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Scientific opinion on Flavouring Group Evaluation 313, (FGE.313): α,β-unsaturated 3(2*H*)-furanone derivatives from chemical group 13

EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF)

Abstract

The Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids of EFSA was requested to evaluate three flavouring substances, 2,5-dimethyl-4-ethoxyfuran-3(2*H*)-one [FL-no: 13.117], 2,5-dimethylfuran-3(2*H*)-one [FL-no: 13.119] and 4-Acetyl-2,5-dimethylfuran-3(2*H*)-one [FL-no: 13.175] in the Flavouring Group Evaluation 313 (FGE.313), using the Procedure in Commission Regulation (EC) No 1565/2000. The substances were considered in FGE.220, and revisions hereof, not to have genotoxic potential. They were evaluated through a stepwise approach that integrates information on the structure–activity relationships, intake from current uses, toxicological threshold of concern, and available data on metabolism and toxicity. The Panel concluded that the two flavouring substances [FL-no: 13.117, 13.119] do not give rise to safety concerns at their level of dietary intake, estimated on the basis of the Maximised Survey-derived Daily Intake (MSDI) approach. For the flavouring substance [FL-no: 13.175], toxicity data are required. Besides the safety assessment of the flavouring substance, the specifications for the materials of commerce have also been considered. Adequate specifications including complete purity criteria and identity for the materials of commerce have been provided for the three flavouring substances. The Panel concluded that for 2,5-dimethyl-4-ethoxyfuran-3(2*H*)-one [FL-no: 13.117], 2,5-dimethylfuran-3(2*H*)-one [FL-no: 13.119] and 4-acetyl-2,5-dimethylfuran-3(2*H*)-one [FL-no: 13.175] for which the Modified Theoretical Added Maximum Daily Intakes (mTAMDIs) are above the thresholds for their structural class, more reliable exposure data are required for a re-evaluation.

Keywords: flavouring, food safety, α,β-unsaturated 3(2*H*)-furanone derivatives, FGE.220, FGE.313, FGE.99

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Summary

Following a request from the European Commission, the European Food Safety Authority (EFSA) Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF Panel) was asked to deliver a scientific opinion on the implications for human health of chemically defined flavouring substances used in or on foodstuffs in the Member States. In particular, the Panel was requested to evaluate three flavouring substances in the Flavouring Group Evaluation 313 (FGE.313), using the Procedure referred to in Commission Regulation (EC) No 1565/2000 '(hereinafter "the Procedure")'. These flavouring substances belong to chemical group 13 of Annex I of the Commission Regulation (EC) No 1565/2000.

The three candidate substances under evaluation are α,β-unsaturated 3(2*H*)-furanone derivatives from chemical group 13: 2,5-dimethyl-4-ethoxyfuran-3(2*H*)-one [FL-no: 13.117], 2,5-dimethylfuran-3(2*H*)-one [FL-no: 13.119] and 4-Acetyl-2,5-dimethylfuran-3(2*H*)-one [FL-no: 13.175].

All three substances possess one chiral centre and their stereoisomeric composition has been specified.

The three candidate substances were assigned to structural class III, according to the decision tree approach presented by Cramer et al., 1978.

[FL-no: 13.117 and 13.119] have been reported to occur naturally in food.

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

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

According to the default MSDI approach, the candidate substances in this group have intakes in Europe from 0.018 to 1.7 μg/capita per day. For all the candidate substances, this is below the threshold of concern value for structural class III (90 μg/person per day). On the basis of the reported annual production volumes in Europe (EFFA, 2004b), the estimated combined daily per capita intake as flavourings of the three candidate substances assigned to structural class III is approximately 3 μg, which does not exceed the threshold of concern for a compound belonging to structural class III of 90 μg/person per day.

The Panel concluded that the two candidate substances for which genotoxicity data were submitted (2,5-dimethylfuran-3(2*H*)-one [FL-no: 13.119] and 4-acetyl-2,5-dimethylfuran-3(2*H*)-one [FL-no: 13.175]) do not give rise to concern with respect to genotoxicity. The same conclusion applies to 2,5-dimethyl-4-ethoxyfuran-3(2*H*)-one [FL-no: 13.117], which is structurally related with respect to the α,β-unsaturated carbonyl moiety. Therefore, these three substances can be evaluated using the Procedure.

The candidate substance [FL-no: 13.117] is expected to be metabolised to innocuous products. Therefore, [FL-no: 13.117] was evaluated via the A-side of the Procedure. [FL-no: 13.119 and 13.175] cannot be anticipated to be metabolised to innocuous products. Consequently, these two substances were evaluated via the B-side of the Procedure.

It is considered that the two candidate substances 2,5-dimethyl-4-ethoxyfuran-3(2*H*)-one [FL-no: 13.117] and 2,5-dimethylfuran-3(2*H*)-one [FL-no: 13.119] evaluated through the Procedure would not give rise to safety concerns at the estimated levels of intake arising from their use as flavouring substances on the basis of the default MSDI approach. Additional toxicity data are required for 4-acetyl-2,5-dimethylfuran-3(2*H*)-one [FL-no: 13.175].

In order to determine whether the conclusion for the candidate substances can be applied to the materials of commerce, it is necessary to consider the available specifications.

Adequate specifications including complete purity criteria and identity for the material of commerce have been provided for the flavouring substances.

The estimated intake based on the mTAMDI approach is 1,000 μg/person per day for each of the candidate substances from structural class III, which is above the threshold of concern for structural class III of 90 μg/person per day. The Panel concluded that for 2,5-dimethyl-4-ethoxyfuran-3(2*H*)-one [FL-no: 13.117], 2,5-dimethylfuran-3(2*H*)-one [FL-no: 13.119] and 4-acetyl-2,5-dimethylfuran-3(2*H*)-one [FL-no: 13.175] for which the mTAMDIs are above the thresholds for their structural class, more reliable exposure data are required. On the basis of such additional data, these flavouring substances should be reconsidered using the Procedure. Subsequently, additional toxicity data might become necessary. For the candidate substance [FL-no: 13.175], toxicity data are required.

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1. Introduction

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

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].

The European Food Safety Authority (EFSA) concluded that a genotoxic potential of five 3(2*H*)-furanones, corresponding to subgroup 4.4a of Flavouring Group Evaluation 19 (FGE.19) in FGE.220Rev1, could not be ruled out.

Information on two representative materials has now been submitted by the European Flavour Association. These are 2,5-dimethylfuran-3(2*H*)-one [FL-no: 13.119] and 4-acetyl-2,5-dimethylfuran-3(2*H*)-one [FL-no: 13.175]. This information is intended to cover the re-evaluation of these two substances and of two substances from FGE.19 subgroup 4.4a: 2,5-dimethyl-4-methoxyfuran-3(2*H*)-one [FL-no: 13.089] and 2,5-dimethyl-4-ethoxyfuran-3(2*H*)-one [FL-no: 13.117].

1.1.1. Terms of Reference as provided by the European Commission

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

1.2. Interpretation of the Terms of Reference

The present scientific opinion FGE.313 covers the safety assessment of two α,β-unsaturated 3(2*H*)-furanone derivatives from chemical group 13: the 2,5-dimethylfuran-3(2*H*)-one [FL-no: 13.119] and 2,5-dimethyl-4-ethoxyfuran-3(2*H*)-one [FL-no: 13.117].

2. Assessment

2.1. Presentation of the substances in Flavouring Group Evaluation 313

2.1.1. Description

The present FGE.313, using the Procedure as referred to in the Commission Regulation (EC) No 1565/2000 (the Procedure – shown in schematic form in Appendix B of this FGE), deals with three flavouring substances (candidate substances) from chemical group 13 of Annex I of Commission Regulation (EC) No 1565/2000[3].

The candidate substances under consideration in the present evaluation, with their chemical Register name, FLAVIS- (FL-), Chemical Abstract Service- (CAS-), Council of Europe- (CoE-) and Flavor and Extract Manufacturers Association (FEMA) numbers, and structures are listed in Table 1.

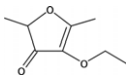
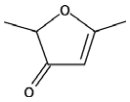
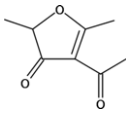
The outcome of the safety evaluation is summarised in Table A.1.

[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.

Table 1: Specification summary of the substances in the Flavouring Group Evaluation 313

FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys. form Mol. formula Mol. weight	Solubility[a] Solubility in ethanol[b]	Boiling point, °C[c] Melting point, °C ID test Assay minimum	Refrac. index[d] Spec. gravity[e]	Specification comments
13.117	2,5-Dimethyl-4-ethoxyfuran-3(2*H*)-one		65330-49-6	Solid $C_8H_{12}O_3$ 156.18	Practically insoluble or insoluble Freely soluble	251 60 MS 95%	n.a. n.a.	Racemate (EFFA, 2015)
13.119	2,5-Dimethylfuran-3(2*H*)-one		11066 14400-67-0	Liquid $C_6H_8O_2$ 112.13	Practically insoluble Freely soluble	68 (16 hPa) IR NMR MS 95%	1.473–1.479 1.050–1.060	Racemate (EFFA, 2013)
13.175	4-Acetyl-2,5-dimethylfuran-3(2*H*)-one			Solid $C_8H_{10}O_3$ 154.17	Very slightly soluble Freely soluble	283 34 NMR MS 95%	n.a. n.a.	Racemate (EFFA, 2015). CASrn 36871-78-0 to be introduced in the Register

FL-no: FLAVIS-number; JECFA-no: The Joint FAO/WHO Expert Committee on Food Additives number; EU: European Union; FEMA no: Flavor and Extract Manufacturers Association number; CoE no: Council of Europe number; CAS no: Chemical Abstract Service number; ID: identity; MS: mass spectrometry; IR: infrared spectroscopy; NMR: nuclear magnetic resonance.
(a): Solubility in water, if not otherwise stated.
(b): Solubility in 95% ethanol, if not otherwise stated.
(c): At 1,013.25 hPa (1 atm), if not otherwise stated.
(d): At 20°C, if not otherwise stated.
(e): At 25°C, if not otherwise stated.

