticipated to be metabolised to only innocuous products. Therefore, and in line with the evaluation of other furan derivatives in FGE.13, 65 and 66, the B-side of the Procedure will be applied.

The Panel further noted that for furan-substituted substances of FGE.67Rev3, analogues of the γ-diketone 2,5-hex-3-enedione can be anticipated to be formed following the opening of the furan ring (Wang et al., 2015). γ-Diketones can react with free protein amines leading to the formation of imines which could cyclise and eventually form stable protein-pyrrole adducts. As described in FGE.07Rev5 (EFSA CEF Panel, 2017), it is well known that saturated γ-diketones may cause neurotoxicity (Topping et al., 1994). For considerations on potential neurotoxicity see Section 3.4.6.6.

3.4.2. Genotoxicity data evaluated by JECFA

3.4.2.1. Genotoxicity studies – text taken[9] from the JECFA (2009a)

Genotoxicity testing has been performed on eight [FL-no: 13.022, 13.029,[10] 13.030,[10] 13.034, 13.044, 13.054, 13.148 and 13.191[11]] representative furan-substituted aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids and related esters, sulphides, disulphides and ethers in this group (JECFA, 2009a). The results of these tests are summarised in Appendix D of this FGE and described below.

In vitro

'In standard *Salmonella* mutagenicity assays, 2,5-dimethylfuran [FL-no: 13.029],[10] 3-methyl-2-(3-methylbut-2-enyl)-furan [FL-no: 13.148], 3-(2-furyl)acrolein [FL-no: 13.034], 4-(2-furyl)-3-buten-2-one [FL-no: 13.044], ethyl 3-(2-furyl)propanoate [FL-no: 13.022] and O-ethyl S-(2-furylmethyl) thiocarbonate [FL-no: 13.191][11] were not mutagenic in *Salmonella typhimurium* strains TA97, TA98, TA100, TA102, TA1535, TA1537 or TA1538 when tested at concentrations of up to 10,000 μg/plate, alone or in the presence of an exogenous rat liver metabolic activation system (S9-mix) (Wild et al., 1983; Mortelmans et al., 1986; Shinohara et al., 1986; Asquith, 1989; Eder et al., 1991; Zeiger et al., 1992; Lee et al., 1994; Verspeek-Rip, 2000). Likewise, with the exception of a single assay in which equivocal results of mutagenicity were reported in *S. typhimurium* strains TA97 and TA107 (Zeiger et al., 1992) methylfuran [FL-no: 13.030][10] was consistently negative in several other strains of *S. typhimurium* (i.e. TA98, TA100, TA102 and TA1535) both alone and with an exogenous rat liver bioactivation system (S9-mix) (Shinohara et al., 1986; Aeschbacher et al., 1989). Evaluated alone and with an exogenous bioactivation system in *S. typhimurium* at concentrations of up to 0.660 μmol/plate (54.2 μg/plate), 2-furyl methyl ketone [FL-no: 13.054] exhibited a significant positive mutagenic potential only in strain TA98 with bioactivation at the two lower concentrations (i.e. 0.165 and 0.330 μmol/plate) (Shinohara et al., 1986). At higher concentrations, significant cytotoxicity was observed, which was reflected by a concentration-dependent decrease in the number of revertants.

Bacterial mutagenicity testing of furans that can be metabolically oxidized to reactive α,β-unsaturated dicarbonyl (2-ene-1,4-dicarbonyl) intermediates is problematic owing to their high

[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.

[10] Substance deleted from the Union list. Commission Regulation (EU) No 246/2014 of 13 March 2014 amending Annex I to Regulation (EC) No 1334/2008 of the European Parliament and of the Council as regards removal from the Union list of certain flavouring substances. OJ L 74, 14.3.2014, p. 58–60.

[11] Substance not supported by industry and not included in the Union List. Commission Implementing Regulation (EU) No 872/2012 of 1 October 2012 adopting the list of flavouring substances provided for by Regulation (EC) No 2232/96 of the European Parliament and of the Council, introducing it in Annex I to Regulation (EC) No 1334/2008 of the European Parliament and of the Council and repealing Commission Regulation (EC) No 1565/2000 and Commission Decision 1999/217/EC. OJ L 267, 2.10.2012, p. 1–161.

bacterial toxicity. The cytotoxicity of these substances is believed to arise from their interactions with protein sulfhydryl and amino groups (Marnett et al., 1985; Eder et al., 1992). Owing to the nature of the glutathione (GSH) conjugation pathway, genotoxicity studies in which high concentrations of α,β-unsaturated carbonyl compounds are formed are likely to create oxidative stress. It is anticipated that cells exposed to high concentrations of these types of substances will rapidly deplete GSH levels, eventually leading to cellular damage and decreased cell viability, as indicated by the above study results.

O-Ethyl S-(2-furylmethyl)thiocarbonate [FL-no: 13.191[11]] showed no mutagenic potential when tested in *Escherichia coli* WP2uvrA at concentrations of up to 3,330 μg/plate, either alone or with a bioactivation system (Verspeek-Rip, 2000). Evaluated in *E. coli* PQ37 under the conditions of the SOS chromotest, 3-(2-furyl)-acrolein [FL-no: 13.034] tested negative (Eder et al., 1991); however, in a subsequent evaluation, 3-(2-furyl)acrolein [FL-no: 13.034] as well as 2-furyl methyl ketone (2-acetylfuran) [FL-no: 13.054] were slightly positive in the SOS chromotest without metabolic activation, as evidenced by 1.72- and 1.75-fold increases, respectively, in the SOS induction factor over a background value of 1 (results were considered to be significant if the induction factor was at least 1.5) (Eder et al., 1993).

In the rec assay, which is based on differential inhibition of growth of repair-deficient strains as a measure of DNA-damaging activity, *Bacillus subtilis* strains H17 (rec+) and M45 (rec-) were incubated with 2-methylfuran [FL-no: 13.030][10], 2,5-dimethylfuran [FL-no: 13.029][10] and 2-furyl methyl ketone (2-acetylfuran) [FL-no: 13.054] at concentrations of up to 55,000 μg/disc, alone and with metabolic activation (Shinohara et al., 1986). 2-Furyl methyl ketone tested negative at a concentration of 550 μg/disc, but was reportedly positive at concentrations of 5,500 μg/disc and greater alone and with metabolic activation. Likewise, 2,5-dimethylfuran was negative at the lowest concentration tested (i.e. 190 μg/disc) with metabolic activation, but tested positive at every concentration tested in the absence of metabolic activation. In contrast, 2-methylfuran was negative with metabolic activation and induced significant differences in the zones of inhibition only without metabolic activation. Additionally, 2-methylfuran and 2-furyl methyl ketone were reported to cleave the double strand of *lambda*-phage DNA in the presence of $Cu2^{+}$; however, a negative control was not included, and, therefore, the statistical significance of these results was not ascertained. Also, it should be noted that potential concomitant cytotoxicity was not monitored in this study.

The potential mammalian cell clastogenicities of 2-methylfuran [FL-no: 13.030][10], 2,5-dimethylfuran [FL-no: 13.029][10] and 2-furyl methyl ketone (2-acetylfuran) [FL-no: 13.054] were evaluated in Chinese hamster ovary (CHO) cells, in which induction of chromosomal aberrations was measured. Cells were exposed to substances from commercial sources (purity not given) for 3 h, followed by 20 h of maintenance. In the absence of exogenous metabolic activation, all three compounds produced increases in the number of chromosomal aberrations, mainly chromatid exchanges; however, in the presence of rat liver metabolic activation, only the clastogenicity of 2-furyl methyl ketone was increased, whereas the clastogenic activities of 2-methylfuran and 2,5-dimethylfuran were reduced in comparison with test systems without metabolic activation. Additionally, the authors noted that when NADP was eliminated from the activation system, the reduction in the chromosomal aberrations observed for 2-methylfuran and 2,5-dimethylfuran and the increase in the clastogenic activity observed with 2-furyl methyl ketone in the presence of the activation system were abolished. This suggests that mixed-function oxidases are integral in the metabolism of alkyl furan derivatives. It should be noted that the experiment with 2-furyl methyl ketone was performed at a limited number of concentrations (two), the active one of which far exceeded (112.6 mmol/L = 13,220 μg/mL) standard concentration limits for this assay and was toxic (Stich et al., 1981).

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

Apoptosis is a type of cell death that occurs under physiological conditions or in response to external stimuli (e.g. DNA-damaging agents, growth factor deprivation or receptor triggering). The mechanism of formation of apoptotic cells includes activation of cysteine proteases (caspases), leading to increased mitochondrial permeability, release of cytochrome c, DNA cleavage and redistribution of phosphatidylserine to the outer layers of the cell membrane, which enhances binding of cells to

phagocytes. DNA cleavage, owing to irreversible activation of endonucleases, is followed by chromatin condensation and oligonucleosomal fragmentation due to double-strand cleavage of DNA in nucleosomal linker regions (Saraste and Pulkki, 2000). During chromatin condensation, the nucleus may split into a number of dense micronuclei. Fragmented DNA and chromatin condensation due to apoptotic events are not easily distinguished from direct action of a specific chemical.

In consideration of such knowledge, findings of chromosomal aberrations must be evaluated in the context of the potential for apoptosis to occur under test conditions. Relatively high concentrations (i.e. up to 1,923–13,220 μg/mL or 20–150 mmol/L) were used in the study conducted by Stich et al. (1981). The K_m for most enzyme kinetic processes is at or below 100 μmol/L (Bu, 2006; Wang and James, 2006), and thus the high concentrations used in this study may not be relevant to the human condition, especially with respect to the low levels of flavouring agents added to food. Furthermore, no information was available on culture conditions that may have promoted apoptosis. Results of chromosomal aberration and micronuclei assays are problematic to interpret in the absence of such information.

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

In vivo

As reported in an abstract, 2-methylfuran [FL-no: 13.030][10] (purity not given) did not induce chromosomal aberrations in bone marrow cells or spermatocytes of Swiss albino mice evaluated at 24-h intervals following administration in the diet at concentrations of 1,000, 2,000 or 4,000 mg/kg (approximately 100, 200 and 400 mg/kg body weight (bw) per day, respectively) for a period of 5 days. No positive control was included. Moreover, the authors noted that 2-methylfuran did not inhibit spindle protein synthesis or cell division in the somatic cells. In the germ cells, which were evaluated at weekly intervals for a period of 5 weeks following final dosing, in order to cover one full spermatogenesis cycle, no structural sperm-head abnormalities were reported (Subramanyam et al., 1989).

2-Furyl methyl ketone (2-acetylfuran) [FL-no: 13.054] was evaluated for clastogenic activity in bone marrow and germ cells of Swiss albino mice. Groups of two per dose per sampling time were administered the compound (99% pure) orally at 0 (control), 1,000, 2,000 or 3,000 mg/L in 0.5 mL of water (approximately 0, 20, 40 and 60 mg/kg bw, respectively) either as a single dose or once daily for 5 consecutive days. No positive control was included. Bone marrow cells were collected periodically for up to 72 h following dosing, and meiotic and sperm preparations from testes and epididymis, respectively, were assessed at 24 h and weekly for a total of 5 weeks post-dosing. In bone marrow cells, the substance at the high dose level was observed to inhibit mitosis beginning at 18 h following single- or multiple-dose treatment. At 24 h, mitodepression was also observed at the high dose level in the single-dose experiment, as well as at the middle and high dose levels in mice administered multiple doses. In the repeat-dose test protocol, the effect remained significant for up to 36 h post-treatment. Mitodepression was accompanied by increases in the frequency of structural chromosomal aberrations, mainly gaps and breaks, in the bone marrow cells. Specifically, at the high dose level (i.e. 3,000 mg/L), between 18 and 24 h following single-dose administration and 12 and 48 h following final treatment of multiple-dose groups, the frequency of aberrations was elevated. Additionally, in animals receiving multiple doses of 2-furyl methyl ketone, significant increases in the number of chromosomal aberrations were also observed at the middle dose level (i.e. 2,000 mg/L) between 24 and 36 h post-treatment. In contrast to the dose- and time-dependent increase in chromosomal aberrations in the somatic cells, only a single isolated increase in structural chromosomal aberrations was observed in mouse spermatocytes 3 weeks following single-dose administrations of the substance, and only at the highest dose level. Following multiple-dose administration, abnormalities in germ cells were limited to significant increases in polyploidy and XY univalents occurring at weeks 3 and 4 at the highest dose level. Furthermore, no sperm-head abnormalities were observed at any dose level, irrespective of the treatment protocol. The absence of sperm-head abnormalities at all dose levels was indicative of a lack of sperm toxicity of the substance. The authors concluded that 2-furyl methyl ketone exhibits only mild clastogenic activity in mouse bone marrow and is not clastogenic in germ cells (Sujatha et al., 1993).

2-Furyl methyl ketone (2-acetylfuran) [FL-no: 13.054] was evaluated for induction of sister chromatid exchanges (SCE) in bone marrow of female Swiss albino mice. Groups of two per dose per exposure regimen were administered compound (99% pure) at 0, 1,000, 2,000 or 3,000 mg/L via gavage either once or for 5 consecutive days. 5-Bromodeoxyuridine was injected intraperitoneally to label chromatids. The mice were sacrificed at 12, 24 or 48 h after receiving the last dose, and slides of bone marrow were prepared and processed for differential staining. A dose-related increase up to about 2-fold in SCE was observed for the 12- and 24-hours groups of both the single-dose regimen and the multiple-dose regimen (Sujatha, 2007).

2-Furyl methyl ketone (2-acetylfuran) [FL-no: 13.054] was evaluated for induction of UDS in hepatocytes isolated from livers of dosed male Sprague-Dawley rats. The assay was conducted according to Good Laboratory Practices (GLP) and OECD guidelines. In a preliminary range-finding toxicity study, lethality was observed at 30 mg/kg bw and greater, and signs of toxicity were observed at 20 mg/kg bw. No sex differences were observed, and therefore only males were used in the main study. Groups of four rats were administered compound (purity not given) at 0, 7 or 21 mg/kg bw via gavage. In experiment 1, the hepatocytes were isolated 16 h post-dosing; in experiment 2, hepatocytes were isolated 2 h post-dosing and cultured for autoradiographic measurement of UDS. No UDS was observed in either experiment, in contrast to the positive controls 2-acetylaminofluorene and *N*,*N*'-dimethylhydrazine (Durward, 2007b).'

Conclusions on genotoxicity

'With few exceptions, eight representative substances of this group were consistently negative in mutation assays conducted in various strains of *S. typhimurium* and *E. coli* under appropriate testing conditions. Negative and positive results were obtained in the rec assay in *B. subtilis* for 2-methylfuran and 2,5-dimethylfuran. In mammalian genotoxicity assays conducted in CHO and V79 cells and human peripheral lymphocytes, study results were inconsistent, with both negative (2,5-dimethylfuran, O-ethyl-S-(2-furylmethyl)thiocarbonate) and positive (2-methylfuran, 2,5-dimethylfuran) results reported. Although positive results were reported in the chromosomal aberration assay in CHO cells with 2-methylfuran and 2,5-dimethylfuran, relatively high concentrations were utilized (i.e. up to 13,220 and 1,923 μg/mL, respectively); the statistical significance of the results was not specified, and the potential cytotoxicity was not monitored in the assay. Moreover, as previously discussed, positive *in vitro* results of chromosomal aberrations are difficult to interpret in the presence of concomitant cytotoxicity and cell cycle delay, which, based on the results of the studies, are a feature of the furan derivatives. Therefore, it may be expected that mammalian cells in culture might not have adequate metabolic capacities to counter this toxicity. In fact, with the exception of one assay in which clastogenic activity was reported for a single compound (i.e. 2-furyl methyl ketone) with a metabolic activation system, results obtained with other representative furan derivatives demonstrated a reduction in the frequency of chromosomal aberrations in the presence of metabolic activation. Furthermore, unlike the positive results reported for 2,5-dimethylfuran among several other compounds evaluated in CHO cells at the high concentrations used in the study of Stich et al. (1981), 2,5-dimethylfuran, tested at lower concentrations in V79 cells, did not exhibit clastogenic activity (Ochi and Ohsawa, 1985). The negative findings in the human hepatocyte DNA damage assay provide evidence that the chromosomal aberration findings are not due to a DNA-reactive mechanism.

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

In conclusion, results of the *in vitro* genotoxicity/mutagenicity tests revealed mixed results, with positive results reported less frequently in the presence of an activation system. This could indicate metabolic detoxication of these substances. The *in vivo* single-dose studies with 2-furyl methyl ketone

did not indicate evidence for genotoxicity, whereas two repeat-dose studies showed weak effects for induction of chromosomal aberrations and SCEs. However, evidence indicates that 2-furyl methyl ketone does not exhibit DNA reactivity. The basis for the positive clastogenicity findings remains unclear'.

For a summary of *in vitro*/*in vivo* genotoxicity data considered by JECFA see Appendix D, Table D.1 of this FGE.

3.4.2.2. Genotoxicity studies – text taken[12] from the JECFA (2012)

At the 69th meeting (JECFA, 2009a), JECFA 'concluded that the Procedure could not be applied to this group, because of unresolved toxicological concerns. Studies that would assist in the safety evaluation included investigations of the influence of the nature and position of ring substitution on metabolism and on covalent binding to macromolecules. Depending on the findings, additional studies might include assays related to the mutagenic and carcinogenic potential of representative members of this group'.

Additional data were submitted for 2-pentylfuran and evaluated by JECFA at the 76th meeting (JECFA, 2012) as reported below.

'The Committee discussed the results of the *in vivo* comet assays. In these studies, the potential genotoxicity of 2-pentylfuran was compared with that of furan using isolated liver cells from male B6C3F1 mice that were gavaged with 2-pentylfuran or furan. Before the conduct of the main studies, pilot comet assays were performed with a single oral furan dose of 250 mg/kg bw, and DNA damage was measured in liver cells at 3, 6 and 24 h. The 3-h time point was found to be optimal for measuring the genotoxic response to furan. Based on this finding, the 3-h time point was used in three separate studies with 2-pentylfuran.

In the first study, 2-pentylfuran (508 mg/kg bw) produced an increase in percent DNA in the comet tail in the absence of proteinase K compared with the vehicle control, indicating DNA damage. In the second study, 2-pentylfuran (762 mg/kg bw) produced no significant changes in the per cent DNA in the comet tail in either the absence or presence of proteinase K, indicating the absence of DNA damage. In the third study, both 2-pentylfuran doses of 508 and 762 mg/kg bw were used. Changes in the comet tail induced by 2-pentylfuran at 508 mg/kg bw in the first study were not observed in the third study. However, in the third study, 2-pentylfuran at 762 mg/kg bw, both without and with proteinase K, provided evidence of DNA damage. The Committee concluded that the combined results of the three comet assays did not allow conclusions to be reached on the genotoxic potential of 2-pentylfuran and its mechanism. Additionally, the Committee also questioned the selection of only a single time point for the analysis of 2-pentylfuran genotoxicity based on the results of the furan'.

JECFA (2012) concluded 'the Procedure could not be applied to this group because of the unresolved toxicological concerns. Studies that could assist in the safety evaluation include investigations of the influence of the nature and position of furan ring substitutions on metabolism and covalent binding to macromolecules, demonstration of the ring opening and reactivity of the resulting products. Depending on the findings, additional genotoxicity or other studies might be needed'.

3.4.2.3. Genotoxicity studies – text taken[12] from the JECFA (JECFA, 2019)

At the 86th meeting JECFA (2019) considered 'Additional studies of genotoxicity on furan-substituted aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids and related esters, sulfides, disulfides and ethers were evaluated for the present meeting. A total of 16 *in vitro* genotoxicity studies were available for seven flavouring agents (Nos 1495, 1497, 1503, 1504, 1511, 1514 and 1520), and a total of eight *in vivo* genotoxicity studies were available for four flavouring agents (Nos 1491, 1497, 1503 and 1511). A positive result was observed for 2-furyl methyl ketone (No. 1503) in an *in vitro* SCE assay. However, an *in vitro* chromosomal aberration assay was negative and *in vivo* studies of DNA damage (comet assays) and micronucleus induction were also negative. All other *in vitro* and *in vivo* genotoxicity assays considered at the present meeting were negative.

The Committee concluded that the newly available *in vitro* and *in vivo* genotoxicity data evaluated at the present meeting allay the previous concerns of the Committee. Those concerns arose primarily from the carcinogenicity of furan itself and from some positive genotoxicity findings for four flavouring agents with short-chain alkyl substituents on the furan ring. Those four flavouring agents, namely 2-methylfuran (No. 1487), 2,5-dimethylfuran (No. 1488), 2-ethylfuran (No. 1489) and 2-butylfuran (No. 1490), are no longer supported by industry, and were not considered in this re-evaluation'.

[12] The text is taken verbatim from the indicated reference source.

3.4.3. Genotoxicity studies – text taken[13] from EFSA FGE.13Rev2 (EFSA CEF Panel, 2011c)

In the following text, which is taken from FGE.13Rev2, the FGE.13-subgrouping is maintained. Only the text related to FGE.13Rev2 subgrop Ib and Ic are reported, because these are relevant for the substances in group IV and in subgroup VI-B of FGE.67Rev3, respectively.

Since the supporting substance for subgroup Ib gives information on genotoxic properties of putative metabolites of the candidate substances in subgroup Ic, the information given for the evaluation of subgroup Ib (see below) is also relevant for subgroup Ic.

Subgroup Ib

No data are available for the one candidate substance in subgroup Ib 2-methyl-5-propionylfuran [FL-no: 13.155]. However, several studies have been carried out with a structurally related flavouring substance, 2-acetylfuran [FL-no: 13.054] (2-furyl methyl ketone).

