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Scientific Opinion on Flavouring Group Evaluation 501 (FGE.501): Grill flavour concentrate (vegetable)

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Abstract

The Panel on Food Additives and Flavourings of the European Food Safety Authority was requested to deliver a scientific opinion on the implications for human health of the product Grill flavour concentrate (vegetable) [FL-no: 21.002] in the Flavouring Group Evaluation 501 (FGE.501), according to Regulation (EC) No 1331/2008 and Regulation (EC) No 1334/2008 of the European Parliament and of the Council. The product is derived from heat-treated canola oil and intended to be used as a food flavouring with grilled aroma in a wide variety of food categories. Information on manufacturing and compositional data was considered adequate to show the reproducibility of the production process. The chronic dietary exposure to the substance estimated using the added portions exposure technique (APET) was calculated to be 0.402 and 0.252 mg/person per day for a 60-kg adult and for a 15-kg child, respectively. Based on exposure estimate and the results from the repeated-dose toxicity studies, a sufficient margin of safety could be calculated. However, the Panel noted that for six constituents of the flavouring there is an indication for genotoxicity. Therefore, these six substances have to be further considered. Until these evaluations have been finalised the safety of Grill flavour concentrate (vegetable) cannot be fully assessed.

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1. Introduction

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

1.1.1. Background

The use of flavourings in food is regulated under Regulation (EC) No 1334/2008¹ of the European Parliament and Council of 16 December 2008 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.

Regulation (EC) No 1331/2008² applies for the evaluation and approval of new 'other' flavouring.

An application for authorisation as a new 'other' flavouring of: Grill flavour concentrate (vegetable) has been submitted to the Commission.

In order for the Commission to be able to consider its inclusion in the Union list of flavourings and source materials (Annex of Regulation (EC) No 1334/2008), EFSA should carry out a safety assessment of this product as a new 'other' flavouring.

1.1.2. Terms of reference

The European Commission requests the European Food Safety Authority to carry out a safety assessment on the product "Grill flavour concentrate (vegetable)" as "other flavouring" in accordance with Regulation (EC) No 1331/2008 establishing a common authorisation procedure for food additives, food enzymes and food flavourings.

1.2. Information on existing authorisation and/or evaluations from other authorities

The Panel is not aware of existing authorisations and/or evaluations of the flavouring 'Grill flavour concentrate (vegetable)' by other authorities.

2. Data and methodologies

2.1. Data

The applicant has submitted a dossier in support of its application for the authorisation of the food flavouring 'Grill flavour concentrate (vegetable)' (see [Documentation provided to EFSA n. 5](#)). The flavouring is intended to be used in meats and meat products, sauces and similar products (ketchups, BBQ sauces), processed cheese and cream cheese, as well as snacks.

Additional information was sought from the applicant during the assessment process in response to requests from EFSA sent on 7 April 2016, 25 October 2016, 8 February 2017, 5 October 2017 and 18 September 2018. The applicant provided the requested information (see [Documentation provided to EFSA n. 6; 7; 8; 9 and 10](#)).

2.2. Methodologies

This assessment was conducted in line with 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 FAF Panel assessed the safety of the 'Grill flavour concentrate (vegetable)' as an example of 'other flavourings' in line with the principles laid down in Regulation (EC) No 1331/2008, Regulation (EC) No 1334/2008, as well as the criteria outlined in the current 'Guidance on the data required for the risk assessment of flavourings to be used in or on foods' (EFSA CEF Panel, 2010), Part B. IV. 'Information to be supplied with an application for the authorisation of Other Flavourings' (see [Appendix D](#)). In addition, the EFSA Scientific Committee Statement on genotoxicity assessment of chemical mixtures (EFSA Scientific Committee, 2019) was also considered in this assessment.

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 Regulation (EC) No 1331/2008 of the European Parliament and of the Council of 16 December 2008 establishing a common authorisation procedure for food additives, food enzymes and food flavourings. OJ L354, 31.12.2008, p. 1–6.

3. Assessment

3.1. Technical data

3.1.1. Identity of the flavouring

The applicant has provided the following information with respect to the identity of the flavouring.

| | |
|---------------------|---|
| Chemical name: | Not applicable |
| FL-no: | 21.002 |
| CAS Number: | Not available |
| EINECS Number: | Not available |
| Synonyms: | Not available |
| Trade name: | The generic name of the flavouring is 'Grill flavour concentrate (vegetable)'. According to the applicant, the product is not marketed as such but used only internally for the manufacturing of flavourings. Therefore, no trade name of the flavouring exists |
| Chemical formula: | Not applicable (complex mixture of volatiles) |
| Structural formula: | Not applicable |
| Molecular weight: | Not applicable |

3.1.2. Specifications

The specifications of 'Grill flavour concentrate (vegetable)' are listed in Table 1.

Table 1: Specifications of 'Grill flavour concentrate (vegetable)'

| 'Grill flavour concentrate (vegetable)' | | |
|---|---|-------------|
| Description of the source and production process | Grill flavour concentrate (vegetable) is derived from rapeseed oil obtained from <i>Brassica napus</i> , low in erucic acid (< 2%) subjected to a heating process and subsequent distillation steps | |
| Composition | Distributions (GC peak area percentages) of the following chemical groups | |
| | Alkanes/alkenes (aliphatic saturated and unsaturated hydrocarbons): 20–25% | |
| | Saturated and unsaturated carboxylic acids: short- and medium-chain (C4–C11) fatty acids 33–34%; long-chain fatty acids 2–4% | |
| | Aromatic compounds: 1–2% | |
| | Saturated and unsaturated aldehydes and ketones: 9–10% | |
| | Acrolein | < 5 mg/kg |
| | Benzo(a)pyrene | < 2 µg/kg |
| Benzo(a)anthracene | < 5 µg/kg | |
| Benzo(c)fluorene | < 20 µg/kg | |
| Sum of 4 PAHs (benzo(a)pyrene, benzo(a)anthracene, benzo(b)fluoranthene and chrysene) | < 10 µg/kg | |
| Sensory profile | Clear, yellow-orange liquid that is oil-soluble. It carries an intensive grill note | |
| Physical characteristics | Water content | < 0.1% |
| | Refractive index 20°C | 1.447–1.467 |
| | Density 20°C | 0.906–0.926 |

GC: gas chromatography; PAHs: polycyclic aromatic hydrocarbons.

3.1.3. Manufacturing process

Source material

The source material of the flavouring 'Grill flavour concentrate (vegetable)' is canola oil. It is derived from the seeds of *Brassica napus* by pressing and solvent extraction, followed by conventionally employed refining steps, i.e. degumming, neutralisation, bleaching and deodorisation. According to a certificate provided by the applicant, the specification of the refined canola oil meets the requirements regarding heavy metals, mycotoxins, dioxins and 3-monochloropropane-1, 2-diol (3-MCPD) according to Commission Regulation (EC) No 1881/2006³ and the requirements regarding pesticide residues according to Regulation (EC) No 396/2005⁴ of the European Parliament and of the Council. Analytical data on tocopherols, heavy metals, polycyclic hydrocarbons and dioxins/polychlorinated biphenyls (PCBs) have been provided for three batches of refined canola oil used as starting material for the production of the flavouring.

Genetically modified organism

The flavouring does not contain and is not produced from genetically modified organisms (GMOs).

Production process

Refined canola oil is pumped together with air at defined flow rates through a stainless steel tube heated at high temperature. Formed solid matter is constantly removed by a wiper rotating inside the tube, and the reaction mixture is passed through water in downstream washing flasks. After combining the aqueous solutions from the washing flasks, the organic phase is separated and subsequently subjected to three sequential fractionated distillations under vacuum (see Appendix F).

Technical details of the production process and key parameters have been made available to the Panel.

3.1.4. Compositional data

The identification of the volatile constituents was based on the analysis of 'Grill flavour concentrate (vegetable)' (batch no. 202509) by gas chromatography and capillary gas chromatography/mass spectrometry (GC/MS). Under the employed conditions, 630 peaks were detected. Of these, 156 (corresponding to 63% of the total peak area) were identified by comparison of the mass spectral data to those of authentic reference compounds or commercially available MS libraries; 88 peaks (corresponding to 21% of the total peak area) were tentatively identified based on fragmentation patterns of homologous compounds, and 386 peaks (corresponding to 16% of the total peak area) remained unidentified (Table 2).

Table 2: Overall composition of Grill flavour concentrate expressed as percentage peak areas determined by GC/MS

| Fraction | Number of peaks | % of total peak area | Relative peak area of single components | |
|---------------------------------------|-----------------|----------------------|---|--------------------|
| | | | Average % of peak area | Max % of peak area |
| All peaks | 630 | 100 | 0.16 | 5.4 |
| Identified ^(a) | 156 | 63 | 0.39 | 5.4 |
| Tentatively identified ^(b) | 88 | 21 | 0.22 | 2.1 |
| Not identified | 386 | 16 | 0.05 | 1.1 |

GC/MS: gas chromatography/mass spectrometry.

(a): By means of MS/Reference library.

(b): Compared with fragmentation pattern of homologous compounds.

³ Regulation (EC) No 1881/2006 of the European Parliament and of the Council of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. OJ L 364, 20.12.2006, p. 5–24.

⁴ Regulation (EC) No 396/2005 of the European Parliament and of the Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC. OJ L 70, 16.3.2005, p. 1–16.

Most of the constituents of the flavouring belong to the chemical classes of saturated and unsaturated short- and medium-chain (C4–C11) fatty acids (33–34%) and long-chain fatty acids (2–4%). They are quantitatively followed by aliphatic saturated and unsaturated hydrocarbons (20–25%), aldehydes/ketones (9–10%) and aromatic compounds (1–2%).

The 20 principal volatile constituents identified in 'Grill flavour concentrate (vegetable)' are shown in Table 3.

Table 3: The 20 principal identified constituents of 'Grill flavour concentrate (vegetable)', expressed as percentage of total peak area determined by GC/MS in batch no. 202509

| Constituent | % of total peak area |
|--------------------|----------------------|
| Octanoic acid | 5.4 |
| Decanoic acid | 5.0 |
| Heptanoic acid | 3.9 |
| 6-Heptenoic acid | 3.5 |
| (Z)-8-Heptadecene | 2.7 |
| Nonanoic acid | 2.6 |
| Hexanoic acid | 2.4 |
| 7-Octenoic acid | 1.9 |
| (E)-2-Decenal | 1.9 |
| 5-Hexenoic acid | 1.4 |
| Nonanal | 1.2 |
| (E)-2-Undecenal | 1.1 |
| 9-Decenoic acid | 0.8 |
| Pentanoic acid | 0.8 |
| 2-Octylfuran | 0.8 |
| 4-Pentenoic acid | 0.7 |
| Pentadecane | 0.7 |
| Heptadecane | 0.7 |
| 10-Undecenoic acid | 0.7 |
| Decan-2-one | 0.4 |
| Total | 38.6 |

GC/MS: gas chromatography/mass spectrometry.

Information on the 156 identified and the 88 tentatively identified substances is presented in Appendix A (Tables A.1, A.2 and A.3). The highest individual percentage peak areas among the fractions of tentatively identified and unidentified constituents were 2.1% and 1.1%, respectively (Table 2).

To demonstrate batch-to-batch variability, the applicant presented a comparison of the GC-FID (gas chromatography–flame ionisation detection) chromatograms of five batches (i.e. batch 202509 produced on 18.10.2012; batch 202510 produced on 24.10.2012; batch 202511 produced on 22.11.2012; batch 202512 produced on 27.11.2012; batch 202513 produced on 29.11.2012) of the 'Grill flavour concentrate (vegetable)'. Despite their complexity, the chromatograms showed high visual similarity, suggesting a good reproducibility of the manufacturing process. This was supported by quantitative comparisons of the peak area percentages for more than 630 peaks; the Panel considered the distributions of the volatiles as sufficiently consistent.

Batch number 202513 was used for toxicological tests.

3.1.5. Stability of the substance, and reaction and fate in food

The stability was tested by monitoring the concentrations of 20 selected constituents (saturated and unsaturated fatty acids, alkanes/alkenes, aldehydes/ketones and an alkylfuran) upon storage of the flavouring. The total amount of the analysed constituents decreased by 2.9% after storage for 4 months at room temperature and by 18% after storage of the flavouring for 7 months in the refrigerator. However, the relative proportions of the 20 analysed representatives of different chemical classes remained sufficiently consistent, indicating that over this storage period there is no significant change in the overall composition of the flavouring.

No data on reactions and fate of the flavouring in food have been provided by the applicant. However, taking into account the nature of the constituents, the Panel considered that the flavouring is not expected to react in food differently from other chemically defined flavourings substances.

3.2. Structural/metabolic similarity to substances present in existing FGEs (Appendix A)

Some of the identified constituents in the Grill flavour concentrate (79 substances out of 156) have been already evaluated by EFSA or are currently being evaluated as chemically defined flavouring substances in one of the Flavouring Group Evaluations (FGE), according to Commission Regulation (EC) No 1565/2000⁵. Their chemical structures, together with their evaluation status, are shown in Appendix A (Table A.1). The remaining identified constituents in the Grill flavour concentrate (i.e. 77 substances out of 156) were not evaluated by EFSA. Their structures, shown in Appendix A (Table A.2), are similar to those of already evaluated chemically defined flavouring substances. The tentatively identified components are listed in Appendix A (Table A.3).

3.3. Information on existing evaluations from EFSA

Grill flavour concentrate (vegetable) has not been evaluated by EFSA before.

3.4. Exposure assessment

The data necessary for the calculation of exposure estimates (i.e. normal and maximum occurrence levels for refined subcategories of foods and beverages) are reported in Appendix B.

3.4.1. Chronic dietary exposure

The exposure assessment to be used for the safety evaluation of the flavouring is the chronic added portions exposure technique (APET) estimate (EFSA, 2010). The chronic APET for [FL-no: 21.002] has been calculated for adults and children (see Table 4). Based on use levels provided by the applicant (see Appendix B), the chronic APET calculation is based on the combined normal occurrence level.

Although the flavouring is not intended to be used in food categories specifically intended for infants and toddlers, these could still be exposed through consumption of foods from the general food categories, which may contain the flavouring. However, at present, there is no generally accepted methodology to estimate chronic dietary exposure in these age groups resulting from consumption of foods from the general categories. Exposure of infants and toddlers is currently under consideration by EFSA.

Table 4: APET – Chronic Dietary Exposure to 'Grill flavour concentrate (vegetable)'

| Chronic APET ^(a) | Added ^(b) | | Other dietary sources ^(c) | | Combined ^(d) | |
|-----------------------------|----------------------|-------------------|--------------------------------------|-------------------|-------------------------|-------------------|
| | µg/kg bw per day | µg/person per day | µg/kg bw per day | µg/person per day | µg/kg bw per day | µg/person per day |
| Adults ^(e) | 6.7 | 402 | 0 | 0 | 6.7 | 402 |
| Children ^(f) | 16.8 | 252 | 0 | 0 | 16.8 | 252 |

(a): APET: added portions exposure technique; bw: body weight: the chronic APET calculation is based on the combined **normal** occurrence level.

(b): APET Added is calculated on the basis of the **normal** amount of flavouring added to a specific food category.

(c): APET Other Dietary Sources is calculated based on the natural occurrence of the flavouring in a specified food category.

(d): APET Combined is calculated based on the combined amount of added flavouring and naturally occurring flavouring in a specified food category.

(e): For the adult APET calculation, a 60-kg person is considered representative.

(f): For the child APET calculation a 3-year-old child with a 15-kg body weight is considered representative.

3.4.2. Acute dietary exposure

The acute APET calculation for [FL-no: 21.002] is based on the combined maximum occurrence level and large portion size, i.e. three times standard portion size (see Appendix B).

⁵ 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 of the European Parliament and of the Council. OJ L 180, 19.7.2000, p. 8–16.

Although the flavouring is not intended to be used in food categories specifically intended for infants and toddlers, these could still be exposed through consumption of foods from the general food categories, which may contain the flavouring. However, at present, there is no generally accepted methodology to estimate acute dietary exposure in these age groups resulting from consumption of foods from the general categories. Exposure of infants and toddlers is currently under consideration by EFSA.

Figures for the chronic APET value for the flavouring are given in Table 5.

Table 5: APET – Acute Dietary Exposure to ‘Grill flavour concentrate (vegetable)’

| Acute APET ^(a) | Added ^(b) | | Other dietary sources ^(c) | | Combined ^(d) | |
|---------------------------|----------------------|-------------------|--------------------------------------|-------------------|-------------------------|-------------------|
| | µg/kg bw per day | µg/person per day | µg/kg bw per day | µg/person per day | µg/kg bw per day | µg/person per day |
| Adults ^(e) | 50 | 3,000 | 0 | 0 | 50 | 3,000 |
| Children ^(f) | 126 | 1,890 | 0 | 0 | 126 | 1,890 |

(a): APET: added portions exposure technique; bw: body weight: the acute APET calculation is based on the combined **maximum** occurrence level.