All three candidate substances are α,β-unsaturated 3(2*H*)-furanones allocated to FGE.220 or the following revisions. In FGE.220Rev2 (EFSA CEF Panel, 2013), one of the candidate substances, 2,5-dimethylfuran-3(2*H*)-one [FL-no: 13.119] was evaluated and it was considered not to be of concern with respect to genotoxicity. The two other candidate substances were considered not to be of concern with respect to genotoxicity based on new data considered in FGE.220Rev3 (EFSA CEF Panel, 2015a). The candidate substances in this FGE are structurally related to furanone derivatives which have previously been considered by EFSA in FGE.99 and FGE.99Rev1 (EFSA CEF Panel, 2015b).

2.1.2. Stereoisomers

It is recognised that geometrical and optical isomers of substances may have different properties. Their flavour may be different; they may have different chemical properties resulting in possible variability in their absorption, distribution, metabolism, elimination and toxicity. Thus, information must be provided on the configuration of the flavouring substance, i.e. whether it is one of the geometrical/optical isomers, or a defined mixture of stereoisomers. The available specifications of purity will be considered in order to determine whether the safety evaluation carried out for candidate substances for which stereoisomers may exist can be applied to the material of commerce. Flavouring substances with different configurations should have individual chemical names and codes (CAS number, FLAVIS number, etc.).

All three candidate substances, possess one chiral centre and the stereoisomeric composition has been specified (EFFA, 2013, 2015) (see Table 1).

2.1.3. Natural occurrence in food

According to TNO (TNO, 2015), two of the three candidate substances have been reported to occur in coffee, guava, mango, passion fruit, wheaten bread and wild rice. Quantitative data on the natural occurrence in food have been reported for both of these substances (see Table 2).

4-Acetyl-2,5-dimethylfuran-3(2*H*)-one [FL-no: 13.175] has not been reported to occur naturally in food (TNO, 2015).

Table 2: Candidate substance reported to occur in food (TNO, 2015)

FL-no	Name	Quantitative data reported
13.117	2,5-Dimethyl-4-ethoxyfuran-3(2*H*)-one	Up to 8 mg/kg in coffee[(a)]
13.119	2,5-Dimethylfuran-3(2*H*)-one	10.7 mg/kg in coffee[(a)]

FL-no: FLAVIS-number.
(a): The database refers to 'coffee' but it is not clear whether this reflects to coffee beans or to the beverage.

2.2. Specifications

Purity criteria for the candidate substance have been provided by the Flavour Industry (EFFA, 2015) (Table 1).

Judged against the requirements in Annex II of Commission Regulation (EC) No 1565/2000[3], this information is adequate for the candidate substances (see Section 2.1.2 and Table 1).

2.3. Intake data

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

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

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

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

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

One option to modify the TAMDI approach is to base the calculation on normal rather than upper use levels of the flavouring substances. This modified approach is less conservative (e.g. it may underestimate the intake of consumers being loyal to products flavoured at the maximum use levels reported).[3] However, it is considered as a suitable tool to screen and prioritise the flavouring substances according to the need for refined intake data (EFSA, 2004).

2.3.1. Estimated daily per capita intake (MSDI approach)

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

Average per capita intake (MSDI) is estimated on the assumption that the amount added to food is consumed by 10% of the population (Eurostat, 1998).[4] This is derived for candidate substances from

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

estimates of annual volume of production provided by Industry and incorporates a correction factor of 0.6 to allow for incomplete reporting (60%) in the Industry surveys (SCF, 1999).

The total annual volume of production of the three candidate substances in the present FGE.313 from use as flavouring substance in Europe has been reported to be approximately 25 kg (EFFA, 2004b, 2015).

On the basis of the annual volume of production reported for the candidate substances, the daily per capita intakes have been estimated. The estimated daily per capita intake from use as a flavouring substance is 0.018 μg for 2,5-Dimethyl-4-ethoxyfuran-3(2*H*)-one [FL-no: 13.117]; 1.7 μg for 2,5-dimethylfuran-3(2*H*)-one [FL-no: 13.119] and 1.3 μg for 4-Acetyl-2,5-dimethylfuran-3(2*H*)-one [FL-no: 13.175] (Table 4).

2.3.2. Intake estimated on the basis of the modified TAMDI (mTAMDI)

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

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

For the three candidate substances, information on food categories and normal and maximum use levels[5,6] was submitted by the Flavour Industry (EFFA, 2004b). The candidate substance is used in flavoured food products divided into the food categories outlined in Annex III of the Commission Regulation (EC) No 1565/2000[3], as shown in Table 3. For the present calculation of mTAMDI, the reported normal use levels were used. In the case where different use levels were reported for different food categories, the highest reported normal use level was used.

Table 3: Use of the candidate substances

Food category	Description	Flavouring used
01.0	Dairy products, excluding products of category 2	Yes
02.0	Fats and oils, and fat emulsions (type water-in-oil)	Yes
03.0	Edible ices, including sherbet and sorbet	Yes
04.1	Processed fruits	Yes
04.2	Processed vegetables, including mushrooms & fungi, roots & tubers, pulses and legumes, and nuts & seeds	No
05.0	Confectionery	Yes
06.0	Cereals and cereal products, including flours & starches from roots & tubers, pulses & legumes, excluding bakery	Yes
07.0	Bakery wares	Yes
08.0	Meat and meat products, including poultry and game	Yes
09.0	Fish and fish products, including molluscs, crustaceans and echinoderms	Yes
10.0	Eggs and egg products	No
11.0	Sweeteners, including honey	No
12.0	Salts, spices, soups, sauces, salads, protein products, etc.	Yes
13.0	Foodstuffs intended for particular nutritional uses	Yes
14.1	Non-alcoholic ('soft') beverages, excluding dairy products	No
14.2	Alcoholic beverages, including alcohol-free and low-alcoholic counterparts	Yes
15.0	Ready-to-eat savouries	Yes
16.0	Composite foods (e.g. casseroles, meat pies, mincemeat) – foods that could not be placed in categories 1–15	Yes

[5] 'Normal use' is defined as the average of reported usages and 'maximum use' is defined as the 95th percentile of reported usages (EFFA, 2002).

[6] The normal and maximum use levels in different food categories[3] have been extrapolated from figures derived from 12 model flavouring substances (EFFA, 2004).

According to the Flavour Industry, the normal use levels for the candidate substance are in the range of 1–5 mg/kg food, and the maximum use levels are in the range of 5–25 mg/kg (EFFA, 2004b) (see Table D.3, Appendix C).

The mTAMDI value is 1,000 μg/person per day for each of the candidate substances from structural class III.

For detailed information on use levels and intake estimations based on the mTAMDI approach, see Section 5 and Appendix C.

3. Absorption, distribution, metabolism and elimination

In FGE.99Rev1, EFSA was in agreement with the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (JECFA, 2006, 63rd meeting, FAS 54) evaluation of the substances 4-hydroxy-2,5-dimethyl-furan-3(2*H*)-one [FL-no: 13.010] and 2,5-dimethyl-4-methoxyfuran-3(2*H*)-one [FL-no: 13.089] in taking them via the A-side of the Procedure. 4-Hydroxy-2,5-dimethyl-furan-3(2*H*)-one [FL-no: 13.010] is expected to form a glucuronic acid conjugate, and to be excreted via the urine. The ether bond of 2,5-dimethyl-4-methoxyfuran-3(2*H*)-one [FL-no: 13.089] is expected to be readily cleaved. The formed hydroxy-furanone can subsequently be conjugated with glucuronic acid and excreted in the urine. In accordance with this, it is expected that the structurally similar candidate substance 2,5-dimethyl-4-ethoxyfuran-3(2*H*)-one [FL-no:13.117], evaluated in this FGE.313, will be oxidised, conjugated with glucuronic acid and excreted in the urine, and may be evaluated via the A-side of the Procedure.

For the two candidate substances, 2,5-dimethylfuran-3(2*H*)-one [FL-no: 13.119] and 4-acetyl-2,5-dimethylfuran-3(2*H*)-one [FL-no: 13.175], the metabolic fate is unclear, and accordingly it cannot be anticipated that these substances will be metabolised to innocuous products.