In vitro *studies*

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

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

In vivo *studies*

2-Acetylfuran [FL-no: 13.054] was also evaluated for induction of SCE in bone marrow of female Swiss albino mice. Groups of two per dose per exposure regimen were administered compound (99% pure) at 0, 1,000, 2,000 or 3,000 mg/L via gavage either once or for 5 consecutive days. 5-Bromodeoxyuridine was injected intraperitoneally to label chromatids. The mice were sacrificed at 12, 24 or 48 h after receiving the last dose, and slides of bone marrow were prepared and processed for differential staining. A dose-related increase up to about 2-fold in SCE was observed for the 12- and 24-h groups of both the single-dose regimen and the multiple-dose regimen (Sujatha, 2007). In an earlier study by the same group (Sujatha et al., 1993) this substance was reported to cause chromosomal aberrations in mouse bone marrow at oral dose levels up to 60 mg/kg bw per day.

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

The candidate and supporting substance in this subgroup are α,β-unsaturated ketones. This structural characteristic has been considered as an additional reason for concern for genotoxic potential of these substances. However, due to structural similarity with acetophenone (i.e. the α,β-unsaturated double bond is part of an aromatic system and therefore less reactive) the concern for genotoxicity, resulting from the formation of such α,β-unsaturated ketones has been lifted.

[13] The text is extracted from the indicated reference source. Text related to substances not included in the present FGE has been removed.

Nevertheless, the experimentally obtained genotoxicity data indicate that the supporting substance may give rise to DNA damage, which may result in chromosomal aberrations rather than gene mutations. Also from Section 4 and Annex III of FGE.13Rev2 (EFSA CEF Panel, 2011c), the formation of DNA-reactive metabolites may be anticipated. In combination with this, the available genotoxicity data are sufficiently strong to raise a concern, which would preclude the evaluation of the candidate substance in subgroup Ib through the Procedure.

Subgroup Ic

No data are available on the genotoxic properties of the two candidate substances in this subgroup, 5-ethyl-5-methylfuran [FL-no: 13.125] and 2-octylfuran [FL-no: 13.162]. Data on *in vitro* genotoxicity were provided for two supporting substances, 2-methylfuran [FL-no: 13.030][10] and 2,5-dimethylfuran [FL-no: 13.029].[10] Data on *in vivo* genotoxicity were only provided for 2-methylfuran [FL-no: 13.030].[10]

Several studies were found with the supporting substances 2-methylfuran [FL-no: 13.030][10] and 2,5-dimethylfuran [FL-no: 13.029].[10] Negative results were obtained in a limited bacterial reverse gene mutation test with *S. typhimurium* (TA97 and TA100 strains only, no data on cytotoxicity, no duplicate trial (Shinohara et al., 1986)). However, a clear concentration-related positive response with limited validity (e.g. no clear data on cytotoxicity; no clear description of scoring criteria) was obtained with both substances in a chromosome aberration test in Chinese hamster ovary cells with and without metabolic activation (Stich et al., 1981). Both substances also gave a positive response in a *rec*-assay for bacterial DNA-repair (Shinohara et al., 1986), but the predictive value of this test system is considered to be limited.

For a 2-alkyl- and 2,5-dialkyl-substituted furans, formation of reactive intermediates cannot be excluded (see Section 4 and Annex III of FGE.13Rev2 (EFSA CEF Panel, 2011c)). These reactive intermediates can bind covalently to DNA, which might result in genotoxic events. In an alternative metabolic pathway, these flavouring substances may also be converted to ketones which are structurally related to the substances in subgroup Ib and for these substances a concern for genotoxicity has been identified. Therefore, owing to the anticipated metabolism of the two candidate substances in subgroup Ic into possible genotoxic metabolites a concern for genotoxicity cannot be excluded. For the two candidate substances in subgroup Ic [FL-no: 13.125 and 13.162] this concern for genotoxicity would preclude their evaluation through the Procedure.

For a summary of *in vitro*/*in vivo* genotoxicity data considered by EFSA, see Appendix D, Table D.1.

3.4.4. EFSA Considerations on genotoxicity in FGE.67Rev2[12]

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

Apart from the alkylfurans (subgroup IV, supported by subgroup Ic from FGE.13rev2), simple hydrolysis/conjugation reactions are also not possible for the substances in subgroup V-A. As ethers

[FL-no: 13.052, 13.061 and 13.123] are more resistant to hydrolysis than the corresponding esters, it may be anticipated that these ethers can also be more prone to ring oxidations and opening than the substances in subgroup Ia in FGE.13Rev2 or group III in FGE.67. Therefore, for the substances in this subgroup a concern for genotoxicity cannot be excluded. The concern is not identified for 2,3-dimethylbenzofuran [FL-no: 13.074] (subgroup V-B), because for this substance furan ring opening is considered unlikely due to the two methyl substituents at the double bond in the furan ring. The other substance in subgroup V-B [FL-no: 13.031] has been allocated to FGE.219 for consideration of genotoxic potential, because this substance is an α,β-unsaturated aldehyde. Afterwards, the CEF Panel considered that since the double bond in α-position to the carbonyl group is part of an aromatic system, the reactivity of this double bond is less than in non-aromatic α,β-unsaturated carbonyls, and for that reason the concern for genotoxicity of this candidate substance [FL-no: 13.031] has been waived and further testing is no longer required (EFSA, 2008b).

Seven of the nine substances in subgroup VI-B are α,β-unsaturated ketones [FL-no: 13.054, 13.066, 13.070, 13.083, 13.101, 13.105 and 13.163]. This structural characteristic has been considered as an additional reason for concern for genotoxic potential of these substances. However, due to structural similarity with acetophenone (i.e. the α,β-unsaturated double bond is part of an aromatic system and therefore less reactive) the concern for genotoxicity, resulting from the formation of such α,β-unsaturated ketones has been lifted (EFSA, 2008b). Nevertheless, the experimentally obtained genotoxicity data indicate that substance [FL-no: 13.054] may give rise to DNA damage, which may result in chromosomal aberrations rather than gene mutations. The formation of DNA-reactive metabolites may be anticipated (EFSA CEF Panel, 2011c). In combination with this, the available genotoxicity data are sufficiently strong to raise a concern, which would preclude the evaluation of the candidate substance in subgroup VI-B through the Procedure. Based on the concern raised by the genotoxicity data on [FL-no: 13.054] and the anticipation that keto-reduction is less favourable for biotransformation than e.g. alcohol or aldehyde oxidation and conjugation, the CEF Panel considered it also necessary to re-evaluate the two remaining alkoyl-substituted furans in subgroup VI-B [FL-no: 13.045 and 13.138]. In similarity with the other ketones in this subgroup VI-B in FGE.67Rev1 (supported by subgroup Ib in FGE.13Rev2), for these two substances also a concern for genotoxicity is identified.

No data on genotoxicity are available for the previously evaluated (FGE.13Rev2) furans with sulphur-containing ring substituents. As it is anticipated that the predominant metabolic attack for these substances will be on the sulphur atom(s), for these substances ring opening is also not considered to be a major metabolic route. Although the genotoxicity of these substances could not be properly assessed, this did not preclude the evaluation of these substances through the Procedure. The same would apply to the two sulphur-containing substances [FL-no: 13.116 and 13.190] in subgroup I in the present FGE.67Rev2.

Thus, in FGE.67Rev2, the CEF Panel concluded that 11 of the 28 substances evaluated in the FGE could be evaluated through the Procedure [FL-no: 13.006, 13.021, 13.022, 13.023, 13.024, 13.031, 13.047, 13.058, 13.074, 13.116 and 13.190]. For all 17 substances in groups IV, V-A and VI-B ([FL-no: 13.045, 13.052, 13.054, 13.059, 13.061, 13.066, 13.069, 13.070, 13.083, 13.101, 13.103, 13.105, 13.106, 13.123, 13.138, 13.148 and 13.163] a concern for genotoxicity was identified, precluding these substances to be evaluated through the Procedure.

3.4.5. Genotoxicity data evaluated by the Panel in FGE.67Rev3

Industry provided genotoxicity data for 2-pentylfuran [FL-no: 13.059] and for 2-acetylfuran [FL-no: 13.054] representative substances of subgroup IV and subgroup VI-B, respectively. The data submitted are listed in Table 4 and summarised in Appendix E.

Table 4: Genotoxicity studies evaluated in FGE.67Rev3

FL-no JECFA-no	Substance name	Data submitted
13.054 1503	2-Acetylfuran	*In vitro* micronucleus assay in human lymphocytes (Charles River, 2020a)
		In vivo gene mutation assay and micronucleus assay in transgenic mice (Covance, 2016)
13.059 1491	2-Pentylfuran	*In vitro* micronucleus assay in human lymphocytes (Charles River, 2020b)
		In vivo comet assay in liver (New York Medical College, 2012)
		In vivo combined bone marrow micronucleus assay and comet assay in liver (Covance, 2014)

FL-No: FLAVIS number; JECFA: The Joint FAO/WHO Expert Committee on Food Additives; FGE: Flavouring Group Evaluation.

The Panel noted that, except for the presence of sulfur in the ring, the substance 3-acetyl-2,5-dimethylfuran [FL-no: 13.066] is structurally identical to 3-acetyl-2,5-dimethylthiophene [FL-no: 15.024], regarding type and positions of the substituents. 3-Acetyl-2,5-dimethylthiophene [FL-no: 15.024] was evaluated as genotoxic *in vivo* in FGE.224 (EFSA CEF Panel, 2013). Therefore, the Panel requested additional data to further investigate the genotoxic potential of flavouring substance [FL-no: 13.066]. However, industry communicated that the evaluation of the substance [FL-no: 13.066] is no longer supported, therefore data were not provided (DG SANTE, 2020b).

3.4.5.1. Genotoxicity studies on 2-acetylfuran [FL-no: 13.054]

From the previous revision of this FGE, the major concerns for the genotoxicity of 2-acetylfuran are related to chromosomal damage. The Panel noted the high concentration (13 mg/mL corresponding to more than 100 mmol/L) used in the positive *in vitro* chromosomal aberration study by Stich et al., 1981. In the *in vivo* study (Sujatha et al., 1993), the increase in the frequency of structural chromosomal aberrations was considered weak by the authors and no positive control was included, as reported by JECFA. Despite the shortcomings of these two studies, and also the limited predictivity of SCE results, reported by Sujatha (2007), they raised sufficient concern to investigate further the possible genotoxicity of this substance including micronucleus assays.

3.4.5.1.1. 2-Acetylfuran [FL-no: 13.054] – in vitro *micronucleus test in human lymphocytes*

Human peripheral blood lymphocytes from healthy donors were treated with 2-acetylfuran [FL-no: 13.054] (purity > 97%) in a concentration range finding assay at concentrations ranging from 2.17 to 1,111 μg/mL. The highest concentration tested is approximately 10 mmol/L, as recommended by OECD test guideline (TG) 487 (OECD, 2016). The *in vitro* micronucleus assay was carried out according to OECD TG 487 (OECD, 2016) and GLP principles. The cytokinesis block micronucleus assay protocol was applied (Charles River, 2020a).

Based on the concentration range-finding results, duplicate cultures of lymphocytes were treated with 2-acetylfuran at concentrations ranging from 34.7 to 1,111 μg/mL in three different testing conditions: 4 h treatment in the presence or in the absence of rat liver S9 metabolic activation and 24 h treatment in the absence of metabolic activation.

The cytotoxicity observed at the highest concentration tested 1111 μg/mL was between 2% and 7% in the three testing conditions. No precipitation of test material was observed at any concentration tested. Therefore, 278, 556 and 1,111 μg/mL were chosen for analysis of micronuclei (MN) (in 2,000 binucleated cells per concentration tested).

2-Acetylfuran did not increase the MN cell frequency compared to vehicle (DMSO) controls in any of the testing conditions.

The Panel concluded that 2-acetylfuran did not increase MN cell frequency under the testing conditions of this study.

Summary of the results are reported in Appendix E, Table E.1.

3.4.5.1.2. 2-Acetylfuran [FL-no: 13.054] – in vivo *gene mutation and micronucleus assays in Muta™ Mice*

2-Acetylfuran (purity 97%) was tested for its ability to induce gene mutations in the *lacZ* transgene within liver and duodenum from treated male Muta™ Mice (CD_2-lacZ80/HazfBR strain), according to OECD TG 488 (OECD, 2013). In addition, an assessment of micronuclei in peripheral blood

reticulocytes from the same animals was included, according to OECD TG 474, albeit without a concurrent positive control (Covance, 2016). The study was carried out according to GLP principles.

2-Acetylfuran was tested in a range-finding experiment at doses of 30, 60 and 90 mg/kg bw per day, for 7 days. Post-dose observations in male animals treated at 60 mg/kg bw per day were limited to semi-closed eyes in two males. In female animals, post-dose observations included hunched posture, reduced activity, semi-closed eyes and raised fur/hair. Due to the poor condition, female mice were terminated early.

Dosing was terminated early in the group of male animals dosed at 90 mg/kg bw per day, due to the severity of observations seen in two animals (including cold to the touch, hunched, prostrate, subdued/sluggish, semi-closed eyes, raised fur and noisy/laboured breathing).

In the group of female animals dosed at 30 mg/kg bw per day, no post-dose observations or clinical signs of toxicity were seen.

The post-dose observations suggested that doses of 90 mg/kg bw per day in male mice and 60 mg/kg bw per day in female mice exceeded the maximum tolerated dose (MTD). The study authors considered that doses of 60 mg/kg bw per day or 30 mg/kg bw per day in male or female mice, respectively, represented the respective MTDs. Although differences in toxicity were observed between male and female animals, these were not considered substantial and therefore male mice only were tested in the main experiment.

Groups of six male mice were administered 2-acetylfuran, by oral gavage, at doses of either 0, 15, 30 or 60 mg/kg bw per day for 28 days. No positive control for mutation frequency was included in the dosing regimen of this study, but positive control DNA (from animals dosed with ethylnitrosourea) from another study of the same laboratory under the same conditions was used for analysis of mutations. The 28-day dosing period was followed by three days expression period before necropsy at day 31. On day 4 and day 29 blood was also collected for the micronucleus assay.

After sacrifice on day 31, animals were macroscopically examined and the liver and duodenum collected for the *lacz* mutation frequency assay. Satellite groups of animals were included for toxicokinetics: 3 animals dosed with corn oil (vehicle) and nine animals dosed with 2-acetylfuran 60 mg/kg bw per day. Blood from these animals was collected at different time points after the last dosing on day 28, but 2 animals were terminated earlier due to poor body condition. Results from the blood analysis are not reported in the study report.

Micronucleus assay

The blood samples were analysed by high speed flow cytometry and at least 20,000 reticulocytes from each sample were scored for MN.

There was no statistically significant increase of MN frequency in peripheral blood reticulocytes compared to the vehicle controls on both days 4 and 29 of the study.

The percentage of reticulocytes in the total erythrocyte population was not affected by the treatment with 2-acetylfuran, providing no evidence of exposure of bone marrow. No data on blood analyses were provided to allow an assessment of systemic exposure. Although there was evidence of clinical toxicity in the range finding study, when male animals were dosed at 90 mg/kg bw per day (cold to the touch, hunched, prostrate, subdued, semi-closed eyes and laboured breathing), this was not considered by the Panel as sufficient evidence of bone marrow exposure.

A further limitation in the analysis of MN in this study is that no positive control was included.

Overall, the Panel considered the results of the *in vivo* MN assay in peripheral blood as inconclusive.

Mutant frequency in the liver and duodenum

DNA was recovered from duodenum and liver tissues from all four treatment groups. In addition, frozen positive control DNA from animals treated with ethylnitrosourea from another study from the same laboratory was analysed. The extracted DNA was packaged into phage heads and transfected in suspensions of *E. coli*, all according to OECD TG 488. The transfected *E. coli* were plated, incubated and scored for plaques.

No statistically significant increases in mutant frequency were observed either in the liver or duodenum of treated Muta™ mice. A trend test was negative for the liver results, but a significant linear trend was observed in the dose response in the duodenum. Nevertheless, there was no statistically significant difference between the mutation frequency at any one dose level compared to that of the concurrent controls. For the liver samples, the values were within the laboratory's historical control data. No adequate historical control data were submitted for duodenum. Considering that for the liver no increase in mutant frequency was observed and that the concern for genotoxicity of 2-acetylfuran is

related to its metabolism, the Panel considered that the equivocal result observed in duodenum (positive trend test and lack of statistically significant increase in mutation frequency) is of no biological relevance given the fact that the liver is metabolically more active than duodenum.

Overall, the Panel concluded that 2-acetylfuran did not induce gene mutations in liver or duodenum in transgenic Muta™ mice.

Summary of the results are reported in Appendix E, Table E.2.

3.4.5.2. Genotoxicity studies on 2-pentylfuran [FL-no: 13.059]

In previous versions of this FGE (EFSA CEF Panel, 2010, 2011a, 2015a), concern for possible genotoxicity of alkyl-furans was raised. The alkyl-furans selected as representative substances for genotoxicity testing were no longer supported by industry and alternatively 2-pentylfuran was selected as representative for this group. However, no prior data on genotoxicity for this substance were available.

3.4.5.2.1. 2-Pentylfuran [FL-no: 13.059] – in vitro *micronucleus test in human lymphocytes*

Human peripheral blood lymphocytes from healthy donors were treated with 2-pentylfuran [FL-no: 13.059] (purity 99.6%) in a concentration range finding assay at concentrations ranging from 2.70 to 1,384 μg/mL. The highest concentration tested is approximately 10 mM, the highest concentration recommended by OECD TG 487 (OECD, 2016). The *in vitro* micronucleus assay was carried out according to OECD TG 487 (OECD, 2016) and GLP principles. The cytokinesis block micronucleus assay protocol was applied (Charles River, 2020b).

Based on the concentration range-finding results, duplicate cultures of lymphocytes were treated with 2-pentylfuran at concentrations ranging from 22 to 131 μg/mL in three different testing conditions: 4 h treatment in the presence or in the absence of rat liver S9 metabolic activation and 24 h treatment in the absence of metabolic activation. No precipitation of test material was observed at any concentration tested.

Cytotoxicity was noted at the end of treatment at concentrations above 86.3 μg/mL in the 4h treatment without metabolic activation, above 60.4 μg/mL in the 4h treatment with metabolic activation and above 69.9 μg/mL in the 24h treatment without metabolic activation.

The concentrations selected for micronucleus analysis were:

- 54.5 μg/mL, 70.4 μg/mL and 86.3 μg/mL for the 4-h treatment without metabolic activation;
- 60.4 μg/mL, 77.9 μg/mL, and 86.3 μg/mL for the 4-h treatment with metabolic activation;
- 56.9 μg/mL, 73.6 μg/mL, and 81.4 μg/mL for the 24-h treatment without metabolic activation.

The highest concentration chosen for the analysis of MN were from cultures with cytotoxicity of approximately 50–60%.

2-Pentylfuran did not increase the MN cell frequency compared to vehicle (DMSO) controls in any of the testing conditions.

The Panel concluded that 2-pentylfuran did not increase MN cell frequency under the testing conditions of this study.

Summary of results are reported in Appendix E, Table E.1.

3.4.5.2.2. 2-Pentylfuran – in vivo *Comet assay in mice*

The aim of the study was to compare the pattern of induced DNA damage in mouse liver by 2-pentylfuran to that of furan (New York Medical College, 2012). The Comet assay was performed before the OECD TG for comet assay was established, however, it was conducted according to the methodology recommended at that time (Tice et al., 2000; Olive and Banath, 2006).

The rationale for the study was based on the evidence of a specific mechanism for genotoxicity of furan revealed by the Comet assay and described in a published paper (Cordelli et al., 2010), cited in the unpublished study report. The paper by Cordelli et al. (2010) describes an increase of DNA damage, as single strand breaks or alkali-labile sites, and of DNA cross-links induced after 3 h of a single administration of furan at the dose of 250 mg/kg bw.

A pilot experiment was carried out with a single oral dose of furan of 250 mg/kg bw and DNA damage was measured at 3, 6 and 24 h. Sampling at three hours after treatment was found to be optimal for furan by the authors and was used for all subsequent studies. This was the only time point used for the assessment of genotoxicity of 2-pentylfuran.

In the main experiment 250 mg/kg bw of furan or an equimolar dose of 2-pentylfuran (purity ≥ 97%; 508 mg/kg bw) were dosed by gavage to male B6C3F1 mice. Liver cell Comet tails were evaluated in the absence and presence of proteinase K (PK), used to digest DNA-protein cross-links.

Furan produced a decrease in % tail DNA of liver cells in the absence of PK, but the authors noted no significant increase in the presence of PK. An increase of % tail DNA indicative of DNA single strand breaks was reported in the liver cells of mice treated with 2-pentylfuran. In the presence of PK, however, %tail DNA was not greater than vehicle control raising an uncertainty in the conclusion of the positive result in the absence of PK.

In the main second experiment, dosing was repeated at a lower dose of furan, 100 mg/kg bw, and a higher dose of 2-pentylfuran, 762 mg/kg bw. The results showed that furan at this dose level did not produce a statistically different response from the corresponding vehicle control either without or with PK. 2-Pentylfuran also produced no significant changes in the % tail DNA either in the absence or presence of PK.

In the main third experiment, furan was applied at the previously positive dose of 250 mg/kg bw, and 2-pentylfuran at both 508 and 762 mg/kg bw, with and without PK. None of the values for either furan or 2-pentylfuran was significantly different from corresponding controls irrespective of the absence or presence of PK via automated slide counting. All slides were then re-analysed three additional times to reduce the variability of results. This additional analysis showed that furan reduced % tail DNA in the absence of PK as shown in the main experiment 1, but induced a statistically significant increase in the presence of PK. 2-Pentylfuran tested at the highest dose, induced a statistically significant reduction of % tail DNA both in the absence and in the presence of PK. The authors noted a diffuse pattern of the measured DNA and smaller nuclear heads, which make quantitation of the DNA from these cells more difficult and proposed that the reduction of % tail DNA may reflect toxicity.