(b): APET Added is calculated on the basis of the **maximum** amount of flavouring added to a specific food category.

(c): APET Other Dietary Sources is calculated based on the natural occurrence of the flavouring in a specified food category.

(d): APET Combined is calculated based on the combined amount of added flavouring and naturally occurring flavouring in a specified food category.

(e): For the adult APET calculation, a 60-kg person is considered representative.

(f): For the child APET calculation, a 3-year-old child with a 15-kg body weight is considered representative.

3.4.3. Exposure via other sources than from the use of the flavouring

According to the information available to the Panel, Grill flavour concentrate (vegetable) is not intended to be used for purposes other than food flavouring.

However, there may be exposure to individual constituents of Grill flavour concentrate (vegetable) via their use as chemically defined flavouring substances.

In addition, several of the constituents of the flavouring are also formed via thermo-oxidation of fatty acids (see Table 3 in Section 3.1.4) and thus humans are exposed to these constituents via consumption of heated lipid-containing foods.

3.5. Biological and toxicological data

3.5.1. Genotoxicity

Grill flavour concentrate (vegetable) is a complex mixture containing approximately 63% as identified components, 21% as tentatively identified components and 16% as unidentified components expressed as relative peak areas.

The recommended approach by the EFSA Scientific Committee (2019) for the genotoxicity assessment of mixtures containing a substantial fraction of unidentified components, foresees that first the genotoxicity of the chemically identified components of the mixture should be assessed individually, using all available information, including read across and QSAR considerations about their potential genotoxicity. The Panel noted that the dossier has been submitted before publication of the EFSA Scientific Committee statement (EFSA, Scientific Committee, 2019), but decided to apply the approach as described in this statement to the compositional data provided by the applicant.

To this end, the identified constituents of the ‘Grill flavour concentrate (vegetable)’ (see Appendix A – Tables A.1 and A.2) were evaluated individually both *in silico* and for 79 components, which were already assessed by EFSA as flavouring substances, also via genotoxicity data made available by industry (see Appendix A – Table A.1). Concerning the *in silico* analysis, the OECD QSAR toolbox (version 4.2) was used for all identified constituents in order to identify structural alerts related to genotoxicity and to derive predictions for specific genotoxicity testing (i.e. Ames test, *in vitro* chromosomal aberration and micronucleus tests profilers). Structural alerts (alpha-beta unsaturated carbonyls and simple aldehyde) were identified in 23 substances.

For 17 of these substances, the genotoxicity concern identified via the *in silico* analysis was ruled out for by the experimental data submitted by industry and assessed by the CEF Panel and by FAF Panel in the context of genotoxicity evaluation of flavourings (EFSA CEF Panel, 2015, 2016, 2018; EFSA FAF Panel, 2018; see Appendix A – Table A.1).

For four substances (i.e. 2-cyclohexen-1-one, 10-oxodecanoic acid, 9-oxononanoic acid and pentadecanal), the Panel noted that structurally related flavouring substances exist in other FGEs for which no safety concerns were identified (see Appendix A – Table A.2). Cyclohexen-1-one has structural similarity to flavouring substances methylcyclohex-2-en-1-one [FL-no: 07.098] and methyl-2-cyclopenten-1-one [FL-no: 07.112]) for which the concern on genotoxicity was ruled out in FGE.212Rev1 (EFSA CEF Panel, 2011a) and in FGE.212Rev3 (EFSA CEF Panel, 2015), respectively. The three aldehydes, 10-oxodecanoic acid, 9-oxononanoic acid and pentadecanal, are structurally similar to a number of linear saturated aldehydes of comparable chain length (i.e. octanal [FL-no: 05.009], nonanal [FL-no: 05.025], undecanal [FL-no: 05.034] and dodecanal [FL-no: 05.011]), which are released as hydrolysis products from several acetal flavouring substances evaluated by EFSA in FGE.03Rev2 (EFSA CEF Panel, 2011d) and as flavouring substances as such by JECFA in 1999 (JECFA, 1999).

For the remaining two identified components, i.e. 2-decen-1,4-lactone and 2-undecen-4-one, the Panel noted that the evaluation of their genotoxic potential is still pending in other FGEs, as additional genotoxicity data have been requested for 2-decen-1,4-lactone in FGE.217Rev2 (EFSA FAF Panel, 2019) (see Appendix A – Table A.1) and for flavouring substances structurally related to 2-undecen-4-one (i.e. non-2-en-4-one [FL-no: 07.187], oct-2-en-4-one [FL-no: 07.082] and hept-2-en-4-one [FL-no: 07.104]) in FGE.204 (EFSA CEF Panel, 2012) (see Appendix A – Table A.2).

In addition, although no structural alerts for genotoxicity were identified by the OECD QSAR Toolbox (version 4.2), the Panel identified a potential genotoxicity concern also for four other identified components of the Grill flavour concentrate (vegetable), i.e. 2-pentylfuran, 2-heptylfuran, 2-octylfuran and 2-hexylfuran (see Appendix A – Table A.1). This is based on the fact that for three of these four components (2-pentylfuran [FL-no: 13.059], 2-heptylfuran [FL-no: 13.069], 2-octylfuran [FL-no: 13.162]) additional genotoxicity data were required by the CEF Panel in FGE.67Rev1 (EFSA CEF Panel, 2011b) and FGE.13Rev2 (EFSA CEF Panel, 2011c), respectively. These data are under evaluation in FGE.67Rev3 and FGE.13Rev3 and are also relevant for the structurally related substance 2-hexylfuran, which is not under assessment as flavouring substance.

With respect to the tentatively identified constituents, the Panel noted that some of them show structural similarity with the identified substances (see Appendix A – Table A.3). However, in the absence of fully confirmatory chemical data, the Panel considered the tentatively identified part of the mixture as uncharacterised.

Regarding the unidentified fraction of the mixture, the EFSA Scientific Committee recommends as first option to test it for genotoxicity separately from the rest of the mixture. Alternatively, if this is not feasible, the testing of the whole mixture should be undertaken (EFSA Scientific Committee, 2019). In the case of Grill flavour concentrate (vegetable), the Panel considered that the separation and testing of the unidentified and/or of the tentatively identified part of the mixture would not be technically feasible. Therefore, the Panel considered not only the available information on individual constituents of the chemically characterised fraction, but also the experimental data on the whole mixture for the genotoxicity assessment of the flavouring.

Bacterial reverse mutation assay

In order to investigate the potential of 'Grill Flavour Concentrate (vegetable)' and/or its metabolites to induce gene mutations in bacteria, an Ames test was performed according to OECD Test Guideline 471 (OECD, 1997) and following Good Laboratory Practice (GLP) in five strains of *Salmonella* Typhimurium (TA97a, TA98, TA100, TA1535 and TA102) both in the presence and absence of metabolic activation (Silesia Gerhard Hanke GmbH & Co. KG, 2013). Two separate experiments were performed applying the plate incorporation method (experiment 1) and the pre-incubation assay (experiment 2). The test article was evaluated in experiment 1 at five concentrations (ranging from 50 to 5,000 µg/plate), and in experiment 2 at seven concentrations (ranging from 79 to 5,000 µg/plate) with and without S9-mix. Appropriate positive controls and ethanol as a vehicle control were evaluated concurrently; all test and control articles were evaluated in quadruplicate plates. All positive controls induced significant increases in revertant colony numbers. The test was considered to fulfil the acceptability criteria.

No precipitate was observed at any tested concentration in any tester strain with or without S9-mix. Toxicity, as evident by the absence or reduction in the mean number of revertant colonies and absence or reduction in the background bacterial lawn, was observed in experiment 2 at the two highest concentrations (2,500 and 5,000 µg/plate) in all tester strains with and without S9-mix. No increase in the mean number of revertant colonies was observed at any tested concentration in any tester strains with or without S9-mix. No mutagenic activity could be observed under the conditions employed in this study.

***In vitro* micronucleus assay**

An *in vitro* micronucleus assay was carried out according to OECD Test Guideline 487 (OECD, 2010) and following GLP (Silesia Gerhard Hanke GmbH & Co. KG, 2014). Whole blood cultures were prepared from healthy donors and peripheral lymphocytes stimulated with phytohaemagglutinin. Based on a preliminary cytotoxicity range-finder experiment, three doses were selected for two independent experiments (from 0.05 to 0.25 µL/mL without S9-mix and from 0.05 to 0.40 µL/mL with S9-mix). Ethanol was used as a vehicle control; mitomycin C (MMC) and cyclophosphamide (CPA) were employed as positive controls in the absence and presence of S9-mix, respectively. Positive controls induced significant increases of micronuclei (MN) and the system was considered sensitive and valid. Cells were treated with the test article after 71 h of culture following two treatment schedules: a short treatment followed by a recovery period (4 + 18 h) both in the presence and absence of metabolic activation, and a continuous treatment for 19 h in the absence of metabolic activation. Cells were harvested about 90 h after culture initiation. Two thousand cells were scored per concentration.

Thirty-four per cent cytotoxicity was observed at the highest concentration after short treatment with and without S9 mix; 40% cytotoxicity was detected at the highest concentration after continuous treatment without S9-mix. No statistically significant increase in the frequency of MN was observed at any concentration analysed. It was concluded that the test article did not induce MN in cultured human peripheral blood lymphocytes under the experimental conditions employed in this study.

The studies are summarised in Table C.1 in Appendix C.

***In vivo* studies**

No data provided.

3.5.2. Conclusion on genotoxicity

Except for six substances (i.e. 2-decen-1,4-lactone, 2-undecen-4-one, 2-pentylfuran, 2-heptylfuran, 2-octylfuran and 2-hexylfuran), the assessment of individual components did not raise a concern for genotoxicity.

In addition, the available experimental data obtained with the whole mixture (Grill flavour concentrate (vegetable), as such) do not indicate a concern for genotoxicity.

Five of the six substances that raised concern regarding genotoxicity (i.e. 2-decen-1,4-lactone, 2-undecen-4-one, 2-pentylfuran, 2-heptylfuran and 2-octylfuran) are currently under consideration in other FGEs (i.e. FGEs 217Rev2, 204Rev1, 67Rev3 and 13Rev3). The remaining substance (i.e. 2-hexylfuran), which is not listed in the Union list of chemically defined flavouring substances,¹ can be covered by the evaluation of the other three 2-alkylfurans.

3.5.3. Absorption, distribution, metabolism and excretion

No information was provided by the applicant on absorption, distribution, metabolism and excretion (ADME) for Grill flavour concentrate.

3.5.4. Acute toxicity

No information was provided by the applicant on acute toxicity for Grill flavour concentrate.

3.5.5. Short-term and subchronic toxicity

Initially, a 28-day repeated-dose toxicity study and a 90-day subchronic toxicity study were conducted by the applicant, in order to identify suitable doses and to test the safety of the Grill flavour concentrate, respectively. To clarify issues raised by the Working group regarding the findings in the 90-day study, the applicant submitted additional toxicity studies; a 14-day dose-range finding (DRF) study and a 28-day repeated-dose toxicity study. All toxicity studies are described below.

3.5.5.1. 28-Day repeated-dose toxicity study in rats (vivo Science GmbH, 2016)

In order to test the palatability of the doses intended for the 90-day repeated-dose toxicity study, a non-GLP 28-day DRF study was performed in Wistar rats. There were four treatment groups, each consisting of six animals each (three males and three females). The control group (0 mg) received basal diet. The Grill flavour concentrate was administered in the feed at doses of 667, 133, 27 and 0 mg/kg of the feed. An estimated food consumption of 90.0 g/kg body weight (bw) per day equals to 60, 12, 2.4 and 0 mg of the test item per kg/bw per day.

However, the actual food consumption was 22% and 30% less than anticipated for female and male rats, respectively, also in the control animals. This resulted in exposures of approximately 47, 9 and 2 mg/kg bw per day for females and 43, 8 and 2 mg/kg bw per day for males over the study period.

There was no difference among animals of any treatment group regarding general clinical signs and motor activity. Body weight gain was within normal range in all treatment groups. The mean food and water consumption of the animals of both sexes was comparable over the duration of the study among all treatment groups. However, there were some differences in water consumption, which was higher in the medium-dose females during the whole study period and for the high-dose animals of both sexes in the beginning of the study. At sacrifice, no difference among the treatment groups were observed during gross examination.

The 28-day study is shown in table format in Appendix C.

3.5.5.2. 90-Day subchronic study (vivo Science GmbH, 2016)

In a 90-day study conducted in compliance with OECD test Guideline 408, (OECD, 1998) and according to GLP, 7- to 8-week-old Wistar Han rats (females were nulliparous and non-pregnant) were grouped in batches of 10 rats/sex per treatment group (randomised by body weight) and were given dietary exposure either to vehicle (mazola corn oil) or to 72, 14.4 and 2.9 mg of Grill flavour concentrate (vegetable)/kg bw per day for 92 days. The Panel noted that the actual food consumption over the study period of 92 days resulted in an exposure that was between 90.8% and 105.5% of the intended exposure. This resulted in a mean exposure of 73.4, 14.5 and 2.9 mg/kg bw per day for females and 71.0, 14.1 and 3.0 for males.

The 90-day study is shown in table format in Appendix C.

No animals died or demonstrated adverse clinical symptoms during the study. Significantly increased body weights (males: day 1; females: days 1–15 were transitory and not dose dependent while food intake and water consumption declined across the study period in all groups and both sexes. One male in the high-dose group also had a renal tumour. No organ weights were significantly altered in treatment groups. No histological abnormalities were reported in the high-dose groups.

A statistically significant increase in plasma creatinine was observed in male rats in the high- (72 mg/kg bw per day) and medium-dose (14.4 mg/kg bw per day) groups compared to the control. The effect was dose-related and associated with a statistically significant increase in blood urea at the top-dose only. The increase in creatinine and urea was not associated with any histological change in the kidney and in the muscles, though the increase in creatinine was higher than the corresponding historical control data for this strain of rats.

Additional changes in biochemical plasma parameters were observed at the top dose (i.e. increase in Cl, Na and Ca and increase in albumin, globulin and total protein), but a correlation with the increase in creatinine and urea could not be established. Similar to male rats, female rats had an increase in globulin and total protein and a decrease in albumin/globulin (A/G) ratio at the top dose. The creatinine level in urine was not measured.

The Panel decided that the observed change in creatinine levels raised a concern for the following reasons: dose-related, statistically significant, outside the historical control range, correlated with an increase in plasma urea (additional marker of renal toxicity) and associated with additional changes in biochemical parameters which were observed at the top dose.

An increase in fatty tissue was observed in both male and female rats. The effect was not dose-related, was not associated with any histological change in any organ and was not associated with an increase in body weight and food consumption. The absence of any correlate, in particular, the absence of any specific organ infiltration by fat tissue, is suggesting that this effect was not adverse; though a relationship with a metabolic imbalance, if any, cannot be excluded as plasma triglycerides were not measured in the study.

The Panel noted that the top dose (i.e. 72 mg/kg bw per day) in the 90-day study was not representing a maximum tolerated dose (MTD) and for this reason a clear correlation with target organ toxicity could not be ruled out as it is possible that at this dose the effect observed indicated a target organ toxicity in the kidney.

In response to the request of the Panel to clarify the toxicological significance of the kidney toxicity observed in the 90-day study, the applicant conducted a 28-day oral toxicity study in rats (see Section 3.5.5.4) preceded by an appropriate 14-day DRF study. The objective of the DRF study was to determine the toxicity of the Grill flavour concentrate (vegetable), following daily oral administration to

the rat for 14 days and to provide the basis for the selection of dose levels up to 350 mg/kg feed, as requested by the Panel, for a subsequent 28-day study.

3.5.5.3. 14-Day dose-range finding study in rats (Covance, 2018)

The report and detailed description of the DRF study was provided by the applicant. This was conducted by Covance, under GLP quality assurance.

Seven- to eight-week-old male and female CrI:WI(Han) rats were grouped into two groups of 10 animals each (5 males/5 females). The control group received diet supplemented with corn oil, whereas the treatment group were given the same diet supplemented with corn oil and the test substance, at a dose to reach the target dose level of 350 mg/kg bw per day.

No mortality occurred, and no extensive test article-related clinical observations were noted. Body weight, body weight gain and food consumption were not adversely affected. Organ weights and organ weights relative to body weights were not significantly altered in the test substance exposed groups (changes < 10%). The incidence of macroscopic and microscopic abnormalities noted did not show any significant relationship to treatment, except for a substance-related increase severity in hyaline droplets, which ranged from minimal in controls to slight/moderate severity grades in males exposed to 350 mg/kg bw per day. No evidence of cell damage was detected. These findings indicate that the dose level of 350 mg of the test substance [FL-no. 21.002]/kg bw per day via dietary (oral intake) is tolerated in rats and a 28-day study could be performed at this dose level.

The 14-day study is shown in table format in Appendix C.