4. Application of the Procedure for the safety evaluation of flavouring substances

The application of the Procedure is based on intakes estimated on the basis of the MSDI approach. Where the mTAMDI approach indicates that the intake of a flavouring substance might exceed its corresponding threshold of concern, a formal safety assessment is not carried out using the Procedure. In these cases, the Panel requires more precise data on use and use levels. For comparison of the intake estimations based on the MSDI approach and the mTAMDI approach, see Section 5.

For the safety evaluation of the three candidate substances from chemical group 13, the Procedure as outlined in Appendix B was applied, based on the MSDI approach. The stepwise evaluation of the substance is summarised in Table A.1.

Step 1

The three candidate substances are all classified according to the decision tree approach by Cramer et al. (1978) into structural class III.

Step 2

One candidate substance 2,5-dimethyl-4-ethoxyfuran-3(2*H*)-one [FL-no:13.117] is expected to be metabolised to innocuous products, and the evaluation proceeds via the A-side of the Procedure.

The two candidate substances, 2,5-dimethylfuran-3(2*H*)-one [FL-no:13.119] and 4-acetyl-2,5-dimethylfuran-3(2*H*)-one [FL-no:13.175], cannot be anticipated to be metabolised to innocuous products and thus the evaluation proceeds via the B-side of the Procedure scheme.

Step A3

The estimated daily per capita intake of the candidate substance 2,5-dimethyl-4-ethoxyfuran-3(2*H*)-one [FL-no:13.117] is 0.018 μg which is below the threshold for its structural class of 90 μg/person per day (class III). Accordingly, the substance is not expected to be of safety concern.

Step B3

The estimated daily per capita intake of the candidate substances, 2,5-dimethylfuran-3(2*H*)-one [FL-no: 13.119] and 4-acetyl-2,5-dimethylfuran-3(2*H*)-one [FL-no: 13.175], are 1.7 and 1.3 μg, respectively, which are below the threshold for their structural class of 90 μg/person per day (class III). Accordingly, the evaluation of the substances proceeds to step B4 of the Procedure.

Step B4

For one of these substances, 2,5-dimethylfuran-3(2*H*)-one [FL-no: 13.119], a margin of safety could be calculated based upon a $BMDL_{05}$ of 0.94 mg/kg body weight (bw) per day for a decrease in total white blood cell count derived from a 90-day oral gavage study (see Section 7 and Appendix F). Compared to the MSDI of 1.7 μg/capita per day equal to 0.028 μg/kg bw or 0.000028 mg/kg bw per day for this compound, this lower 95% confidence limit (one-sided) for the BMD (BMDL) provides an adequate margin of safety of 33×10^3.

Therefore, the Panel concluded that 2,5-dimethylfuran-3(2*H*)-one is not of safety concern based on the MSDI approach.

For the candidate substance 4-acetyl-2,5-dimethylfuran-3(2*H*)-one [FL-no: 13.175], no adequate study from which a no observed adverse effect level (NOAEL) or BMDL could be derived was available neither for the candidate substance nor for a sufficiently structurally related substance. Therefore, the Panel concluded that additional data are required for the candidate substance 4-acetyl-2,5-dimethylfuran-3(2*H*)-one [FL-no: 13.175].

5. Comparison of the intake estimations based on the MSDI approach and the mTAMDI approach

The estimated intake of each of the candidate substances assigned to structural class III, based on the mTAMDI, is 1,000 μg/person per day, which is above the threshold of concern for structural class III of 90 μg/person per day.

Thus, for all three candidate substance, further information is required. This would include more reliable intake data and then, if required, additional toxicological data.

For comparison of the MSDI and mTAMDI values, see Table 4.

The estimated intake of each of the candidate substances assigned to structural class III, based on the mTAMDI, is 1,000 μg/person per day, which is above the threshold of concern for structural class III of 90 μg/person per day.

Thus, for all three candidate substance further information is required. This would include more reliable intake data and then, if required, additional toxicological data.

Table 4: Estimated intakes based on the MSDI approach and the mTAMDI approach

FL-no	EU Register name	MSDI (μg/capita per day)	mTAMDI (μg/person per day)	Structural class	Threshold of concern (μg/person per day)
13.119	2,5-Dimethylfuran-3(2*H*)-one	1.7	1,000	Class III	90
13.117	2,5-Dimethyl-4-ethoxyfuran-3(2*H*)-one	0.018	1,000	Class III	90
13.175	4-Acetyl-2,5-dimethylfuran-3(2*H*)-one	1.3	1,000	Class III	90

MSDI: Maximised Survey-derived Daily Intake; mTAMDI: Modified Theoretical Added Maximum Daily Intake; FL-no: FLAVIS number; EU: European Union.

6. Considerations of combined intakes from use as flavouring substances

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

On the basis of the reported annual production volumes in Europe (EFFA, 2004b, 2015), the estimated combined daily per capita intake as flavourings of the three candidate substances assigned to structural class III is approximately 3 μg, which does not exceed the threshold of concern for a compound belonging to structural class III of 90 μg/person per day.

The three candidate substances are structurally related to five supporting substances (structural class III) considered by EFSA in FGE.99 Revision 1 (EFSA CEF Panel, 2015b). Based on reported production volumes, European per capita intakes (MSDI) could be estimated for all five supporting substances.

The total combined intake of the candidate and supporting substances belonging to structural class III is approximately 1,540 μg/capita per day, which exceeds the threshold of concern for a compound belonging to structural class III of 90 μg/person per day. The main contribution to this MSDI originates from three supporting substances, 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one [FL-no: 13.010], ethyl-4-hydroxy-5-methyl-3(2*H*)-furanone [FL-no: 13.084] and acetoxy-2,5-dimethylfuran-3(2*H*)-one [FL-no: 13.099] for which the estimated daily per capita intake is 1,520 μg, of which approximately 60% comes from [FL-no: 13.010] for which a NOAEL of 200 mg/kg bw per day was determined in a chronic toxicity study (Kelly and Bolte, 2003). This provides an adequate margin of safety for the combined exposure of 8,000.

7. Toxicity

7.1. Acute toxicity

No data are available for the candidate substances.

7.2. Subacute, subchronic, chronic and carcinogenicity studies

7.2.1. 2,5-dimethylfuran-3(2*H*)-one [FL-no: 13.119]

7.2.1.1. 14-day dietary range-finding study

In a preliminary palatability and range-finding study, 2,5-dimethylfuran-3(2*H*)-one [FL-no: 13.119] was administered to male and female CRL Sprague–Dawley® CD®IGS rats (5/sex per group) for 14 consecutive days in the diet at dietary concentrations of 3,000, 6,000 and 12,000 mg/kg for target dose levels of 250, 500 and 1,000 mg/kg bw per day (Bauter, 2015a). Homogeneity analysis showed that the substance was homogeneously distributed in the feed. Stability analysis was performed on diets sampled on 0, 4, 7 and 10 days after preparation and revealed considerable loss of 2,5-dimethylfuran-3(2*H*)-one, resulting in dietary levels that were 47.5%, 46.1% and 42.9% of the target dietary concentrations on average over the course of the study. Based on the results of stability analysis and on feed consumption data, the mean daily intake was calculated to be 125, 210 and 329 mg/kg bw per day, respectively, for males, and 138, 239 and 366 mg/kg bw per day, respectively, for females, over the course of the study.

There were no mortalities during this study. Feed consumption was significantly reduced over the course of the study at all intake levels, and resulted in decreased overall body weights and body weight gains in all treatment groups. Clinical observations included slightly thin appearance in one high-dose female and reduced faecal volume in all high-dose animals, both considered to be associated with reduced feed consumption. No other clinical observations were reported or any abnormal macroscopic findings at scheduled necropsy attributable to administration of 2,5-dimethylfuran-3(2*H*)-one.

Due to the observed instability in the feed matrix and lack of palatability, dietary administration of 2,5-dimethylfuran-3(2*H*)-one was not considered feasible for a longer duration study. The dose levels for the 90-day follow-up study (see below) were based on the adjusted mean daily intakes for male and female rats in the low-dose group of approximately 125 and 138 mg/kg bw per day in this 14-day range-finding study, respectively (Bauter, 2015a).

7.2.1.2. 90-day oral gavage study

In an OECD Section 4 (part 408) compliant subchronic toxicity study, 2,5-dimethylfuran-3(2*H*)-one [FL-no: 13.119] was administered to Sprague–Dawley Crl:CD (SD) IGS BR strain rats by oral gavage (10/sex per dose) at dose levels of 15, 45 and 135 mg/kg bw per day for 90 days (Bauter, 2015b). Administration of the test substance by oral gavage was selected in this study in the light of significant

instability in the feed and lack of palatability. Test suspensions were prepared daily, were kept on a stir plate during dosing and were used within 2 h. Samples of the neat test substance were taken at the beginning, middle and end of the study, and were found to be stable. Samples of the dosing preparations were taken on days 1, 43 and 93 of the study to verify dosing concentrations, and were found to be between 95.1% and 109.6% of the target concentrations of 0.3%, 0.9% and 2.7% of test substance in vehicle (distilled water).