The authors noted that the study results were inconsistent and difficult to interpret.

The Panel considered that there were a number of shortcomings in the study. In particular, a positive control agent producing single strand breaks as shown by an increase in % tail DNA was not used. Furthermore, the responses seen were not consistent among experiments. Some responses were considered likely to be related to cell toxicity but this was not measured. Finally, the Comet protocol applied using the proteinase K is only useful when assessing DNA-protein cross links specifically. Moreover, DNA damage in vehicle controls, expressed as % tail DNA were high, ranging from 27% and 36% in the different experiments.

In conclusion, the Panel considered that the study was not sufficiently reliable to conclude on the genotoxicity of 2-pentylfuran in mice.

3.4.5.2.3. 2-Pentylfuran – in vivo *combined comet and micronucleus assay in rats*

2-Pentylfuran [FL-no: 13.059] (purity 99.2%) was tested *in vivo* using the bone marrow micronucleus assay combined with the comet assay in liver of rats (Covance, 2014). The micronucleus assay was conducted in accordance with GLP and OECD TG 474 (OECD, 1997). The comet assay was conducted before the publication of the first relevant OECD test guideline (OECD TG 489, 2014), but it was based on the guidance provided by the Comet Workshop (Tice et al., 2000; Hartmann et al., 2003), International Workshops on Genotoxicity Testing (Burlinson et al., 2007), the international validation of the *in vivo* comet assay by the JaCVAM and literature (Hartmann et al., 2004; Smith et al., 2008) available at that time. However, measurements of achieved concentrations, stability and homogeneity were not included in the study. The study complied with GLP guideline.

Based on a range-finding experiment, a dose of 170 mg/kg bw was taken as the maximum tolerated dose in rats.

In the main experiment, groups of six male rats were administered doses of 42.5, 85 and 170 mg/kg bw of 2-pentylfuran by oral gavage three times at 0, 24 and 45 h. Rats were sacrificed and sampled at 48 h post the initial dose. No clinical signs of toxicity were observed in any animals in the study. A slight dose related decrease in percentage bodyweight was observed in all dosed groups when compared with the vehicle control group. Liver samples were processed for the Comet assay. Blood samples were collected for bioanalysis, but no results were given in the study report to inform on systemic availability.

A dose related increase in blood aspartate transaminase and alanine transaminase was observed in animals from all groups treated with 2-pentylfuran. Alkaline phosphatase activity and total bilirubin levels were increased in the highest dose group. In the same animals a decrease in urea was observed.

The necropsy did not show any macroscopic findings related to the administration of 2-pentylfuran. The histopathological analysis of the liver showed a centrilobular hepatocyte eosinophilia in animals from all groups given 2-pentylfuran and a centrilobular degeneration/necrosis in animals treated with 2-pentylfuran at 85 and 170 mg/kg bw.

Micronucleus assay

2-Pentylfuran did not increase mean frequencies of micronucleated polychromatic erythrocytes in bone marrow smears of any treated groups. However, 2-pentylfuran did not reduce the percentage of polychromatic erythrocytes among total erythrocytes, therefore, there was no indication of bone marrow toxicity. The clinical signs of toxicity observed are not sufficient to demonstrate the systemic exposure to the tested substance. Moreover, no other data are provided to demonstrate systemic availability at the doses tested.

Therefore, the Panel concluded that results from this *in vivo* micronucleus test are inconclusive.

Comet assay

Despite some increases of up to 2.3-fold in the mean tail intensity of the treated groups compared to values in the control group, no statistically significant increase in mean tail intensity and no dose-related response were observed in animals treated with 2-pentylfuran.

There was no dose-related increase in % clouds in liver cells following treatment with 2-pentylfuran, thus demonstrating that treatment did not cause excessive DNA damage that could have interfered with Comet analysis.

Based on the available data from this study, the Panel concluded that 2-pentylfuran did not induce DNA strand breaks in the liver when analysed in the Comet assay at dose levels that were associated with liver toxicity.

Summary of the results are reported in Appendix E, Table E.2.

3.4.5.3. Discussion on genotoxicity data

The CEF Panel considered that the 2-alkyl-substituted furans in subgroup IV (FGE. 67) and in subgroup Ic (FGE.13) can share similar metabolic pathways with the substances in subgroup VI-B (FGE.67) and in subgroup Ib (FGE.13). For 2-alkyl-substituted furans (subgroup IV in FGE.67 and subgroup Ic in FGE.13), formation of reactive metabolites cannot be excluded. These reactive intermediates can bind covalently to DNA, which might result in genotoxic events.

In the present revision of FGE.67 (FGE.67Rev3), additional genotoxicity data are evaluated for the representative substances 2-acetylfuran [FL-no: 13.054] (subgroup VI-B) and 2-pentylfuran [FL-no: 13.059] (subgroup IV).

3.4.5.3.1. 2-Acetylfuran [FL-no: 13.054]

From studies considered in previous versions of this FGE, a concern for genotoxicity was raised for 2-acetylfuran (see Section 3.4.4). The studies showed limitations and the CEF Panel decided that additional information was necessary for its evaluation. Following the request of the Panel, an *in vivo* combined gene mutation and micronucleus assay in Muta™ mice was submitted. 2-Acetylfuran did not increase the mutation frequency in liver and duodenum, indicating that the testing substance does not induce gene mutations. In the same study, 2-acetylfuran did not increase the percentage of micronucleated cells in peripheral blood. However, the study data did not sufficiently demonstrate target tissue exposure. The clinical signs of toxicity observed in this study and in a 28/90-day repeated dose toxicity study in rats (Bio-Research Laboratories, 1985) were not sufficient to demonstrate the systemic exposure to the tested substance in mice. The use of toxicity data from a different species as evidence of systemic exposure is not recommended by the EFSA Scientific Committee (EFSA Scientific Committee, 2017a).

Therefore, the Panel requested to test 2-acetylfuran in an *in vitro* micronucleus assay and this study showed that 2-acetylfuran did not induce chromosomal damage and is not aneugenic.

Based on the available data, the Panel concluded that for 2-acetylfuran [FL-no: 13.054] the concern for genotoxicity is ruled out.

3.4.5.3.2. 2-Pentylfuran [FL-no: 13.059]

For 2-pentylfuran [FL-no: 13.059], industry submitted results of an *in vivo* comet assay in mice (New York Medical College, 2012). The Panel considered that this study was not sufficiently reliable to conclude on the genotoxicity of 2-pentylfuran in mice.

Subsequently, 2-pentylfuran was tested *in vivo* in a combined comet assay and micronucleus assay in rats. Results from the comet assay in liver were negative, suggesting that 2-pentylfuran did not induce gene mutations or clastogenic effects. In the same study, 2-pentylfuran did not increase MN cell frequency in bone marrow, but target tissue exposure was not substantiated from the available data.

By request of the Panel, industry also submitted an *in vitro* micronucleus assay for 2-pentylfuran [FL-no: 13.059] in human peripheral blood lymphocytes with FISH analysis. In this *in vitro* test, 2-pentylfuran did not induce chromosomal damage and was not aneugenic.

Based on the available data, the Panel concluded that for 2-pentylfuran [FL-no: 13.059] the concern for genotoxicity is ruled out.

3.4.5.4. Conclusion on genotoxicity

Based on the additional genotoxicity data, the Panel concluded that the concern for genotoxicity is ruled out for the two representative substances 2-acetylfuran [FL-no: 13.054] and 2-pentylfuran [FL-no: 13.059]. Therefore, the concern for genotoxicity is ruled out also for the substances in subgroup IV (2-heptylfuran [FL-no: 13.069], 2-decylfuran [FL-no: 13.106], 3-methyl-2(3-methylbut-2-enyl)furan [FL-no: 13.148]) and in subgroup VI-B of FGE.67 (1-(2-furyl)-propan-2-one [FL-no: 13.045], 2-hexanoylfuran [FL-no: 13.070], 2-acetyl-5-methylfuran [FL-no: 13.083], 2-acetyl-3,5-dimethylfuran [FL-no: 13.101], 2-butyrylfuran [FL-no: 13.105], 1-(2-furyl)butan-3-one [FL-no: 13.138], and 2-pentanoylfuran [FL-no: 13.163]). All these 12 substances can be evaluated through the Procedure.

Based on these experimental data on 2-acetylfuran [FL-no: 13.054] and 2-pentylfuran [FL-no: 13.059] the concern for genotoxicity can be ruled out also for the structurally related substances in FGE.13Rev2 [FL-no: 13.125, 13.162].

3.4.6. Toxicity data evaluated by the Panel in FGE.67Rev3

Industry provided toxicity data for 2-pentylfuran [FL-no: 13.059] and for 2-acetyfuran [FL-no: 13.054] representative substances of subgroup IV and subgroup VI-B, respectively. The data submitted are listed in Table 5 and summarised in Appendix F.

Table 5: Toxicity studies evaluated in FGE.67Rev3

FL-no JECFA-no	Substance name	Data submitted
13.054 1503	2-Acetylfuran	Combined 28-day and 90-day toxicity study in rats (Bio-Research Laboratories, 1985)
13.059 1491	2-Pentylfuran	Summary of an acute toxicity study (Gulf South Research Institute, 1971a)
		90-day toxicity study in rats (Gulf South Research Institute, 1971b)
		14-day toxicity/palatability study in rats (Product Safety Labs, 2016)
		90-day study in rats (Product Safety Labs, 2017)

FL-No: FLAVIS number; JECFA: The Joint FAO/WHO Expert Committee on Food Additives; FGE: Flavouring Group Evaluation.

3.4.6.1. 2-Acetylfuran – Repeated-dose toxicity studies

2-Acetylfuran was tested in Sprague–Dawley CD rats in a combined 28-day and 90-day toxicity study (Bio-Research Laboratories, 1985). The study was conducted in compliance with the United States Food and Drug Administration's Good Laboratory Practice Regulations.

Groups of male and female Sprague–Dawley rats were exposed through the diet to 0, 5, 25 or 100 mg 2-acetylfuran/kg bw per day (nominal dosing). The study was performed under GLP control. The study animals were allocated to two lots: animals of lot one were sacrificed after 28 days, and those of lot two after 90 days of exposure. Thirty two animals per sex were administered control feed and the same numbers of males and females received feed that provided 5 mg/kg bw per day. Twelve animals per sex were exposed to 25 mg/kg bw per day and 10 animals per sex were exposed to 100 mg/kg bw per day. After 28 days, half of the animals in the control and 5 mg/kg groups and all animals in the 100 mg/kg group were sacrificed. The remaining animals (16 per sex in control and low dose groups; 12 per sex in the 25 mg/kg bw group) were sacrificed after 90 days of exposure.

Animal feeds were prepared weekly and concentrations in feed were adjusted, based on body weights and feed consumption to maintain the exposure at the same daily level throughout the experiment. Feed analyses demonstrated stability and homogeneity of the substance in the feed. Average levels of exposure over the course of the experiment were 91, 90 or 77% of target values for

males and 108, 108 and 82% of target values for females. The nominal dose levels of 5, 25 and 100 mg/kg bw per day are equal to 4.6, 22.6 or 77 mg/kg bw per day for males and 5.4, 27 or 82 mg/kg bw per day for females.

Animals were inspected daily for clinical signs and twice daily for mortality. Body weights and feed intake were monitored once per week during the study. All animals were examined at schedule for haematology, blood biochemistry and urinalysis (all conventional parameters, except blood clotting parameters, reticulocytes and thrombocytes) and organ weights of adrenals, heart, kidney, liver, ovaries, testes, epididymis, thyroid and parathyroids were determined. Samples of 28 tissues plus additional abnormalities were collected.

One female animal in the control group died in week 7 of the study. No other deaths were recorded. There were no clinical observations that were attributable to treatment with the test substance.

Results after 28 days of exposure

In the animals of the 100 mg/kg group, a statistically significant decrease in body weight was observed in males and females at week 3 (–15 or –35%, respectively). In these animals also a decreased feed consumption (–15% in the males and –17% in the females) was observed. At week 4, the animals were sacrificed together with half of the 5 mg/kg bw and control group animals. In the 100 mg/kg male animals, glucose and alkaline phosphatase in serum were significantly decreased and blood urea nitrogen (BUN) was significantly increased. For glucose and alkaline phosphatase in the females a similar change was observed as in the males, but statistical significance was not reached. In females, BUN was not affected. A significant increase in relative liver weight was observed in males and females dosed at 100 mg/kg per day. In males also an increase of relative gonad (testis + epididymis) weight was observed. Other parameters in haematology, urinalysis, clinical chemistry or organ weights and histopathology were not affected. No adverse effects were observed in the animals from the 5 mg/kg group.

Results after 90 days of exposure

At study termination, the body weights of the males and females in the 25 mg/kg bw dose group was statistically significantly lower (by 10%) than those of the control animals. The decreases in body weight were rather limited and connected to a reduced feed consumption (–4% in the males and –15% in the females). Since no findings attributable to treatment were noted in the clinical, haematological and histopathological examinations or in the clinical chemistry and urinalysis parameters, the Panel identified a NOAEL of 25 mg/kg bw per day (nominal value) from this study, which is the highest dose tested up to 90-day of exposure. This NOAEL is equal to 22.6 mg/kg bw per day in males and 27 mg/kg bw per day in females.

3.4.6.2. 2-Pentylfuran – acute oral toxicity study in mice

2-Pentylfuran was tested in a single dose oral acute toxicity study in Swiss Webster mice (Gulf South Research Institute, 1971a, only data summary available). 2-Pentylfuran in corn oil solution was administered via intubation to male and female mice (5 animals for each group) at these doses: 0, 800, 1,000, 1,260, 1,600, 2,000 mg/kg. Dose-related effects included depression and lethargy, tachypnoea. Surviving animals generally appeared normal within 2 to 4 days after intubation. The LD_{50} derived was 1,185 mg/kg for males and 1,220 mg/kg for females.

3.4.6.3. 2-Pentylfuran – 90-day toxicity study in rats (Gulf South Research Institute, 1971b)

2-Pentylfuran was tested in Sprague–Dawley rats (Gulf South Research Institute, 1971b), administered in the diet at 25.6 mg/kg per day.

No clinical signs of toxicity were observed and no significant changes in body weight and feed consumption were observed, nor any significant changes in haematological and biochemical parameters. After 13 weeks, serum alkaline phosphatase levels were higher than control, but were within the historical control range of the laboratory. Average liver weights of males and liver and kidney weights of female were statistically significant higher than control. The study authors considered that this result was due to a low average organ weight in control and not to a real enlargement of tissues in treated animals. Histological lesions were observed in lungs, which were associated to a virus infection. The histological analysis showed no significant difference between treated and control animals.

3.4.6.4. 2-Pentylfuran – 14-day range-finding study in rats

Sprague–Dawley rats (5 per sex per group) were exposed to 2-pentylfuran in a 14-day dietary range-finding and palatability study (Product Safety Labs, 2016). Dietary concentrations (0, 1,200, 3,000 and 6,000 mg/kg feed) aimed at exposure levels of 0, 100, 250 and 500 mg/kg bw per day. The substance was homogeneously distributed in the feed (Product Safety Labs, 2016). Exposure levels calculated from feed consumption, body weights and nominal dietary 2-pentylfuran concentration were 0, 106, 258 and 477 mg/kg bw per day for males and 112, 263 and 491 mg/kg bw per day for females. However, upon inclusion into the feed, the concentration of 2-pentylfuran declined down to 59% or 63% on day 0, down to 34% or 54% on day 4, down to 35% or 34% on day 7 and down to 33% or 29% of target concentrations for the low-dose and the high-dose group, respectively. Upon request from the Panel the applicant demonstrated that the neat test substance was stable under the conditions of storage over the course of this study and that the loss of the substance from the feed was caused by volatilisation. Since the feed was replaced every 7 days, the actual exposures, taking into account the loss of 2-pentylfuran during feed mixing and the reduction in feed concentration over a 7-days period, were 65% of the estimated exposure based on nominal concentrations i.e. 0, 70, 168, and 310 mg/kg bw per day for males and 0, 73, 171 and 319 mg/kg bw per day for females. The only adverse effects reported were decreased fecal volume in 2 males and 4 females in the high dose group and one the mid-dose group and transient decreased body weight and body weight gain in high dose animals. These effects were attributed to reduced feed consumption. In the mid-dose males and females these effects were also observed in the beginning of the study, but there was no impact on the overall body weight. According to the study authors, decreased feed consumption was attributed to the lack of palatability of 2-pentylfuran. Macroscopic examination at scheduled necropsy revealed no further peculiarities. The study authors concluded that animals are expected to tolerate nominal dietary concentrations up to 3,000 ppm of 2-pentylfuran, in a study of longer duration, which would correspond to an actual exposure of approximately 170 mg/kg bw per day, taking into account the loss in the feed.

3.4.6.5. 2-Pentylfuran – 90-day oral gavage study in rats (Product Safety Labs, 2017)

Based on the observations in the 14-day study, 2-pentylfuran (95.7% purity) was given by oral gavage to individually housed male and female CRL Sprague–Dawley® CD® IGS rats (10/sex/dose group) for 90 days (Product Safety Labs, 2017). The study was compliant to OECD TG 408 and GLP principles. 2-Pentylfuran was formulated in corn oil and administered at dose levels of 0, 30, 100 and 150 mg/kg bw per day. The test substance (neat) was stable throughout the length of the study and homogeneity of test substance formulations was demonstrated. The test formulations met the target concentrations for the mid- and high-dose groups, but for the low-dose group the concentrations of the formulations were 125%, 53% and 96% of the target concentrations at beginning, mid-term or end of the study, respectively.

Ophthalmoscopy was carried out on all animals at the start and at the end of the study. Animals were inspected for clinical signs, daily during handling and weekly in more detail. Animals were weighed during acclimation, on study day 1 and weekly thereafter and prior to sacrifice. Feed consumption and efficiency were measured and calculated weekly. Samples for blood biochemistry, haematology and urinalysis were collected at the end of the study. Full necropsy and collection of tissues and organ weights was performed on all animals weighed and tissues preserved for histopathological examination in accordance with OECD TG 408 (1998). A Functional Observational Battery examination for neurological damage was not included, but this is only optional in OECD TG 408.

All animals survived until the end of the study. Ophthalmological examination and gross observation at necropsy revealed no abnormalities in any of the animals that could be attributed to the treatment.

In-life daily clinical observations showed a dose-related increased incidence and severity of hypersalivation and increases in staining of fur in various regions of the head and legs and of the anogenital region in males and females of the mid- and high-dose groups. Contrary to this, for the weekly detailed clinical observations, these conditions were only reported incidentally and without clear dose relationship.

Throughout the study, all dose groups (males and females) showed a slight but dose-related reduced mean body weight as compared to the controls, but statistical significance was only reached for the high-dose male group in the last 2 weeks of the study. In the males the group mean terminal body weights were reduced by 4%, 6% and 14% (low-mid-high). Feed consumption was slightly reduced in the high-dose male group, but statistical significance was not reached (over the entire

study 7% less feed consumption than the control animals). Feed consumption in the female dose groups was not affected. In the males, feed efficiency was statistically significantly reduced in mid- and high-dose (dose-related) with 20% reduction in the high-dose group. A reduced feed efficiency was also observed in the females, but the data were more variable in time and less clearly dose-related than for the males. At the end of the study statistical significance was only reached for the mid-dose females (–20%).

Changes in haematology parameters included decreases in haematocrit ($p < 0.05$; high-dose males; mid- and high-dose females), haemoglobin, mean corpuscular volume, mean corpuscular haemoglobin, erythrocyte haemoglobin concentration and increases in platelet counts ($p < 0.05$; mid- and high-dose males and females), red cell distribution width ($p < 0.05$; high-dose females only) and reticulocyte count ($p < 0.05$ high-dose males but not significant in females).

Increased plasma sorbitol dehydrogenase (SDH) and total bilirubin levels were observed in mid- and high-dose males, and in low- mid- and high-dose males and mid- and high-dose females, respectively. In mid- and high dose males, also the prothrombin time ($p < 0.05$) was statistically significantly increased by up to 10% in the high-dose group. Further changes in clinical chemistry parameters for which statistical significance ($p < 0.05$) was reached included decreased glucose and triglycerides (mid- and high-dose males), increases in total protein mid-dose females and high-dose males and females), albumin (mid- and high-dose males and females), globulin (high-dose females), phosphorous levels (high dose males), calcium (high dose females) and decreased chloride levels (high dose males).

Changes in urinalysis parameters included increases in urinary volume (high-dose males; all dose groups in females) and urobilinogen (mid- and high-dose males and females) accompanied by decreases in urine specific gravity (mid- and high-dose males and all dose groups in females) and urine protein (high dose males and low-dose females). These observations were also seen in the female low dose group, but were within the laboratory's historical control ranges, and were not correlated with serum chemistry or histopathology findings.

Histopathologic examination of the spleen revealed an increase in pale-brown pigment-laden macrophages in the spleen of the mid (6/10 score minimal) and high dose males (1/10 score minimal; 6/10 score mild and 3/10 score moderate). In the control and low-dose animals this lesion was not observed. In all females in all groups (including the controls) pigment-laden macrophages in the spleen were observed, but the severity of the scores increased with the dose (from 7 score minimal and 3 score mild in the control group to 1 score minimal, 3 score mild and 6 score moderate in the high-dose group). In the livers minimal centrilobular hepatocellular hypertrophy was observed in all males in all exposure groups, in 8/10 females from the low-dose group and in all females in the mid- and high-dose groups. In addition, in two males and one female of the high-dose groups increased hepatic cell apoptosis (score minimal) was observed.