3.5.5.4. 28-Day repeated-dose toxicity study in rats (Covance, 2018)

In a 28-day study conducted in compliance with OECD test Guideline 407 (OECD, 2008) and according to GLP, nine- to 10-week-old CrI:WI(Han) rats were randomised to treatment groups (5 males and 5 females in each group except 'second maximum dose group' for which 2 males and 2 females were included) and provided with *ad libitum* access to water and feed. The vehicle was corn oil and animals were administered either vehicle only (controls) or test substance in corn oil by oral gavage for 28 days. Five principal treatment groups were included, with dose levels of the test substance at 0, 2.9, 14.4, 72 and 350 mg of the test substance (FL-no. 21.002)/kg bw per day. Two secondary groups (controls and maximum dose) were used for recovery testing, with treatment stopping at the end of week 4, allowing 2 weeks recovery prior to necropsy (n = 5/sex per group for all groups, except n = 2 for a second maximum dose group).

Target concentrations of the test substance in corn oil were achieved. Detailed observations of weight, clinical observations, functional observational battery and motor activity were made, blood and over-night urine were collected for detailed analyses (including measurement of kidney damage indicators) in week 4.

No incidences of mortality and no effects on body weight (apart from a significant, 8%, increase in terminal body weights of recovery phase females at the highest dose level) or body weight gain or food consumption were reported in dosing or recovery groups.

A few functional or observational parameters were significantly affected by treatment but none of these showed a dose dependency (e.g. significantly increased foot splaying in females at 14.4 mg/kg bw per day).

Exposure to 350 mg/kg bw per day increased absolute and relative kidney weight for both male and female rats (i.e. 20% and 14% increase in relative kidney weight, respectively) compared to controls. Recovery testing showed that the increase in kidney weight was partly and fully reversible in male and female rats, respectively.

A substance-related and dose-dependent increase in albumin:creatinine ratio was observed in male rats administered 72 and 350 mg/kg bw per day. There was an increase for urine kidney injury marker-1 (KIM-1): creatinine ratios in male rats administered 350 mg/kg bw per day compared to controls. Enlarged kidneys were recorded for males administered 350 mg/kg bw per day and pale and/or mottled kidneys for males exposed to 72 and 350 mg/kg bw per day upon macroscopic examination. Lower urine volumes were recorded on day 28 for male rats administered 2.9, 72 or 350 mg/kg bw per day. In addition, male rats administered 350 mg/kg bw per day had a small increase in total plasma protein, which were attributed to a slightly higher increase in globulin and urea. No other substance-related haematological/clinical biochemical changes were observed.

No treatment-related changes in the amounts of adipose tissue were reported. In addition, no increase in plasma triglyceride levels was observed for any of the dose groups.

Males exposed to 72 or 350 mg/kg bw per day showed increased severity of hyaline droplets and tubular basophilia. In addition, males exposed to the top dose, had an increase in hyaline/granular casts. Immunohistochemical staining indicated the presence of α 2-microglobulin. The Panel notes that the identification of α 2-microglobulin only was performed for controls and the top dose. In addition, some positive staining of α 2-microglobulin was detected in kidney of female rats, which indicates cross-reaction, and the level of this cross-reactivity compared to the level in male kidney has not been described.

The applicant argues that formation of hyaline droplets and the concurrent kidney damage is due to the naturally occurring α 2u-globulin and that the substance-related effects on kidney is therefore not relevant for humans. Despite the limitations pointed out concerning the immunohistochemistry, the Panel concurs with the view of the applicant that the findings in kidney of male rats are not relevant for human risk assessment. However, a kidney effect (increase in relative and absolute kidney weight) was also observed in female rats at the top dose and this was reversible.

Overall, the additional 28-day study with the increased dose level of 350 mg of the test substance [FL-no: 21.002]/kg bw per day confirms α 2u-globulin accumulation indicating potential kidney pathology in male rats.

The 28-day study is shown in table format in Appendix C.

3.5.6. Chronic toxicity and carcinogenicity

Chronic toxicity/carcinogenicity studies are not available for the safety evaluation of the Grill flavour concentrate (vegetable).

3.5.7. Reproductive and developmental toxicity

Developmental toxicity studies are not available for the safety evaluation of the Grill flavour concentrate (vegetable).

3.5.8. Conclusion on toxicity

In the absence of the data falling under Sections 3.5.6 and 3.5.7, the general toxicity of the Grill flavour concentrate (vegetable) will be based on the available subchronic oral toxicity studies.

From the additional 28-day study, the Panel concluded that the changes in the male kidney are caused by the α 2-microglobulin accumulation, resulting in hyaline droplets formation and hyaline casts in the kidney. This mechanism is irrelevant for humans (Hard et al., 1993; Swenberg, 1993), and therefore, the renal changes in the male kidneys in the 90-day study are not further considered.

Similarly, the absence of changes in serum triglycerides levels in the 28-day study indicates that Grill flavour concentrate (vegetable) [FL-no: 21.002] does not cause metabolic imbalance, thus the increase in the amount of adipose tissue in the 90-day study is not considered adverse.

On the other hand, in the 90-day study, the Panel noted that Grill flavour concentrate (vegetable) [FL-no: 21.002] induced statistically significant changes in serum plasma globulin, A/G ratio and total protein also in female animals. The Panel considered that these effects were adverse. Benchmark dose (BMD) analyses were applied for these effects in rats and the lower 95% confidence limit (single sided) of the benchmark dose for a 5% effect (BMDL₀₅) were calculated to be 12.8 to 76.7 mg/kg bw per day. The BMDL₀₅ was calculated in accordance to the update on the use of the BMD approach (EFSA Scientific Committee, 2017) using the EFSA web-tool (Appendix E). The models used in the analysis were consistent and passed statistical validation.

The Panel decided that the BMDL₀₅ value of 12.8 mg/kg bw per day for the parameter A/G ratio was the most appropriate departure point for the calculation of margin of safety.

3.6. Discussion

Grill flavour concentrate (vegetable) is a complex mixture resulting from the heating of canola oil and subsequent distillation steps. It contains approximately 63% of identified components, 21% of tentatively identified components and 16% of unidentified components expressed as relative peak areas (GC-MS).

Out of 156 identified constituents in the Grill flavour concentrate, 79 have been already evaluated by EFSA or are currently being evaluated as chemically defined flavouring substances in one of the FGE. Their chemical structures, together with their evaluation status, are shown in Appendix A (Table A.1).

Except for six substances (i.e. 2-decen-1,4-lactone, 2-undecen-4-one, 2-pentylfuran, 2-heptylfuran, 2-octylfuran and 2-hexylfuran), the assessment of individual components did not raise a concern for genotoxicity. Additional data have been requested to finalise the evaluation of the genotoxic potential of these six substances. The available experimental data obtained with the whole mixture (Grill flavour concentrate (vegetable) as such) do not indicate a concern for genotoxicity.

The chronic dietary exposure to the substance estimated using the APET was calculated to be 0.402 mg/person per day for a 60-kg adult and 0.252 mg/person per day for a 15-kg child.

Based on the BMDL₀₅ value of 12.8 mg/kg bw per day for the parameter A/G ratio and the exposure estimate mentioned above, the Panel calculated margins of safety of 1,910 for adults and 762 for children (see Table 6).

Table 6: Margins of safety

| Consumer | Study type | BMDL ₀₅ mg/kg bw per day | Add APET µg/kg bw per day (mg/kg bw per day) | Margin of Safety |
|----------|--|---|--|---------------------|
| Adults | 90-Day feeding study in rats on Grill flavour concentrate (vegetable) [FL-no:12.002] | 12.8 | 6.7 (0.0067) | 1,910 |
| Children | | | 16.8 (0.0168) | 762 |

BMDL₀₅: benchmark dose for a 5% effect; APET: added portions exposure technique; bw: body weight.

The Panel considers these margins of safety calculated for Grill flavour concentrate (vegetable) [FL-no: 21.002] as sufficient.

4. Conclusions

Based on exposure estimate and the results from the repeated-dose toxicity studies, sufficient margins of safety could be calculated.

However, the Panel noted that for six constituents of the flavouring (i.e. 2-decen-1,4-lactone, 2-undecen-4-one, 2-pentylfuran, 2-heptylfuran, 2-octylfuran and 2-hexylfuran) there is an indication for genotoxicity. Therefore, these six substances have to be evaluated. Until these evaluations have been finalised in the context of FGE.217, FGE.204, FGE.67 and FGE.13, the safety of Grill flavour concentrate (vegetable) cannot be fully assessed.

5. Recommendations

The Panel recommends that on receipt of genotoxicity data, that will cover the six constituents for which there is an indication for genotoxicity, this opinion should then be reconsidered.

Documentation provided to EFSA

- 1) Covance, 2018. Grill flavour concentrate (vegetable): 28 Day Oral (Gavage) Administration Toxicity Study in the Rat Followed by a 2 Week Recovery Period. Covance Study Number 8384830, December 2018. Unpublished study report submitted by Silesia Gerhard Hanke GmbH & Co. KG.
- 2) Covance, 2018. Grill flavour concentrate (vegetable): 14 Day Range-finding Study in the Rat. Covance Study Number 8384829, July 2018. Unpublished study report submitted by Silesia Gerhard Hanke GmbH & Co. KG.
- 3) Silesia Gerhard Hanke GmbH & Co. KG, 2013. Test report: Ames test Determination of the mutagenic potential of Grill Flavour Concentrate (vegetable) Material number: 33500103 with the Bacterial Reverse Mutation Test. Study number 13071001G803, October 2013. Unpublished study report submitted by Silesia Gerhard Hanke GmbH & Co. KG.
- 4) Silesia Gerhard Hanke GmbH & Co. KG, 2014. Test report: Micronucleus test Determination of the genotoxic potential of Grill Flavour Concentrate (vegetable) Material number: 33500103 with the In Vitro Mammalian Micronucleus Test in Human Lymphocytes. Study number 13071001G860, January 2014. Unpublished study report submitted by Silesia Gerhard Hanke GmbH & Co. KG.
- 5) Silesia Gerhard Hanke GmbH & Co. KG, Oct 2015. Application for authorisation of Grill flavour concentrate (vegetable) in accordance with Regulation (EC) No 1331/2008 according to the Regulation (EC) No 1334/2008. Submitted by Silesia Gerhard Hanke GmbH and Co. KG.

- 6) Silesia Gerhard Hanke GmbH and Co. KG, May 2016. Responses to the request for additional information on the product Grill flavour concentrate (vegetable) [FL-no: 21.002], (EFSA-Q-2015-00821). Submitted by Silesia Gerhard Hanke GmbH and Co. KG on 31/5/2016 in reply to EFSA letter dated 7/4/2016.
- 7) Silesia Gerhard Hanke GmbH and Co. KG, Jan 2017. Responses to the request for additional information on the product Grill flavour concentrate (vegetable) [FL-no: 21.002], (EFSA-Q-2015-00821). Submitted by Silesia Gerhard Hanke GmbH and Co. KG on 9/1/2017 in reply to EFSA letter dated 25/10/2016.
- 8) Silesia Gerhard Hanke GmbH and Co. KG, May 2017. Responses to the request for additional information on the product Grill flavour concentrate (vegetable) [FL-no: 21.002], (EFSA-Q-2015-00821). Unpublished data submitted by Silesia Gerhard Hanke GmbH and Co. KG on 30/05/2017 in reply to EFSA letter dated 8/2/2017.
- 9) Silesia Gerhard Hanke GmbH and Co. KG, Aug 2018. Responses to the request for additional information on the product Grill flavour concentrate (vegetable) [FL-no: 21.002], (EFSA-Q-2015-00821). Submitted by Silesia Gerhard Hanke GmbH and Co. KG on 3/8/2018 in reply to EFSA letter dated 05/10/2017, which was followed by a clarification teleconference on 13/7/2018.
- 10) Silesia Gerhard Hanke GmbH and Co. KG, Dec 2018. Responses to the request for additional information on the product Grill flavour concentrate (vegetable) [FL-no: 21.002], (EFSA-Q-2015-00821). Submitted by Silesia Gerhard Hanke GmbH and Co. KG on 10/12/2018 in reply to EFSA letter dated 18/9/2018.
- 11) Vivo Science GmbH, 2015. Dose range finding study in Wistar rats (28d) with Grill flavour concentrate. Summary Report L09-030 DRF. Study number L09-030 DRF, May 2015. Unpublished study report submitted by Silesia Gerhard Hanke GmbH and Co. KG.
- 12) Vivo Science GmbH, 2016. Repeated dose toxicity study of Grill flavour concentrate. Study number L09-030, July 2016. Unpublished study report submitted by Silesia Gerhard Hanke GmbH and Co. KG.

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





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





| | |
|--------------------|---|
| ADME | absorption, distribution, metabolism and excretion |
| A/G | albumin and globulin |
| APET | added portions exposure technique |
| BMD | benchmark dose |
| BMDL ₀₅ | benchmark dose for a 5% effect |
| BMR | benchmark response |
| BW | body weight |
| CAS | Chemical Abstract Service |
| CEF | Panel on Scientific Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids |
| CPA | cyclophosphamide |


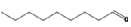




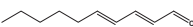
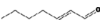
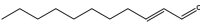
| | |
|-------------|---|
| DRF | dose-range finding |
| EINECS | European Inventory of Existing Commercial chemical Substances |
| FAF | EFSA Panel on Food Additives and Flavourings |
| FAO | Food and Agriculture Organization of the United Nations |
| FGE | Flavouring Group Evaluation |
| FID | flame ionisation detection |
| FLAVIS (FL) | Flavour Information System |
| GC | gas chromatography |
| GLP | good laboratory practice |
| GMO | genetically modified organisms |
| JECFA | The Joint FAO/WHO Expert Committee on Food Additives |
| KIM-1 | kidney injury marker-1 |
| MMC | mitomycin C |
| MN | micronuclei |
| MS | mass spectrometry |
| MSDI | maximised survey-derived daily intake |
| MTD | maximum tolerated dose |
| NOAEL | no observed adverse effect level |
| OECD | Organisation for Economic Co-operation and Development |
| PAHs | polycyclic aromatic hydrocarbons |
| PCB | polychlorinated biphenyl |
| QSAR | quantitative structure–activity relationship |
| SPET | single portion exposure technique |
| WHO | World Health Organization |



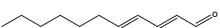

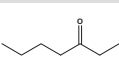
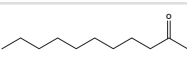
Appendix A – Constituents of Grill concentrate






Table A.1: Summary of 79 constituents identified in 'Grill flavour concentrate (vegetable)' which have also been evaluated as chemically defined flavouring substances

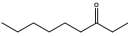
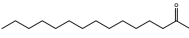
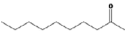
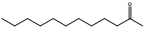

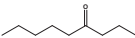
| FL-no FGE | CASrn | Union list substance name | Structural formula | % of peak area | Cramer class | α,β - subgroup | Structural alert for genotoxicity | EFSA Evaluation status |
|------------------|------------|------------------------------|---|----------------|-----------------|------------------------------|--|---|
| 01.037 25 | 112-41-4 | Dodec-1-ene |  | 0.37 | Class I | | | No longer supported by Industry Toxicity data required |
| 01.038 25 | 112-40-3 | Dodecane |  | 0.12 | Class I | | | No safety concern at the estimated level of intake based on the MSDI approach |
| 01.051 25 | 91-57-6 | 2-Methylnaphthalene |  | 0.07 | Class III | | | No longer supported by Industry Toxicity data required |
| 01.054 25 | 629-62-9 | Pentadecane |  | 0.71 | Class I | | | No safety concern at the estimated level of intake based on the MSDI approach |
| 01.057 25 | 629-59-4 | Tetradecane |  | 0.11 | Class I | | | No safety concern at the estimated level of intake based on the MSDI approach |
| 02.187 205/07 | 21964-44-3 | Non-1-en-3-ol |  | 0.03 | Class II | 1.2.2 | | No safety concern at the estimated level of intake based on the MSDI approach |
| 04.026 58 | 108-39-4 | 3-Methylphenol |  | 0.14 | Class I | | | No safety concern at the estimated level of intake based on the MSDI approach |
| 04.027 58 | 95-48-7 | 2-Methylphenol |  | 0.02 | Class I | | | No safety concern at the estimated level of intake based on the MSDI approach |





| FL-no FGE | CASrn | Union list substance name | Structural formula | % of peak area | Cramer class | α,β - subgroup | Structural alert for genotoxicity | EFSA Evaluation status |
|--------------|----------|------------------------------|---|----------------|-----------------|------------------------------|--|--|
| 04.028 58 | 106-44-5 | 4-Methylphenol |  | 0.09 | Class I | | | No safety concern at the estimated level of intake based on the MSDI approach |
| 04.041 88 | 108-95-2 | Phenol |  | 0.13 | Class I | | | No safety concern at the estimated level of intake based on the MSDI approach |
| 04.046 58 | 644-35-9 | 2-Propylphenol |  | 0.12 | Class I | | | No safety concern at the estimated level of intake based on the MSDI approach |
| 04.070 22 | 90-00-6 | 2-Ethylphenol |  | 0.12 | Class I | | | No safety concern at the estimated level of intake based on the MSDI approach |
| 05.008 – | 66-25-1 | Hexanal |  | 0.005 | Class I | | (d) | No safety concern at the estimated level of intake based on the MSDI approach Evaluated by JECFA before 2000 – No EFSA considerations required |
| 05.009 – | 124-13-0 | Octanal |  | 0.29 | Class I | | (d) | No safety concern at the estimated level of intake based on the MSDI approach Evaluated by JECFA before 2000 – No EFSA considerations required |

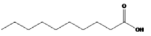
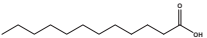
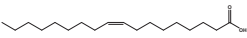
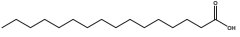
| FL-no FGE | CASrn | Union list substance name | Structural formula | % of peak area | Cramer class | α,β - subgroup | Structural alert for genotoxicity | EFSA Evaluation status |
|---------------|--------------------------|--|---|----------------|-----------------|------------------------------|--|--|
| 05.013 54 | 100-52-7 | Benzaldehyde |  | 0.03 | Class I | | (d) | No safety concern at the estimated level of intake based on the MSDI approach |
| 05.025 – | 124-19-6 | Nonanal |  | 1.16 | Class I | | (d) | No safety concern at the estimated level of intake based on the MSDI approach Evaluated by JECFA before 2000 – No EFSA considerations required |
| 05.029 20 | 104-87-0 | <i>p</i> -Tolualdehyde |  | 0.006 | Class I | | (d) | No safety concern at the estimated level of intake based on the MSDI approach |
| 05.031 – | 111-71-7 | Heptanal |  | 0.09 | Class I | | (d) | No safety concern at the estimated level of intake based on the MSDI approach Evaluated by JECFA before 2000 – No EFSA considerations required |
| 05.072 200 | 18829-56-6 | <i>trans</i> -2-Nonenal |  | 0.13 | Class I | 1.1.1 | (c) | No safety concerns for genotoxicity |
| 05.084 203 | 4313-03-5 ^(a) | Hepta-2,4-dienal |  | 0.06 | Class I | 1.1.4 | (c) | No safety concerns for genotoxicity |
| 05.140 203 | 25152-84-5 | Deca-2(<i>trans</i>),4(<i>trans</i>)-dienal |  | 0.25 | Class I | 1.1.4 | (c) | No safety concerns for genotoxicity |
| 05.150 200 | 18829-55-5 | Hept-2(<i>trans</i>)-enal |  | 0.16 | Class I | 1.1.1 | (c) | No safety concerns for genotoxicity |
| 05.184 200 | 53448-07-0 | Undec-2(<i>trans</i>)-enal or (<i>E</i>)-2-Undecenal |  | 1.15 | | 1.1.1 | (c) | No safety concerns for genotoxicity |

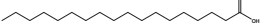



| FL-no FGE | CASrn | Union list substance name | Structural formula | % of peak area | Cramer class | α,β - subgroup | Structural alert for genotoxicity | EFSA Evaluation status |
|---------------|------------|---|---|----------------|-----------------|------------------------------|--|--|
| 05.190 200 | 2548-87-0 | <i>trans</i> -2-Octenal |  | 0.43 | | 1.1.1 | (c) | No safety concerns for genotoxicity |
| 05.191 200 | 3913-81-3 | <i>trans</i> -2-Decenal or (<i>E</i>)-2-Decenal |  | 1.92 | | 1.1.1 | (c) | No safety concerns for genotoxicity |
| 05.196 203 | 30361-29-6 | <i>tr</i> -2, <i>tr</i> -4-Undecadienal |  | 0.15 | | 1.1.4 | (c) | No safety concerns for genotoxicity |
| 07.002 – | 110-43-0 | Heptan-2-one |  | 0.03 | Class II | | | No safety concern at the estimated level of intake based on the MSDI approach Evaluated by JECFA before 2000 – No EFSA considerations required |
| 07.003 – | 106-35-4 | Heptan-3-one |  | 0.005 | Class II | | | No safety concern at the estimated level of intake based on the MSDI approach Evaluated by JECFA before 2000 – No EFSA considerations required |
| 07.016 – | 112-12-9 | Undecan-2-one |  | 0.36 | Class II | | | No safety concern at the estimated level of intake based on the MSDI approach Evaluated by JECFA before 2000 – No EFSA considerations required |

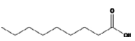
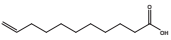

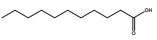
| FL-no FGE | CASrn | Union list substance name | Structural formula | % of peak area | Cramer class | α,β - subgroup | Structural alert for genotoxicity | EFSA Evaluation status |
|------------------|-----------|------------------------------|---|----------------|-----------------|------------------------------|--|---|
| 07.019 – | 111-13-7 | Octan-2-one |  | 0.13 | Class II | | | No safety concern at the estimated level of intake based on the MSDI approach Evaluated by JECFA before 2000 – No EFSA considerations required |
| 07.020 – | 821-55-6 | Nonan-2-one |  | 0.24 | Class II | | | No safety concern at the estimated level of intake based on the MSDI approach. Evaluated by JECFA before 2000 – No EFSA considerations required |
| 07.062 – | 106-68-3 | Octan-3-one |  | 0.02 | Class II | | | No safety concern at the estimated level of intake based on the MSDI approach Evaluated by JECFA before 2000 – No EFSA considerations required |
| 07.098 212/51 | 1193-18-6 | 3-Methylcyclohex-2-en-1-one |  | 0.12 | Class II | 2.6 | (c) | No safety concern at the estimated level of intake based on the MSDI approach |
| 07.112 212/51 | 2758-18-1 | 3-Methyl-2-cyclopenten-1-one |  | 0.10 | Class II | 2.6 | (c) | No safety concern at the estimated level of intake based on the MSDI approach |

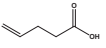
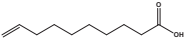
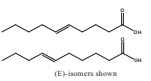

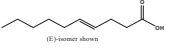
| FL-no FGE | CASrn | Union list substance name | Structural formula | % of peak area | Cramer class | α,β - subgroup | Structural alert for genotoxicity | EFSA Evaluation status |
|------------------|-----------|------------------------------|---|---|-----------------|------------------------------|--|---|
| 07.113 – | 925-78-0 | Nonan-3-one |  | 0.03 | Class II | | | No safety concern at the estimated level of intake based on the MSDI approach. Evaluated by JECFA before 2000 – No EFSA considerations required |
| 07.137 – | 2345-28-0 | Pentadecan-2-one |  | 0.13 (5-Pentylidihydrofuran-2-(3H)-one + Pentadecan-2-one) ^(e) | Class II | | | No safety concern at the estimated level of intake based on the MSDI approach. Evaluated by JECFA before 2000 – No EFSA considerations required |
| 07.150 07 | 693-54-9 | Decan-2-one |  | 0.45 | Class II | | | No safety concern at the estimated level of intake based on the MSDI approach |
| 07.158 07 | 6175-49-1 | Dodecan-2-one |  | 0.19 | Class II | | | No safety concern at the estimated level of intake based on the MSDI approach |
| 07.161 205/07 | 1629-60-3 | Hex-1-en-3-one |  | 0.005 (Hexanal + Hex-1-en-3-one) ^(e) | Class II | 1.2.2 | ^(c) | No safety concern at the estimated level of intake based on the MSDI approach |
| 07.189 07 | 4485-09-0 | Nonan-4-one |  | 0.04 | Class II | | | No safety concern at the estimated level of intake based on the MSDI approach. |


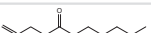
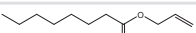

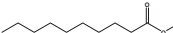
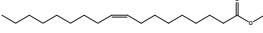
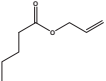
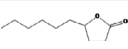

| FL-no FGE | CASrn | Union list substance name | Structural formula | % of peak area | Cramer class | α,β - subgroup | Structural alert for genotoxicity | EFSA Evaluation status |
|--------------|----------|--------------------------------|---|----------------|-----------------|------------------------------|--|--|
| 08.005 – | 107-92-6 | Butyric acid |  | 0.40 | Class I | | | No safety concern at the estimated level of intake based on the MSDI approach Evaluated by JECFA before 2000 – No EFSA considerations required |
| 08.007 – | 109-52-4 | Valeric acid or Pentanoic acid |  | 0.8 | Class I | | | No safety concern at the estimated level of intake based on the MSDI approach Evaluated by JECFA before 2000 – No EFSA considerations required |
| 08.009 – | 91-57-6 | Hexanoic acid |  | 2.45 | Class I | | | No safety concern at the estimated level of intake based on the MSDI approach Evaluated by JECFA before 2000 – No EFSA considerations required |
| 08.010 – | 124-07-2 | Octanoic acid |  | 5.38 | Class I | | | No safety concern at the estimated level of intake based on the MSDI approach Evaluated by JECFA before 2000 – No EFSA considerations required |





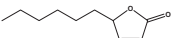

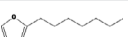

| FL-no FGE | CASrn | Union list substance name | Structural formula | % of peak area | Cramer class | α,β - subgroup | Structural alert for genotoxicity | EFSA Evaluation status |
|--------------|----------|------------------------------|---|----------------|-----------------|------------------------------|--|--|
| 08.011 – | 334-48-5 | Decanoic acid |  | 4.96 | Class I | | | No safety concern at the estimated level of intake based on the MSDI approach Evaluated by JECFA before 2000 – No EFSA considerations required |
| 08.012 – | 143-07-7 | Dodecanoic acid |  | 0.33 | Class I | | | No safety concern at the estimated level of intake based on the MSDI approach Evaluated by JECFA before 2000 – No EFSA considerations required |
| 08.013 – | 112-80-1 | Oleic acid |  | 0.66 | Class I | | | No safety concern at the estimated level of intake based on the MSDI approach Evaluated by JECFA before 2000 – No EFSA considerations required |
| 08.014 – | 57-10-3 | Hexadecanoic acid |  | 0.74 | Class I | | | No safety concern at the estimated level of intake based on the MSDI approach Evaluated by JECFA before 2000 – No EFSA considerations required |

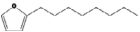
| FL-no FGE | CASrn | Union list substance name | Structural formula | % of peak area | Cramer class | α, β - subgroup | Structural alert for genotoxicity | EFSA Evaluation status |
|--------------|----------|------------------------------|--|----------------|-----------------|-------------------------------|--|--|
| 08.015 – | 57-11-4 | Octadecanoic acid |  | 0.04 | Class I | | | No safety concern at the estimated level of intake based on the MSDI approach Evaluated by JECFA before 2000 – No EFSA considerations required |
| 08.016 – | 544-63-8 | Tetradecanoic acid |  | 0.22 | Class I | | | No safety concern at the estimated level of intake based on the MSDI approach Evaluated by JECFA before 2000 – No EFSA considerations required |
| 08.021 54 | 65-85-0 | Benzoic acid |  | 0.14 | Class I | | | No safety concern at the estimated level of intake based on the MSDI approach |
| 08.028 – | 111-14-8 | Heptanoic acid |  | 3.93 | Class I | | | No safety concern at the estimated level of intake based on the MSDI approach Evaluated by JECFA before 2000 – No EFSA considerations required |

| FL-no FGE | CASrn | Union list substance name | Structural formula | % of peak area | Cramer class | α,β - subgroup | Structural alert for genotoxicity | EFSA Evaluation status |
|--------------|----------|--|--|----------------|-----------------|------------------------------|--|--|
| 08.029 – | 112-05-0 | Nonanoic acid |  | 2.62 | Class I | | | No safety concern at the estimated level of intake based on the MSDI approach Evaluated by JECFA before 2000 – No EFSA considerations required |
| 08.039 – | 112-38-9 | Undec-10-enoic acid or 10-Undecenoic acid |  | 0.74 | Class I | | | No safety concern at the estimated level of intake based on the MSDI approach |
| 08.041 – | 60-33-3 | Octadeca-9,12-dienoic acid |  | 0.04 | Class I | | | No safety concern at the estimated level of intake based on the MSDI approach Evaluated by JECFA before 2000 – No EFSA considerations required |
| 08.042 – | 112-37-8 | Undecanoic acid |  | 0.65 | Class I | | | No safety concern at the estimated level of intake based on the MSDI approach Evaluated by JECFA before 2000 – No EFSA considerations required |

| FL-no FGE | CASrn | Union list substance name | Structural formula | % of peak area | Cramer class | α,β - subgroup | Structural alert for genotoxicity | EFSA Evaluation status |
|-----------------------------|------------|--|---|----------------|-----------------|------------------------------|--|--|
| 08.048 – | 591-80-0 | Pent-4-enoic acid or 4-Pentenoic acid |  | 0.73 | Class I | | | No safety concern at the estimated level of intake based on the MSDI approach Evaluated by JECFA before 2000 – No EFSA considerations required |
| 08.065 – | 14436-32-9 | Dec-9-enoic acid or 9-Decenoic acid |  | 0.79 | Class I | | | No safety concern at the estimated level of intake based on the MSDI approach Evaluated by JECFA before 2000 – No EFSA considerations required |
| 08.068 – | 72881-27-7 | Dec-(5- and 6)-enoic acid |  | 0.02 | Class I | | | No safety concern at the estimated level of intake based on the MSDI approach Evaluated by JECFA before 2000 – No EFSA considerations required |
| 08.072 ^(b) 05 | 3724-65-0 | But-2-enoic acid (<i>cis</i> and <i>trans</i>) |  | 0.06 | Class I | | | No safety concern at the estimated level of intake based on the MSDI approach |
| 08.075 ^(b) 62 | 26303-90-2 | Dec-4-enoic acid |  | 0.27 | Class I | | | No safety concern at the estimated level of intake based on the MSDI approach |

| FL-no FGE | CASrn | Union list substance name | Structural formula | % of peak area | Cramer class | α,β -subgroup | Structural alert for genotoxicity | EFSA Evaluation status |
|-----------------|------------|--------------------------------|---|----------------|--------------|--------------------------|-----------------------------------|--|
| 08.123 71/96 | 10352-88-2 | <i>trans</i> -2-Heptenoic acid |  | 0.23 | Class I | | | No safety concern at the estimated level of intake based on the MSDI approach |
| 09.097 200 | 142-19-8 | Allyl heptanoate |  | 0.07 | Class II | 1.1.1 | | No safety concerns for genotoxicity |
| 09.119 200 | 4230-97-1 | Allyl octanoate |  | 0.51 | Class II | 1.1.1 | | No safety concerns for genotoxicity |
| 09.244 200 | 123-68-2 | Allyl hexanoate |  | 0.10 | Class II | 1.1.1 | | No safety concerns for genotoxicity |
| 09.251 – | 110-42-9 | Methyl decanoate |  | 0.04 | Class I | | | No safety concern at the estimated level of intake based on the MSDI approach Evaluated by CoE before 2000 – No EFSA considerations required |
| 09.652 05 | 112-62-9 | Methyl oleate |  | 0.15 | Class I | | | No safety concern at the estimated level of intake based on the MSDI approach |
| 09.866 200 | 6321-45-5 | Allyl valerate |  | 0.01 | | 1.1.1 | | No safety concerns for genotoxicity |
| 10.001 – | 104-61-0 | Nonano-1,4-lactone |  | 0.12 | Class I | | | No safety concern at the estimated level of intake based on the MSDI approach |
| 10.013 – | 108-29-2 | Pentano-1,4-lactone |  | 0.06 | Class I | | | No safety concern at the estimated level of intake based on the MSDI approach |

| FL-no FGE | CASrn | Union list substance name | Structural formula | % of peak area | Cramer class | α,β - subgroup | Structural alert for genotoxicity | EFSA Evaluation status |
|---------------|------------|------------------------------|---|----------------|-----------------|------------------------------|--|---|
| 10.020 – | 105-21-5 | Heptano-1,4-lactone |  | 0.12 | Class I | | | No safety concern at the estimated level of intake based on the MSDI approach |
| 10.021 – | 695-06-7 | Hexano-1,4-lactone |  | 0.16 | Class I | | | No safety concern at the estimated level of intake based on the MSDI approach |
| 10.022 – | 104-50-7 | Octano-1,4-lactone |  | 0.12 | Class I | | | No safety concern at the estimated level of intake based on the MSDI approach |
| 10.055 10 | 542-28-9 | Pentano-1,5-lactone |  | 0.03 | Class I | | | No safety concern at the estimated level of intake based on the MSDI approach |
| 10.060 217 | 2518-53-8 | 2-Decen-1,4-lactone |  | 0.05 | Class III | 4.1 | (c) | Evaluated in FGE.217Rev2, additional genotoxicity data required |
| 13.059 67 | 3777-69-3 | 2-Pentylfuran |  | 0.14 | Class II | | | Evaluated in FGE.67Rev1, additional genotoxicity data are required |
| 13.069 67 | 3777-71-7 | 2-Heptylfuran |  | 0.08 | Class II | | | Evaluated in FGE.67Rev1, additional genotoxicity data are required |
| 13.095 75 | 41239-48-9 | 2,5-Diethyltetrahydrofuran |  | 0.004 | Class II | | | No safety concern at the estimated level of intake based on the MSDI approach |

| FL-no FGE | CASrn | Union list substance name | Structural formula | % of peak area | Cramer class | α,β - subgroup | Structural alert for genotoxicity | EFSA Evaluation status |
|--------------|-----------|------------------------------|---|----------------|-----------------|------------------------------|--|--|
| 13.162 13 | 4179-38-8 | 2-Octylfuran |  | 0.80 | Class II | | | Evaluated in FGE.13Rev2, additional genotoxicity data are required |

CASrn: Chemical Abstracts Service Registry Number; MSDI: Maximised Survey-derived Daily Intake; CoE: Council of Europe; FGE: Flavouring Group Evaluation.

(a): CAS no 4313-03-5 in Union list refers to (*E,E*)-isomer.




















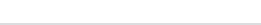






(b): The unsaturated acid component identified in Grill concentrate is the (*E*)-isomer whereas the Union list substance is the mixture.

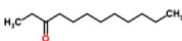
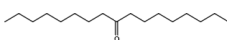
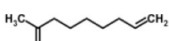
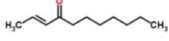
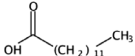
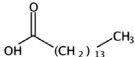
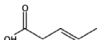



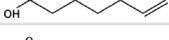
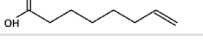
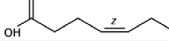
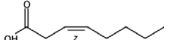
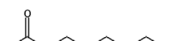

(c): Structural alert related to genotoxicity (α - β unsaturated carbonyl) identified via OECD QSAR Toolbox (version 4.2).

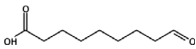
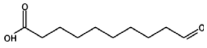
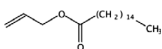
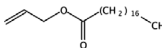
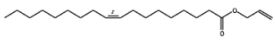
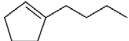
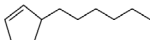
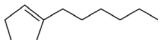
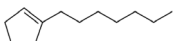

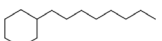
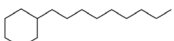
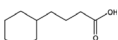
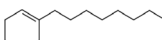
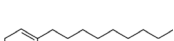
(d): Structural alert related to genotoxicity (simple aldehyde) identified via OECD QSAR Toolbox (version 4.2).

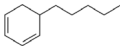
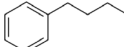
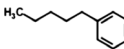
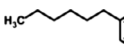
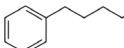
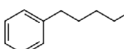
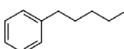
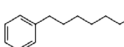
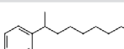
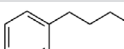
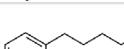
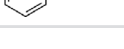
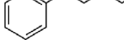

(e): Peaks could not be separated in all chromatograms.

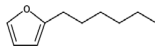
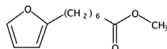



Table A.2: Summary of 77 constituents identified in 'Grill flavour concentrate (vegetable)' which have not been evaluated as flavouring substances by EFSA

| CAS no | Chemical name | Structural formula | Relative peak area % | Structural alert for genotoxicity |
|------------|---------------------------------|--|--|---|
| 124-18-5 | Decane |  | 0.02 | |
| 1120-21-4 | <i>N</i> -Undecane |  | 0.06 | |
| 629-50-5 | <i>n</i> -Tridecane |  | 0.06 | |
| 544-76-3 | Hexadecane |  | 0.04 | |
| 629-78-7 | Heptadecane |  | 0.72 | |
| 593-45-3 | Octadecane |  | 0.03 | |
| 629-92-5 | Nonadecane |  | 0.19 | |
| 124-11-8 | 1-Nonene |  | 0.004 | |
| 872-05-9 | 1-Decene |  | 0.07 | |
| 7433-56-9 | (<i>E</i>)-5-Decene |  | 0.02 | |
| 821-95-4 | 1-Undecene |  | 0.33 | |
| 2437-56-1 | 1-Tridecene |  | 0.40 | |
| 1120-36-1 | 1-Tetradecene |  | 0.63 | |
| 41446-63-3 | (<i>E</i>)-7-Tetradecene |  | 0.08 | |
| 13360-61-7 | 1-Pentadecene |  | 0.41 | |
| 629-73-2 | <i>n</i> -Hexadec-1-ene |  | 0.61 | |
| 16369-12-3 | (<i>Z</i>)-8-Heptadecene |  | 2.70 | |
| 16416-42-5 | (<i>E</i>)-8-Heptadecene |  | 1.82 ((<i>E</i>)-8-Heptadecene + Octanoic acid) ^(a) | |
| 112-88-9 | 1-Octadecene |  | 0.17 | |
| 51865-02-2 | (<i>Z</i>)-9-Nonadecene |  | 0.11 | |
| 13688-67-0 | 1,10-Undecadiene |  | 0.01 | |
| 56134-02-2 | (<i>Z</i>)-1,8-Heptadecadiene |  | 0.22 | |
| 2765-11-9 | Pentadecanal |  | 0.24 | Structural alert related to genotoxicity (simple aldehyde) identified via OECD QSAR Toolbox (version 4.2) |
| 591-78-6 | 2-Hexanone |  | 0.004 | |
| 589-63-9 | Octan-4-one |  | 0.02 | |
| 2216-87-7 | Undecan-3-one |  | 0.08 | |

| CAS no | Chemical name | Structural formula | Relative peak area % | Structural alert for genotoxicity |
|------------|----------------------|--|---|--|
| 1534-27-6 | Dodecan-3-one |  | 0.06 | |
| 540-08-9 | 9-Heptadecanone |  | 0.14 | |
| – | 6,7-Dodecadione | – | 0.01 | |
| 5009-32-5 | 8-Nonen-2-one |  | 0.13 | |
| 62485-94-3 | 2-Undecen-4-one |  | 0.45 (3-Nonyl-cyclohexene + 2-Undec-4-one) ^(a) | Structural alert related to genotoxicity (α - β unsaturated carbonyl) identified via OECD QSAR Toolbox (version 4.2) |
| 638-53-9 | Tridecanoic acid |  | 0.22 | |
| 1002-84-2 | Pentadecanoic acid |  | 0.07 | |
| 5204-64-8 | (E)-3-Pentenoic acid |  | 0.10 | |
| 35194-36-6 | (Z)-4-Hexenoic acid |  | 0.06 | |
| 1577-22-6 | 5-Hexenoic acid |  | 1.43 | |
| 1119-60-4 | 6-Heptenoic acid |  | 3.48 | |
| 18719-24-9 | 7-Octenoic acid |  | 1.88 | |
| 18654-81-4 | (Z)-4-Octenoic acid |  | 0.16 | |
| 5169-51-7 | (Z)-3-Octenoic acid |  | 0.05 | |
| 31642-67-8 | 8-Nonenoic acid |  | 1.31 | |
| 31502-23-5 | (Z)-6-Nonenoic acid |  | 0.21 | |
| 57602-94-5 | (E)-4-Decenoic acid |  | 0.33 | |

| CAS no | Chemical name | Structural formula | Relative peak area % | Structural alert for genotoxicity |
|------------|---------------------------------|--|----------------------|---|
| 2553-17-5 | 9-Oxo-Nonanoic acid |  | 0.42 | Structural alert related to genotoxicity (simple aldehyde) identified via OECD QSAR Toolbox (version 4.2) |
| 5578-80-3 | 10-Oxodecanoic acid |  | 0.20 | Structural alert related to genotoxicity (simple aldehyde) identified via OECD QSAR Toolbox (version 4.2) |
| 43211-62-7 | Prop-2-enyl hexadecanoate |  | 0.21 | |
| 6289-31-2 | Prop-2-enyl octadecanoate |  | 0.03 | |
| 19855-52-8 | Prop-2-enyl (Z)-9-octadecanoate |  | 0.16 | |
| 2423-01-0 | 1-Butyl-cyclopentene |  | 0.005 | |
| 37689-18-2 | 3-Hexyl-cyclopentene |  | 0.05 | |
| 4291-99-0 | 1-Hexyl-cyclopentene |  | 0.06 | |
| 4292-00-6 | 1-Heptyl-cyclopentene |  | 0.25 | |
| 1678-93-9 | Butylcyclohexane |  | 0.009 | |
| 1795-15-9 | Octyl-cyclohexane |  | 0.27 | |
| 2883-02-5 | Nonyl-cyclohexane |  | 0.66 | |
| 4441-63-8 | Cyclohexanebutyric acid |  | 0.07 | |
| 15232-87-8 | 1-Octyl-cyclohexene |  | 0.47 | |
| 15232-88-9 | 1-Nonyl-cyclohexene |  | 0.09 | |

| CAS no | Chemical name | Structural formula | Relative peak area % | Structural alert for genotoxicity |
|------------|-----------------------------|---|----------------------|-----------------------------------|
| 56318-84-4 | 5-Pentyl-1,3-cyclohexadiene |  | 0.03 | |
| 104-51-8 | <i>n</i> -Butylbenzene |  | 0.07 | |
| 538-68-1 | <i>n</i> -Pentylbenzene |  | 0.24 | |
| 1077-16-3 | <i>n</i> -Hexylbenzene |  | 0.10 | |
| 1078-71-3 | Heptylbenzene |  | 0.40 | |
| 2189-60-8 | <i>n</i> -Octylbenzene |  | 0.11 | |
| 1081-77-2 | Nonyl-benzene |  | 0.08 | |
| 104-72-3 | Decylbenzene |  | 0.06 | |
| 4536-88-3 | (1-Methyldecyl)-Benzene |  | 0.07 | |
| 1821-12-1 | 4-Phenylbutyric acid |  | 0.09 | |
| 2270-20-4 | 5-Phenylpentanoic acid |  | 0.06 | |
| 5581-75-9 | 6-Phenylhexanoic acid |  | 0.09 | |
| 40228-90-8 | 7-Phenylheptanoic acid |  | 0.08 | |
| – | 2-Pentyl-tetrahydrofuran |  | 0.07 | |

| CAS no | Chemical name | Structural formula | Relative peak area % | Structural alert for genotoxicity |
|------------|-------------------------------------|---|----------------------|--|
| 3777-70-6 | 2-Hexylfuran |  | 0.07 | Alert for genotoxicity due to structural similarity with other 2-alkylfurans (see Table A1: 2-pentylfuran, 2-heptylfuran and 2-octylfuran) for which additional genotoxicity data are required |
| 98188-02-4 | 2-Furanheptanoic acid, methyl ester |  | 0.04 | |
| 123-31-9 | Hydroquinone |  | 0.009 | |
| 930-68-7 | 2-Cyclohexen-1-one |  | 0.09 | Structural alert related to genotoxicity (α - β unsaturated carbonyl) identified via OECD QSAR Toolbox (version 4.2) |
| 119-64-2 | 1,2,3,4-Tetrahydronaphthalene |  | 0.02 | |

OECD: Organisation for Economic Co-operation and Development; QSAR: quantitative structure-activity relationship.

(a): Peaks could not be separated in all chromatograms.

Table A.3: Summary of 88 constituents tentatively identified in 'Grill flavour concentrate (vegetable)' based on fragmentation pattern of homologous compounds

| Chemical name | % of peak area |
|-----------------------------|---|
| 2-Propyl-tetrahydrofuran | 0.001 |
| 1-Propyl-cyclohexene | 0.01 |
| Decene isomer | 0.01 |
| Undecene isomer | 0.02 |
| 5-Undecene isomer | 0.03 |
| 5-Undecene isomer | 0.01 |
| Undecene isomer | 0.04 |
| 1,3-Decadiene isomer | 0.03 |
| Undecene isomer | 0.42 |
| Undecene isomer | 0.24 |
| 2,4-Decadiene isomer | 0.011 |
| 2,4-Decadiene isomer | 0.03 |
| Undecadiene isomer | 0.03 |
| Dodecene isomer | 0.06 |
| Dodecene isomer | 0.16 |
| 1,3-Undecadiene isomer | 0.46 |
| Dodecene isomer | 0.16 |
| Dodecene isomer | 0.08 |
| 1-Butyl-cyclohexene | 0.08 |
| 2,4-Undecadiene isomer | 0.04 |
| 2,4-Undecadiene isomer | 0.53 |
| 1,3-Dodecadiene isomer | 0.914 |
| 1-Hexyl-cyclohexene | 0.107 |
| 2-Hexyltetrahydrofuran | 0.054 |
| Tridecene isomer | 0.231 |
| Dodecadiene isomer | 0.198 |
| 2,4-Dodecadiene isomer | 0.181 (Nonan-2-one + 2,4-Docecadiene) ^(a) |
| 2,4-Dodecadiene isomer | 0.120 |
| 2,4-Dodecadiene isomer | 0.555 |
| 2,4-Dodecadiene isomer | 0.016 |
| 3-Heptyl-cyclohexene | 0.071 |
| 1-Octyl-1-cyclopentene | 1.0051 (1-Octyl-1-cyclopenten + 1-Tetradecene) ^(a) |
| 1,3-Tridecadiene isomer | 0.135 |
| 1-Heptyl-cyclohexene | 0.037 |
| 2-Heptyltetrahydrofuran | 0.052 |
| 2-Butyl-cyclopentanone | 0.048 |
| Tetradecene isomer | 0.5741 (Tetradecene + Tridecadiene) ^(a) |
| Tridecadiene isomer | 0.5741 (Tetradecene + Tridecadiene) ^(a) |
| Tridecadiene isomer | 0.122 |
| Tetradecadiene isomer | 0.115 |
| Pentadecene isomer | 0.263 |
| Nonylcyclopentene isomer | 0.598 |
| 1-Nonyl-cyclopentene | 0.585 |
| Tridecatriene isomer | 0.041 |
| 2-Methyl-1 <i>H</i> -Indene | 0.0051 (2-Methyl-1 <i>H</i> -indene + Methyl oleate) ^(a) |
| Pentylcyclopentanone isomer | 0.054 |
| Pentadecene isomer | 0.079 |
| Methylhexylbenzol isomer | 0.041 |

| Chemical name | % of peak area |
|--------------------------------|--|
| Pentadecadiene isomer | 0.438 |
| Hexadecene isomer | 0.065 |
| 2-Nonylfuran | 0.507 |
| 6,8-Tetradecadiene isomer | 0.393 |
| 1,9-hexadecadiene isomer | 0.031 |
| 1,13-Hexadecadiene isomer | 2.135 |
| 3-Nonyl-cyclohexene | 0.4451 (3-Nonyl-1-cyclohexene + 2-Undecene-4-one) ^(a) |
| Hexadecadiene isomer | 0.472 |
| Heptadecene isomer | 0.106 |
| Heptadecene isomer | 0.7231 (Heptadecan + Heptadecene) ^(a) |
| Methylheptylbenzol | 0.055 |
| Tetradecan-5-one | 0.189 |
| Heptadecene isomer | 0.208 |
| Heptadecadiene isomer | 0.245 |
| Heptadecadiene isomer | 0.178 |
| 1-Decyl-cyclohexene | 0.106 |
| Octadecene | 0.325 |
| 3-Octadecene isomer | 0.211 |
| Tetradecan-5-one | 0.037 |
| Heptadecadiene isomer | 0.091 |
| Octadecadiene isomer | 0.072 |
| Octadecadiene isomer | 0.070 |
| Heptanoic acid, 1,1'-anhydride | 0.123 |
| (9E)-9-Nonadecene | 0.082 |
| 7-Pentadecanone | 0.054 |
| 2-Nonyl-cyclopentanone | 0.067 |
| Eicosadiene isomer | 0.1441 (Eicosadiene + <i>m</i> -Cresol) ^(a) |
| 2-Nonyl-2-cyclopenten-1-one | 0.153 |
| Octanoic acid anhydride | 0.091 |
| 4-Butyl-phenol | 0.081 |
| 9-Heptadecenal isomer | 1.175 |
| 1-Nonenylcyclohexane isomer | 0.118 |
| 8,11-Heptadecadienal isomer | 0.220 |
| 5-Oxo-hexanoic acid | 0.128 |
| Undecylenic acid isomer | 0.812 |
| Undecylenic acid isomer | 0.599 |
| Dodecenoic acid isomer | 0.280 |
| Dodecenoic acid isomer | 0.148 |
| Tridecenoic acid isomer | 0.207 |
| Pentadecadiene isomer | 0.032 |

(a): Peaks could not be separated in all chromatograms.

Appendix B – Use levels and exposure calculations

Table B.1: Normal and Maximum Occurrence Levels for Refined Categories of Foods and Beverages for 'Grill flavour concentrate (vegetable)'

| CODEX code | Food categories ^(a) | Standard portions ^(b) (g) | Occurrence level as added flavouring substance (mg/kg) | | Occurrence level from other sources ^(c) (mg/kg) | | Combined occurrence level from all sources ^(e) (mg/kg) | |
|------------|--|--------------------------------------|--|---------|--|---------|---|---------|
| | | | Normal | Maximum | Average ^(d) | Maximum | Normal | Maximum |
| 01.6 | Cheese and analogues | 40 | 1 | 5 | 0 | 0 | 1 | 5 |
| 08.2 | Processed meat, poultry and game products in whole pieces or cuts | 100 | 3 | 10 | 0 | 0 | 3 | 10 |
| 08.3 | Processed comminute meat, poultry and game products | 100 | 4 | 10 | 0 | 0 | 4 | 10 |
| 09.2 | Processed fish and fish products, including molluscs, crustaceans and echinoderms | 100 | 3 | 8 | 0 | 0 | 3 | 8 |
| 12.2 | Herbs, spices, seasonings and condiments (e.g. seasoning for instant noodles) | 1 | 10 | 100 | 0 | 0 | 10 | 100 |
| 12.6 | Sauces and like products | 30 | 3 | 10 | 0 | 0 | 3 | 10 |
| 15.1 | Snacks, potato-, cereal-, flour- or starch-based (from roots and tubers, pulses and legumes) | 30 | 2 | 5 | 0 | 0 | 2 | 5 |
| 15.2 | Processed nuts, including coated nuts and nut mixtures (with e.g. dried fruit) | 30 | 2 | 5 | 0 | 0 | 2 | 5 |
| 15.3 | Snacks – fish based | 30 | 2 | 5 | 0 | 0 | 2 | 5 |

(a): Most of the categories reported are the subcategories of Codex GSFA (General Standard for Food Additives) used by the JECFA in the SPET technique (JECFA, 2008). In the case of category 13.2 (complementary foods for infants and young children), further refined categories have been created so that a specific assessment of dietary exposure can be performed in young children.

(b): For adults. In case of foods marketed as powder or as concentrates, occurrence levels must be reported for the reconstituted product, considering the instructions reported on the product label or one of the standard dilution factors established by the JECFA (FAO/WHO 2008):

- 1/25 for powder used to prepare water-based drinks such as coffee, containing no additional ingredients,
- 1/10 for powder used to prepare water-based drinks containing additional ingredients such as sugars (ice tea, squashes, etc.),
- 1/7 for powder used to prepare milk, soups and puddings,
- 1/3 for condensed milk.

(c): As natural constituent and/or developed during the processing and/or as carry-over resulting from their use in animal feed.

(d): In order to estimate normal values in each category, only foods and beverages in which the substance is present in significant amount will be considered (e.g. for the category 'Fresh fruit' 04.1.1., the normal concentration will be the median concentration observed in all kinds of fruit where the flavouring substance is known to occur).

(e): As added flavouring or from other sources. The normal and maximum combined occurrence levels of the substance will be assessed by the applicant either by adding up occurrence levels from added use to that from other sources or by expert judgment based on the likelihood of their concomitant presence. This will be done both for normal use levels and for maximum use levels.

Calculation of the dietary exposure – APET

Chronic dietary exposure – ‘Added Portions Exposure Technique’ (APET)⁶

The chronic APET calculations are based on the normal combined occurrence level by adding the highest contributing portion of food and highest contributing portion of beverages (either among soft drinks or alcoholic beverages) (see Table 4). The APET calculation for children is performed by adding the highest contributing portion of food and the highest contributing portion of beverages (among soft drinks). Furthermore, in the APET calculation for children the portion sizes listed in Table B.1 are adjusted by a factor 0.63 to take into account the smaller portion sizes consumed by the child.

Adults (‘Added Portions Exposure Technique’ (APET))

On the basis of normal occurrence level from added flavouring

Solid Food: The maximum intake will be from category 8.3 (Processed comminute meat, poultry and game products) with the normal combined occurrence level of 0.4 mg/adult per day.

Beverage: the flavouring is not used in beverages.

The total APET will be 0.4 mg/adult per day corresponding to 0.0067 mg/kg bw per day for a 60-kg person.

Children (3-year-old child of 15-kg body weight)

Solid Food: The maximum intake will be from category 8.3 (Processed comminute meat, poultry and game products) with the normal combined occurrence level of $0.4 \times 0.63 = 0.252$ mg/child per day.

Beverage: the flavouring is not used in beverages.

The total APET will be 0.252 mg/child per day corresponding to 0.0168 mg/kg bw per day for a 15-kg child.

Conclusion

The higher of the two values among adults and children, expressed per kg/bw per day, should be used as the basis for the safety evaluation of the candidate substance, i.e. the value of 0.0168 mg/kg bw per day for a 15-kg child should be compared to the appropriate NOAEL for the candidate substance.

Infants and young children

The estimate to infant exposure is currently under revision in the DATA Unit of EFSA.

Acute dietary exposure

The calculation was based on the maximum use levels and large portion size, i.e. three times standard portion size (see Table 5). Although the substance is not intended to be used in food categories specifically intended for infants and toddlers, these could still be exposed through consumption of foods from the general food categories, which may contain the substance. However, at present there is no generally accepted methodology to estimate exposure in these age groups resulting from consumption of foods from the general categories. The APET calculation for children the portion sizes listed in Table B.1 is adjusted by a factor 0.63 to take into account the smaller portion sizes consumed by the child.

Adults

The highest contribution comes from 3 portions of category 8.3 (Processed comminute meat, poultry and game products) and is $(3 \times 100 \text{ g}) \times 10 \text{ mg/kg} = 3$ mg/adult per day.

Children⁷

The highest contribution comes from 3 portions of category 8.3 (Processed comminute meat, poultry and game products) and is $(3 \times 100 \text{ g}) \times 0.63 \times 10 \text{ mg/kg} = 1.89$ mg/child per day.

Infants and young children (0–1 year)

Acute dietary exposure is not calculated for infants and young children.

⁶ The APET has been calculated based on the occurrence levels in the food subcategories reported in the above table, with the exclusion of categories 13.2 (complementary foods for infants and young children).

⁷ Based on the same considerations as for adults but using the special factors used for chronic exposure to infants.

Appendix C – Toxicological data

Table C.1: *In vitro* genotoxicity data on 'Grill flavour concentrate (vegetable)' as such. Genotoxicity studies for individual components have been addressed in various FGEs, as far as data are available

| Chemical name FL-no | Test system <i>in vitro</i> | Test object | Concentrations of substance and test conditions | Result | Reference | Comments |
|--|--------------------------------|---|---|----------|---|---|
| Grill flavour concentrate (vegetable) [21.002] | Reverse mutation | <i>Salmonella</i> Typhimurium TA97a, TA98, TA100, TA102 and TA1535 | 50–5,000 µg/plate ^(a) | Negative | Silesia Gerhard Hanke GmbH & Co. KG (2013) | Reliable without restrictions. Study performed under GLP and in accordance with OECD TG 471. Two experiments were performed – the plate incorporation and preincubation methods |
| | Micronucleus induction | Human peripheral blood lymphocytes | 0.05–0.4 µL/mL ^(a) | Negative | Silesia Gerhard Hanke GmbH & Co. KG (2014) | Reliable without restrictions. Study performed under GLP and in accordance with OECD TG 487 |

FL-No: FLAVIS number; FGE: Flavouring Group Evaluation; GLP: Good Laboratory Practice; OECD: Organisation for Economic Co-operation and Development.

(a): With and without metabolic activation.

Table C.2: Toxicity data

| Chemical name [FL-no] | Species; sex no./group | Route | Dose levels (mg/kg bw per day) | Duration (days) | BMDL ₀₅ (mg/kg bw per day) | Reference | Comments |
|--|---------------------------------------|-------|--------------------------------------|--------------------|---|-----------------------------|---|
| Grill flavour concentrate (vegetable) [21.002] | Wistar Han rats; M + F 3 + 3/4 | Diet | 0, 2.4, 12 and 60* | 28 | NA | vivo Science GmbH (2015) | Dose-range finding study, not performed under GLP |
| | Wistar Han rats 10 M + 10 F | Diet | 0, 2.9, 14.4 and 72** | 92 | 11.1 | vivo Science GmbH (2016) | Study performed under GLP and in accordance with OECD TG 408 |
| | Wistar Han rats; M + F 10 M + 10 F | Diet | 0, 350 | 14 | NA | Covance (2018) | Dose-range finding study performed under GLP |
| | Wistar Han rats; M + F 27 M + 27 F | Diet | 0, 2.9, 14.4, 72.0, 350 | 28 | NA | Covance (2018) | Study performed under GLP and in accordance with OECD TG 407 A substance-related increase in hyaline droplets was observed in the kidneys of male rats, associated with α ₂ -globulin hyaline droplet nephropathy specific for adult male rats and not relevant for human risk assessment |

FL-No: FLAVIS number; GLP: Good Laboratory Practice; BMDL₀₅: benchmark dose for a 5% effect; bw: body weight; OECD: Organisation for Economic Co-operation and Development; M: male; F: female.

*: Estimated doses based on a food consumption of 90 g/day. The actual doses were approximately 46.8, 9.4, 1.9 mg/kg bw per day for females and 42, 8.4, 1.7 mg/kg bw per day for males.

** : The mean actual food consumption over 92 days were, respectively, 73.4, 14.5, 2.9 mg/kg bw per day for females and 71.0, 14.1, 3.0 mg/kg bw per day for males.

Appendix D – Methodology

The definition of 'other flavouring', referred to in Article 3(2)(h) of Regulation (EC) No 1334/20081 is 'a flavouring added or intended to be added to food in order to impart odour and/or taste and which does not fall under the definitions of Article 3(2)(b) – (g) of Regulation (EC) No 1334/2008', and the data requirements for its safety evaluation can be found in the EFSA scientific opinion: 'Guidance on the data required for the risk assessment of flavourings to be used in or on foods' (EFSA CEF Panel, 2010), Part B. IV. 'Information to be supplied with an application for the authorisation of Other Flavourings'.

It is difficult to anticipate what kind of materials will undergo an evaluation as 'Other Flavourings', which suggests that the standard evaluation template is flexible. (...) As a general approach, the following data should be provided:

- *full description of the production process, with emphasis on the parameters that might influence the composition of the flavouring;*
- *identification and quantification of the substances present in the flavouring;*
- *specifications of the flavouring;*
- *exposure and toxicological data required to perform a risk assessment of the flavouring.*

Appendix E – Benchmark dose modelling: report

Data Description

The endpoints to be analysed are: globulin, albumin and globulin ratio (A/G) and total proteins.

Data used for the analysis:

| act_dose | Globulin | AG ratio | Total protein | Gender |
|----------|----------|----------|---------------|--------|
| 0.0 | 31.8 | 1.2 | 70 | F |
| 0.0 | 33.4 | 1.2 | 74 | F |
| 0.0 | 31.2 | 1.2 | 70 | F |
| 0.0 | 33.2 | 1.1 | 70 | F |
| 0.0 | 33.8 | 1.2 | 76 | F |
| 0.0 | 31.2 | 1.2 | 68 | F |
| 0.0 | 32.2 | 1.4 | 76 | F |
| 0.0 | 30.6 | 1.2 | 68 | F |
| 0.0 | 33.6 | 1.3 | 76 | F |
| 0.0 | 32.0 | 1.3 | 72 | F |
| 2.9 | 36.8 | 1.2 | 82 | F |
| 2.9 | 34.4 | 1.2 | 74 | F |
| 2.9 | 38.0 | 1.2 | 84 | F |
| 2.9 | 38.4 | 1.0 | 78 | F |
| 2.9 | 35.4 | 1.3 | 80 | F |
| 2.9 | 34.6 | 1.3 | 80 | F |
| 2.9 | 34.4 | 1.2 | 74 | F |
| 2.9 | 37.6 | 1.1 | 80 | F |
| 2.9 | 35.6 | 1.0 | 72 | F |
| 2.9 | 31.2 | 1.2 | 68 | F |
| 14.9 | 34.6 | 1.2 | 76 | F |
| 14.9 | 36.4 | 1.2 | 80 | F |
| 14.9 | 36.0 | 1.2 | 78 | F |
| 14.9 | 31.4 | 1.2 | 70 | F |
| 14.9 | 34.6 | 1.1 | 74 | F |
| 14.9 | 36.6 | 1.1 | 76 | F |
| 14.9 | 38.0 | 1.2 | 84 | F |
| 14.9 | 33.6 | 1.3 | 76 | F |
| 14.9 | 32.6 | 1.1 | 70 | F |
| 73.4 | 43.4 | 1.0 | 86 | F |
| 73.4 | 46.4 | 0.9 | 90 | F |
| 73.4 | 46.4 | 0.9 | 88 | F |
| 73.4 | 43.2 | 1.0 | 86 | F |
| 73.4 | 44.8 | 1.0 | 88 | F |
| 73.4 | 46.4 | 0.9 | 90 | F |
| 73.4 | 40.2 | 0.9 | 78 | F |
| 73.4 | 30.8 | 0.9 | 60 | F |
| 73.4 | 41.0 | 0.9 | 78 | F |
| 73.4 | 39.6 | 0.9 | 76 | F |
| 0.0 | 36.0 | 0.9 | 70 | M |
| 0.0 | 38.2 | 0.9 | 74 | M |
| 0.0 | 32.2 | 1.0 | 66 | M |
| 0.0 | 37.0 | 0.9 | 72 | M |
| 0.0 | 34.6 | 1.0 | 68 | M |

| act_dose | Globulin | AG ratio | Total protein | Gender |
|----------|----------|----------|---------------|--------|
| 0.0 | 38.2 | 0.9 | 74 | M |
| 0.0 | 34.2 | 1.0 | 68 | M |
| 0.0 | 36.6 | 0.9 | 70 | M |
| 0.0 | 34.6 | 1.0 | 68 | M |
| 0.0 | 36.8 | 1.0 | 74 | M |
| 3.0 | 37.2 | 1.0 | 74 | M |
| 3.0 | 37.0 | 0.9 | 72 | M |
| 3.0 | 35.2 | 1.0 | 70 | M |
| 3.0 | 36.4 | 1.0 | 72 | M |
| 3.0 | 38.6 | 0.9 | 74 | M |
| 3.0 | 35.6 | 1.0 | 70 | M |
| 3.0 | 37.8 | 1.0 | 74 | M |
| 3.0 | 35.6 | 1.0 | 72 | M |
| 3.0 | 36.6 | 0.9 | 70 | M |
| 3.0 | 38.4 | 0.9 | 72 | M |
| 14.1 | 36.4 | 0.9 | 70 | M |
| 14.1 | 34.8 | 1.0 | 68 | M |
| 14.1 | 36.8 | 1.0 | 72 | M |
| 14.1 | 38.2 | 0.9 | 72 | M |
| 14.1 | 33.8 | 1.0 | 66 | M |
| 14.1 | 38.2 | 0.9 | 74 | M |
| 14.1 | 35.8 | 1.0 | 70 | M |
| 14.1 | 37.8 | 0.9 | 72 | M |
| 14.1 | 37.4 | 0.9 | 72 | M |
| 14.1 | 35.8 | 0.9 | 68 | M |
| 71.0 | 50.6 | 0.8 | 90 | M |
| 71.0 | 49.8 | 0.8 | 90 | M |
| 71.0 | 47.0 | 0.8 | 86 | M |
| 71.0 | 47.4 | 0.8 | 86 | M |
| 71.0 | 46.2 | 0.9 | 86 | M |
| 71.0 | 46.2 | 0.8 | 84 | M |
| 71.0 | 45.6 | 0.8 | 82 | M |
| 71.0 | 42.6 | 0.8 | 78 | M |
| 71.0 | 46.8 | 0.8 | 82 | M |
| 71.0 | 49.4 | 0.7 | 86 | M |

Response variable: Globulin

The following outliers were identified for Expon. m3-abv:

| act_dose | Gender | Globulin | AGratio | Totalprotein |
|----------|--------|----------|---------|--------------|
| 72 | F | 30.8 | 0.9 | 60 |

The following outliers were identified for Hill m3-abv:

| act_dose | Gender | Globulin | AGratio | Totalprotein |
|----------|--------|----------|---------|--------------|
| 72 | F | 30.8 | 0.9 | 60 |

The following outliers were identified for Inv.Expon. m3-abv:

| act_dose | Gender | Globulin | AGratio | Totalprotein |
|----------|--------|----------|---------|--------------|
| 72 | F | 30.8 | 0.9 | 60 |

The following outliers were identified for LN m3-abv:

| act_dose | Gender | Globulin | AGratio | Totalprotein |
|----------|--------|----------|---------|--------------|
| 72 | F | 30.8 | 0.9 | 60 |

Response variable: Totalprotein

The following outliers were identified for Expon. m3-abv:

| act_dose | Gender | Globulin | AGratio | Totalprotein |
|----------|--------|----------|---------|--------------|
| 72 | F | 30.8 | 0.9 | 60 |

The following outliers were identified for Hill m3-abv:

| act_dose | Gender | Globulin | AGratio | Totalprotein |
|----------|--------|----------|---------|--------------|
| 72 | F | 30.8 | 0.9 | 60 |

The following outliers were identified for Inv.Expon. m3-abv:

| Dose | Gender | Globulin | AGratio | Totalprotein |
|------|--------|----------|---------|--------------|
| 72 | F | 30.8 | 0.9 | 60 |

The following outliers were identified for LN m3-abv:

| Dose | Gender | Globulin | AGratio | Totalprotein |
|------|--------|----------|---------|--------------|
| 72 | F | 30.8 | 0.9 | 60 |

Selection of the BMR

The benchmark response (BMR) used is a 5% change in mean response compared to the controls. The benchmark dose (BMD) is the dose corresponding with the BMR of interest.

A 90% confidence interval around the BMD will be estimated, the lower bound is reported by BMDL and the upper bound by BMDU.

Software used

Results are obtained using the EFSA web-tool for BMD analysis, which uses the R-package PROAST, version 66.24, for the underlying calculations.

Specification of Deviations from Default Assumptions

None.

Dose-response models

Default set of fitted models:

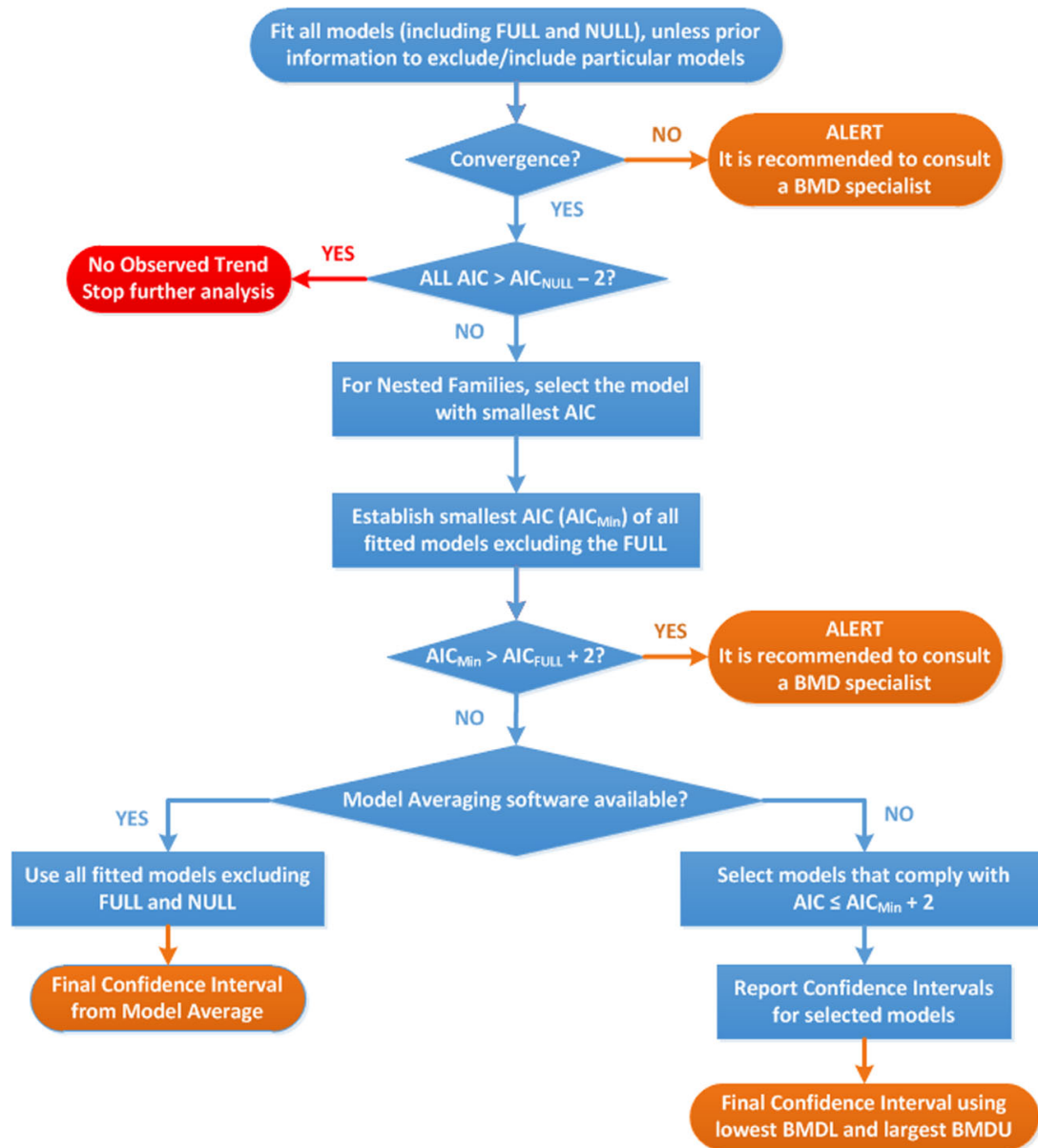
| Model | Number of parameters | Formula |
|-------------|----------------------|---|
| Null | 1 | $y = a$ |
| Full | No. of groups | $y = \text{group mean}$ |
| Exp model 3 | 3 | $y = a \cdot \exp(bx^d)$ |
| Exp model 4 | 4 | $y = a \cdot (c - (c - 1) \exp(-bx^d))$ |

| Model | Number of parameters | Formula |
|---------------------|----------------------|---|
| Hill model 3 | 3 | $y = a \cdot \left(1 - \frac{x^d}{b^d + x^d}\right)$ |
| Hill model 4 | 4 | $y = a \cdot \left(1 - \frac{(c-1)x^d}{b^d + x^d}\right)$ |
| 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

Flow chart for selection of BMDL:



Results

Response variable: Globulin

Fitted Models

| model | converged | loglik | npar | AIC |
|-------------------|-----------|--------|------|---------|
| full model | yes | 109.87 | 9 | -201.74 |
| full-v | yes | 115.55 | 10 | -211.10 |
| null modelv | yes | 51.17 | 3 | -96.34 |
| null model-a-v | yes | 54.71 | 4 | -101.42 |
| Expon. m3-v | yes | 98.98 | 5 | -187.96 |
| Expon. m3-av | yes | 109.22 | 6 | -206.44 |
| Expon. m3-abv | yes | 110.42 | 7 | -206.84 |
| Expon. m5-av | yes | 109.22 | 7 | -204.44 |
| Expon. m5-abv | yes | 110.42 | 8 | -204.84 |
| Hill m3-av | yes | 109.22 | 6 | -206.44 |
| Hill m3-abv | yes | 110.42 | 7 | -206.84 |
| Hill m5-av | yes | 109.22 | 7 | -204.44 |
| Hill m5-abv | yes | 110.42 | 8 | -204.84 |
| Inv.Expon. m3-av | yes | 109.17 | 6 | -206.34 |
| Inv.Expon. m3-abv | yes | 110.35 | 7 | -206.70 |
| Inv.Expon. m5-av | yes | 109.16 | 7 | -204.32 |
| Inv.Expon. m5-abv | yes | 110.34 | 8 | -204.68 |
| LN m3-av | yes | 109.18 | 6 | -206.36 |
| LN m3-abv | yes | 110.37 | 7 | -206.74 |
| LN m5-av | yes | 109.18 | 7 | -204.36 |
| LN m5-abv | yes | 110.36 | 8 | -204.72 |

OF NOTE: the AIC of the best model (minimum AIC) is more than two units larger than that of the full model. However, considering that the fitted models are quite close to the full model, the results can be still considered acceptable.

Estimated Model Parameters

EXP

estimate for var-F : 0.006715
 estimate for var-M : 0.001934
 estimate for a-F : 34.05
 estimate for a-M : 36.2
 estimate for CED-F : 33.82
 estimate for CED-M : 28.96
 estimate for d- : 1.882

HILL

estimate for var-F : 0.006715
 estimate for var-M : 0.001934
 estimate for a-F : 34.05
 estimate for a-M : 36.2
 estimate for CED-F : 33.83
 estimate for CED-M : 28.95
 estimate for d- : 1.884

INVEXP

estimate for var-F : 0.006751
 estimate for var-M : 0.001932
 estimate for a-F : 34.1
 estimate for a-M : 36.25

estimate for CED-F : 33.59
 estimate for CED-M : 29.19
 estimate for d- : 0.3172

LOGN

estimate for var-F : 0.006741
 estimate for var-M : 0.001933
 estimate for a-F : 34.09
 estimate for a-M : 36.24
 estimate for CED-F : 33.88
 estimate for CED-M : 29.74
 estimate for d- : 0.6196

The following outliers were identified:

| model | act_dose | Gender | Globulin | AGratio | Totalprotein |
|-------------------|----------|--------|----------|---------|--------------|
| Expon. m3-abv | 72 | F | 30.8 | 0.9 | 60 |
| Hill m3-abv | 72 | F | 30.8 | 0.9 | 60 |
| Inv.Expon. m3-abv | 72 | F | 30.8 | 0.9 | 60 |
| LN m3-abv | 72 | F | 30.8 | 0.9 | 60 |

Weights for Model Averaging

| EXP | HILL | INVEXP | LOGN |
|------|------|--------|------|
| 0.26 | 0.26 | 0.24 | 0.24 |

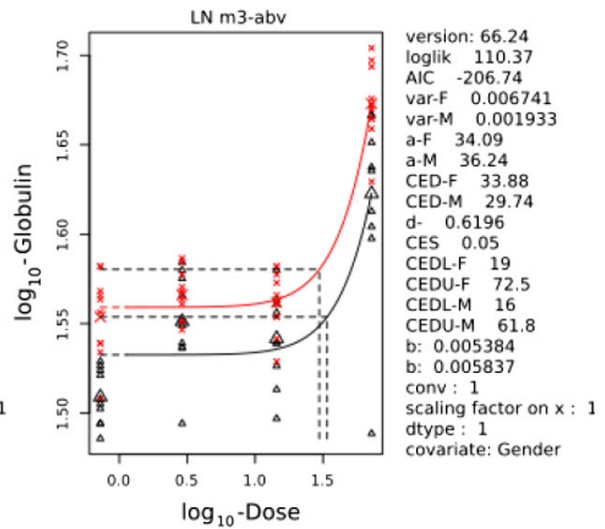
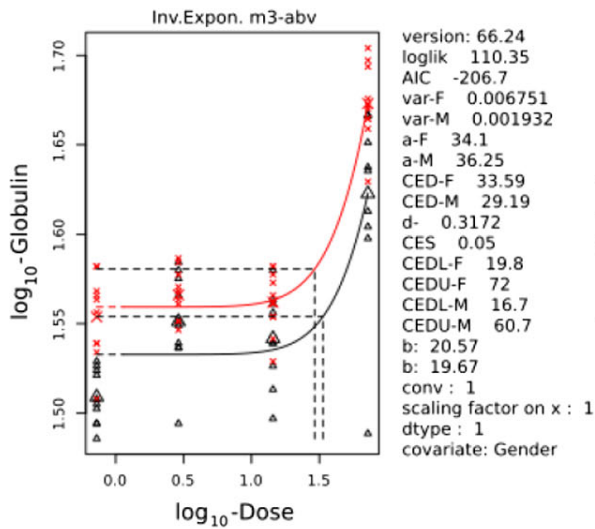
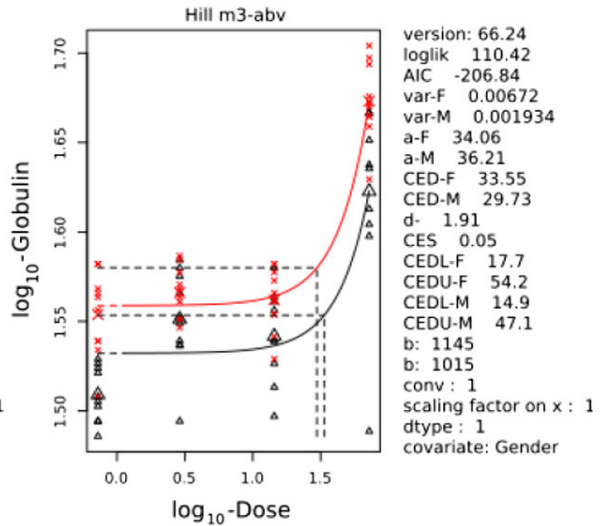
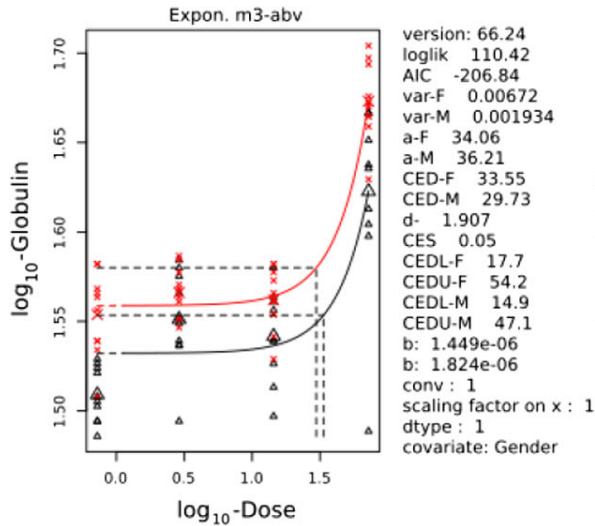
Final BMD Values

| endpoint | subgroup | BMDL | BMDU |
|----------|----------|------|------|
| Globulin | F | 22.0 | 55.0 |
| Globulin | M | 18.1 | 51.7 |

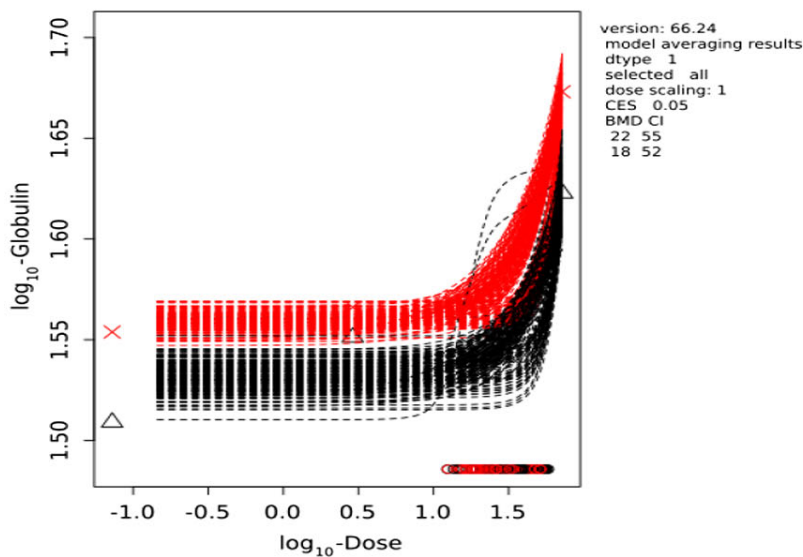
Confidence intervals for the BMD are based on 200 bootstrap data sets.

Visualisation

Plot for response 'Globulin'.



Bootstrap curves based on model averaging



Response variable: AGratio

Fitted Models

| model | converged | loglik | npar | AIC |
|------------------|-----------|--------|------|---------|
| full model | yes | 110.94 | 9 | -203.88 |
| full-v | yes | 111.78 | 10 | -203.56 |
| null model | yes | 37.00 | 2 | -70.00 |
| null model-a | yes | 61.53 | 3 | -117.06 |
| Expon. m3- | yes | 54.65 | 4 | -101.30 |
| Expon. m3-a | yes | 106.34 | 5 | -202.68 |
| Expon. m3-ab | yes | 109.11 | 6 | -206.22 |
| Expon. m5-a | yes | 106.34 | 6 | -200.68 |
| Expon. m5-ab | yes | 109.11 | 7 | -204.22 |
| Hill m3-a | yes | 106.34 | 5 | -202.68 |
| Hill m3-ab | yes | 109.11 | 6 | -206.22 |
| Hill m5-a | yes | 106.34 | 6 | -200.68 |
| Hill m5-ab | yes | 109.10 | 7 | -204.20 |
| Inv.Expon. m3-a | yes | 106.28 | 5 | -202.56 |
| Inv.Expon. m3-ab | yes | 109.01 | 6 | -206.02 |
| Inv.Expon. m5-a | yes | 106.27 | 6 | -200.54 |
| Inv.Expon. m5-ab | yes | 109.00 | 7 | -204.00 |
| LN m3-a | yes | 106.30 | 5 | -202.60 |
| LN m3-ab | yes | 109.05 | 6 | -206.10 |
| LN m5-a | yes | 106.29 | 6 | -200.58 |
| LN m5-ab | yes | 109.04 | 7 | -204.08 |

Estimated Model Parameters

EXP

estimate for var- : 0.003699
 estimate for a-F : 1.2
 estimate for a-M : 0.9544
 estimate for CED-F : 24.61
 estimate for CED-M : 30.47
 estimate for d- : 1.473

HILL

estimate for var- : 0.003699
 estimate for a-F : 1.2
 estimate for a-M : 0.9544
 estimate for CED-F : 24.61
 estimate for CED-M : 30.47
 estimate for d- : 1.475

INVEXP

estimate for var- : 0.003706
 estimate for a-F : 1.198
 estimate for a-M : 0.953
 estimate for CED-F : 23.35
 estimate for CED-M : 31.04
 estimate for d- : 0.2428

LOGN

estimate for var- : 0.003703
 estimate for a-F : 1.199
 estimate for a-M : 0.9536
 estimate for CED-F : 23.58

estimate for CED-M : 30.85

estimate for d- : 0.4686

Weights for Model Averaging

| EXP | HILL | INVEXP | LOGN |
|------|------|--------|------|
| 0.26 | 0.26 | 0.24 | 0.24 |

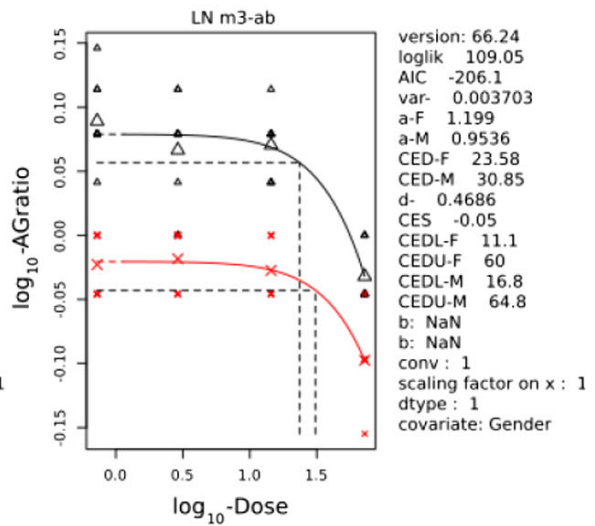
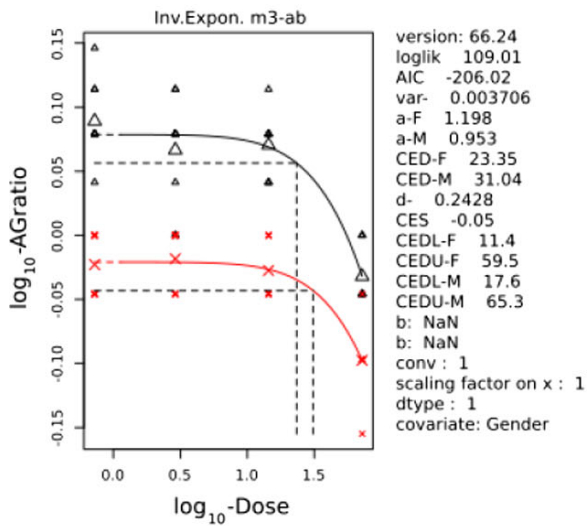
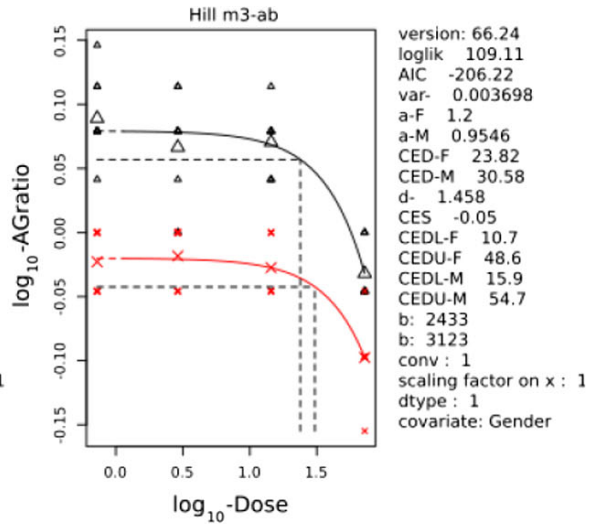
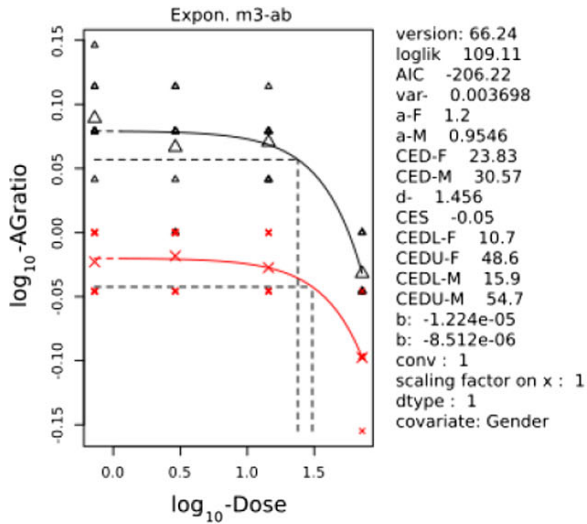
Final BMD Values

| endpoint | subgroup | BMDL | BMDU |
|----------|----------|------|------|
| AGratio | F | 12.8 | 51.5 |
| AGratio | M | 18.4 | 56.6 |

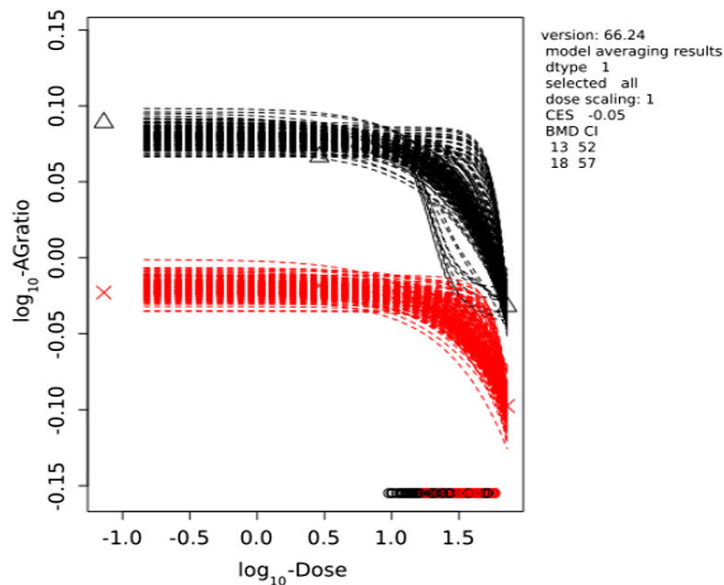
Confidence intervals for the BMD are based on 200 bootstrap data sets.

Visualisation

Plot for response 'AGratio'.



Bootstrap curves based on model averaging



Response variable: Total proteins**Fitted Models**

| model | converged | loglik | npar | AIC |
|-------------------|-----------|--------|------|---------|
| full model | yes | 111.68 | 9 | -205.36 |
| full-v | yes | 122.63 | 10 | -225.26 |
| null modelv | yes | 79.41 | 3 | -152.82 |
| null model-a-v | yes | 80.66 | 4 | -153.32 |
| Expon. m3-v | yes | 113.08 | 5 | -216.16 |
| Expon. m3-av | yes | 114.97 | 6 | -217.94 |
| Expon. m3-bv | yes | 113.97 | 6 | -215.94 |
| Expon. m3-abv | yes | 119.11 | 7 | -224.22 |
| Expon. m5-v | yes | 113.08 | 6 | -214.16 |
| Expon. m5-av | yes | 114.97 | 7 | -215.94 |
| Expon. m5-bv | no | 113.97 | 7 | -213.94 |
| Expon. m5-abv | yes | 119.11 | 8 | -222.22 |
| Hill m3-v | yes | 113.08 | 5 | -216.16 |
| Hill m3-av | yes | 114.97 | 6 | -217.94 |
| Hill m3-bv | yes | 113.97 | 6 | -215.94 |
| Hill m3-abv | yes | 119.11 | 7 | -224.22 |
| Hill m5-v | no | 113.08 | 6 | -214.16 |
| Hill m5-av | no | 114.97 | 7 | -215.94 |
| Hill m5-bv | yes | 113.97 | 7 | -213.94 |
| Hill m5-abv | yes | 119.11 | 8 | -222.22 |
| Inv.Expon. m3-v | yes | 113.09 | 5 | -216.18 |
| Inv.Expon. m3-av | yes | 114.98 | 6 | -217.96 |
| Inv.Expon. m3-bv | yes | 113.99 | 6 | -215.98 |
| Inv.Expon. m3-abv | yes | 119.12 | 7 | -224.24 |
| Inv.Expon. m5-v | yes | 113.09 | 6 | -214.18 |
| Inv.Expon. m5-av | yes | 114.98 | 7 | -215.96 |
| Inv.Expon. m5-bv | yes | 113.99 | 7 | -213.98 |
| Inv.Expon. m5-abv | yes | 119.12 | 8 | -222.24 |
| LN m3-v | yes | 113.09 | 5 | -216.18 |
| LN m3-av | yes | 114.98 | 6 | -217.96 |
| LN m3-bv | yes | 113.99 | 6 | -215.98 |
| LN m3-abv | yes | 119.12 | 7 | -224.24 |
| LN m5-v | yes | 113.09 | 6 | -214.18 |
| LN m5-av | yes | 114.98 | 7 | -215.96 |
| LN m5-bv | yes | 113.99 | 7 | -213.98 |
| LN m5-abv | yes | 119.12 | 8 | -222.24 |

Estimated Model Parameters**EXP**

estimate for var-F : 0.00643
 estimate for var-M : 0.001307
 estimate for a-F : 74.89
 estimate for a-M : 70.89
 estimate for CED-F : 64.04
 estimate for CED-M : 51.16
 estimate for d- : 4

HILL

estimate for var-F : 0.00643
 estimate for var-M : 0.001307
 estimate for a-F : 74.89
 estimate for a-M : 70.89
 estimate for CED-F : 64.03
 estimate for CED-M : 51.14
 estimate for d- : 4

INVEXP

estimate for var-F : 0.006431
 estimate for var-M : 0.001306
 estimate for a-F : 74.89
 estimate for a-M : 70.89
 estimate for CED-F : 65.45
 estimate for CED-M : 56.38
 estimate for d- : 0.8822

LOGN

estimate for var-F : 0.006431
 estimate for var-M : 0.001306
 estimate for a-F : 74.89
 estimate for a-M : 70.89
 estimate for CED-F : 66.79
 estimate for CED-M : 59.71
 estimate for d- : 2.235

The following outliers were identified:

| model | act_dose | Gender | Globulin | AGratio | Totalprotein |
|-------------------|----------|--------|----------|---------|--------------|
| Expon. m3-abv | 72 | F | 30.8 | 0.9 | 60 |
| Hill m3-abv | 72 | F | 30.8 | 0.9 | 60 |
| Inv.Expon. m3-abv | 72 | F | 30.8 | 0.9 | 60 |
| LN m3-abv | 72 | F | 30.8 | 0.9 | 60 |

Weights for Model Averaging

| EXP | HILL | INVEXP | LOGN |
|------|------|--------|------|
| 0.25 | 0.25 | 0.25 | 0.25 |

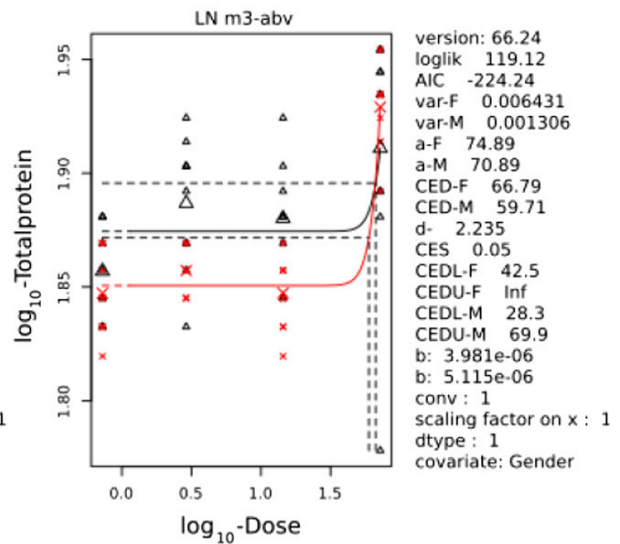
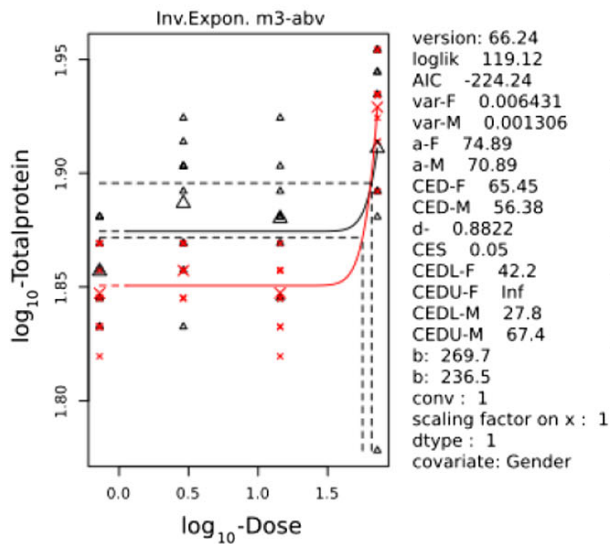
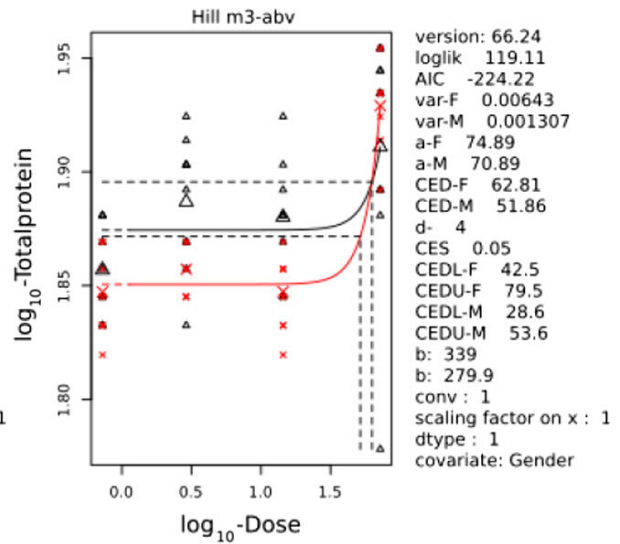
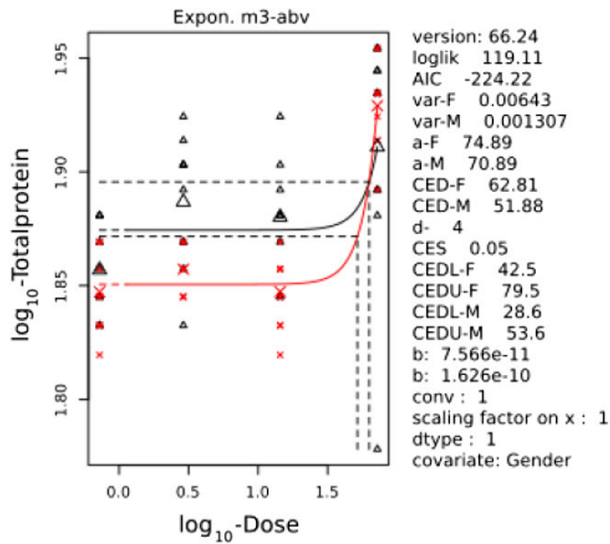
Final BMD Values

| endpoint | subgroup | BMDL | BMDU |
|--------------|----------|------|------|
| Totalprotein | F | 42.3 | 76.7 |
| Totalprotein | M | 25.9 | 56.2 |

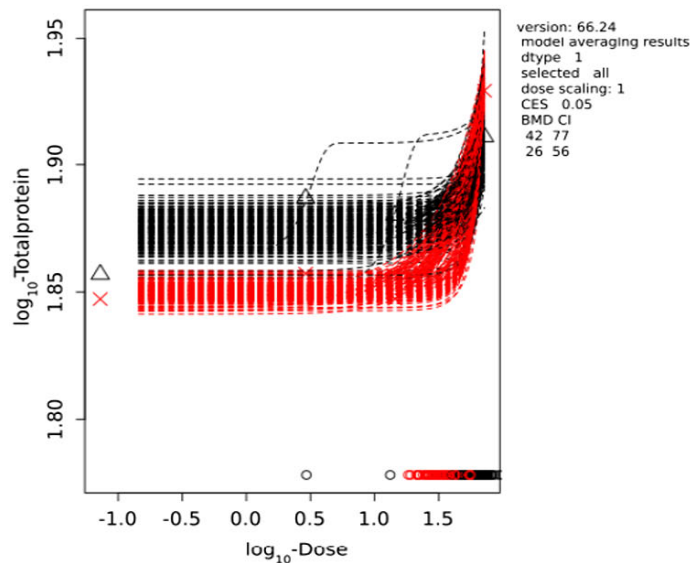
Confidence intervals for the BMD are based on 200 bootstrap data sets.

Visualisation

Plot for response 'Total proteins'.



Bootstrap curves based on model averaging



Appendix F – Schematic representation of the production process of Grill flavour concentrate (vegetable) (confidential)