Animals were observed during daily handling, and individual detailed clinical observations were made weekly. Very few observations have been recorded from the weekly detailed clinical observations, and none of these give any indication of being related to dosage and/or length of treatment. However, observations made during daily handling have been recorded that may give reason to assume a dose–effect relationship. In particular, during daily handling, hypersalivation has been recorded in the mid-dose and the high-dose groups, the first occasions on day 8 of the study and the last on day 72. Notations of hypersalivation in males and females of the high-dose group are about four times more frequent than in the mid-dose group; 14 separate observations have been made in the mid-dose group (in 6/10 males and in 4/10 females) and 57 separate observations in the high-dose group (in 8/10 males and 7/10 females). Contrary to the observations during daily handling, only two observations of hypersalivation have been made during the weekly individual detailed clinical observations; both of these on day 8 of the study; one in a female of the mid-dose group and one in a female of the high-dose group.

The Panel noted that most of the notations of salivation made during daily handling have been made on days when the animals were not subject to detailed clinical observation. Ten of the daily handling notations of salivation have been made on days when the animals indeed were also subjected to detailed clinical observations, but for these 10 observations no recordings were mentioned in the report of the detailed clinical observations. This might suggest that the salivation was of short duration otherwise it is difficult to explain this discrepancy. The study authors argued that as findings of hypersalivation was resolved by day 72 and had no histopathological correlate, hypersalivation was not an adverse effect. However, given that a dose–effect relationship exists for hypersalivation and no hypersalivation was observed in the control and low-dose groups, this effect cannot be explained by handing or gavage dosing and must be substance related. Therefore, the Panel decided not to disregard this observation.

Changes in haematology parameters included statistically significant ($p < 0.05$, Dunnett's two-sided or Dunnett's non-parametric two-sided test) decreases in total white blood cell counts and neutrophils, in males only, of the mid- and high-dose groups, with the decrease in white blood cell counts being slightly more severe in the mid-dose group. Other statistically significant changes included, decreased absolute lymphocyte, eosinophil and basophil counts in males at mid-dose only. As the neutrophils are part of the total white blood cell counts, the latter was submitted for BenchMark Dose (BMD) analysis.[7] In the high-dose females, an increased absolute reticulocyte count was observed (28% up) but without any other major changes in red blood cells or bone marrow cellularity, this observation in females only is without biological relevance. There were no statistically significant changes in coagulation parameters.

Statistically significant changes in clinical chemistry parameters included increases in alkaline phosphatase (~ 28%) and total bilirubin (~ 40%) in high-dose group males and decreased aspartate aminotransferase (~ 28%) in high-dose group females, which latter change has no toxicological relevance. Changes reaching statistical significance were observed in males only in some clinical chemistry parameters (increased cholesterol, total protein, albumin, Ca, P, K and decreased Cl). However, the latter changes were of limited magnitude without toxicological relevance, as was the limited increase in alkaline phosphatase. Because the increase in plasma total bilirubin is more indicative of liver damage than the small changes in circulating liver enzymes, plasma total bilirubin was chosen for the BMDL calculation.

There were no changes in urine analysis parameters between treated and control animals.

Body weights, body weight gain, feed consumption and feed efficiency were overall comparable between low-dose animals and control animals, as well as between mid-dose females and control. Body weight and body weight gain were generally lower in mid- and high-dose males compared to

[7] BMDL: lower 95% confidence limit (one-sided) for the BMD. BMD is the dose which is associated with a predefined risk level (i.e. Benchmark response (BMR)). According to the guidance (EFSA Scientific Committee, 2009), the default BMR is an extra risk of 10% in incidence (compared to the incidence in the control animals) for quantal response data or a change of 5% in the population geometric mean for continuous response data.

control, reaching statistical significance for the study overall. High-dose females had lower body weight and body weight gain compared to control, but did not reach statistical significance. Feed consumption was decreased in mid- and high-dose group males and feed efficiency was decreased in mid- and high-dose males, reaching statistical significance for the study overall.

In males, the relative liver weights (relative to body weight) were statistically significantly increased at all doses levels, whereas the relative kidney, testes and epididymides were only statistically significantly increased at the high dose. In females, only the relative liver weights (relative to body weight) were statistically significantly increased at the high-dose level. Increased ratios of kidney and liver weights relative to brain weight were also reported at the 135 mg/kg bw per day dose level for both males and females. Statistically significant decreases in absolute organ weights were reported in females for adrenal glands at the high dose, and brain at mid- and high doses, and in males, for brain at high dose, heart at mid- and high doses, and spleen at mid dose. As these changes did not show a dose response, or were not reflected in organ weights relative to body- and or brain weight, these absolute organ weight changes were not considered of toxicological relevance. Relative organ weight changes (to body weight) were considered for BMDL calculation.

No test substance-related macroscopic findings were found in the study. Animals from control and high-dose groups were subject to full histopathological examinations, as were any gross lesions found in animals from low- and mid-dose groups. No microscopic findings related to the test substance were identified.

The Panel decided to derive a BMDL from this study, rather than the NOAEL, in line with the Opinion of the EFSA Scientific Committee (EFSA Scientific Committee, 2009). Details of the BMDL estimation have been presented in Appendix F. The following observations were the most relevant: hypersalivation in the mid- and high-dose groups, changes in white blood cell counts, changes in body weight changes in relative organ weight (liver, kidneys, epididymides and testes) and changes in plasma total bilirubin. For the various toxicity parameters analysed, the $BMDL_{05}$ of 0.94 mg/kg bw per day for a 5% decrease in total white blood cell count was the lowest value obtained. This BMDL is used for the evaluation of 2,5-dimethylfuran-3(2*H*)-one.

Toxicity data are summarised in Table F.1.

7.3. Developmental/reproductive toxicity studies

No data on developmental toxicity and reproductive toxicity are available for the candidate substances.

7.4. Genotoxicity

2,5-Dimethylfuran-3(2*H*)-one [FL-no: 13.119] was evaluated in FGE.220Rev2 (EFSA CEF Panel, 2013) and 4-acetyl-2,5-dimethylfuran-3(2*H*)-one [FL-no: 13.175] was evaluated in FGE.220Rev3 (EFSA CEF Panel, 2015a) with respect to genotoxicity.

They were both tested in *Salmonella* Typhimurium strains TA98, TA100, TA1535, TA1537 and TA102, both in the absence and presence of S9-mix (Sokolowski, 2007; Bowen, 2011). Both candidate substances were also tested in an *in vitro* micronucleus assay in human peripheral blood lymphocytes, with and without metabolic activation, for their ability to induce chromosomal damage or aneuploidy (Lloyd, 2011, 2012, 2014). The two candidate substances [FL-no: 13.119 and 13.175] did not induce mutations and did not induce increased levels of micronuclei in these valid studies.

The Panel therefore concluded that the two candidate substances for which genotoxicity data were submitted (2,5-dimethylfuran-3(2*H*)-one [FL-no: 13.119] and 4-acetyl-2,5-dimethylfuran-3(2*H*)-one [FL-no: 13.175]) do not give rise to concern with respect to genotoxicity. The same conclusion applies to 2,5-dimethyl-4-ethoxyfuran-3(2*H*)-one [FL-no: 13.117], which is structurally related with respect to the α,β-unsaturated carbonyl moiety. Therefore, these three substances can be evaluated using the Procedure. Genotoxicity data are summarised in Table F.2.

8. Conclusion

The FGE.313 deals with the evaluation of three α,β-unsaturated 3(2*H*)-furanone derivatives from chemical group 13. These three candidate substances are 2,5-dimethyl-4-ethoxyfuran-3(2*H*)-one [FL-no: 13.117], 2,5-dimethylfuran-3(2*H*)-one [FL-no: 13.119] and 4-acetyl-2,5-dimethylfuran-3(2*H*)-one [FL-no: 13.175].

All three substances possess one chiral centre and their stereoisomeric composition has been specified.

The three candidate substances were assigned to structural class III, according to the decision tree approach presented by Cramer et al. (1978).

[FL-no: 13.117 and 13.119] have been reported to occur naturally in food.

According to the default MSDI approach, the candidate substances in this group have intakes in Europe from 0.018 to 1.7 μg/capita per day. For all the candidate substances, this is below the threshold of concern value for structural class III (90 μg/person per day). On the basis of the reported annual production volumes in Europe (EFFA, 2004b), the estimated combined daily per capita intake as flavourings of the three candidate substances assigned to structural class III is approximately 3 μg, which does not exceed the threshold of concern for a compound belonging to structural class III of 90 μg/person per day.

The Panel concluded that the two candidate substances for which genotoxicity data were submitted (2,5-dimethylfuran-3(2*H*)-one [FL-no: 13.119] and 4-acetyl-2,5-dimethylfuran-3(2*H*)-one [FL-no: 13.175]) do not give rise to concern with respect to genotoxicity. The same conclusion applies to 2,5-dimethyl-4-ethoxyfuran-3(2*H*)-one [FL-no: 13.117], which is structurally related with respect to the α,β-unsaturated carbonyl moiety. Therefore, these three substances can be evaluated using the Procedure.

The candidate substance [FL-no: 13.117] is expected to be metabolised to innocuous products. Therefore, [FL-no: 13.117] was evaluated via the A-side of the Procedure. [FL-no: 13.119 and 13.175] cannot be anticipated to be metabolised to innocuous products. Consequently, these two substances were evaluated via the B-side of the Procedure.