In the mid- and high-dose groups, increases in mean absolute and relative (to body and brain weight) liver weights were observed in both males and females. Increased mean organ-to-body weight ratio for brain and testes of high dose males and for kidney (male and female) and spleen (males only) of mid and high dose rats were also observed.

Discussion and conclusion of the study results:

The main effects of 2-pentylfuran in the rat are haematological effects, in particular on the red blood cells and, related to this, effects on reticulocyte counts and on spleen. The test substance appears to increase the turn-over of erythrocytes. It is not clear from the data whether this is caused by a direct haemolytic activity or by subcytotoxic damage to the erythrocytes resulting in a shortening of their life-span. The increase in splenic macrophage pigmentation and the increase in platelet and reticulocyte counts are also linked to this, since these changes may indicate increased erythrocyte scavenging in the spleen and increased production of new erythrocytes. Furthermore, the haematological changes were accompanied by increased plasma total bilirubin levels, which may be linked to increased haemoglobin break-down following erythrocytes destruction. Despite the effects on the red blood cells, no indications of changes in bone marrow histology or extramedullary haematopoiesis were found.

The main effect in the liver was centrilobular hepatocellular hypertrophy, which was accompanied by an increase in relative and absolute liver weights. Some indications for liver toxicity was obtained from the increase in plasma SDH, but other clinical chemistry parameters and histopathology did not support this. The changes in plasma albumin and total plasma protein, which are strongly correlated

do not reflect an adverse effect. The decreased levels of plasma glucose and triglycerides are probably related to reduced feed consumption and therefore are of limited toxicological relevance.

Increases in mean organ-to-body weight ratio were found for brain, testes, kidney and spleen. Since the weight changes in brain, testes and kidney were not accompanied by histopathological changes or by increases in absolute organ weights or organ weights-relative-to-brain weights, they were considered of no toxicological relevance.

The changes in urinalysis were mainly due to increased urinary volume, except for the increase in urobilinogen content. This substance is a break-down product of bilirubin, which is formed in the GI-tract and excreted in the urine after re-absorption. An increased turn-over of haemoglobin, which results in increased production of bilirubin may also result in a higher renal excretion of urobilinogen.

Calculation of the Point of Departure for the assessment

The results for all haematological, clinical chemistry and urinalysis parameters were subjected to dose-response modelling, using the EFSA PROAST web-tool,[14] in line with the EFSA SC guidance document (EFSA Scientific Committee, 2017b). Instead of using the default value of 5% for the BMR, the Panel employed endpoint-specific benchmark responses (BMRs), based on the theory developed by Slob (2017). This theory takes better account of the natural variability in the measured parameters, than the default BMRs. This results in biologically more plausible BMRs and subsequently more plausible BMDLs. These endpoint-specific BMRs were calculated with the RIVM PROAST webtool.[15] The data on liver and spleen histopathology were not submitted to dose–response analysis because in the EFSA tool for ordinal data this is not possible.

Results of the dose–response modelling

For the haematological data, no dose-related trend was observed for the erythrocyte counts and the haemoglobin concentrations and for prothrombin time (PTT)[16] in the females. For the other parameters dose related trends were identified and BMDLs could be estimated (see Appendix G, Table G.1). The BMDL for mean corpuscular volume (MCV) was the lowest BMDL identified among this set of parameters (14 and 25.4 mg/kg bw per day for males and females, respectively) based on a BMR of 2.6%.

Among the clinical chemistry and urinalysis no dose-related trend could be identified for the following parameters: aspartate aminotransferase (AST), alkaline phosphatase (ALKP), BUN, creatinine (CREA), cholesterol (CHOL), potassium (K), urinary pH and globulin (GLOB) in males. Extremely wide BMD confidence intervals or very deviant results for the four Dose-Response models evaluated in the dose-response modelling process were identified for alanine aminotransferase (ALT), triglycerides (TRIG) in males, glucose (GLUC) in females, and urinary volume (UVOL), urinary specific gravity (SG) and urinary total protein (UMTP) in males and females resulting from insufficient information in the data. These endpoints are not suitable for derivation of a reference point for the evaluation of 2-pentylfuran. The dose-response curves for urobilinogen (URO) showed an unexpected levelling off at the highest dose and a wide BMD confidence interval. In addition, no correction was applied for urinary volume. Also, the increased excretion of URO in the urine is related to the increase in plasma total bilirubin (BILI). For these reasons, for the clinical chemistry data the preference is given to the more reliable BMDL of plasma total bilirubin, an indicator for red blood cell damage, noting the absence of evidence for liver damage. This BMDL amounted to 8.51 or 18.3 mg/kg bw per day for males and females, respectively, based on a BMR of 22%.

For the decrease in body weights and the increases in absolute and relative liver weights, BMDLs could be estimated. For the spleen, there was no dose-related trend in the absolute weight. The increase in relative spleen weight showed large model uncertainty resulting in very wide confidence intervals for the BMD. The dose-related trend in these data is likely to be dependent on the trend in the body weight data rather than on an effect on the spleen. The lowest reliable BMDL for these parameters were 52.6 and 29.9 mg/kg bw per day for increased absolute liver weight in males and females, respectively, based on a BMR of 15%.

[14] https://efsa.openanalytics.eu/ freely available.

[15] https://proastweb.rivm.nl/

[16] Abbreviations used in these sections: ALKP: Alkaline phosphatase; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; BILI: Bilirubin; BMDL: Benchmark Dose lower confidence interval limit (95% single sided); BUN: Blood urea nitrogen; CHOL: Cholesterol; CL: Chloride; CREA: Creatinine; GLOB: Globulin; GLUC: Glucose; K: Potassium; MCV: Mean corpuscular volume; pH: Urinary pH; PTT: Prothrombin time; SDH: Sorbitol dehydrogenase; SG: Urinary specific gravity; TRIG: Triglycerides; UMTP: Urinary total protein; URO: Urobilinogen; UVOL: Urinary volume.

Overall, for the evaluation of 2-pentylfuran and the structurally related flavouring substances in this FGE the BMDL of 8.51 mg/kg bw per day in males will be used as the reference point. All other reliable BMDLs were higher than this BMDL for BILI.

A graphical representation of the respective BMD confidence intervals is given in Appendix G (Figures G.2–G.4). Note that these graphs have a 10-log horizontal axis. This appendix includes also the report for the dose–response modelling for plasma total bilirubin and a table (Table G.1) with the numerical values of the endpoint-specific BMRs with the BMD confidence intervals for the parameters that were included in this assessment, where applicable.

3.4.6.6. Considerations on potential neurotoxicity

Furan-substituted substances of FGE.67Rev3 might be metabolised into analogues of the γ-diketone 2,5-hex-3-enedione, following the opening of the furan ring (Wang et al., 2015). As described in FGE.07Rev5 (EFSA CEF Panel, 2017), it is well known that saturated γ-diketones may cause neurotoxicity (Topping et al., 1994). Such γ-diketones can react with free protein amines, leading to the formation of imines which could cyclise and eventually form stable protein-pyrrole adducts (Sanz et al., 1995). If formed in sufficient amounts, these adducts may cause neurotoxicity (i.e. axonal swelling, axonal atrophy, etc.) (Couri and Milks, 1982). When considering the chemical structures of the furan-substituted substances in FGE.67Rev3, the Panel noted that the ring closure (pyrrolisation) of the imines (which would result from the nucleophilic attack of the analogues of γ-diketone 2,5-hex-3-enedione by a free protein amine) would not be energetically favoured due to conformational constraints around the double (C=C) bond, thus preventing the generation of potential neurotoxic protein-pyrrole adducts.

The Panel took note that the substances in FGE.67Rev3 are furans substituted with functional groups. Therefore, there are additional possibilities for metabolic pathways which would limit the extent of the ring opening, and thus limit the formation of reactive metabolites, e.g. γ-3-enediones potentially resulting from some of the candidate substances.

The Panel also noted that, based on the current knowledge and the most up-to-date peer reviewed literature, the reactive products of furan and furan derivatives target the liver, with some minor kidney effects too, but no neurotoxic effects have been described (EFSA CONTAM Panel, 2017).

Overall, the Panel concluded that there is no solid ground to raise a concern for potential formation of neurotoxic protein adducts from the substituted furans in FGE.67Rev3.

3.4.6.7. Conclusions on toxicity studies

EFSA Considerations

The Panel considered that the toxicity data available for 2-acetylfuran and 2-pentylfuran are suitable for the calculation of a margin of safety (MOS) for the flavouring substances that they represent. For the substances that are represented by 2-acetylfuran a NOAEL of 22.6 mg/kg bw per day identified from a 90-day oral toxicity study with 2-acetylfuran can be used. The flavouring substances for which 2-pentylfuran is considered to be representative can be evaluated against the BMDL of 8.51 mg/kg bw per day derived from a 90-day oral toxicity study with this substance.

3.5. Application of the Procedure

Application of the Procedure to 12 substances from the JECFA group of 40 diverse furan derivatives (JECFA, 2006a,b, 2009a,b, 2012, 2019)

At the 65th, 69th and 76th JECFA meetings (JECFA, 2006a,b, 2009a,b, 2012), JECFA concluded that the furan derivatives under consideration could not be evaluated through the JECFA procedure due to unresolved issues with respect to genotoxicity. In the toxicological monograph, drafted for the 69th meeting[17] (JECFA, 2009a), JECFA did not give an allocation to structural classes according to Cramer et al. (1978) to any of the candidate flavouring substances. Also, no indication was given on the evaluation pathway ("A-side or B-side").

At the 86th meeting the JECFA (2019) concluded that based on newly submitted data on genotoxicity for [FL-no: 13.054 and 13.059], the concern with respect to genotoxicity was no longer an issue for this group of flavouring substances, and evaluated these candidate substances through

[17] A monograph was prepared for the 65th meeting, but this was withdrawn at the 69th meeting where a new monograph was prepared. At the 76th and 86th meetings only an addendum was prepared to the monograph for the 69th meeting.

the procedure as modified at the 82nd JECFA meeting (JECFA, 2016). All substances were allocated to structural class III. For the alkylfuran 2-heptylfuran [FL-no: 13.069] and the alkoylfuran 2-hexanoylfuran [FL-no: 13.070] the highest exposure estimates (SPET) were below the TTC for their structural class III (90 μg/person per day), and JECFA concluded that these were not of safety concern. For the remaining three alkylfurans [FL-no: 13.059, 13.106, 13.148] and seven alkoylfurans [FL-no: 13.045, 13.054, 13.083, 13.101, 13.105, 13.138 and 13.163] the highest exposure estimates (SPET) were above the TTC for structural class III substances.

For two alkylfurans [FL-no: 13.059 and 13.106], JECFA calculated Margins of Safety (MOSs) by comparing their exposure estimates with the NOAEL of 30 mg/kg bw per day for 2-pentylfuran [FL-no: 13.059] (for study description see Section 3.4.6.5). For the remaining alkylfuran [FL-no: 13.148], a MOS was calculated by comparison of its exposure estimate with the NOAEL for 2-furylacrylaldehyde [FL-no: 13.034].[21]

For four alkoylfurans [FL-no: 13.045, 13.054, 13.105, 13.163], JECFA calculated MOSs by comparing their exposure estimates with the NOAEL of 25 mg/kg bw per day for 2-acetylfuran [FL-no: 13.054] (for study description see Section 3.4.6.1). For two alkoylfurans [FL-no: 13.083 and 13.101], JECFA calculated MOSs by comparing their exposure estimates with the NOAEL of 10 mg/kg bw per day for 3-acetyl-2,5-dimethylfuran [FL-no: 13.066],[18] derived from a 14-day oral toxicity study (see Appendix F). For the remaining alkoylfuran [FL-no: 13.138] JECFA calculated a MOS by comparing its exposure estimate with the NOAEL of 30 mg/kg bw per day for 4-(2-furyl)-3-buten-2-one [FL-no: 13.044],[19] derived from a 14-day oral toxicity study (see Appendix F). For all 12 flavouring substances JECFA reached the conclusion that they were not of safety concern.

The JECFA safety evaluations of the 12 flavouring substances are summarised in Appendix H – Table H.1.

EFSA considerations

The Panel noted that JECFA applied the revised Procedure scheme (JECFA, 2016) to the evaluation of this group of substances at the 86th meeting (JECFA, 2019). This is consistent with the TTC approach for evaluation of substances, which has been adopted by EFSA Scientific Committee (2019). However, taking into consideration the requirements set out in Commission Regulation (EC) No 1565/2000, the Panel followed the strategy as specified in the Opinion on a Programme for the Evaluation of Flavouring substances of the Scientific Committee on Food (SCF, 1999) (for details see Appendix A).

The Panel agrees with JECFA with respect to the allocation of the candidate flavouring substances in structural class III. Based on the strategy of the Scientific Committee (SCF, 1999) the FAF Panel concluded that metabolites of the candidate substances in this FGE cannot be considered as innocuous. Therefore, the candidate substances have to be evaluated along the B-side of the Procedure. Since the exposure estimates, based on the MSDI approach, are all below the TTC of 90 μg/capita per day, at step B4 of the Procedure a NOAEL or BMDL is needed to calculate a MOS.

The Panel noted that 4-(2-furyl)but-3-en-2-one [FL-no: 13.044][19] is a ketone and cannot be considered as a representative for [FL-no: 13.148], which is an alkylfuran. The FAF Panel also considered that for the four alkylfurans in group IV, [FL-no: 13.059, 13.069, 13.106 and 13.148], the BMDL of 8.51 mg/kg bw per day calculated from the results of a 90-day oral toxicity study with 2-pentylfuran [FL-no: 13.059] is more suitable than the NOAEL of 30 mg/kg bw per day obtained from the same study with 2-pentylfuran. At step B4, the FAF Panel calculated adequate MOSs of 1.3×10^5, 4.3×10^7, 4.3×10^7 and 4.3×10^6 for [FL-no: 13.059, 13.069, 13.106 and 13.148], respectively.

The Panel considered that the NOAEL of 10 mg/kg bw per day for 3-acetyl-2,5-dimethylfuran [FL-no: 13.066][18] and the NOAEL of 30 mg/kg bw per day for 4-(2-furyl)-3-buten-2-one [FL-no: 13.044][19] were not suitable for use in the Procedure, since these two NOAELs were obtained from studies of too short duration. The Panel also considered that [FL-no: 13.066][18] is not a representative for [FL-no: 13.083 and 13.101] since these have the alkoyl function at position 2 of the furan ring, rather than at position 3. Therefore, for all eight alkoylfurans the FAF Panel decided to use the NOAEL of 22.6 mg/kg bw per day obtained from a 90-day oral toxicity study with 2-acetylfuran for the calculation of MOSs for all substances from group VI-B. At step B4 of the Procedure the FAF Panel calculated adequate MOSs of 1.8×10^7, 2.3×10^4, 1.1×10^8, 2.0×10^6, 1.1×10^8, 1.1×10^7, 5.6×10^6, and 2.2×10^7 for [FL-no: 13.045, 13.054, 13.070, 13.083, 13.101, 13.105, 13.138 and 13.163], respectively.

[18] 3-Acetyl-2,5-dimethylfuran [FL-no: 13.066] is no longer supported by industry (DG SANTE, 2020b).
[19] Substance no longer supported by industry (DG SANTE, 2019).

Thus, for the 12 candidate substances considered in FGE.67Rev3, the FAF Panel concluded that these are not of safety concern, when based on the MSDI approach.

For all 12 candidate flavouring substances considered in this revision of FGE.67 and for all 11 flavouring substances considered in FGE.67Rev2, use levels are available and mTAMDI values have been calculated (see Appendix C, Tables C.1 and C.4). For five substances, [FL-no: 13.006, 13.058, 13.069, 13.070 and 13.166], the mTAMDI intake estimates are below the TTC for their structural classes (III). For the remaining 18 substances, the mTAMDI intake estimates are above the TTC for the structural classes (III).

4. Discussion

This revision 3 of FGE.67 comprises in total 23 flavouring substances, which have been evaluated by JECFA at the 86th meeting. Eleven of these substances have already been considered in FGE.67Rev2. The remaining 12 substances [FL-no: 13.045, 13.054, 13.059, 13.069, 13.070, 13.083, 13.101, 13.105, 13.106, 13.138, 13.148 and 13.163] could not be evaluated in FGE.67Rev2, because of concerns with respect to genotoxicity. In the current revision of FGE.67, these concerns have been ruled out based on evaluation of newly submitted *in vitro* and *in vivo* genotoxicity data for two representative substances, 2-acetylfuran [FL-no: 13.054] and 2-pentylfuran [FL-no:13.059].

Based on considerations of structural class, metabolism data and absence of genotoxic potential *in vivo*, and the MSDI exposure estimates, the FAF Panel concludes that the 12 flavouring substances considered in FGE.67Rev3 do not raise a safety concern at step B4 of the Procedure, when based on MSDI approach.

Normal and maximum use levels for different food categories according to Commission Regulation (EC) No 1565/2000 are available, allowing for the calculation of mTAMDI intake estimates for all 23 flavouring substances in this group. For five substances, [FL- no: 13.006, 13.058, 13.069, 13.070 and 13.116], the mTAMDI intake estimates are below the TTC for their structural classes (III). No further data are required for these five substances. For 18 substances [FL-no: 13.021, 13.022, 13.023, 13.024, 13.031, 13.045, 13.047, 13.054, 13.059, 13.074, 13.083, 13.101, 13.105, 13.106, 13.138, 13.148, 13.163 and 13.190] the mTAMDI intake estimates are above the TTC for their structural classes (III). For these substances more detailed data on uses and use levels should be provided in order to refine the exposure assessment and to finalise their safety evaluation.

The conclusions for the 23 JECFA-evaluated substances can be applied to the materials of commerce, since adequate specifications, including complete purity criteria and identity, are available for all 23 flavouring substances.

5. Conclusions

In conclusion, for all 23 flavouring substances in FGE.67Rev3 the Panel agrees with JECFA conclusions 'No safety concern at estimated levels of intake as flavouring substances' when based on the MSDI approach.

For five substances, [FL-no: 13.006, 13.058, 13.069, 13.070 and 13.116], there is no concern when the exposure was estimated based on the mTAMDI approach.

For 18 substances more detailed information on uses and normal and maximum use levels are needed to refine the mTAMDI estimates in order to finalise their evaluation. Upon submission of such data, additional data on toxicity may become necessary.

Adequate specifications including complete purity criteria and identity for the materials of commerce have been provided for all 23 flavouring substances evaluated through the Procedure.

6. Recommendation

The Panel recommends the European Commission to consider:

- to request more reliable data on uses and use levels for [FL-no: 13.021, 13.022, 13.023, 13.024, 13.031, 13.045, 13.047, 13.054, 13.059, 13.074, 13.083, 13.101, 13.105, 13.106, 13.138, 13.148, 13.163 and 13.190], as the mTAMDI exposure estimates are above the threshold of concern for their structural class III. When these data are received, the assessment for these flavouring substances should be updated accordingly and expanded if necessary (i.e. request of additional toxicology data);

- to change in the Union List the name of the substance [FL-no: 13.023] from isopentyl 3-(2-furan)propionate to isopentyl 3-(2-furyl)propionate.

7. Documentation provided to EFSA

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9) DG SANTE (Directorate General for Health and Food Safety), 2019. Letter from European Commission DG-SANTE (Ares (2019) 7681587) informing of the withdrawal from the Union List of six flavouring substances from FGE.222.
10) DG SANTE (Directorate General for Health and Food Safety), 2020a. Letter from European Commission DG-SANTE (Ares (2020) 3647124) informing of the withdrawal from the Union List of the flavouring substance 2-butylfuran [FL-no: 13.103].
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Abbreviations

ALKP	alkaline phosphatase
ALT	alanine aminotransferase
AST	aspartate aminotransferase
BILI	bilirubin
BMD	benchmark dose
BMDL	benchmark dose lower boundary of confidence interval (95% single sided)
BMDU	benchmark dose upper boundary of confidence interval (95% single sided)
BMR	benchmark response
BUN	blood urea nitrogen
bw	body weight
CAS	Chemical Abstract Service
CEF	Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CHO	Chinese hamster ovary cells
CHOL	cholesterol
Cl	chloride

CREA	creatinine
CoE	Council of Europe
CYP	cytochrome P450
EFFA	European Flavour Association
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)
GLOB	globulin
GLP	Good Laboratory Practice
GLUC	glucose
GSH	glutathione
ID	identity
IOFI	International Organization of the Flavour Industry
IR	infrared spectroscopy
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
K	potassium
MCV	mean corpuscular volume
MN	micronuclei
MOS	margin of Safety
MS	mass spectrometry
MSDI	maximised survey-derived daily intake
mTAMDI	modified theoretical added maximum daily intake
MTD	maximum tolerated dose
NMR	nuclear magnetic resonance
No	number
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Cooperation and Development
pH	urinary pH
PK	proteinase K
PTT	prothrombin time
(Q)SAR	(quantitative) structure–activity relationship
SCE	sister Chromatid Exchanges
SCF	Scientific Committee on Food
SDH	sorbitol dehydrogenase
SG	urinary specific gravity
SPET	single-portion exposure technique
TG	test guideline
TTC	threshold of toxicological of concern
TRIG	triglycerides
UDS	unscheduled DNA Synthesis
UMTP	urinary total protein
URO	urobilinogen
UVOL	urinary volume
WHO	World Health Organization

Appendix A – Procedure of the safety evaluation

The approach for a safety evaluation of chemically defined flavouring substances as referred to in Commission Regulation (EC) No 1565/2000, named the 'Procedure', is shown in schematic form in Figure A.1. The Procedure is based on the Opinion of the Scientific Committee on Food expressed on 2 December 1999 (SCF, 1999), which is derived from the evaluation Procedure developed by the Joint FAO/WHO Expert Committee on Food Additives at its 44th, 46th and 49th meetings (JECFA, 1995, 1996, 1997, 1999), hereafter named the 'JECFA Procedure'.[20]

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

Class I contains flavourings that have simple chemical structures and efficient modes of metabolism, which would suggest a low order of oral toxicity. Class II contains flavourings that have structural features that are less innocuous but are not suggestive of toxicity. Class III comprises flavourings that have structural features that permit no strong initial presumption of safety, or may even suggest significant toxicity (Cramer et al., 1978). The TTCs for these structural classes of 1,800, 540 or 90 μg/person per day, respectively, are derived from a large database containing data on subchronic and chronic animal studies (JECFA, 1996).

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

- Can the flavourings be predicted to be metabolised to innocuous products[21] (step 2)?
- Do their exposures exceed the TTC for the structural class (steps A3 and B3)?
- Are the flavourings or their metabolites endogenous[22] (step A4)?
- Does a NOAEL exist on the flavourings or on structurally related substances (steps A5 and B4)?

In addition to the data provided for the flavouring substances to be evaluated (candidate substances), toxicological background information available for compounds structurally related to the candidate substances is considered (supporting substances), in order to assure that these data are consistent with the results obtained after application of the Procedure. The Procedure is not to be applied to flavourings with existing unresolved problems of toxicity. Therefore, the right is reserved to use alternative approaches if data on specific flavourings warranted such actions.

[20] The FAF Panel is aware that a Revised Procedure for the Safety Evaluation of Flavouring agents has been agreed by JECFA (JECFA, 2016). The EFSA Scientific Committee has developed a modified procedure for evaluation of substances based on the TTC approach (EFSA Scientific Committee, 2019). However, these developments have no impact on the present evaluation, which should follow the requirements as set out in Commission Regulation (EC) No 1565/2000.

[21] *Innocuous products*: products that are known or readily predicted to be harmless to humans at the estimated intake of the flavouring agent (JECFA, 1997).

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

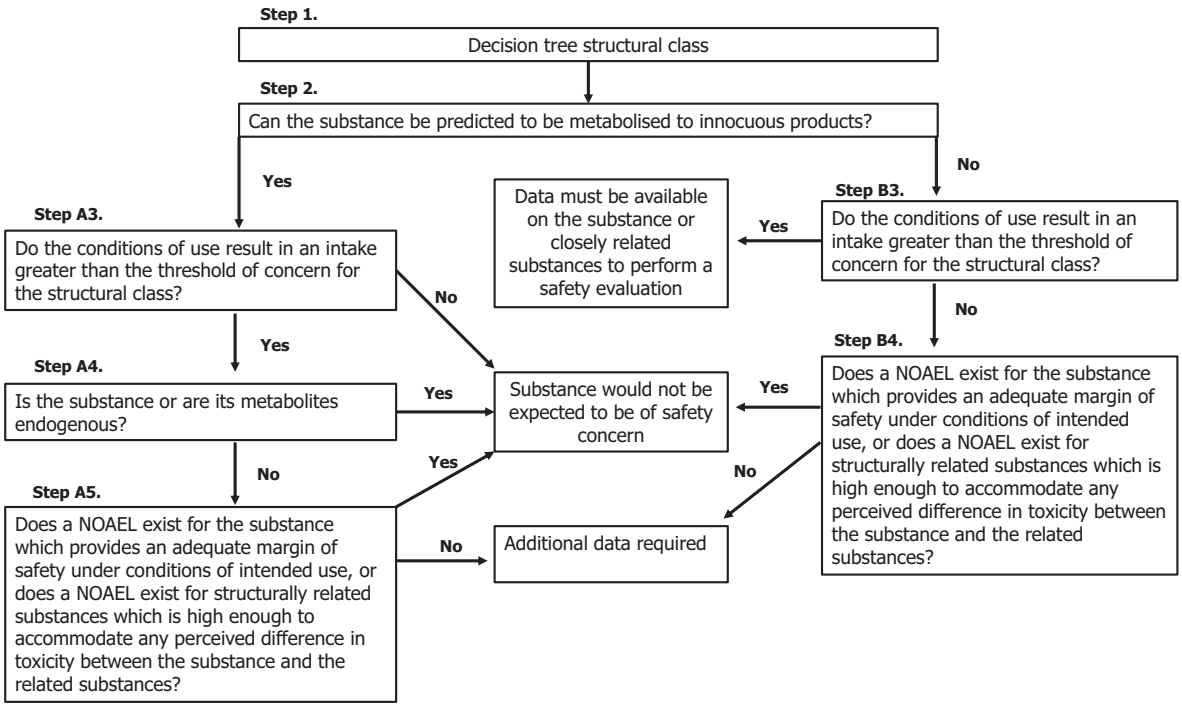


Figure A.1: Procedure for the safety evaluation of chemically defined flavouring substances

For the flavouring substances considered in this Flavouring Group Evaluation (FGE), the FAF 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 considerations 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 evaluated through the EFSA Procedure.

The following issues are of special importance:

a) *Intake*

In its evaluation, the Panel as a default uses the 'maximised survey-derived daily intake' (MSDI)[23] approach to estimate the per capita intakes of the flavouring substances in Europe.

In its evaluation, 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 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 JECFA only on the basis of these figures. For substances in the Union List[2] of flavouring substances for which this is the case, the Panel will need European Union (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 levels 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 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, 2006a,b).

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 (see Appendix C).

[23] EU MSDI: Amount added to food as flavour in (kg/year) × 10^9/(0.1 × population in Europe (= 375 × 10^6) × 0.6 × 365) = μg/capita per day.

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

b) Threshold of 1.5 μg/person per day (step B5) used by JECFA

JECFA uses the threshold of concern of 1.5 μg/person per 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 μg/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, should be amended to include the last step on the right-hand side of the original procedure ('Do the conditions of use result in an intake greater than 1.5 μg per day?')' (JECFA, 1999).

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 μg per person per day.

C) Genotoxicity

As reflected in the opinion of SCF (1999), 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.

d) Specifications

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

e) 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.

Revised JECFA Procedure for the safety evaluation of flavouring substances (JECFA, 2016)

Based on the recommendations from the joint EFSA/WHO experts workshop (EFSA and WHO, 2016) on how the existing TTC framework may be improved and how to develop a globally harmonized decision-tree for a tiered approach on the application of the TTC in the risk assessment of chemicals, the JECFA Committee proposed a revised Procedure for the safety evaluation of flavouring substances in its 82nd meeting (JECFA, 2016).

In the revised JECFA Procedure scheme an initial question with respect to the genotoxicity potential of the substance is introduced (i.e. if a concern for genotoxicity is identified the Procedure cannot be applied). As a consequence, also step B5 of the old Procedure ("Do the conditions of use result in an intake greater than 1.5 μg/day?") is removed as it was considered of little practical application. Moreover, the Cramer class thresholds as applied would be adequately protective for a non-genotoxic cancer endpoint. Another important change is the deletion of the question, at step 2 of the old Procedure, related to the expected metabolites ("Can the substance be predicted to be metabolized to innocuous products?") and therefore to combine the A- and B- side of the old Procedure. The evaluation is then based on the comparison of the highest predicted dietary exposure estimate (based on MSDI and single-portion exposure technique (SPET) approach) of the flavouring substance with the corresponding TTC value for its structural class.

The updated JECFA Procedure scheme is reported in the Figure A.2.

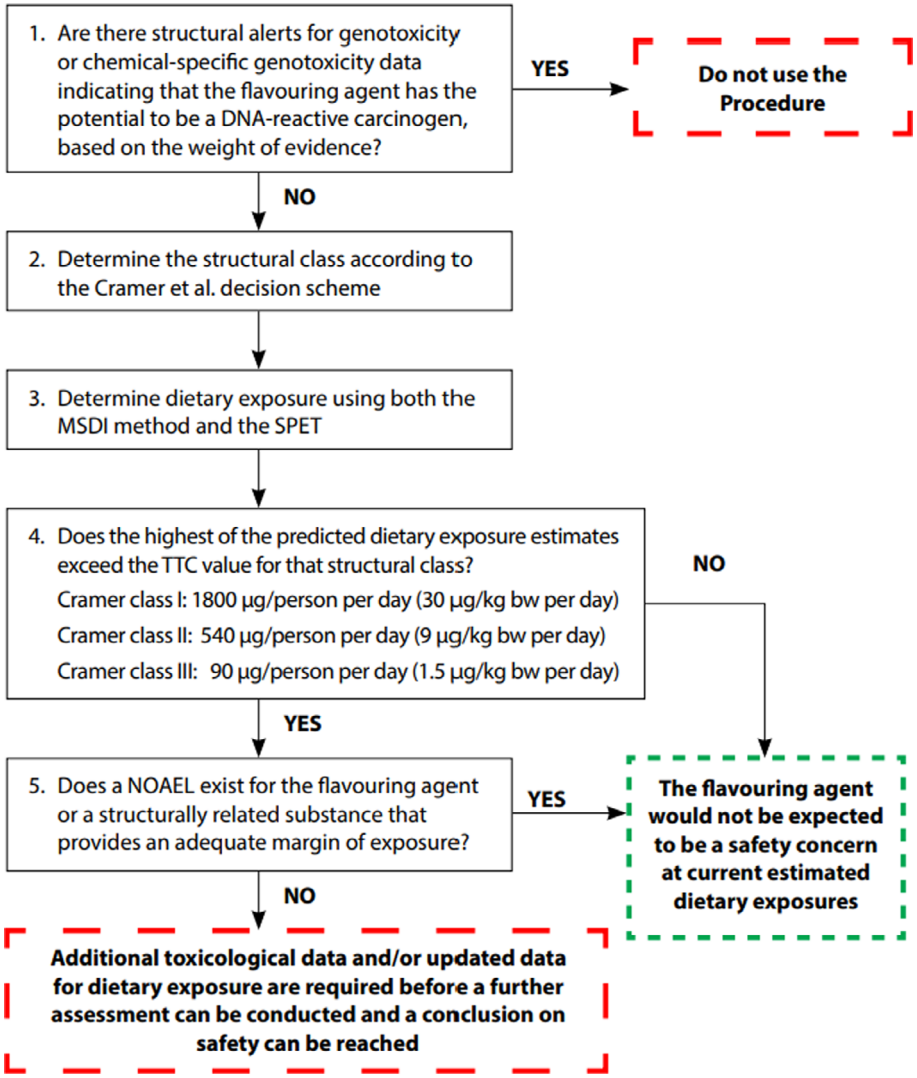


Figure A.2: Revised JECFA Procedure for the safety evaluation of flavouring substances

Appendix B – Specifications

Table B.1: Summary table on specifications data for flavouring substances in FGE.67Rev3 (for chemical structures see Appendix H) that are included in the Union List. Substances which are no longer supported by industry and which have been deleted from the EU Union list [FL-no 13.029, 13.030, 13.052, 13.061, 13.092, 13.107, 13.123, 13.191] are referenced in the previous version, FGE.67Rev2 (EFSA CEF Panel, 2015a)

Information included in the EU Union list Regulation No (EU) 1334/2008 as amended			Most recent available specifications data[a]				
FL-no JECFA-no FEMA no CoE no CAS no	**Chemical name**	**Purity of the named compound**	**Phys. form Mol. formula Mol. weight**	**Solubilityc[c] Solubility in ethanol[d]**	**Boiling point, °C[e] Melting point, °C ID test Assay minimum (isomers distribution/ secondary components)**	**Refrac. Index[f] Spec. gravity[g]**	**EFSA comments**
13.006 1517 2865 362 7149-32-8	Phenethyl 2-furoate	(b)	Liquid $C_{13}H_{12}O_3$ 216.24	Insoluble Soluble in oils Soluble	275 n.a. NMR 96%	1.585–1.593 1.136–1.142	
13.021 1516 2070 2080 7779-66-0	Isopentyl 4-(2-furan)butyrate	(b)	Liquid $C_{13}H_{20}O_3$ 224.30	Insoluble soluble	263-265 n.a. NMR 95%	1.551–1.555 0.975–0.981	
13.022 1513 2435 2091 10031-90-0	Ethyl 3(2-furyl)propionate	(b)	Solid $C_9H_{12}O_3$ 168.19	Very slightly soluble Soluble	n.a. 24-25 NMR 95%	n.a. n.a.	
13.023 1515 2071 2092 7779-67-1	Isopentyl 3-(2-furan) propionate	(b)	Liquid $C_{12}H_{18}O_3$ 210.27	Insoluble Soluble	258 n.a. NMR 96%	1.549–1.557 0.987–0.993	Name in the Union List to be changed to isopentyl 3-(2-furyl) propionate

Information included in the EU Union list Regulation No (EU) 1334/2008 as amended			Most recent available specifications data[a]				EFSA comments
FL-no JECFA-no FEMA no CoE no CAS no	**Chemical name**	**Purity of the named compound**	**Phys. form Mol. formula Mol. weight**	**Solubilityc[c] Solubility in ethanol[d]**	**Boiling point, °C[e] Melting point, °C ID test Assay minimum (isomers distribution/ secondary components)**	**Refrac. Index[f] Spec. gravity[g]**	
13.024 1514 2198 2093 105-01-1	Isobutyl 3-(2-furyl)propionate	(b)	Liquid $C_{11}H_{16}O_3$ 196.25	Very slightly soluble Soluble	105 (4 hPa) n.a. NMR 96%	1.531-1.537 1.007-1.013	
13.031 751 3128 2247 4265-16-1	2-Benzofurancarboxaldehyde	(b)	Solid $C_9H_6O_2$ 146.15	Insoluble Slightly soluble	130-131 (17hPa) 195-198 (cryst) MS 96%	n.a. n.a.	
13.045 1508 2496 11837 6975-60-6	1-(2-Furyl)-propan-2-one	(b)	Liquid $C_7H_8O_2$ 124.14	Very slightly soluble Soluble (EFFA, 2014)	179-180 n.a. NMR 97%	1.499–1.505 1.074–1.080	
13.047 1518 2945 11842 623-22-3	Propyl 3-(2-furyl)acrylate	(b)	Liquid $C1_0H_{12}O_3$ 180.20	Insoluble Soluble	119 (9 hPa) n.a. NMR 97%	1.520–1.526 1.071–1.077 (20°)	Mixture of isomers: 50–80% *E* and 20–50% *Z* (EFFA, 2014)
13.054 1503 3163 11653 1192-62-7	2-Acetylfuran	(b)	Liquid $C_6H_6O_2$ 110.11	Very slightly soluble; Soluble slightly soluble in propylene glycol, vegetable oils	67 (13 hPa) n.a. IR 97%	1.505–1.510 1.102–1.107	

Information included in the EU Union list Regulation No (EU) 1334/2008 as amended			Most recent available specifications data[a]				EFSA comments
FL-no JECFA-no FEMA no CoE no CAS no	**Chemical name**	**Purity of the named compound**	**Phys. form Mol. formula Mol. weight**	**Solubilityc[c] Solubility in ethanol[d]**	**Boiling point, °C[e] Melting point, °C ID test Assay minimum (isomers distribution/ secondary components)**	**Refrac. Index[f] Spec. gravity[g]**	
13.058 1500 3307 10355 31704-80-0	3-(5-Methyl-2-furyl) butanal	(b)	Liquid $C_9H_{12}O_2$ 152.19	Insoluble Soluble Soluble in oils	88-91 (16 hPa) n.a. NMR 98% Racemate	1.575–1.581 1.006–1.012	
13.059 1491 3317 10966 3777-69-3	2-Pentylfuran	(b)	Liquid $C_9H_{14}O$ 138.21	Slightly soluble Soluble	58-60 (13 hPa) n.a. NMR 99%	1.443–1.449 0.886–0.893	
13.066 1506 3391 10921 10599-70-9	3-Acetyl-2,5-dimethylfuran	(b)	Liquid $C_8H_{10}O_2$ 138.17	Slightly soluble Soluble Soluble in propylene glycol, most fixed oils	83 (14 hPa) n.a. NMR 99%	1.488–1.490 1.037–1.039	Substance not supported by industry (DG SANTE, 2020b)
13.069 1492 3401 10952 3777-71-7	2-Heptylfuran	(b)	Liquid $C_{11}H_{18}O$ 166.26	Insoluble Soluble	209-210 n.a. NMR 99%	1.446–1.452 0.860–0.866	
13.070 1512 3418 11180 14360-50-0	2-Hexanoylfuran	(b)	Liquid $C_{10}H_{14}O_2$ 166.22	Slightly soluble Soluble	65-67 (0.7 hPa) n.a. NMR 99%	1.490–1.496 0.992–0.998	
13.074 1495 3535 11913 3782-00-1	2,3-Dimethylbenzofuran	(b)	Liquid $C_{10}H_{10}O$ 146.19	Insoluble Soluble Soluble in fats	96-98 (20 hPa) n.a. NMR 97%	1.554–1.563 1.031–1.037	

Information included in the EU Union list Regulation No (EU) 1334/2008 as amended			Most recent available specifications data[a]				EFSA comments
FL-no JECFA-no FEMA no CoE no CAS no	**Chemical name**	**Purity of the named compound**	**Phys. form Mol. formula Mol. weight**	**Solubilityc[c] Solubility in ethanol[d]**	**Boiling point, °C[e] Melting point, °C ID test Assay minimum (isomers distribution/ secondary components)**	**Refrac. Index[f] Spec. gravity[g]**	
13.083 1504 3609 11038 1193-79-9	2-Acetyl-5-methylfuran	(b)	Liquid $C_7H_8O_2$ 124.14	Slightly soluble Soluble Soluble in corn oil	71-72 (10 hPa) n.a. IR NMR 99%	1.511–1.517 1.066–1.072 (20°)	
13.101 1505 4071 - 22940-86-9	2-Acetyl-3,5-dimethylfuran	(b)	Liquid $C_8H_{10}O_2$ 138.17	Insoluble Soluble	195 18 MS 95%	1.494–1.500 1.041–1.047	
13.103 1490 4081 10927 4466-24-4	2-Butylfuran	(b)	Liquid $C_8H_{12}O$ 124.18	Insoluble Soluble	139 n.a. MS 95%	1.444–1.450 0.884–0.890	Substance not supported by industry (DG SANTE, 2020a)
13.105 1507 4083 11045 4208-57-5	2-Butyrylfuran	(b)	Liquid $C_8H_{10}O_2$ 138.17	Insoluble Soluble	195 n.a. NMR MS 95%	1.489–1.495 1.050–1.056	
13.106 1493 4090 - 83469-85-6	2-Decylfuran	(b)	Solid $C_{14}H_{24}O$ 208.34	Insoluble Soluble	n.a. 30 NMR 95%	n.a. n.a.	
13.116 1523 4034 - 55764-22-2	2,5-Dimethyl-3-thioacetoxyfuran	(b)	Liquid $C_8H_{10}O_2S$ 170.23	Practically insoluble Soluble Soluble in diethylether	230 n.a. IR NMR MS 98%	1.527–1.533 1.137–1.143	

Information included in the EU Union list Regulation No (EU) 1334/2008 as amended			Most recent available specifications data[a]				EFSA comments
FL-no JECFA-no FEMA no CoE no CAS no	**Chemical name**	**Purity of the named compound**	**Phys. form Mol. formula Mol. weight**	**Solubilityc[c] Solubility in ethanol[d]**	**Boiling point, °C[e] Melting point, °C ID test Assay minimum (isomers distribution/ secondary components)**	**Refrac. Index[f] Spec. gravity[g]**	
13.138 1510 4120 11084 699-17-2	1-(2-Furyl)butan-3-one	(b)	Solid $C_8H_{10}O_2$ 138.17	Slightly soluble Soluble	n.a. 37 MS 95%	n.a. n.a.	
13.148 1494 4174 - 15186-51-3	3-Methyl-2(3-methylbut-2-enyl)furan	(b)	Liquid $C_{10}H_{14}O$ 150.22	Slightly soluble Soluble	70 (15 hPa) n.a. MS 98%	1.473–1.479 0.998–1.004	
13.163 1509 4192 - 3194-17-0	2-Pentanoylfuran	(b)	Liquid $C_9H_{12}O_2$ 152.19	Slightly soluble Soluble	101 (13 hPa) n.a. MS 95%	1.486–1.492 1.009–1.015	
13.190 1525 4056 - 61295-44-1	3-((2-Methyl-3-furyl)thio)-2-butanone	(b)	Liquid $C_9H_{12}O_2S$ 184.25	Practically insoluble Soluble Soluble in ethyl acetate,triacetin	70 (1 hPa) n.a. IR NMR MS 99% (racemate)	1.510–1.516 1.104–1.110	

n.a.: not applicable; FL-No: FLAVIS number; JECFA: The Joint FAO/WHO Expert Committee on Food Additives; FEMA: Flavor and Extract Manufacturers Association; CoE: Council of Europe; CAS: Chemical Abstract Service; ID: identity; IR: infrared spectroscopy; NMR: nuclear magnetic resonance; MS: mass spectrometry,
(a): JECFA, 2005, 2018 and documentation provided to EFSA (EFFA, 2014).
(b): At least 95% unless otherwise specified.
(c): Solubility in water, if not otherwise stated.
(d): Solubility in 95% ethanol, if not otherwise stated.
(e): At 1,013.25 hPa, if not otherwise stated.
(f): At 20°C, if not otherwise stated.
(g): At 25°C, if not otherwise stated.