It is considered that the two candidate substances, 2,5-dimethyl-4-ethoxyfuran-3(2*H*)-one [FL-no: 13.117] and 2,5-dimethylfuran-3(2*H*)-one [FL-no: 13.119], evaluated through the Procedure would not give rise to safety concerns at the estimated levels of intake arising from their use as flavouring substances on the basis of the default MSDI approach. Additional toxicity data are required for 4-acetyl-2,5-dimethylfuran-3(2*H*)-one [FL-no: 13.175].

In order to determine whether the conclusion for the candidate substances can be applied to the materials of commerce, it is necessary to consider the available specifications.

Adequate specifications including complete purity criteria and identity for the material of commerce have been provided for the flavouring substances.

The estimated intake based on the mTAMDI approach is 1,000 μg/person per day for each of the candidate substances from structural class III, which is above the threshold of concern for structural class III of 90 μg/person per day. The Panel concluded that for 2,5-dimethyl-4-ethoxyfuran-3(2*H*)-one [FL-no: 13.117], 2,5-dimethylfuran-3(2*H*)-one [FL-no: 13.119] and 4-acetyl-2,5-dimethylfuran-3(2*H*)-one [FL-no: 13.175] for which the mTAMDIs are above the thresholds for their structural class, more reliable exposure data are required. On the basis of such additional data, these flavouring substances should be reconsidered using the Procedure. Subsequently, additional toxicity data might become necessary. For the candidate substance [FL-no: 13.175], toxicity data are required.

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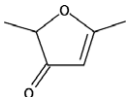
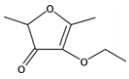
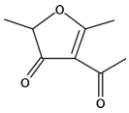
Abbreviations

BMD	BenchMark Dose
BMDL	lower 95% confidence limit (one-sided) for the BMD
BMDU	upper 95% confidence limit (one-sided) for the BMD
BMR	Benchmark response
bw	body weight
CAS	Chemical Abstract Service
CEF	EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids Chemical
CoE	Council of Europe
EFFA	European Flavour and Fragrance 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)
GLP	good laboratory practice
GUI	graphical user interface
ID	identity
IOFI	International Organization of the Flavour Industry
IR	infrared spectroscopy
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
MNBN	micronucleated binucleated cells
MS	mass spectrometry
MSDI	Maximised Survey-derived Daily Intake
mTAMDI	Modified Theoretical Added Maximum Daily Intake

NMR	nuclear magnetic resonance
NOAEL	no observed adverse effect level
NTP	National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
SCF	Scientific Committee on Food
TAMDI	Theoretical Added Maximum Daily Intake
WBC	white blood cell
WHO	World Health Organization

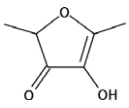
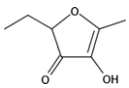
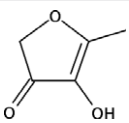
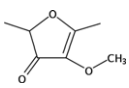
Appendix A – Summary of the safety evaluation

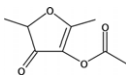
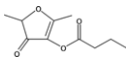
Table A.1: Summary of the safety evaluation for the substances in FGE.313 applying the Procedure

FL-no	EU Register name	Structural formula	MSDI[(a)] (μg/capita per day)	Class[(b)] Evaluation procedure path[(c)]	Outcome on the named compound [[(d)] or [(e)]]	Outcome on the material of commerce [[(f)], [(g)] or [(h)]]	Evaluation remarks
13.119	2,5-Dimethylfuran-3(2*H*)-one		1.7	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	d	F	Evaluated in FGE.220Rev2, genotoxicity concern could be ruled out
13.117	2,5-Dimethyl-4-ethoxyfuran-3(2*H*)-one		0.018	Class III A3: Intake below threshold	d	F	Evaluated in FGE.220Rev3, genotoxicity concern could be ruled out
13.175	4-Acetyl-2,5-dimethylfuran-3(2*H*)-one		1.3	Class III B3: Intake below threshold, B4: Toxicity data are required	d	F	Evaluated in FGE.220Rev3, genotoxicity concern could be ruled out

FGE: Flavouring Group Evaluation; FL-no: FLAVIS number; MSDI: Maximised Survey-derived Daily Intake; NOAEL: No Observed Adverse Effect Level.
(a): EU MSDI: Amount added to food as flavour in (kg/year) × 10E9/(0.1 × population in Europe (= 375 × 10E6) × 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.
(d): No safety concern based on intake calculated by the MSDI approach of the named compound.
(e): Data must be available on the substance or closely related substances to perform a safety evaluation.
(f): No safety concern at the estimated level of intake of the material of commerce meeting the specification requirement (based on intake calculated by the MSDI approach).
(g): Tentatively regarded as presenting no safety concern (based on intake calculated by the MSDI approach) pending further information on the purity of the material of commerce and/or information on stereoisomerism.
(h): No conclusion can be drawn due to lack of information on the purity of the material of commerce.

Table A.2: Summary of supporting substances evaluated in FGE.99 (EFSA CEF Panel, 2012)

FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU)[(a)] (μg/capita per day)	SCF status[(b)] JECFA status[(c)] CoE status[(d)]
13.010	4-Hydroxy-2,5-dimethyl-furan-3(2*H*)-one		3174 536 3658-77-3	1446 JECFA specification (JECFA, 2005)	960	No safety concern (JECFA, 2005) Category B (CoE, 1992)
13.084	2-Ethyl-4-hydroxy-5-methyl-3(2*H*)-furanone		3623 27538-09-6[(e)]	1449 JECFA specification (JECFA, 2005)	160	No safety concern (JECFA, 2005)
13.085	4-Hydroxy-5-methylfuran-3(2*H*)-one		3635 11785 19322-27-1	1450 JECFA specification (JECFA, 2005)	5.6	No safety concern (JECFA, 2005)
13.089	2,5-Dimethyl-4-methoxyfuran-3(2*H*)-one		3664 4077-47-8	1451 JECFA specification (JECFA, 2005)	19	No safety concern (JECFA, 2005)

FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU)[a] (μg/capita per day)	SCF status[b] JECFA status[c] CoE status[d]
13.099	4-Acetoxy-2,5-dimethylfuran-3(2*H*)-one		3797 4166-20-5	1456 JECFA specification (JECFA, 2005)	400	No safety concern (JECFA, 2005)
13.176	Furaneyl butyrate		3970	1519 JECFA specification (JECFA, 2006)	12	No evaluation (JECFA, 2009)

FL-no: FLAVIS-number; EU: European Union; FEMA: Flavor and Extract Manufacturers Association number; CoE: Council of Europe number; CAS: Chemical Abstract Service number; JECFA: The Joint FAO/WHO Expert Committee on Food Additives number; MSDI: Maximised Survey-derived Daily Intake; SCF: Scientific Committee on Food.

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

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

(c): No safety concern at estimated levels of intake.

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

(e): CASrn in the Register to be changed to 27538-10-9.

Appendix B – Procedure for the safety evaluation

The approach for a safety evaluation of chemically defined flavouring substances as referred to in Commission Regulation (EC) No 1565/2000[3], named the 'Procedure', is shown in schematic form in Figure C.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).

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 thresholds of concern (human exposure thresholds) have been specified. Exposures below these thresholds are not considered to present a safety concern.

Class I contains flavourings that have simple chemical structures and efficient modes of metabolism, which would suggest a low order of oral toxicity. Class II contains flavourings that have structural features that are less innocuous, but are not suggestive of toxicity. Class III comprises flavourings that have structural features that permit no strong initial presumption of safety, or may even suggest significant toxicity (Cramer et al., 1978). The thresholds of concern for these structural classes of 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[8] (Step 2)?
- Do their exposures exceed the threshold of concern for the structural class (Step A3 and B3)?
- Are the flavourings or their metabolites endogenous[9] (Step A4)?
- Does a NOAEL exist on the flavourings or on structurally related substances (Step A5 and B4)?

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

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

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

[9] '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).

Appendix C – Procedure for safety evaluation of chemically defined flavouring substances

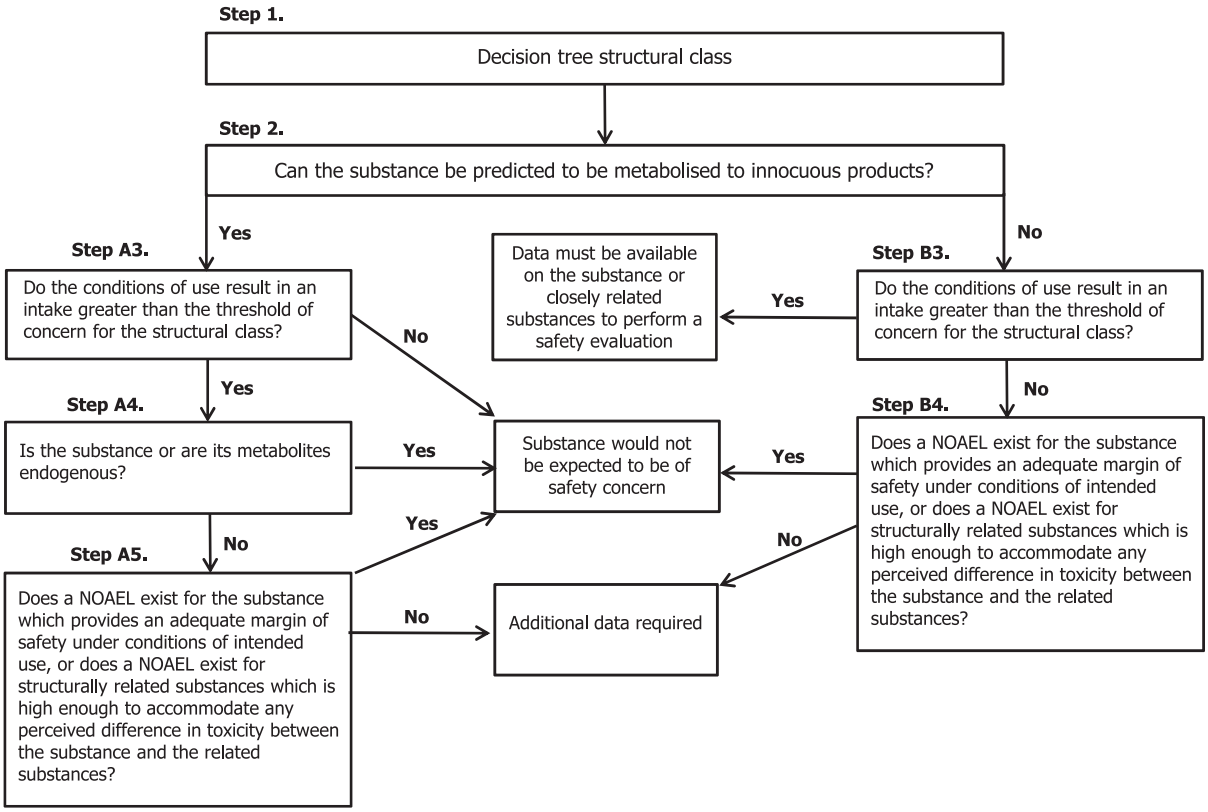


Figure C.1: Procedure for safety evaluation of chemically defined flavouring substances

Appendix D – Use levels/mTAMDI

D.1. Normal and maximum use levels

For each of the 18 Food categories (Table A.2) in which the candidate substances are used, Flavour Industry reports a 'normal use level' and a 'maximum use level'.[3] According to the Industry, the 'normal use' is defined as the average of reported usages and 'maximum use' is defined as the 95th percentile of reported usages (EFFA, 2002). The normal and maximum use levels in different food categories have been extrapolated from figures derived from 12 model flavouring substances (EFFA, 2004a).

Table D.1: Food categories according to Commission Regulation (EC) No 1565/2000

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

The 'normal and maximum use levels' are provided by Industry for the candidate substances in the present flavouring group (Table D.1).

D.2. mTAMDI calculations

The method for calculation of modified Theoretical Added Maximum Daily Intake (mTAMDI) values is based on the approach used by SCF up to 1995 (SCF, 1995). The assumption is that a person may consume the amount of flavourable foods and beverages listed in Table D.1. These consumption estimates are then multiplied by the reported use levels in the different food categories and summed up.

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 D.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 D.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	2.0 (chewing gum)

The mTAMDI value (see Table D.2) is presented for the flavouring substances in the present flavouring group (EFFA, 2004b). The mTAMDI value is only given for the highest reported normal use levels.

Table D.3: Estimated intakes based on the mTAMDI approach

FL-no	EU Register name	mTAMDI (μg/person per day)	Structural class	Threshold of concern (μg/person per day)
13.119	2,5-Dimethylfuran-3(2*H*)-one	1,000	Class III	90
13.117	2,5-Dimethyl-4-ethoxyfuran-3(2*H*)-one	1,000	Class III	90
13.175	4-Acetyl-2,5-dimethylfuran-3(2*H*)-one	1,000	Class III	90

FL-no: FLAVIS number; EU: European Union; mTAMDI: Modified Theoretical Added Maximum Daily Intake.

Table D.4: Normal and maximum use levels (mg/kg) for the candidate substances in FGE.313 (EFFA, 2004b)

FL-no	Food categories Normal use levels (mg/kg) Maximum use levels (mg/kg)																	
	01.0	02.0	03.0	04.1	04.2	05.0	06.0	07.0	08.0	09.0	10.0	11.0	12.0	13.0	14.1	14.2	15.0	16.0
13.117	3	2	3	2	–	4	2	5	1	1	–	–	2	3	–	4	5	2
	15	10	15	10	–	20	10	25	5	5	–	–	10	15	–	20	25	10
13.119	3	2	3	2	–	4	2	5	1	1	–	–	2	3	–	4	5	2
	15	10	15	10	–	20	10	25	5	5	–	–	10	15	–	20	25	10
13.175	3	2	3	2	–	4	2	5	1	1	–	–	2	3	–	4	5	2
	15	10	15	10	–	20	10	25	5	5	–	–	10	15	–	20	25	10

FL-no: FLAVIS number.

Table D.5: Distribution of the 18 food categories listed in Commission Regulation (EC) No 1565/2000 into the seven SCF food categories used for TAMDI calculation

Key	Food categories according to Commission Regulation (EC) No 1565/2000	Distribution of the seven SCF food categories		
	Food category	Food	Beverages	Exceptions
01.0	Dairy products, excluding products of category 02.0	Food		
02.0	Fats and oils, and fat emulsions (type water-in-oil)	Food		
03.0	Edible ices, including sherbet and sorbet	Food		
04.1	Processed fruit	Food		
04.2	Processed vegetables (including mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds	Food		

Key	Food categories according to Commission Regulation (EC) No 1565/2000	Distribution of the seven SCF food categories		
	Food category	**Food**	**Beverages**	**Exceptions**
05.0	Confectionery			Exception a
06.0	Cereals and cereal products, including flours & starches from roots & tubers, pulses & legumes, excluding bakery	Food		
07.0	Bakery wares	Food		
08.0	Meat and meat products, including poultry and game	Food		
09.0	Fish and fish products, including molluscs, crustaceans and echinoderms	Food		
10.0	Eggs and egg products	Food		
11.0	Sweeteners, including honey			Exception a
12.0	Salts, spices, soups, sauces, salads, protein products, etc.			Exception d
13.0	Foodstuffs intended for particular nutritional uses	Food		
14.1	Non-alcoholic ('soft') beverages, excl. dairy products		Beverages	
14.2	Alcoholic beverages, including alcohol-free and low-alcoholic counterparts			Exception c
15.0	Ready-to-eat savouries			Exception b
16.0	Composite foods (e.g. casseroles, meat pies, mincemeat) – foods that could not be placed in categories 01.0–15.0	Food		

SCF: Scientific Committee on Food; TAMDI: Theoretical Added Maximum Daily Intake.

Appendix E – Absorption, distribution, metabolism and elimination

According to JECFA (2009), data on furan-substituted aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids, and related esters, sulfides, disulfides and ethers with an oxygenated alkyl substituent indicate that the furyl derivatives in this group are rapidly absorbed, metabolised and excreted from animals.

In FGE.99Rev1, EFSA was in agreement with the JECFA (JECFA, 2006, 63rd meeting, FAS 54) evaluation of the substances, 4-hydroxy-2,5-dimethyl-furan-3(2*H*)-one [FL-no: 13.010] and 2,5-dimethyl-4-methoxyfuran-3(2*H*)-one [FL-no: 13.089], in taking them via the A-side of the Procedure. 4-Hydroxy-2,5-dimethyl-furan-3(2*H*)-one [FL-no: 13.010] is expected to form a glucuronic acid conjugate, and to be excreted via the urine. The ether bond of 2,5-dimethyl-4-methoxyfuran-3(2*H*)-one [FL-no: 13.089] is expected to be readily cleaved. The formed hydroxy-furanone can subsequently be conjugated with glucuronic acid and excreted in the urine. In accordance with this, it is expected that the structurally similar candidate substance, 2,5-dimethyl-4-ethoxyfuran-3(2*H*)-one [FL-no:13.117], evaluated in this FGE.313, will be oxidised, conjugated with glucuronic acid and excreted in the urine, and may be evaluated via the A-side of the Procedure.

For the two candidate substances, 2,5-dimethylfuran-3(2*H*)-one [FL-no: 13.119] and 4-acetyl-2, 5-dimethylfuran-3(2*H*)-one [FL-no: 13.175], the metabolic fate is unclear, and accordingly, it cannot be anticipated that these substances will be metabolised to innocuous products.

Appendix F – Toxicity

Table F.1: Toxicity data considered by the Panel in FGE.313

Chemical name [FL-no:]	Species; sex no./group	Route	Doses (mg/kg bw per day)	Duration (days)	NOAEL (mg/kg bw per day)	References	Comments
2,5-Dimethylfuran-3(2*H*)-one [13.119]	Rats; M,F 3/6	Diet	0, 125, 210, 329 (M) and 0, 138, 239, 366 (F)	14		Bauter (2015a)	Dose-range finding study
	Rats; M,F 3/20	Gavage	15, 45, 135	90	15	Bauter (2015b)	NOAEL was suggested by the author of the study report The Panel calculated a $BMDL_{05}$ of 0.94 mg/kg bw per day for this study

FGE: Flavouring Group Evaluation; FL-no: FLAVIS number; bw: body weight; NOAEL: No Observed Adverse Effect Level.

Table F.2: Genotoxicity data (*in vitro*) considered by the Panel in FGE.220Rev2 (EFSA CEF Panel, 2013) and FGE.220Rev3 (EFSA CEF Panel, 2015a)

Chemical name [FL-no]	Test system	Test object	Dose	Reported result	Reference	Comments
2,5-Dimethylfuran-3(2*H*)-one [13.119]	Reverse mutation	*Salmonella* Typhimurium TA98, TA100, TA102, TA1535 and TA1537	3–5,000 μg/plate[(a),(b)]	Negative	Sokolowski (2007)	All strains were negative. Study design complied with current GLP and OECD recommendations. Acceptable top concentration was achieved
		S. Typhimurium TA98, TA100, TA102, TA1535 and TA1537	33–5,000 μg/plate[(a),(c)]	Negative		
	Micronucleus Assay	Human peripheral blood lymphocytes	900–1,120 μg/mL[(a),(f)] 900–1,120 μg/mL[(d),(g)]	Negative	Lloyd (2011)	The MNBN cell frequencies in all treated cultures fell within the normal range. Complies with draft OECD Guideline 487 and GLP recommendations

Chemical name [FL-no]	Test system	Test object	Dose	Reported result	Reference	Comments
4-Acetyl-2,5-dimethylfuran-3(2*H*)-one [13.175]	Reverse Mutation	*S.* Typhimurium TA98, TA100, TA102, TA1535 and TA1537	0.32–5,000 μg/plate[(a),(b)]	Negative	(Bowen, 2011)	Evidence of toxicity was observed at 5,000 μg/plate in all strains in the absence and presence of S9. Study design complied with current GLP and OECD recommendations
			78.13–5,000 μg/plate[(b),(d)] 78.13–5,000 μg/plate[(c),(d)]	Negative		
	Micronucleus Assay	Human peripheral blood lymphocytes	1,000–1,542 μg/mL[(a),(f)] 400–900 μg/mL[(d),(g)]	Negative	Lloyd (2012)	Study in compliance with GLP and OECD recommendations. Statistical significant increase, dose-related, in the presence of S9-mix at all three concentrations in a first experiment. Lower statistical significance at the two highest concentrations in an enlarged scoring, carried out with an unjustified approach. Mean MNBN cell frequencies fell within the historical control range with exception of a single replicate (see main text)
			1,000, 1,250 and 1,542 μg/mL[(e),(f)]	Negative	Lloyd (2014)	Follow-up study in compliance with GLP and OECD recommendations. Statistically significant increase ($p \leq 0.05$) in the mean frequency of micronuclei was reported only at the lowest of the three concentrations tested (1,000 μg/mL) but remained well within the normal historical control range values (0.1–0.9%) for both replicate cultures

FGE: Flavouring Group Evaluation; FL-no: FLAVIS number; GLP: Good Laboratory Practice; OECD: Organisation for Economic Co-operation and Development; MNBN: micronucleated binucleated cells.
(a): With and without S9 metabolic activation.
(b): Plate incorporation method.
(c): Preincubation method.
(d): Without S9 metabolic activation.
(e): With S9 metabolic activation.
(f): 3-h incubation with 21-h recovery period.
(g): 24-h incubation with no recovery period.

Appendix G – Dose–response modelling for 2,5-dimethylfuran-3(2*H*)-one [FL-no: 13.119]

In compliance with the Opinion of the EFSA Scientific Committee on the use of the Benchmark dose (BMD)[7] approach in Risk Assessment (EFSA Scientific Committee, 2009), the results obtained in the subchronic study with 2,5-dimethylfuran-3(2*H*)-one [FL-no: 13.119] (Bauter, 2015b) have been submitted to statistical dose–response modelling. This study has been summarised in Section 7.2 of the main document, where it was explained that the following observations were used for the calculation of the BMDL from this study: hypersalivation, body weight changes, changes in total white blood cell count (WBC), relative liver weight changes, relative kidney weight changes, changes in relative weight of testes and epididymis, and changes in plasma total bilirubin.

For all modelling, the statistical package PROAST (version 61.5) has been used in graphical user interface mode (GUI-mode). This package is available via: http://www.rivm.nl/en/foodnutritionandwater/foodsafety/proast.jsp; the version mentioned can be requested directly from the authors. Using this statistical package, 95% lower confidence limit (single-sided) of the BMDLs were calculated (see EFSA Scientific Committee, 2009) for the various effects. For each evaluation, all statistical models available in PROAST (i.e. the 'EPA-models' plus the Exponential and Hill families of models) were used.

The dose–response modelling was carried out using means, SDs and group sizes as provided in the study report. The data used have been presented in Table G.1.

The evaluations were carried out in GUI-mode with the following settings:

- Benchmark response: According to the Guidance (EFSA Scientific Committee, 2009), the default Benchmark response is an extra risk of 10% in incidence (compared to the incidence in the control animals) for quantal response data or a change of 5% in the population geometric mean for continuous response data. However, the Panel notes that these default values might be more conservative than necessary for individual toxicity parameters.
- No restrictions for model parameters to limit e.g. steepness of the fitted dose–response curves (default option; cannot be modified in GUI-mode).
- Sex was used as a covariate.

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

- p-value for goodness of fit: 0.05.
- For continuous variables (i.e. all except hypersalivation), the Exponential and Hill nested model families were used. Dose–response modelling for hypersalivation (quantal data) was carried out using the EPA-models as well as the Exponential and Hill model families.

Table G.1: Experimental data from Bauter (2015b) used for dose–response modelling and calculation of a BMDL for 2,5-dimethylfuran-3(2*H*)-one [FL-no: 13.119]

Dose (mg/kg bw)	Sex	General number of animals per group (N)	General number of animals per group for WBC (N)[a]	Observations													
				Hypersalivation (N)	bw (g)		Plasma total bilirubin (mg/dl)		Relative liver weight (g/kg bw)		Relative kidney weight (g/kg bw)		Relative epididymis weight (g/kg bw)		Relative testes weight (g/kg bw)		Total white blood cell count (WBC) ($\times 10^3/\mu l$)	
					Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
0	m	10	10	0	493	32	0.16	0.03	24.8	1.4	6.74	0.47	3.20	0.31	7.89	0.65	14.64	3.07
15	m	10	10	0	527	60	0.17	0.02	27.2	1.8	6.81	0.61	3.15	0.47	7.45	1.16	13.27	1.94
45	m	10	9	6	447	52	0.19	0.03	27.6	2.0	7.34	0.38	3.53	0.49	8.30	1.08	9.95	1.12
135	m	10	10	8	423	27	0.23	0.01	33	1.3	8.36	0.26	3.73	0.19	8.88	0.49	11.05	2.20
0	f	10	10	0	302	21	0.20	0.03	28.4	2.3	7.01	0.38	NA	NA	NA	NA	9.87	3.68
15	f	10	10	0	317	14	0.20	0.04	28.5	2.5	7.05	0.58	NA	NA	NA	NA	9.47	2.24
45	f	10	10	5	286	31	0.20	0.03	28.2	2.4	6.95	0.47	NA	NA	NA	NA	9.82	2.22
135	f	10	10	7	281	23	0.24	0.05	31.7	1.8	7.55	0.58	NA	NA	NA	NA	8.46	2.19

BMDL: lower 95% confidence limit (one-sided) for the BMD; bw: body weight; SD: standard deviation.
(a): For the white blood cell count (WBC), the number of animals in one of the groups is one less than the general number of animals because of a missing value for one animal.

G.1. Quantal responses: hypersalivation

The results of the BMD analysis for hypersalivation have been presented in Table G.2.

Table G.2: Results of a BMD analysis of the data from Bauter (2015b): occurrence of hypersalivation in animals dosed via gavage with 2,5-dimethylfuran-3(2*H*)-one

Model	n.par	loglik	Accepted	BMDL[(a)]	BMDU[(a)]	BMD[(a)]	sens.subgr
null	1	−50.45					
full	8	−25.01					
two.stage-	3	−29.37	Yes	6.96	22.3	10.1	–
log.logist-	3	−28.07	Yes	8.31	27.0	17.3	–
Weibull-	3	−29.12	Yes	4.89	24.8	13.6	–
log.prob-	3	−27.81	Yes	9.62	28.1	18.6	–
gamma-	3	−28.92	Yes	5.32	27.1	15.7	–
logistic-	2	−33.71	No			28.5	–
LVM_Exp: m4-	3	−25.06	Yes	18.40	33.3	28.1	–
LVM_Hill: m2-	2	−28.00	Yes	10.60	21.9	16.0	–

BMDL: lower 95% confidence limit (one-sided) for the BMD; BMDU: upper 95% confidence limit (one-sided) for the BMD; BMD: BenchMark Dose.
(a): BMD, BMDL and BMDU are in mg/kg bw per day; 5 days/week.

From Table G.2, it can be concluded that all models, except the logistic model gave an acceptable fit. As none of the models is a priori preferable above one of the others, the BMDL, which can be selected from this table, is 4.89 mg/kg bw per day from the EPA Weibull model. This BMDL is the lowest BMDL calculated. The BMDU for this parameter is obtained from the Exponential model (m4). Note that the 90% confidence intervals (two-sided) around the BMDs are rather small, and that also the differences between the various models are relatively limited. A graphical representation of the modelled dose–response curve is given in Figure G.1. Note that in this plot, the maximal observable incidence of the effect is normalised to 1, which would represent response in all animals of the group.

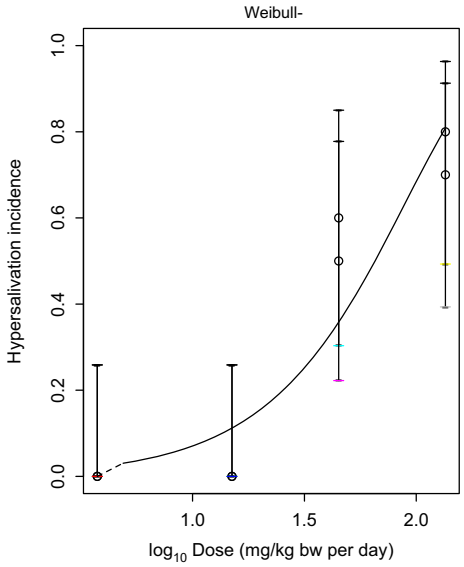


Figure G.1: Weibull dose–response analysis for incidence of hypersalivation as reported in a 90-day oral toxicity study with 2,5-dimethylfuran-3(2*H*)-one [FL-no:13.119] (Bauter, 2015b). Lower squares are the incidences observed in the females, upper squares are the incidences observed in the males

G.2. Continuous responses

All other responses mentioned above were continuous responses, for which group means, standard deviations and groups size were used as input parameters for the BMDL calculations (see Table G.3). For continuous responses, only the Hill and Exponential nested model families are applicable. In GUI-mode, PROAST has two options for dose–response modelling: either selection of the minimum model or selection of the maximum model. The minimum model is the model that uses the smallest number of model parameters that result in a statistically acceptable description of the empirical data. Adding more model parameters would not statistically improve the quality of the fitted dose–response curve, in the light of the degrees of freedom available. The maximum model always provides estimates for all model parameters irrespective of whether a higher number of model parameters actually improves the quality of the modelling. This is a deviation from the approach described in the EFSA opinion of 2009. The current view is that the use of the maximum model takes a better account of the uncertainty in the dose–response, and therefore should be preferable to the minimal model approach (Slob and Setzer, 2014; EFSA Scientific Committee, 2016). For the sake of completeness, the BMDLs from both approaches have been provided in Table G.3. Note that the BMDL and BMDU values may come from different models, even within one parameter (as is the case for hypersalivation).

Table G.3: Overview of BMDL and BMDU values for 2,5-dimethylfuran-3(2*H*)-one [FL-no: 13.119]. The values are for continuous parameters and obtained with either Hill or Exponential nested model families

Parameter	Direction of change	Minimal model		Maximal model	
		$BMDL_{05}$	$BMDU_{05}$	$BMDL_{05}$	$BMDU_{05}$
Body weight changes	Decrease	F+M: 18.77	43.11	F+M: 19.46	42.95
Total white blood cell count (WBC) changes	Decrease	F: 8.07	Inf	F: 36.28	Inf
		M: 0.94	9.22	M: 4.54	35.60
Relative liver weight changes	Increase	F: 40.06	93.40	F: 27.07	94.46
		M: 21.38	33.64	M: 7.30	54.83
Relative kidney weight changes	Increase	F: 48.42	117.10	F: 53.80	134.46
		M: 27.24	42.65	M: 25.27	62.07
Relative testes weight of changes	Increase	28.80	87.43	18.60	91.08
Relative epididymis weight changes	Increase	26.59	72.70	10.80	53.49
Plasma total bilirubin changes	Increase	F+M: 18.56	36.23	F: 11.16	132.29
				M: 3.16	45.68

BMDL: lower 95% confidence limit (one-sided) for the BMD; BMDU: upper 95% confidence limit (one-sided) for the BMD.

For all parameters, for both model families acceptable fits were obtained. For almost all parameters, the 90% confidence interval (two-sided) around the BMD (BMDL–BMDU) was not extremely wide, (usually less than a factor of 10). However, for the total white blood cell counts, in the females no upper bound estimate for the BMD (BMDU) could be calculated. Nevertheless, the more relevant lower bound estimate (BMDL) could be estimated. As can be seen in the main text, the female response for this parameter was less pronounced than in the males, which is also reflected in the results of the dose–response modelling.

The figure below (Figure G.2) gives the graphical representation of the fitted dose–response curves for the total white blood cell counts (upper panel; minimal model). The lower panel gives the dose–response curves for plasma total bilirubin (maximum model).

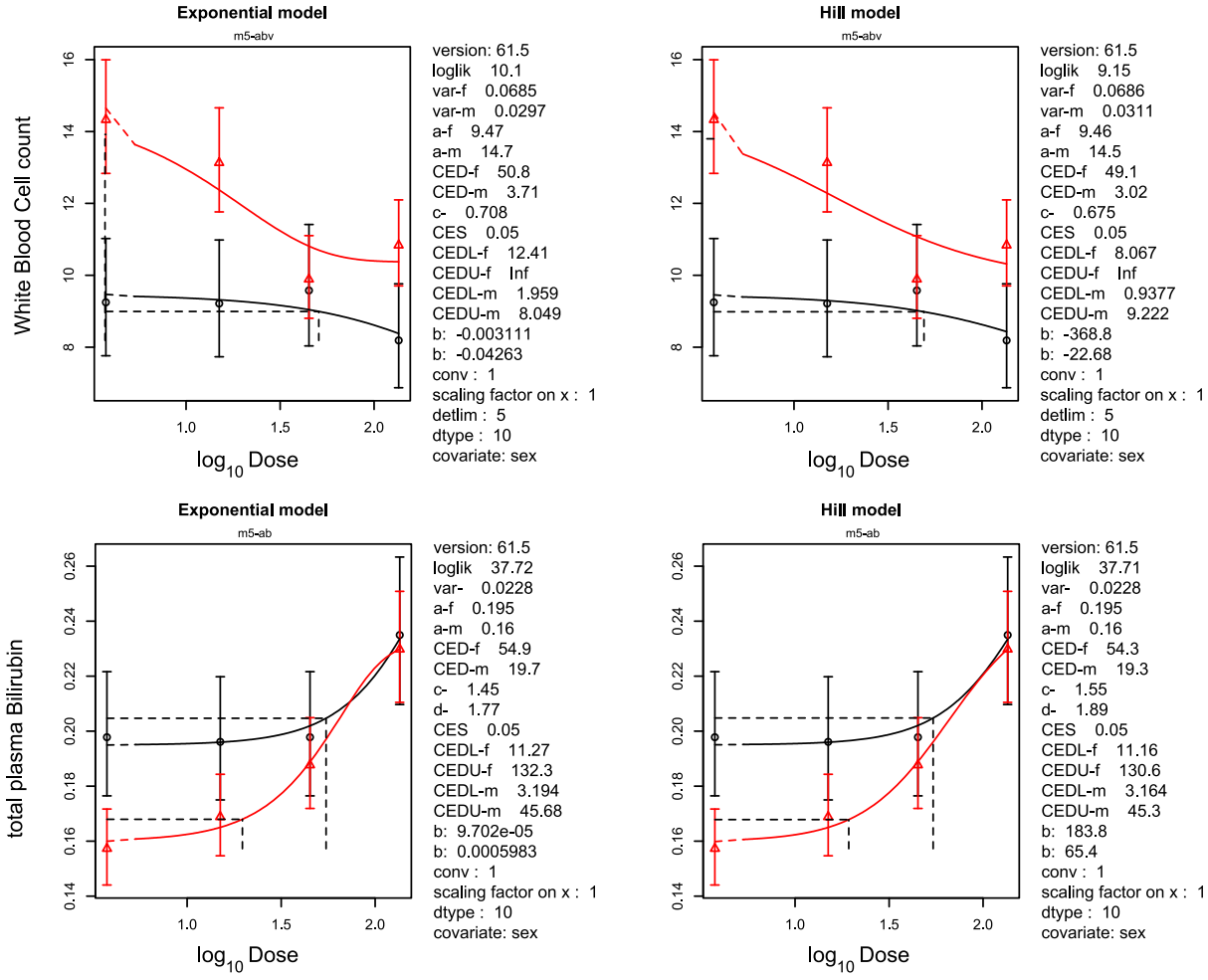


Figure G.2: Dose–response modelling for 2,5-dimethylfunan-3(2*H*)-one [FL-no: 13.119]. Upper Panel: total white blood cell counts (minimal model). Lower panel: plasma total bilirubin (maximum model). The black curves represent the females, the red curves represent the males

When the maximum model is used, the lowest BMDL (3.16 mg/kg bw per day) is connected to increased plasma total bilirubin.

According to the EFSA guidance (EFSA Scientific Committee, 2009), the minimal model is the one to be used for BMDL calculations. As the dose–response modelling for the total white blood cell counts in the males resulted in the lowest BMDL of 0.94 mg/kg bw per day, this BMDL will be used for the assessment of [FL-no: 13.119].