Appendix C – Exposure estimates

C.1. Normal and Maximum Use Levels

Table C.1: Normal and maximum use levels (mg/kg food) for JECFA-evaluated substances in FGE.67Rev3

FL-no	Food Categories																	
	Normal use levels[a] (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
13.006[c]								0.02 –										
13.021[c]								3 –							0.3 –			
13.022[c]	3.7 –					3.75 –		4.26 –							0.98 –	1 –		
13.023[c]								8 –										
13.024[c]								8 –								2 –		
13.031[d]			2.5 –			5 –							5 –					
13.045[b]	1 11.5						1 20	5 20	1 20									
13.047[d]	2 –					8.8 18	0.74 9.9						0.0044 –		0.013 0.11		0.015 –	
13.054[b]	1 5.6						1 4	5 20	3.5 20						1 3			
13.058[d]		0.25 1.8																
13.059[b]	1.5 3						1 3	1 3	1.8 3				2.5 3					
13.069[b]	0.05 0.5						0.02 0.2	0.06 0.2	0.02 0.2						0.02 0.2			
13.070[b]	0.04 0.1						0.04 0.1	0.04 0.1	0.04 0.1									

FL-no	Food Categories Normal use levels[a] (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**
13.074[d]	0.43 –	0.5 1.4	0.17 –			0.017 0.043							0.43 –		0.23 1.4	0.54 2.9		
13.083[b]	0.5 1.5			0.5[e] 1.5			0.5 1.5	0.5 1.5	0.5 1.5				1 1.5				1 2	
13.101[b]	1 35	3.5 25					1 25	1 50	2 100				5 25					
13.105[b]	5 15	2 10	3 15	2 10		4 20	2 10	5 25	1 5	1 5			2 10				5 25	
13.106[b]	2 15	5 10					1 10	1 25	3 5									
13.116[c]									0.02 –				0.02 – –				0.01 –	
13.138[b]	2.5 15			2 10			1 10	1 25	1 5						0.3 25			
13.148[b]	3.5 15	1.7 10					1 10	1 25	3 5									
13.163[b]	1 35						1 25	8 50	10 100									
13.190[c]								0.001 –	0.001 –				10 –				0.001 –	

FL-No: FLAVIS number; JECFA: The Joint FAO/WHO Expert Committee on Food Additives; FGE: Flavouring Group Evaluation.
(a): 'Normal use' is defined as the average of reported usages and 'maximum use' is defined as the 95th percentile of reported usages (EFFA, 2002)
(b): Use levels data provided by industry (EFFA, 2020b).
(c): Use levels data provided by industry (EFFA, 2018).
(d): Use levels data submitted by EFFA to European Commission (DG SANCO, 2014).

C.2. mTAMDI calculation

The method for calculation of modified Theoretical Added Maximum Daily Intake (mTAMDI) values is based on the approach used by the SCF up to 1995 (SCF, 1995). The assumption is that a person may consume the amount of flavourable foods and beverages listed in Table C.2. These consumption estimates are then multiplied by the reported use levels in the different food categories and summed up.

Table C.2: Estimated amount of flavourable foods, beverages and exceptions assumed to be consumed per person per day (SCF, 1995)

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

The mTAMDI calculations are based on the normal use levels reported by industry. The seven food categories used in the SCF TAMDI approach (SCF, 1995) correspond to the 18 food categories as outlined in Commission Regulation (EC) No 1565/2000 and reported by the Flavour Industry in the following way (see Table C.3):

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

Table C.3: Distribution of the 18 food categories listed in Commission Regulation (EC) No 1565/2000 into the seven SCF food categories used for mTAMDI calculations (SCF, 1995)

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

Table C.4: Estimated intakes based on the MSDI approach and the mTAMDI approach (DG SANCO, 2014; EFFA, 2018, 2020b; JECFA, 2001, 2019)

Estimated intakes based on the MSDI, SPET and the mTAMDI approaches						
FL-no	**EU Union List name**	**MSDI – EU (μg/capita per day)**	**SPET[(c)] (μg/capita per day)**	**mTAMDI (μg/person per day)**	**Structural class**	**TTC (μg/person per day)**
13.006	Phenethyl 2-furoate	0.012	2 (MSDI)	2.7[(b)]	Class III	90
13.021	Isopentyl 4-(2-furan)butyrate	0.24	150	500[(b)]	Class III	90
13.022	Ethyl 3(2-furyl)propionate	0.012	463	1,000[(b)]	Class III	90
13.023	Isopentyl 3-(2-furan)propionate	0.24	400	1,100[(b)]	Class III	90
13.024	Isobutyl 3-(2-furyl)propionate	0.12	400	1,100[(b)]	Class III	90
13.031	2-Benzofurancarboxaldehyde	0.012	n.a.[(d)]	570	Class III	90
13.045[(a)]	1-(2-Furyl)-propan-2-one	0.074	150	670	Class III	90
13.047	Propyl 3-(2-furyl)acrylate	2.2	18	510	Class III	90
13.054[(a)]	2-Acetylfuran	60	350	990	Class III	90
13.058	3-(5-Methyl-2-furyl) butanal	0.0012	50	33	Class III	90
13.059[(a)]	2-Pentylfuran	3.8	180	290	Class III	90
13.069[(a)]	2-Heptylfuran	0.012	6	14	Class III	90
13.070[(a)]	2-Hexanoylfuran	0.012	15	5.3	Class III	90
13.074	2,3-Dimethylbenzofuran	0.52	100	160	Class III	90
13.083[(a)]	2-Acetyl-5-methylfuran	0.67	100	110	Class III	90
13.101[(a)]	2-Acetyl-3,5-dimethylfuran	0.012	1000	570	Class III	90
13.105[(a)]	2-Butyrylfuran	0.12	625	1,000	Class III	90
13.106[(a)]	2-Decylfuran	0.012	300	670	Class III	90
13.116	2,5-Dimethyl-3-thioacetoxyfuran	3	2	3.3[(b)]	Class III	90
13.138[(a)]	1-(2-Furyl)butan-3-one	0.24	400	430	Class III	90
13.148[(a)]	3-Methyl-2(3-methylbut-2-enyl)furan	0.12	300	470	Class III	90
13.163[(a)]	2-Pentanoylfuran	0.061	1000	1,300	Class III	90
13.190	3-((2-Methyl-3-furyl)thio)-2-butanone	0.012	0.3	200[(b)]	Class III	90

MSDI: maximised survey-derived daily intake; mTAMDI: modified theoretical added maximum daily intake; n.a.: not available.
(a): Updated data provided by industry (EFFA, 2020b).
(b): Use levels data provided by industry (EFFA, 2018).
(c): Values for SPET reported by JECFA (JECFA, 2019).
(d): SPET was not calculated by JECFA for 2-benzofurancarboxaldehyde [FL-no: 13.031] because this substance was evaluated by JECFA in 2001.

C.3. Natural occurrence

JECFA status

JECFA reported that 20 of the 39 flavouring substances evaluated at the 86th meeting have been reported to occur as natural components in foods including cheese, chicken, cocoa, coffee, honey, rye bread, spirituous beverages, tomatoes, wheaten bread, wine and other foods (JECFA, 2019).

Information provided by industry (EFFA, 2020a)

Some of the candidate chemicals have been reported to occur in foods as described below (Nijssen et al., 2020). 1-(2-Furyl)-propan-2-one [FL-no: 13.045] has been found in allium species, beef, coffee, pork, potato, sherry, soybean, sukiyaki and wheat bread.

2-Acetylfuran [FL-no: 13.054] has been found in a large number of foods, including beef, beer, cabbage, calamus (sweet flag), chicken, cocoa, coconut, coffee, grape brandy, guava, honey, malt, plum, pork, potato chips, rum, tamarind, tomato, garlic, fruits, nuts, vegetables, shellfish, fish, milk and milk products, and wine.

2-Pentylfuran [FL-no: 13.059] has been found in a large number of foods, including artichoke, asparagus, avocado, beans, beef, capsicum species, cauliflower, celery, chicken, coffee, egg, fig, fish, grape, melon, mushroom, olive, okra, parsley, peach, peanut, pistachio, plum, pork, potato, tea and tomato.

2-Heptylfuran [FL-no: 13.069] has been found in beef, chicken, endive, hazelnut, potato and tomato.

2-Hexanoylfuran [FL-no: 13.070] has been found in fried potato (French fries).

2-Acetyl-5-methylfuran [FL-no: 13.083] has been found in a large number of foods, including allium species, beef, beer, cocoa, coffee, fish, grape, licorice, malt, mate, mushroom, peanut, plum, popcorn, pork, potato, soybean, squid, tea, tomato, truffle and wild rice.

2-Acetyl-3,5-dimethylfuran [FL-no: 13.101] has been found in rum.

2-Butylfuran [FL-no: 13.103] has been found in avocado, beef, chicken, coffee, egg, hazelnut, licorice, milk, miso, musk melon, oats, okra, peanut, pork, potato, rice, soybean, tea, tomato, *Vaccinium* species (American cranberry) and walnut.

2-Butyrylfuran [FL-no: 13.105] has not been reported to occur in nature.

2-Decylfuran [FL-no: 13.106] has been found in coriander seed.

1-(2-Furyl)butan-3-one [FL-no: 13.138] has been found in beef and coffee.

3-Methyl-2(3-methylbut-2-enyl)furan [FL-no: 13.148] has been found in Asian mint, Calabash nutmeg, ginger and capsicum species.

2-Pentanoylfuran [FL-no: 13.163] has been found in grilled or roasted beef.

Appendix D – Summary of the Genotoxicity data considered in FGE.67Rev2

Table D.1: *In vitro* and *in vivo* genotoxicity data for furan-substituted substances evaluated by the JECFA at the 65th (JECFA, 2006b) and 69th meeting (JECFA, 2009a)

Chemical name [FL-no:] JECFA-no.	Test system	Test object	Concentration/dose and test conditions	Results	Reference
In vitro					
2-Methylfuran [13.030](cc) 1487	Reverse mutation	*S.* Typhimurium TA98 and TA100	0.165, 0.330, 0.495 or 0.660 μmol/plate (13.5, 27.1, 40.6 or 54.2 μg/plate)(a)	Negative(b)	Shinohara et al. (1986)
	Reverse mutation	*S.* Typhimurium TA98, TA100, TA102 and TA1535	Up to 10,000 μg/plate	Negative(b),(c),(d)	Zeiger et al. (1992)
	Reverse mutation	*S.* Typhimurium TA97 and TA104	Up to 10,000 μg/plate	Equivocal(b),(c),(d)	Zeiger et al. (1992)
	Reverse mutation	*S.* Typhimurium TA98, TA100 and TA102	11 nmol/plate to 1.1 mmol/plate (0.9–90310 μg/plate)(a)	Negative(b)	Aeschbacher et al. (1989)
	DNA damage	*B. subtilis* H17 (rec^+) and M45 (rec^-)	0.16, 16 or 1,600 μg/disc	Negative Positive(b),(e)	Shinohara et al. (1986)
	Chromosomal aberration	CHO cells	0–150 mmol/L (0–12,315 μg/mL)(a)	Positive(b),(f)	Stich et al. (1981)
2,5-Dimethylfuran [13.029](cc) 1488	Reverse mutation	*S.* Typhimurium TA98 and TA100	0.165, 0.330, 0.495 or 0.660 μmol/plate (13.5, 27.1, 40.6 or 54.2 μg/plate)(g)	Negative(b)	Shinohara et al. (1986)
	Reverse mutation	*S.* Typhimurium TA98 and TA100	Not specified	Negative(b)	Lee et al. (1994)
	Reverse mutation	*S.* Typhimurium TA97, TA98, TA100 and TA1535	Up to 3,333 μg/plate	Negative(b),(c),(d)	Zeiger et al. (1992)
	DNA damage	*B. subtilis* H17 (rec^+) and M45 (rec^-)	190, 1900 or 9500 μg/disc	Negative Positive(b),(h)	Shinohara et al. (1986)
	Chromosomal aberration	Chinese hamster V79 cells	1 mmol/L (96.13 μg/mL)(g)	Negative	Ochi and Ohsawa (1985)
	Chromosomal aberration	CHO cells	0–20 mmol/L (0–1,923 μg/mL)(g)	Positive(b),(f)	Stich et al. (1981)

Chemical name [FL-no:] JECFA-no.	Test system	Test object	Concentration/dose and test conditions	Results	Reference
3-Methyl-2-(3-methylbut-2-enyl)-furan [13.148] 1494	Reverse mutation	*S.* Typhimurium TA98, TA100, TA1535 and TA1537	3.2, 16, 80, 400 or 2,000 μg/ plate	Negative[(b)]	Asquith (1989)
3-(2-Furyl)acrolein [13.034][(dd)]	Reverse mutation	*S.* Typhimurium TA100	Not specified	Negative[(b),(c)]	Eder et al. (1991)
	DNA damage (SOS chromotest)	*E. coli* PQ37	Not specified	Negative[(i)]	Eder et al. (1991)
	DNA damage (SOS chromotest)	*E. coli* PQ37	Not specified	Weakly positive[(j)]	Eder et al. (1993)
2-Acetylfuran [13.054] 150	Reverse mutation	*S.* Typhimurium TA98 and TA100	0.165, 0.330, 0.495 or 0.660 μmol/plate (13.5, 27.1, 40.6 or 54.2 μg/plate)[(j)]	Negative Positive[(b),(k)]	Shinohara et al. (1986)
	DNA damage	*E. coli* PQ37 (SOS chromotest)	Not specified	Slightly positive[(j)]	Eder et al. (1993)
	DNA damage	*B. subtilis* H17 (rec^+) and M45 (rec^-)	550, 5,500 or 55,000 μg/disc	Negative Positive[(b),(L)]	Shinohara et al. (1986)
	Chromosomal aberration	CHO cells	0–112.6 mmol/ L (0–13,220 μg/ mL)[(j)]	Positive[(b),(m),(n)]	Stich et al. (1981)
	UDS	Human hepatocytes	2.19, 4.38, 8.75, 17.5, 35, 70, 140 or 280 μg/ mL	Negative	Durward (2007a)
4-(2-Furyl)-3-buten-2-one [13.044][(dd)] 1511	Reverse mutation	*S.* Typhimurium TA98, TA100, TA1535 and TA1537	33, 100, 333, 1,000, 2,166 or 3,333 μg/plate	Negative[(b),(c),(o)]	Mortelmans et al. (1986)
Ethyl 3-(2-furyl)propanoate [13.022] 1513	Reverse mutation	*S.* Typhimurium TA98, TA100, TA1535, TA1537 and TA1538	Up to 3,600 μg/plate	Negative[(b)]	Wild et al. (1983)

Chemical name [FL-no:] JECFA-no.	Test system	Test object	Concentration/dose and test conditions	Results	Reference
O-Ethyl-S-(2-furylmethyl)thio-carbonate [13.191][(ee)] 1526	Reverse mutation	*S.* Typhimurium TA98, TA100, TA1535 and TA1537	33, 100, 333, 1,000 or 3,330 μg/ plate	Negative[(b),(p)]	Verspeek-Rip (2000)
	Reverse mutation	*E. coli* WP2uvrA	33, 100, 333, 1,000 or 3,330 μg/ plate	Negative[(b),(q)]	Verspeek-Rip (2000)
	Chromosomal aberration	Human peripheral lymphocytes	150, 300 or 350 μg/mL	Negative[(b),(r)]	Meerts (2000)
	Chromosomal aberration	Human peripheral lymphocytes	130, 240 or 280 μg/mL	Positive[(i),(s)]	Meerts (2000)
	Chromosomal aberration	Human peripheral lymphocytes	100, 130 or 240 μg/mL	Positive[(i),(t)]	Meerts (2000)
	Chromosomal aberration	Human peripheral lymphocytes	150, 325 or 375 μg/mL	Negative Positive[(r),(u),(v)]	Meerts (2000)
In vivo					
2-Methylfuran [13.030][(cc)] 1487	Chromosomal aberration	Mouse bone marrow cells and spermatocytes	1,000, 2,000 or 4,000 mg/kg (100, 200 or 400 mg/kg bw per day)[(w)]	Negative	Subramanyam et al. (1989)
2-Acetylfuran [13.054] 1503	Chromosomal aberration	Mouse bone marrow	1,000, 2,000 or 3,000 mg/ L (20, 40 or 60 mg/kg bw)[(x)]	Positive[(y),(z)]	Sujatha et al. (1993)
	Chromosomal aberration	Mouse spermatocytes	1,000, 2,000 or 3,000 mg/ L (20, 40 or 60 mg/kg bw)[(x)]	Negative[(aa)]	Sujatha et al. (1993)
	SCE	Mouse bone marrow	1,000, 2,000 or 3,000 mg/ L (20, 40 or 60 mg/kg bw)[(x)]	Positive	Sujatha (2007)
	UDS	Rat liver	7 or 21 mg/kg bw	Negative	Durward (2007b)
O-Ethyl-S-(2-furylmethyl)thio-carbonate [13.191][(ee)] 1526	Micronucleus induction	Mouse bone marrow	100, 250 or 500 mg/kg bw[(bb)]	Negative	Verspeek-Rip (2001)

CHO: Chinese hamster ovary; SCE: sister chromatid exchange; UDS: unscheduled DNA synthesis.
(a): Calculated using relative molecular mass of 2-methylfuran = 82.1.
(b): With and without metabolic activation.
(c): Pre-incubation method.
(d): Occasional incidences of slight to complete clearing of the background lawn at the higher concentrations.
(e): Negative at all concentrations with metabolic activation; positive without metabolic activation.

(f): Clastogenic activity decreased with metabolic activation (statistical significance of results was not specified).
(g): Calculated using relative molecular mass of 2,5-dimethylfuran = 96.13.
(h): Positive at every concentration without metabolic activation; with metabolic activation, negative at 190 μg/disc, but positive at higher concentrations.
(i): Without metabolic activation.
(j): Calculated using relative molecular mass of 2-furyl methyl ketone = 110.11.
(k): Positive only in strain TA98 with an increase in the presence of metabolic activation.
(l): Negative at 550 μg/disc; positive at 5,500 and 55,000 μg/disc (with and without metabolic activation).
(m): Cytotoxicity was observed at 12,398 μg/ mL (112.6 mmol/ L) in the presence of metabolic activation.
(n): Clastogenic activity increased with metabolic activation (statistical significance of results was not specified).
(o): Cytotoxicity was observed at 3,333 μg/plate in all S. typhimurium strains and at 2166 μg/plate in S. typhimurium strains TA100 and TA1537.
(p): Cytotoxicity was observed at the 3,330 μg/plate level in all S. typhimurium strains and at 1,000 μg/plate in S. typhimurium strains TA100 and TA1535.
(q): Cytotoxicity was observed at 3,330 μg/plate in the absence of metabolic activation.
(r): 3-h continuous exposure time.
(s): 24-h continuous exposure time.
(t): 48-h continuous exposure time.
(u): With metabolic activation.
(v): Statistically significant dose-dependent increases in chromosomal aberrations were seen at the two highest concentrations only, 325 and 375 μg/ mL.
(w): Mice received 2-methylfuran in the diet for 5 consecutive days at 24-h intervals.
(x): Two experimental protocols were utilised. In one experiment, animals received single oral dose administrations of the test compound. In the other experiment, the test compound was orally administered once per day at the same concentrations as in the single-dose study for 5 consecutive days with 24-h intervals between doses.
(y): No effects observed at 20 mg/kg bw dose level and only mild, but significant ($p < 0.05$) effects seen at higher concentrations in bone marrow cells.
(z): Chromosomal aberrations were observed in the presence of significant mitodepression.
(aa): A single statistically significant occurrence of increased chromosomal aberrations observed 3 weeks following a single dose administration in the 60 mg/kg bw test group; statistically significant increases in polyploidy and XY univalents observed at weeks 3 and 4 at 60 mg/kg bw in multipledose-treated rats.
(bb): Single dose administered by gavage.
(cc): Substance deleted from the Union list. Commission Regulation (EU) No 246/2014 of 13 March 2014 amending Annex I to Regulation (EC) No 1334/2008 of the European Parliament and of the Council as regards removal from the Union list of certain flavouring substances. OJ L74, 14.3.2014, p. 58–60.
(dd): Substance not supported by industry (DG SANTE, 2019).
(ee): Substance not supported by industry and not included in the Union List. Commission Implementing Regulation (EU) No 872/2012 of 1 October 2012 adopting the list of flavouring substances provided for by Regulation (EC) No 2232/96 of the European Parliament and of the Council, introducing it in Annex I to Regulation (EC) No 1334/2008 of the European Parliament and of the Council and repealing Commission Regulation (EC) No 1565/2000 and Commission Decision 1999/217/EC. OJ L 267, 2.10.2012, p. 1–161.

Appendix E – Additional Genotoxicity data evaluated in FGE.67Rev3

Table E.1: Summary of *in vitro* genotoxicity data evaluated in FGE.67Rev3

Chemical name [FL-no]	Test system	Test object	Concentrations of substance and test conditions (μg/mL)	Result	Reference	Comments
2-Acetylfuran [13.054]	Micronucleus assay	Human peripheral blood lymphocytes	From 34.7 to 1,111[(a),(b),(c)]	Negative	Charles River (2020a)	Reliable without restrictions. Study performed in compliance with OECD TG 487 and GLP
2-Pentylfuran [13.059]	Micronucleus assay	Human peripheral blood lymphocytes	From 54.5 to 100.8[(a),(b)] From 51.4 to 91.1[(c)]	Negative	Charles River (2020b)	Reliable without restrictions. Study performed in compliance with OECD TG 487 and GLP

FL-No: FLAVIS number; FGE: Flavouring Group Evaluation; OECD: Organization for Economic Cooperation and Development; TG: test guideline; GLP: Good Laboratory Practice.
(a): Without S9 metabolic activation, 4 + 20 h treatment.
(b): With S9 metabolic activation, 4 + 20 h treatment.
(c): Without S9 metabolic activation, 24 h treatment.

Table E.2: Summary of *in vivo* genotoxicity data evaluated in FGE.67Rev3

Chemical name [FL-no]	Test system	Test object	Route	Dose (mg/kg bw per day)	Result	Reference	Comments
2-Acetylfuran [13.054]	Micronucleus assay (peripheral blood)	Muta® Mice, M	Gavage	0, 15, 30 and 60	Negative	Covance (2016)	Reliable with restrictions (no clear evidence of bone marrow exposure). Study performed in compliance with GLP and according to OECD TG 474, but positive control was not included
	Gene mutation assay in liver and duodenum				Negative		Reliable without restrictions. Study performed in compliance with GLP and according to OECD TG 488
2-Pentylfuran [13.059]	Comet assay (liver)	B6C3F1 mice, M	Gavage	508	Positive Negative in the presence of proteinase K	New York Medical College, 2012	Insufficient reliability The study aimed at comparing furan and 2-pentylfuran. Liver was sampled 3h after treatment
				762	Negative both with and without proteinase K		
				508, 762	Negative both with and without proteinase K Re-estimate: reduction of tail length at the highest dose, both with and without proteinase K		
2-Pentylfuran [13.059]	Micronucleus assay (bone marrow)	Han Wistar rats, M	Gavage	42.5, 85, 170[(a)]	Negative	Covance (2014)	Reliable with restrictions (no clear evidence of bone marrow exposure). Study performed in compliance with GLP and according to OECD TG 474
	Comet assay (liver)				Negative		Reliable without restrictions. The study was performed in compliance with recommendations of the Comet and IWGT workshop, Japanese Center for the Validation of Alternative Methods (JaCVAM) and current literature

FL-No: FLAVIS number; FGE: Flavouring Group Evaluation; OECD: Organization for Economic Cooperation and Development; TG: test guideline; GLP: Good Laboratory Practice; M: male.
(a): Administered via gavage in 3 doses at times 0, 24 and 45 h with sacrifice and harvest at 48 h.

Appendix F – Toxicity data evaluated in FGE.67Rev3

Table F.1: Summary of toxicity data evaluated by the Panel in FGE.67Rev3 or by JECFA at the 86th meeting

Chemical name [FL-no:]	Species; Sex No/group	Route	Doses (mg/kg bw per day)	Duration (days)	NOAEL or BMDL (mg/kg bw per day)	Reference	Comments
4-(2-Furyl)-3-buten-2-one [13.044]	Rat; M & F; 5/sex/group	Diet	0, 30	14	30	Gill and Van Miller (1987) as cited by JECFA (2009a)	Substance no longer supported by industry (DG SANTE, 2019)
3-Acetyl-2,5-dimethylfuran [13.066]	Rat; M & F 5/sex/dose level	Diet	0, 10	14	10	Van Miller and Weaver, (1987)	Substance no longer supported by industry (DG SANTE, 2020b)
2-Acetylfuran [13.054]	Rat; M & F; 32 rats for control and lowest dose; 12 rats for the mid dose; 10 rats for the highest dose	Diet	0, 5, 25, 100 (nominal)	28		Bio-Research Laboratories (1985)	
			0, 5, 25 (nominal, equal to 22.6 in males and 27 in females)	90	22.6		Study conducted according to OECD TG 408 and GLP
2-Pentylfuran [13.059]	Mice; M & F; 5 animals/group	Gavage	0, 800, 1,000, 1,260, 1,600, 2,000	Single dose acute toxicity study	LD_{50} about 1,200 mg/kg	Gulf South Research Institute (1971a)	Only summary available
	Rats; M & F; 23 animals/group	Diet	0, 25.6	90	–	Gulf South Research Institute (1971b)	
	Rat; M & F 5/sex/dose level	Diet	0, 100, 250 and 500	14	–	Product Safety Labs (2016)	
	Rat; M & F 10/sex/dose level	Oral gavage	0, 30, 100 and 150	90	8.51	Product Safety Labs (2017)	Study conducted according to OECD TG 408 and GLP. Value 8.51 is $BMDL_{22}$. The study authors proposed a NOAEL of 30 mg/kg bw per day

FL-No: FLAVIS number; FGE: Flavouring Group Evaluation; JECFA: The Joint FAO/WHO Expert Committee on Food Additives; bw: body weight; NOAEL: no observed adverse effect level; BMDL: benchmark Dose lower boundary of confidence interval (95% single sided); OECD: Organization for Economic Cooperation and Development; TG: test guideline; GLP: Good Laboratory Practice.

Appendix G – Benchmark Dose Modelling: Report (Bilirubin)

G.1. Data description

The endpoint to be analysed is: plasma total bilirubin concentration.
Data used for analysis:

	Dose	BILI_mn	BILI_SD	BILI_N	Sex
5	0	0.19	0.02	10	f
6	30	0.23	0.04	10	f
7	100	0.32	0.06	10	f
8	150	0.35	0.06	10	f
1	0	0.19	0.02	10	m
2	30	0.25	0.06	10	m
3	100	0.45	0.15	10	m
4	150	0.50	0.11	10	m

As can be observed from the table above, plasma total bilirubin concentration increases in a dose dependent way in males and to a lesser extent in females. From observations in the haematological investigations it is clear that this increase is related to an increased turn-over of red blood cells. The data in the table represent average group plasma total bilirubin concentration (BILI_mn), standard deviation (BILI_SD) and number of animals (BILI_N).

G.2. Selection of the BMR

The BMR (benchmark response) is the change in mean response compared to the controls. The BMD (benchmark dose) is the dose corresponding with the BMR of interest. A two-sided 90% confidence interval around the BMD will be estimated, the lower bound is reported by BMDL and the upper bound by BMDU.

The BMR used for the evaluation is an endpoint specific BMR, which is calculated from the within group variance of the BILI parameter doses and genders considering log-normality. One BMR of 22% change is derived, that is applicable for males as well as females. The derivation is based on a theoretical consideration by Slob (2017). For further clarification see also the ANS opinion on the re-evaluation of sodium and potassium nitrite (EFSA ANS Panel, 2017).

Software Used

The endpoint specific BMR for BILI was calculated using RIVM PROAST Webtool.[15]

Results are obtained using the EFSA web-tool for BMD analysis, which uses the R-package PROAST, version 69.0, for the underlying calculations.

G.3. Specification of deviations from default assumptions

None, except for the use of an endpoint-specific BMR, rather than the default of 5% for continuous endpoints (see EFSA Scientific Committee, 2017b).

Dose–response models

Default set of fitted models:

Model	Number of parameters	Formula
Null	1	$y = a$
Full	No. of groups	$y =$ group mean
Exp model 3	3	$y = a \cdot \exp(bx^d)$
Exp model 4	4	$y = a \cdot (c - (c - 1)\exp(-bx^d))$
Hill model 3	3	$y = a \cdot \left(1 - \frac{x^d}{b^d + x^d}\right)$

Model	Number of parameters	Formula
Hill model 4	4	$y = a \cdot \left(1 - \frac{(c-1)\cdot x^d}{b^d + x^d}\right)$
Inverse Exponential	4	$y = a \cdot (1 + (c-1)\exp(-bx^{-d}))$
Log-Normal Family	4	$y = a \cdot (1 + (c-1)\phi(\ln b + d\ln x))$

As a covariate is included in the analysis, these models will also be fitted assuming that some of the parameters [background response parameter (a), potency parameter (BMD) and/or variance (var)] depend on the subgroup defined by the covariate. Therefore, the number of parameters in each model might be larger than indicated in the table above.

Procedure for selection of BMDL

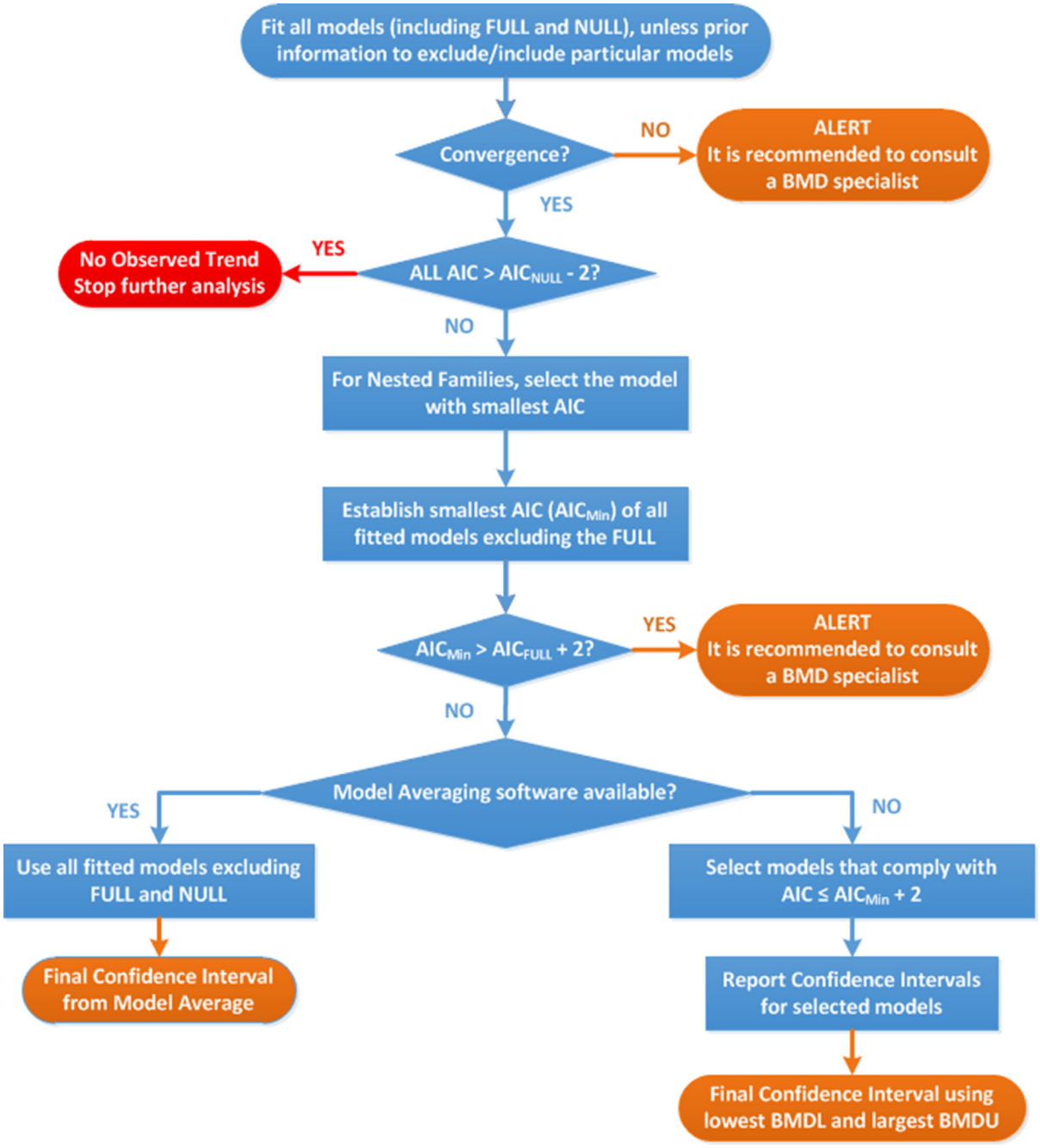


Figure G.1: Flow chart for selection of BMDL

G.4. Results

G.4.1. Response variable: BILI_mn

G.4.1.1. Fitted Models

Model	Converged	loglik	npar	AIC
full model	Yes	18.96	9	−19.92
full-v	Yes	21.67	10	−23.34
null model-v	Yes	−33.95	3	73.90
null model-a-v	Yes	−31.73	4	71.46
Expon. m3-v	Yes	8.30	5	−6.60
Expon. m3-av	Yes	14.93	6	−17.86
Expon. m3-abv	Yes	20.11	7	−26.22
Expon. m5-av	Yes	16.09	7	−18.18
Expon. m5-abv	Yes	20.61	8	−25.18
Hill m3-av	Yes	14.94	6	−18.18
Hill m3-abv	Yes	20.13	7	−25.18
Hill m5-av	Yes	16.09	7	−18.58
Hill m5-abv	Yes	20.59	8	−26.90
Inv.Expon. m3-av	Yes	15.29	6	−18.18
Inv.Expon. m3-abv	Yes	20.45	7	−25.06
Inv.Expon. m5-av	Yes	16.09	7	−18.30
Inv.Expon. m5-abv	Yes	20.53	8	−25.06
LN m3-av	Yes	15.15	6	−18.30
LN m3-abv	Yes	20.34	7	−26.68
LN m5-av	Yes	16.09	7	−18.18
LN m5-abv	Yes	20.55	8	−25.10

G.4.1.2. Estimated Model Parameters

EXP

estimate for var-f : 0.0241
estimate for var-m : 0.05204
estimate for a-f : 0.1886
estimate for a-m : 0.1857
estimate for CED-f : 29.13
estimate for CED-m : 14.93
estimate for d- : 0.7126

HILL

estimate for var-f : 0.02409
estimate for var-m : 0.05202
estimate for a-f : 0.1887
estimate for a-m : 0.1857
estimate for CED-f : 29.25
estimate for CED-m : 14.96
estimate for d- : 0.7176

INVEXP

estimate for var-f : 0.02399
estimate for var-m : 0.0514
estimate for a-f : 0.1901
estimate for a-m : 0.1852
estimate for CED-f : 33.77

estimate for CED-m : 16.29
estimate for d- : 0.1631

LOGN

estimate for var-f : 0.02402
estimate for var-m : 0.05163
estimate for a-f : 0.1895
estimate for a-m : 0.1854
estimate for CED-f : 31.86
estimate for CED-m : 15.77
estimate for d- : 0.2754

G.4.1.3. Weights for Model Averaging

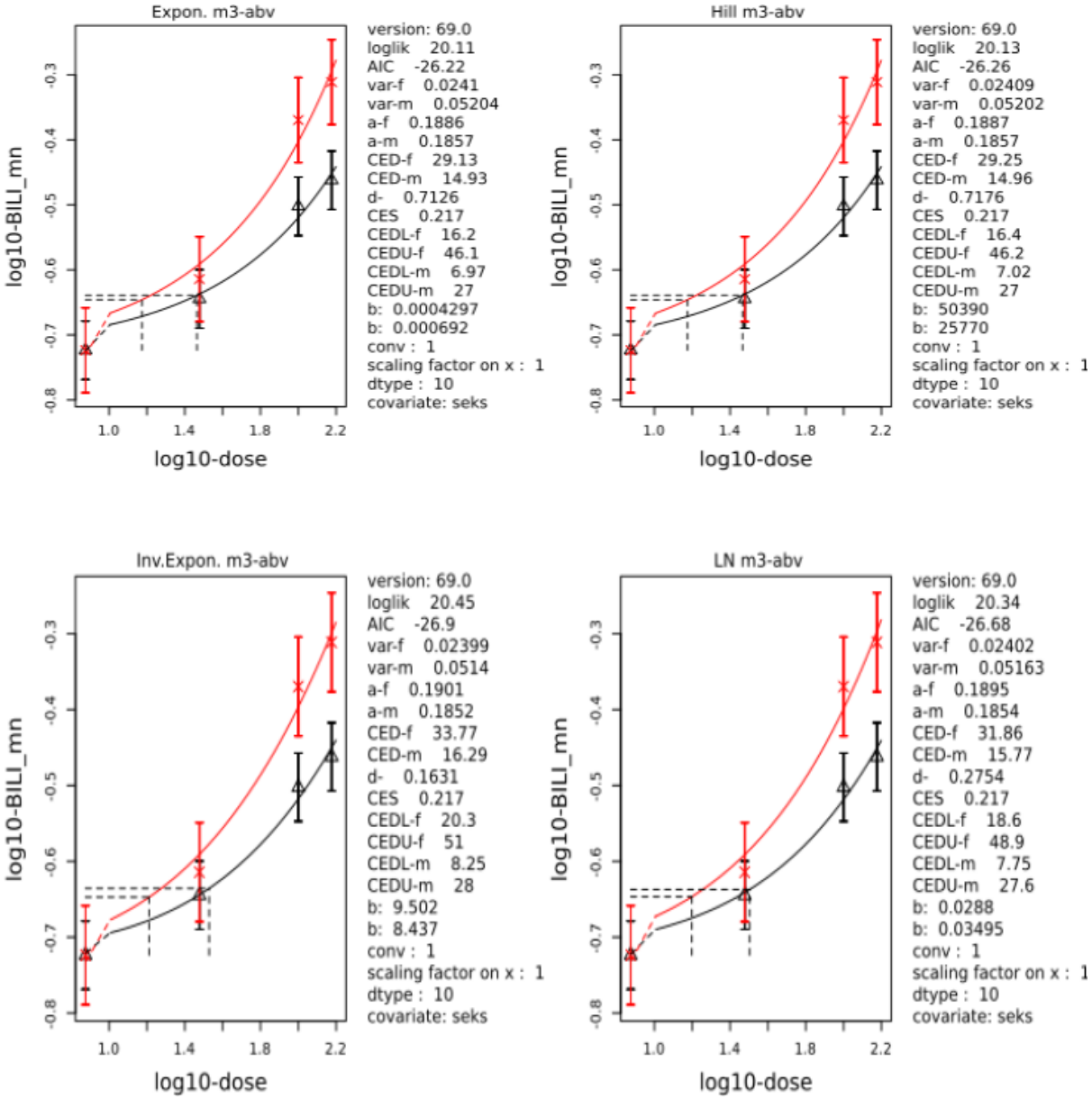
EXP	HILL	INVEXP	LOGN
0.21	0.22	0.3	0.27

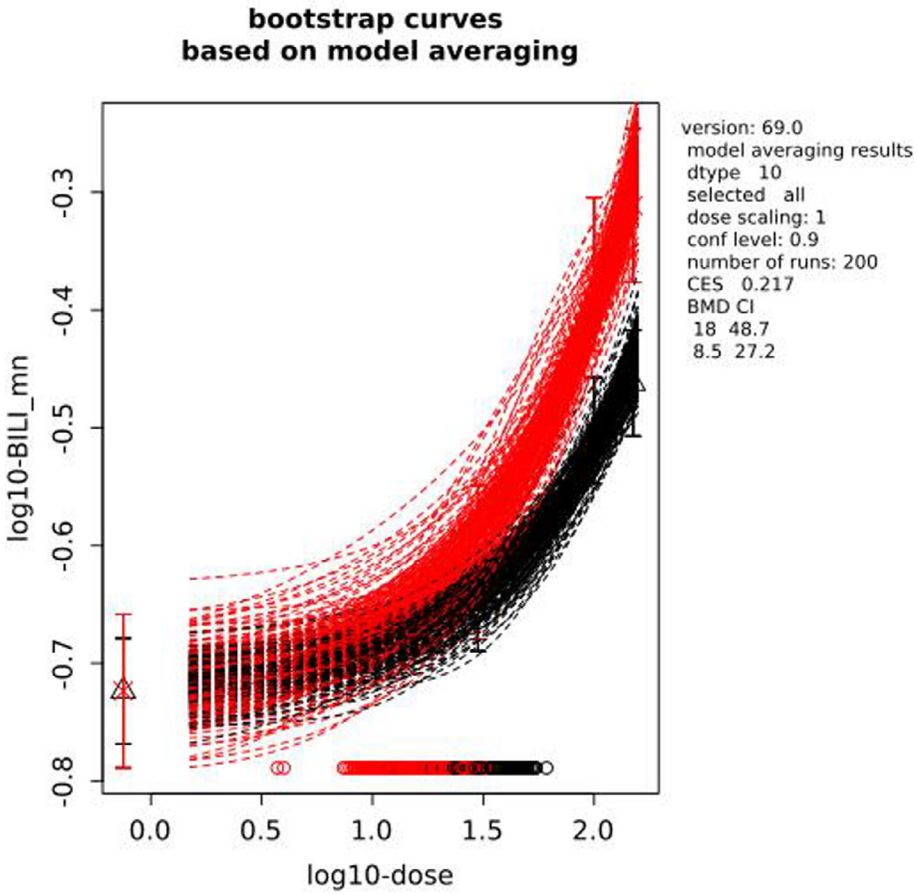
G.4.1.4. Final BMD Values

Endpoint	Subgroup	BMDL	BMDU
BILI_mn	f	18.30	48.7
BILI_mn	m	8.51	27.2

Confidence intervals for the BMD are based on 200 bootstrap data sets.

G.4.1.5. Visualization





G.5. Conclusions

The dose–response analysis for BILI resulted in a BMDL of 8.51 mg/kg bw per day based on an end-point-specific BMR of 22% increase. There is a high concordance between the results of the four underlaying models and the BMDL estimated is close to the lowest dose level in the study.

G.6. Overview of DR modelling results for all other parameters

Table G.1: BMDL–BMDU intervals for the haematological, clinical chemistry, urinalysis and organ and body weight parameters from the 90-day oral toxicity study with 2-pentylfuran. In the table also the respective endpoint-specific benchmark responses (rounded to 2 significant digits) have been specified

Parameter[1]	ES-BMR[2]	Males		Females	
		BMDL[3]	BMDU[3]	BMDL	BMDU
Haematology					
RBC		No trend		No trend	
HGB		No trend		No trend	
HCT	4.3 (d)	21.1	219	27.9	124
MCV	2.6 (d)	14	58.7	25.4	72.5
MCH	7.4 (d)	71.6	125	82.3	125
MCHC	1.4 (d)	38.2	115	38.3	109
RDW	4.4 (i)	73.7	179	87.3	146
PLT	11 (i)	14.7	108	14.6	106
ARET	18 (i)	Large model uncertainty		121	958

Parameter[1]	ES-BMR[2]	Males		Females	
		BMDL[3]	BMDU[3]	BMDL	BMDU
PTT	2.3 (i)	27.6	123	No trend	
Clinical Chemistry					
ALT	25 (d)	Large model uncertainty		Large model uncertainty	
AST		No trend		No trend	
SDH	60 (i)	42.3	141	97.9	4840
ALKP		No trend		No trend	
BILI	22 (i)	8.51	27.2	18.3	48.7
BUN		No trend		No trend	
CREA		No trend		No trend	
CHOL		No trend		No trend	
TRIG	29 (d)	1.87	143	No trend	
GLUC	11 (d)	23.2	109	Large model uncertainty	
TP	5.3 (i)	53.6	130	48.2	128
ALB	5.5 (i)	47.3	109	50.6	111
GLOB	6.2 (i)	No trend		78	143
CALC	3.6 (i)	131	1250	98.2	153
IPHS	8.7 (i)	132	152	132	1167
Na	1.3 (d)	41	1180	51.1	1120
K		No trend		No trend	
CL	1.7 (d)	64.6	331	40.4	126
Urinalysis					
UVOL	68 (i)	Large model uncertainty		Large model uncertainty	
pH		No trend		No trend	
SG	19 (d)	Large model uncertainty		Large model uncertainty	
UMTP	42 (d)	Large model uncertainty		Large model uncertainty	
URO	55 (i)	17	89.1	13.8	93.7
Body and organ weight changes					
bw	10 (d)	42.3	3400	56.9	452
lw-a	15 (i)	52.6	225	29.9	92.3
lw-r	8.7 (i)	30.4	77.3	21.3	43.2
spl-a		no trend		no trend	
spl-r	12 (i)	0.51	112	Large model uncertainty	

(1): Abbreviations of parameters: RBC: Red blood cell count; HGB: Plasma haemoglobin concentration; HCT: Haematocrit; MCV: Mean corpuscular volume; MCH: Mean corpuscular haemoglobin contents; MCHC: Mean corpuscular haemoglobin concentration; RDW: Red cell distribution width; PLT: Platelet count; ARET: Absolute reticulocytes count; PTT: Prothrombin time; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; SDH: Sorbitol dehydrogenase; ALKP: Alkaline phosphatase; BILI: Bilirubin; BUN: Blood urea nitrogen; CREA: Creatinine; CHOL: Cholesterol; TRIG: Triglycerides; GLUC: Glucose; TP: Total protein; ALB: Albumin; GLOB: Globulin; CALC: Calcium; IPHS: Inorganic phosphorous; Na: Sodium; K: Potassium; CL: Chloride; UVOL: Urinary volume; pH: Urinary pH; SG: Urinary specific gravity; UMTP: Urinary total protein; URO: Urobilinogen; Bw: Body weight; lw-a: Absolute liver weight; lw-r: Relative liver weight; spl-a: Absolute spleen weight; spl-r: Relative spleen weight.

(2): ES-BMR: endpoint-specific Benchmark Response (% change) (d) and (i): decrease or increase.

(3): BMDL and BMDU (Benchmark Dose lower and upper confidence interval (90% two-sided) limits); values are given in mg/kg bw per day. Large model uncertainty means that for the respective parameters BMDL-BMDU intervals were estimated, that were extremely wide (e.g. 6 or more orders of magnitude) or for which the individual models produce strongly deviating results.

G.7. Graphical representations of BMDL–BMDU intervals

In the graphs below, for the intervals with dotted lines the upper limit is beyond the extent of the horizontal axis. Missing lines and parameters indicate that for that parameter no reliable BMD confidence interval could be estimated for the respective sex. Note that the horizontal axis is logarithmic.

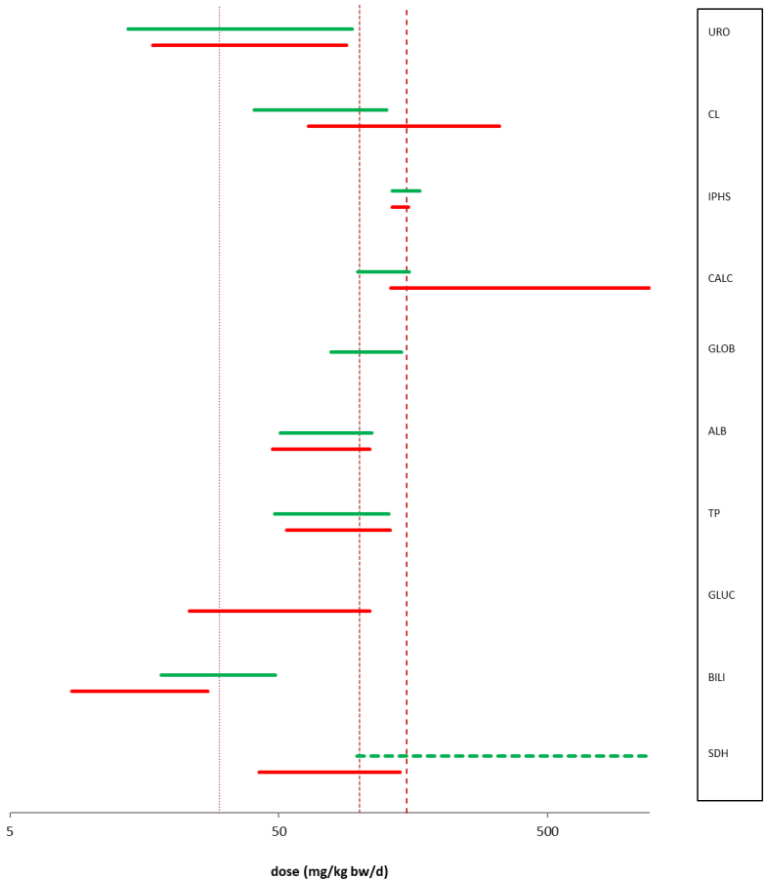


Figure G.2: Model-averaged BMD confidence intervals for clinical chemistry and urinalysis parameters. males: red lower line; females: green upper line. Note that BMRs are endpoint specific and differ for each parameter (see Table G.1). For the identification of the parameters (panel on the right), see the note to Table G.1

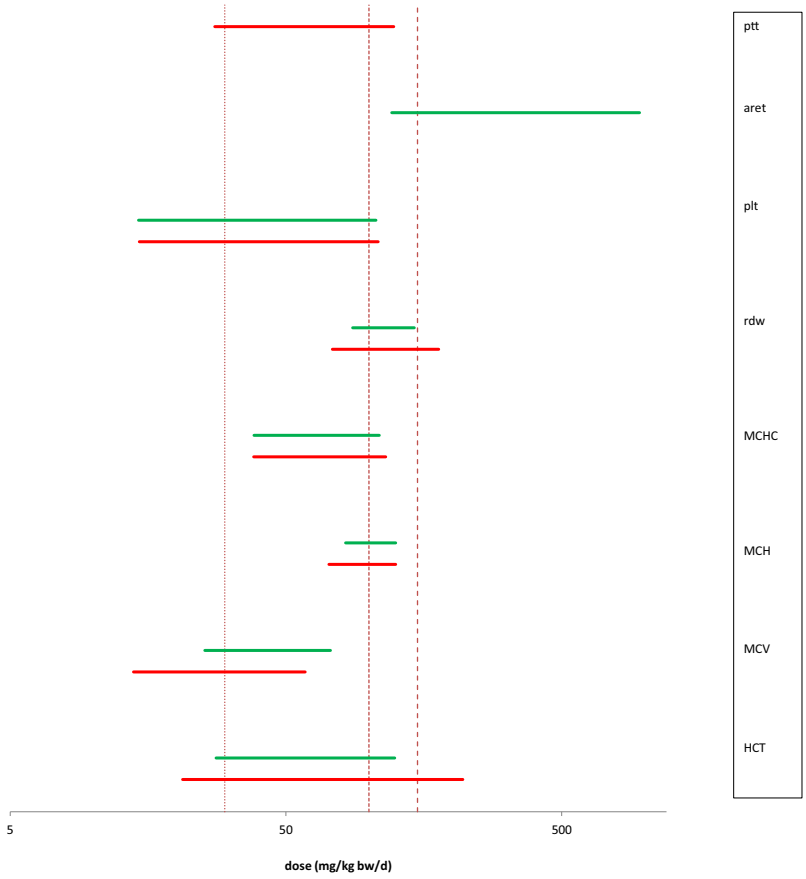


Figure G.3: Model-averaged BMD confidence intervals for haematological parameters. males: red lower line; females: green upper line. Note that BMRs are endpoint specific and differ for each parameter (see Table G.1). For the identification of the parameters (panel on the right), see the note to Table G.1

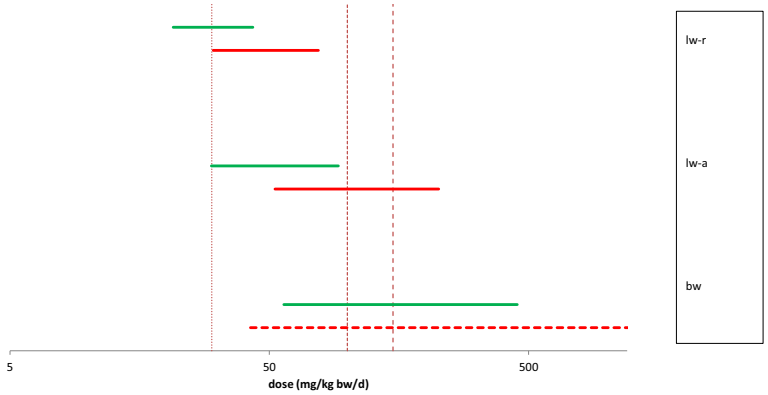
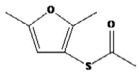
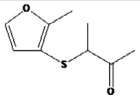
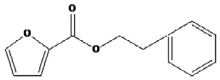
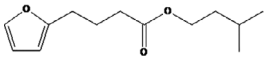
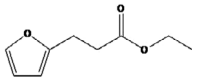


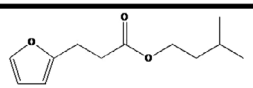
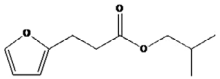
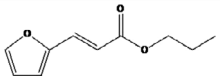
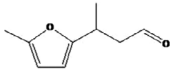
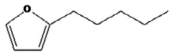
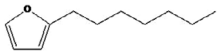
Figure G.4: Model-averaged BMD confidence intervals for liver weight (absolute and relative) and body weight. males: red lower line; females: green upper line. Note that BMRs are endpoint specific and differ for each parameter (see Table G.1). For the identification of the parameters (panel on the right), see the note to Table G.1

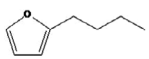
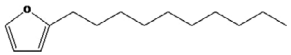
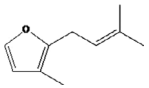
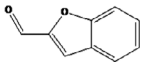
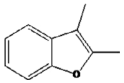
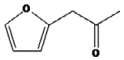
Appendix H – Summary of safety evaluations

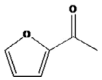
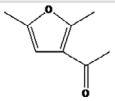
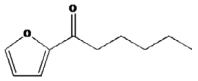
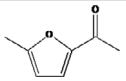
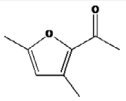
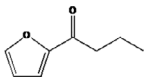
The conclusions for the substances in the various subgroups are summarized in Table H.1.

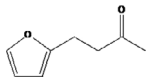
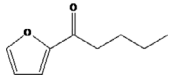
Table H.1: Summary of Safety Evaluation performed by JECFA and EFSA conclusions on flavouring substances in FGE.67Rev3

FL-no JECFA-no	EU Union List chemical name	Structural formula	JECFA conclusions **Class[a]** **Evaluation procedure path[b]** **Outcome on the named compound based on the MSDI/ SPET[c] approach**	EFSA conclusions **Procedural path if different from JECFA, Conclusion based on the MSDI[d],[e] approach on the named compound and on the material of commerce**
		Subgroup I		
13.116 1523	2,5-Dimethyl-3-thioacetoxyfuran		Class III 4: Intake below threshold	Class III B3: Intake below threshold B4: Adequate NOAEL exists. No safety concern concluded in FGE. 67
13.190 1525	3-((2-Methyl-3-furyl)thio)-2-butanone		Class III 4: Intake below threshold	Class III B3: Intake below threshold B4: Adequate NOAEL exists. No safety concern concluded in FGE.67Rev1
		Subgroup II		
13.006 1517	Phenethyl 2-furoate		Class III 4: Intake below threshold	Class III B3: Intake below threshold B4: Adequate NOAEL exists. No safety concern concluded in FGE.67
		Subgroup III		
13.021 1516	Isopentyl 4-(2-furan)butyrate		Class III 4: Intake above threshold, 5: Adequate NOAEL exists	Class III B3: Intake below threshold B4: Adequate NOAEL exists. No safety concern concluded in FGE.67
13.022 1513	Ethyl 3(2-furyl)propionate		Class III 4: Intake above threshold, 5: Adequate NOAEL exists	Class III B3: Intake below threshold B4: Adequate NOAEL exists. No safety concern concluded in FGE.67

FL-no JECFA-no	EU Union List chemical name	Structural formula	JECFA conclusions Class[a] Evaluation procedure path[b] Outcome on the named compound based on the MSDI/ SPET[c] approach	EFSA conclusions Procedural path if different from JECFA, Conclusion based on the MSDI[d],[e] approach on the named compound and on the material of commerce
13.023 1515	Isopentyl 3-(2-furan)propionate		Class III 4: Intake above threshold, 5: Adequate NOAEL exists	Class III B3: Intake below threshold B4: Adequate NOAEL exists. No safety concern concluded in FGE.67; Name in the Union List to be changed to isopentyl 3-(2-furyl)propionate
13.024 1514	Isobutyl 3-(2-furyl)propionate		Class III 4: Intake above threshold, 5: Adequate NOAEL exists	Class III B3: Intake below threshold B4: Adequate NOAEL exists. No safety concern concluded in FGE.67
13.047 1518	Propyl 3-(2-furyl)acrylate		Class III 4: Intake below threshold	Class III B3: Intake below threshold B4: Adequate NOAEL exists No safety concern concluded in FGE.67
13.058 1500	3-(5-Methyl-2-furyl) butanal		Class III 4: Intake below threshold	Class III B3: Intake below threshold B4: Adequate NOAEL exists. No safety concern concluded in FGE.67Rev2
		Subgroup IV		
13.059 1491	2-Pentylfuran		Class III 4: Intake above threshold, 5: Adequate NOAEL (30 mg/kg bw per day) exists	Class III B3: Intake below threshold, B4: Adequate BMDL[e] (8.51 mg/kg bw per day) exists No safety concern concluded in FGE.67Rev3
13.069 1492	2-Heptylfuran		Class III 4: Intake below threshold	Class III B3: Intake below threshold, B4: Adequate BMDL (8.51 mg/kg bw per day) exists No safety concern concluded in FGE.67Rev3

FL-no JECFA-no	EU Union List chemical name	Structural formula	JECFA conclusions Class[a] Evaluation procedure path[b] Outcome on the named compound based on the MSDI/ SPET[c] approach	EFSA conclusions Procedural path if different from JECFA, Conclusion based on the MSDI[d],[e] approach on the named compound and on the material of commerce
13.103 1490	2-Butylfuran		Class II No evaluation	No longer supported by industry (DG SANTE, 2020a)
13.106 1493	2-Decylfuran		Class III 4: Intake above threshold, 5: Adequate NOAEL (30 mg/kg bw per day) exists	Class III B3: Intake below threshold, B4: Adequate BMDL (8.51 mg/kg bw per day) exists. No safety concern concluded in FGE.67Rev3
13.148 1494	3-Methyl-2(3-methylbut-2-enyl)furan		Class III 4: Intake above threshold, 5: Adequate NOAEL (45 mg/kg bw per day) exists	Class III B3: Intake below threshold, B4: Adequate BMDL (8.51 mg/kg bw per day) exists. No safety concern concluded in FGE.67Rev3
		Subgroup V-B		
13.031 751	2-Benzofurancarboxaldehyde		Class III B3: Intake below threshold, B4: Adequate NOAEL exists (Evaluation according to previous JECFA Procedure)	Class III No safety concern concluded in FGE.67Rev1.
13.074 1495	2,3-Dimethylbenzofuran		Class III 4: Intake above threshold, 5: Adequate NOAEL exists	Class III B3: Intake below threshold, B4: Adequate NOAEL exists No safety concern concluded in FGE.67.
		Subgroup VI-B		
13.045 1508	1-(2-Furyl)-propan-2-one		Class III 4: Intake above threshold, 5: Adequate NOAEL (25 mg/kg bw per day) exists	Class III B3: Intake below threshold, B4: Adequate NOAEL (22.6 mg/kg bw per day) exists. No safety concern concluded in FGE.67Rev3

FL-no JECFA-no	EU Union List chemical name	Structural formula	JECFA conclusions Class[a] Evaluation procedure path[b] Outcome on the named compound based on the MSDI/ SPET[c] approach	EFSA conclusions Procedural path if different from JECFA, Conclusion based on the MSDI[d],[e] approach on the named compound and on the material of commerce
13.054 1503	2-Acetylfuran		Class III 4: Intake above threshold, 5: Adequate NOAEL (25 mg/kg bw per day) exists	Class III B3: Intake below threshold, B4: Adequate NOAEL[e] (22.6 mg/kg bw per day) exists. No safety concern concluded in FGE.67Rev3
13.066 1506	3-Acetyl-2,5-dimethylfuran		Class III 4: Intake above threshold, 5: Adequate NOAEL (10 mg/kg bw per day) exists	Substance no longer supported by industry (DG SANTE, 2020b)
13.070 1512	2-Hexanoylfuran		Class III 4: Intake below threshold	Class III B3: Intake below threshold, B4: Adequate NOAEL (22.6 mg/kg bw per day) exists. No safety concern concluded in FGE.67Rev3.
13.083 1504	2-Acetyl-5-methylfuran		Class III 4: Intake above threshold, 5: Adequate NOAEL (10 mg/kg bw per day) exists	Class III B3: Intake below threshold, B4: Adequate NOAEL (22.6 mg/kg bw per day) exists. No safety concern concluded in FGE.67Rev3.
13.101 1505	2-Acetyl-3,5-dimethylfuran		Class III 4: Intake above threshold, 5: Adequate NOAEL (10 mg/kg bw per day) exists	Class III B3: Intake below threshold, B4: Adequate NOAEL (22.6 mg/kg bw per day) exists. No safety concern concluded in FGE.67Rev3.
13.105 1507	2-Butyrylfuran		Class III 4: Intake above threshold, 5: Adequate NOAEL (25 mg/kg bw per day) exists	Class III B3: Intake below threshold, B4: Adequate NOAEL (22.6 mg/kg bw per day) exists. No safety concern concluded in FGE.67Rev3.

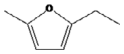
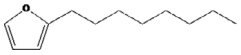
FL-no JECFA-no	EU Union List chemical name	Structural formula	JECFA conclusions Class[a] Evaluation procedure path[b] Outcome on the named compound based on the MSDI/ SPET[c] approach	EFSA conclusions Procedural path if different from JECFA, Conclusion based on the MSDI[d],[e] approach on the named compound and on the material of commerce
13.138 1510	1-(2-Furyl)butan-3-one		Class III 4: Intake above threshold, 5: Adequate NOAEL (30 mg/kg bw per day) exists	Class III B3: Intake below threshold, B4: Adequate NOAEL (22.6 mg/kg bw per day) exists. No safety concern concluded in FGE.67Rev3.
13.163 1509	2-Pentanoylfuran		Class III 4: Intake above threshold, 5: Adequate NOAEL (25 mg/kg bw per day) exists	Class III B3: Intake below threshold, B4: Adequate NOAEL (22.6 mg/kg bw per day) exists. No safety concern concluded in FGE.67Rev3.

FL-No: FLAVIS number; FGE: Flavouring Group Evaluation; JECFA: The Joint FAO/WHO Expert Committee on Food Additives; NOAEL: No observed adverse effect level; bw: body weight.
(a): Thresholds of concern: Class I = 1,800 μg/person per day, Class II = 540 μg/person per day, Class III = 90 μg/person per day.
(b): WHO technical Report Series 1014. Evaluation of certain food additives. Eighty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives.
(c): The highest intake estimate based on either the MSDI or SPET approach will be used in the comparison to the TTC.
(d): EU MSDI: Amount added to food as flavouring in (kg/year) × $10E^9$/(0.1 × population in Europe (= 375 × $10E^6$) × 0.6 × 365) = μg/capita per day.
(e): NOAEL or BMDL derived by EFSA see Section 3.5.

Appendix I – Summary of safety evaluations for structurally related substances from FGE.13Rev3

Only information related to the supporting substances relevant for the current evaluation is reported. Information on the full list of substances in FGE.13 can be retrieved in FGE.13Rev2 (EFSA CEF Panel, 2011c).

Table I.1: Summary of safety evaluation applying the Procedure for the structurally related substances in FGE.13Rev3

FL-no	EU Union List chemical name	Structural formula	MSDI[(a)] (μg/capita per day)	Class[(b)] Evaluation procedure path[(c)] Outcome on the named compound and on the material of commerce	EFSA comments
13.125	2-Ethyl-5-methylfuran		0.06	Class III B3: Intake below threshold B4: Adequate NOAEL exists No safety concern based on intakes calculated by the MSDI approach	Concluded in FGE.13Rev3
13.162	2-Octylfuran		0.12	Class III B3: Intake below threshold B4: Adequate NOAEL exists No safety concern based on intakes calculated by the MSDI approach	Concluded in FGE.13Rev3

FL-No: FLAVIS number; FGE: Flavouring Group Evaluation; MSDI: maximised survey-derived daily intake.
(a): EU MSDI: Amount added to food as flavour in (kg/year) × $10E^9$/(0.1 × population in Europe (= 375 × $10E^6$) × 0.6 × 365) = μg/capita per day.
(b): Thresholds of concern: Class I = 1,800 μg/person per day, Class II = 540 μg/person per day, Class III = 90 μg/person per day.
(c): Procedure path A, substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot.