SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 18 May 2022

doi: 10.2903/j.efsa.2022.7355

Scientific opinion on flavouring group evaluation 415 (FGE.415): (*E*)-3-benzo[1,3]dioxol-5-yl-*N*,*N*-diphenyl-2-propenamide

EFSA Panel on Food Additives and Flavourings (FAF),
Maged Younes, Gabriele Aquilina, Laurence Castle, Gisela Degen, Karl-Heinz Engel, Paul J Fowler, Maria Jose Frutos Fernandez, Peter Fürst, Ursula Gundert-Remy, Rainer Gürtler, Trine Husøy, Melania Manco, Peter Moldeus, Sabina Passamonti, Romina Shah, Ine Waalkens-Berendsen, Matthew Wright, Romualdo Benigni, Claudia Bolognesi, Kevin Chipman, Eugenia Cordelli, Karin Nørby, Camilla Svendsen, Maria Carfí, Giorgia Vianello and Wim Mennes

Abstract

The EFSA Panel on Food Additives and Flavourings (FAF) was requested to evaluate the safety of the substance (*E*)-3-benzo[1,3]dioxol-5-yl-*N*,*N*-diphenyl-2-propenamide [FL-no: 16.135] as a new flavouring substance, in accordance with Regulation (EC) No 1331/2008. The substance has not been reported to occur naturally and it is chemically synthesised. It is intended to be used as a flavouring substance in specific categories of food, but not intended to be used in beverages. The chronic dietary exposure to [FL-no: 16.135] estimated using the added portions exposure technique (APET), is calculated to be 780 μg/person per day for a 60-kg adult and 480 μg/person per day for a 15-kg 3-year-old child. [FL-no: 16.135] did not show genotoxic effects in bacterial mutagenicity and mammalian cell micronucleus assays *in vitro*. Developmental toxicity was not observed in a study in rats at the dose levels up to 1,000 mg/kg body weight (bw) per day. The Panel derived a BMDL of 101 mg/kg bw per day from a 90-day toxicity study. Based on this BMDL, adequate margins of exposure of 7,800 and 3,200 could be calculated for adults and children, respectively. The Panel concluded that there is no safety concern for [FL-no: 16.135], when used as a flavouring substance at the estimated level of dietary exposure calculated using the APET approach, based on the intended uses and use levels as specified in Appendix B. The Panel further concluded that the combined exposure to [FL-no: 16.135] from its use as a food flavouring substance and from its presence in toothpaste is also not of safety concern.

© 2022 Wiley-VCH Verlag GmbH & Co. KgaA on behalf of the European Food Safety Authority.

Keywords: (*E*)-3-benzo[1,3]dioxol-5-yl-*N*,*N*-diphenyl-2-propenamide, FGE.415, flavouring, FL-no: 16.135

Requestor: European Commission

Question number: EFSA-Q-2020-00572

Correspondence: fip@efsa.europa.eu

Panel members: Maged Younes, Gabriele Aquilina, Laurence Castle, Gisela Degen, Karl-Heinz Engel, Paul J Fowler, Maria Jose Frutos Fernandez, Peter Fürst, Ursula Gundert-Remy, Rainer Gürtler, Trine Husøy, Melania Manco, Wim Mennes, Peter Moldeus, Sabina Passamonti, Romina Shah, Ine Waalkens-Berendsen and Matthew Wright.

Declarations of interest: The declarations of interest of all scientific experts active in EFSA's work are available at https://ess.efsa.europa.eu/doi/doiweb/doisearch.

Acknowledgements: The Panel wishes to thank the following for the support provided to this scientific output: the FAF Panel WG on Specifications of food additives and EFSA staff Ana Maria Rincon and Alkiviadis Stagkos-Georgiadis.

Suggested citation: EFSA FAF Panel (EFSA Panel on Food Additives and Flavourings), Younes M, Aquilina G, Castle L, Degen G, Engel K-H, Fowler PJ, Frutos Fernandez MJ, Fürst P, Gundert-Remy U, Gürtler R, Husøy T, Manco M, Moldeus P, Passamonti S, Shah R, Waalkens-Berendsen I, Wright M, Benigni R, Bolognesi C, Chipman K, Cordelli E, Nørby K, Svendsen C, Carfí M, Vianello G and Mennes W, 2022. Scientific opinion on flavouring group evaluation 415 (FGE.415): (*E*)-3-benzo[1,3]dioxol-5-yl-*N*,*N*-diphenyl-2-propenamide. EFSA Journal 2022;20(7):7355, 30 pp. https://doi.org/10.2903/j.efsa.2022.7355

ISSN: 1831-4732

© 2022 Wiley-VCH Verlag GmbH & Co. KgaA on behalf of the European Food Safety Authority.

This is an open access article under the terms of the Creative Commons Attribution-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited and no modifications or adaptations are made.

The EFSA Journal is a publication of the European Food Safety Authority, a European agency funded by the European Union.

Table of contents

1. Introduction

The present scientific opinion deals with the safety assessment of (*E*)-3-benzo[1,3]dioxol-5-yl-*N*,*N*-diphenyl-2-propenamide [FL-no: 16.135] to be used as a new flavouring substance in and on food.

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

Background

The use of flavourings in and on food is regulated under Regulation (EC) No 1334/2008[1] 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 1331/2008[2] applies for the evaluation and approval of new flavouring substances.

The applicant has submitted an application for authorisation of the substance mentioned above as a new flavouring substance in 2019. The application has been examined for administrative completeness and it is considered complete.

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

Terms of Reference

The European Commission requests the European Food Safety Authority to carry out the safety assessment of the substance (*E*)-3-benzo[1,3]dioxol-5-yl-*N*,*N*-diphenyl-2-propenamide (CAS 1309389–73-8) as a new flavouring substance in accordance with Regulation (EC) No 1331/2008 establishing a common authorisation procedure for food additives, food enzymes and food flavourings.

1.2. Existing authorisations and evaluations

JECFA evaluated (*E*)-3-benzo[1,3]dioxol-5-yl-*N*,*N*-diphenyl-2-propenamide (JECFA no. 2228) as flavouring substance at the 82nd meeting (JECFA, 2016a,b, 2017) according to the JECFA Procedure (JECFA, 1999). The substance was evaluated by JECFA in the group of aliphatic and aromatic amines and amides. According to JECFA, it is reported to be a flavour modifier. JECFA concluded that (*E*)-3-benzo[1,3]dioxol-5-yl-*N*,*N*-diphenyl-2-propenamide would not pose a safety concern at current estimated dietary exposures (see Appendix F).

A dossier for (*E*)-3-benzo[1,3]dioxol-5-yl-*N*,*N*-diphenyl-2-propenamide has been registered in the framework of REACH[3] (Registration, Evaluation, Authorisation and Restriction of Chemicals) Regulation.

2. Data and methodologies

2.1. Data

The present evaluation is based on data on (*E*)-3-benzo[1,3]dioxol-5-yl-*N*,*N*-diphenyl-2-propenamide [FL-no: 16.135] provided by the applicant in a dossier (Documentation provided to EFSA No.1) to support its evaluation as a food flavouring substance. Additional information was provided by the applicant during the risk assessment process on 8 October 2021 (Documentation provided to EFSA No. 2) and on 19 January 2022 (Documentation provided to EFSA No. 3) in response to requests from EFSA sent on 2 March 2021 and on 11 November 2021, respectively.

2.2. Methodologies

This opinion was prepared following the principles described in the EFSA Guidance of the Scientific Committee on transparency with regard to scientific aspects of risk assessment (EFSA Scientific

[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 L 354, 31.12.2008, p. 1–6.

[3] https://echa.europa.eu/it/registration-dossier/-/registered-dossier/17599

Committee, 2009) and following the relevant existing Guidance documents from the EFSA Scientific Committee.

The safety assessment of (*E*)-3-benzo[1,3]dioxol-5-yl-*N*,*N*-diphenyl-2-propenamide [FL-no: 16.135] was carried out in accordance with the procedure as outlined 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) and the EFSA technical report '*Proposed template to be used in drafting scientific opinions on flavouring substances (explanatory notes for guidance included)*' (EFSA, 2012).

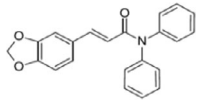
3. Assessment

3.1. Technical data

3.1.1. Identity of the substance

The chemical structure of the flavouring substance (*E*)-3-benzo[1,3]dioxol-5-yl-*N*,*N*-diphenyl-2-propenamide and the specification data provided by the applicant are shown in Table 1. The flavouring substance has been allocated the FLAVIS number [FL-no: 16.135].

Table 1: Specification data for (*E*)-3-benzo[1,3]dioxol-5-yl-*N*,*N*-diphenyl-2-propenamide as provided by the applicant in the original dossier (Documentation provided to EFSA No. 1)

Chemical name	**CAS no** **EC no** **CoE no** **JECFA no** **FL-no** **FEMA no**	**Chemical formula** **MW**	**Structural formula**	**Physical form**	**Solubility data**	**ID test**	**Purity**	**Impurities**	**Boiling point[(a)]** **Melting point** **Specific gravity[(b)]** **Refractive index[(c)]**
(*E*)-3-benzo [1,3]dioxol-5-yl-*N*,*N*-diphenyl-2-propenamide	1309389-73-8 811-467-2 - 2228 16.135 4788	$C_{17}H_{22}NO_3$ 343.39		Colourless crystals. When ground, a solid, white powder	Water: insoluble Ethanol: > 2%	GC, HPLC, IR, NMR, MS	> 95%	3,4-(methylenedioxy) cinnamic acid < 3%; (*Z*)-3-benzo[1,3]dioxol-5-yl-*N*,*N*-diphenyl-2-propenamide < 3%	n.a. 145°C n.a. n.d.

CAS: Chemical Abstract Service; EC: European Commission; CoE: Council of Europe; JECFA: Joint FAO/WHO Expert Committee on Food Additives; FL-no: FLAVIS number; FEMA: Flavour and Extract Manufactures Association; HPLC: High-Performance Liquid Chromatography; MW: Molecular Weight; ID: Identity; GC: Gas Chromatography; MS: Mass Spectrometry; IR: infrared; n.a.: not applicable; n.d.: not determined; NMR: Nuclear magnetic Resonance.
(a): At 1,013.25 hPa, if not otherwise stated.
(b): At 20°C, unless otherwise stated.
(c): At 25°C, unless otherwise stated.

The Panel noted that the substance is formally a derivative of 2-propenamide (also known as acrylamide), but 2-propenamide is not used in the synthesis and it will not be present as an impurity.

In the original dossier, the applicant reported that the analysis of a commercial batch of the flavouring substance revealed less than 3% 3,4-(methylenedioxy)cinnamic acid and less than 3% (*Z*)-3-benzo[1,3]dioxol-5-yl-*N*,*N*-diphenyl-2-propenamide, and that no other impurities were detected (no analytical data substantiating this statement had been provided) (Documentation provided to EFSA No. 1). Upon request by EFSA, the applicant clarified that the purity of the flavouring substance has been determined via GC by reporting relative area percentages using flame ionisation detection without applying substance-specific correction factors and by HPLC using the UV signal at 280 nm, which is suitable for detection of aromatic substances. In the response, the applicant also reported that the flavouring substance has been in use for oral care applications for several years and that the optimisation of the production and purification process led to consistently higher purities above 99% without further detectable impurities. Especially traces of unreacted free 3,4-(methylenedioxy)cinnamic acid are removed by the purification step employed after the amide formation. Accordingly, the applicant declared that as regulatory specification a purity ≥ 98%, without further specifications for impurities, would be suggested (Documentation provided to EFSA No. 2). The Panel agrees with this suggestion.

3.1.2. Organoleptic characteristics

According to the applicant, the flavouring substance is intended to be used as a flavouring substance with cooling properties adding minty, burning, tingling, fresh and fruity notes (Documentation provided to EFSA No.1).

3.1.3. Manufacturing process

The approach employed to synthesise the flavouring substance is outlined in Figure 1. The applicant presented details on the key parameters of the different steps of the production process, including the purification steps of the substance, and on the purity specifications for the employed starting materials, reagents and process solvents. The information provided was found to be adequate by the Panel.

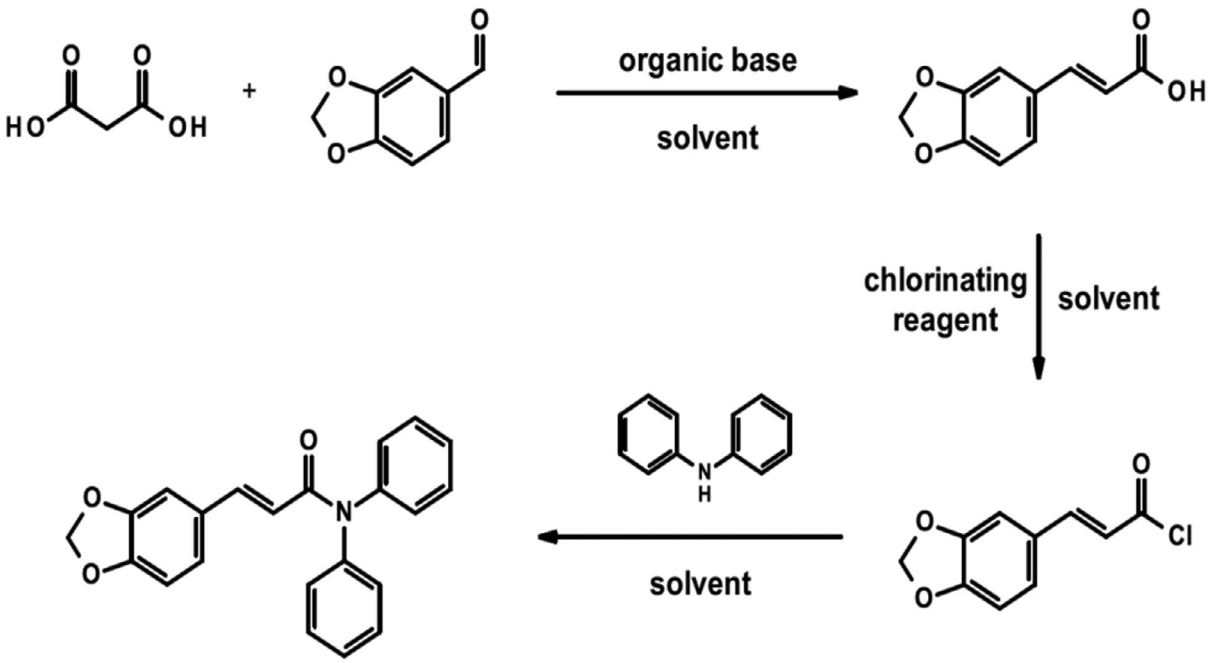


Figure 1: Approach employed to synthesise the flavouring substance (Documentation provided to EFSA No. 1)

3.1.4. Proposed specifications

The specifications proposed by the applicant for (*E*)-3-benzo[1,3]dioxol-5-yl-*N*,*N*-diphenyl-2-propenamide [FL-no: 16.135] are shown in Table 1. With regard to the purity assay, i.e. above 95%, the Panel noted that a higher minimum purity assay would be technologically achievable as declared by the applicant (i.e. $\geq$ 98%, see also Section 3.1.1). Otherwise, the Panel found the proposed specifications acceptable.

3.1.5. Solubility and particle size

The applicant provided the solubility data presented in Table 2 (Documentation provided to EFSA No. 1 and 2).

In the original dossier, the applicant had reported the flavouring substance to be 'insoluble' in water (unbuffered). Upon request by EFSA, the applicant provided the results of a solubility test according to OECD TG 105 (OECD, 1995), demonstrating a solubility of the flavouring substance in water of 0.124 mg/L at 20°C (Documentation provided to EFSA No. 2). The Panel noted that the solubility of the substance in water does not meet the criteria established in the EFSA Scientific Committee Guidance on technical requirements for regulated food and feed product applications to establish the presence of small particles including nanoparticles (EFSA SC Guidance on particle-TR) (EFSA Scientific Committee, 2021a).

In relation to the reported solubility data in the other organic solvents and solvent mixtures, the applicant clarified that the values presented in Table 2 reflect the concentrations of (*E*)-3-benzo[1,3]dioxol-5-yl-*N*,*N*-diphenyl-2-propenamide, which have been found 'stable' in terms of solubility, i.e. clear soluble by visual control for at least 8 h at 4°C (Documentation provided to EFSA No. 3).

The applicant also provided information on the octanol–water partition coefficient (Ko/w) of the candidate substance. The Ko/w was determined in a GLP study employing HPLC according to OECD TG 117 (OECD, 2004) and EU method A.8,[4] resulting in a log Ko/w of 3.4 $\pm$ 0.2 (Documentation provided to EFSA No. 3).

Table 2: Solubility data for the flavouring substance (Documentations provided to EFSA No. 2 and No. 3)

Solvent 1	Solvent 2	Solvent 3	Maximum concentration of substance (%)
Water (unbuffered)			1.24×10^{-5}(a)
Ethanol			2
Benzyl Acetate			5
Benzyl Alcohol			8
Triethyl Citrate (50%)	Peppermint Oil (20%)	Triacetin (25%)	5
Triethyl Citrate (50%)	Triacetin (45%)		5
Triethyl Citrate (31.6%)	Peppermint Oil (31.6%)	Triacetin (31.6%)	5
Triethyl Citrate			5
Triethyl Citrate (47.5%)	Peppermint Oil (47.5%)		5
Peppermint Oil (47.5%)	Triacetin (47.5%)		5

(a): 0.124 mg/L at 20°C, solubility in water determined in accordance to OECD TG 105.

Particle size

The particle size distribution of the flavouring substance was analysed by laser diffraction and electron microscopy (EM) (Documentations provided to EFSA No. 1 and 2). An SEM report with the result of the analysis of (*E*)-3-(1,3-benzodioxol-5-yl)-*N*,*N*-diphenylprop-2-enamide was submitted. However, the number-based distribution of the minimal external particle size of the constituent particles of the examined material, both in the aqueous dispersion and in the dry form, was not reported. In addition, the corresponding descriptive statistics, which should at least include the median size, the number of analysed particles and the percentage of the fraction of constituent particles in the

[4] Council Regulation (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH).

sub-500 nm fraction with a minimal external dimension that is smaller than 250 nm, was lacking. Without this information, based on the available SEM report, it was not possible to conclude if the material would require specific assessment of properties at the nanoscale (EFSA Scientific Committee, 2021a).

In line with the EFSA SC Guidance on particle-TR (EFSA Scientific Committee, 2021a), the Panel compared the reported solubilities of [FL-no: 16.135] in the organic solvents and solvent mixtures, respectively, in which it is dissolved for technological purposes (Table 2), with the intended concentrations of the substance in the proposed food matrices (i.e. the intended use levels, see Appendix B). The Panel noted that the intended maximum use levels of the flavouring substance [FL-no: 16.135] for various food categories range between 150 and 500 mg/kg. Taking this into account, the Panel considered that the flavouring substance can be reasonably anticipated to be fully dissolved when added to the proposed foods. Moreover, the provided information on the log Ko/w and the solubility in water indicate that the flavouring substance will behave as expected for a low molecular weight lipophilic substance, i.e. partitioning in food and in the GIT in the lipidic fractions. Therefore, the Panel concluded that the EFSA Guidance on Nanotechnology (EFSA Scientific Committee, 2021b) is not applicable. Consequently, the risk assessment of the flavouring substance can be performed following the Guidance on risk assessment of flavourings to be used in or on food (EFSA CEF Panel, 2010).

3.1.6. Stability and fate in food

According to the applicant, the shelf-life stability of the neat product is at least 8 months at room temperature. Limited stability was observed in concentrated solutions in organic solvents under the effect of sunlight. According to the applicant, under these conditions, a photolytic degradation via isomerisation of the double bond can be observed, and such solutions should be stored in the dark (Documentation provided to EFSA No. 1).

In the original dossier, the applicant had stated that the substance is considered stable in all media and in all foods in which it has been incorporated. In a request for additional information, the Panel noted that no analytical data substantiating this statement had been provided and that based on the molecular structure of the flavouring substance and the reported information, hydrolysis might occur when the compound is in solution. In the response to this request, the applicant stated that due to the low solubility of the flavouring substance in beverage-type applications, it has not been possible to derive meaningful stability data in the respective food matrices. Consequently, the applicant withdrew from the application the use in food categories 14.1 (non-alcoholic ('soft') beverages) and 14.2.1 (beer and malt beverages) (Documentation provided to EFSA No. 2).

No analytical data regarding the stability of the flavouring substance in the food categories 05.2 (confectionery including hard and soft candy, nougats, etc., other than food categories 05.1, 05.3 and 05.4), 05.3 (chewing gum) and 05.4 (decorations (e.g. for fine bakery wares), toppings (non-fruit) and sweet sauces) have been provided by the applicant.

Since hydrolysis of amides requires strongly acidic or alkaline conditions mostly in combination with elevated temperatures, the Panel considered that hydrolytic degradation of the flavouring substance in the proposed food categories (Appendix B, Table B.1) is not to be expected. Taking into account the limited penetration depth of UV-radiation, in particular in solid food matrices, the Panel considered the *cis-trans* isomerisation of the flavouring substance reported by the applicant to occur in concentrated organic solutions in sunlight, not to be of relevance in the proposed food categories.

No trials have been performed regarding the reaction of the flavouring substance with other food components. Taking into account the structure of the flavouring substance, the Panel considered that no reaction products of potential safety concern would be expected upon its use in the proposed food categories. In particular, the Panel considered that neither acid hydrolysis nor *cis-trans* isomerisation of the substance can give rise to 2-propenamide.

Uses and use levels proposed by the applicant are listed in Appendix B (Table B.1). Following the proposal from the applicant to remove the food categories 14.1 and 14.2 from the application, these have not been considered in the exposure assessment (see Section 3.3).

3.2. Structural/metabolic similarity to flavouring substances in existing FGE

No flavouring substances structurally related to (*E*)-3-benzo[1,3]dioxol-5-yl-*N*,*N*-diphenyl-2-propenamide were identified in existing FGEs.

3.3. Exposure assessment

3.3.1. Natural occurrence in food

(*E*)-3-Benzo[1,3]dioxol-5-yl-*N*,*N*-diphenyl-2-propenamide has not been reported to occur naturally in any food or food source (volatile compounds in food (VCF, 2021) database, version 16.8). Therefore, the only occurrence levels in food arise from its use as added flavouring substance.

3.3.2. Non-food sources of exposure

(*E*)-3-Benzo[1,3]dioxol-5-yl-*N*,*N*-diphenyl-2-propenamide is used in oral care formulations, mainly in toothpaste. An estimated exposure from this use was provided by the applicant (Table 3).

Table 3: Calculation of exposure to (*E*)-3-benzo[1,3]dioxol-5-yl-*N*,*N*-diphenyl-2-propenamide via exposure to toothpaste as provided by the applicant

Level of (*E*)-3-benzo [1,3]dioxol-5-yl-*N*,*N*-diphenyl-2-propenamide in toothpaste	Amount applied per use day (g)	Frequency of applications (n. per day)	Retention factor	Daily exposure to toothpaste (g/day)	Exposure (μg/kg bw per day)
200–800 ppm	2.75[(a)]	2	0.05[(a)]	0.275	1–3.6

(a): Values reported in the dossier reflect the default values used by the Scientific Committee on Consumer Safety (SCCS, 2018).

In the calculation of the exposure from the use of toothpaste, the applicant considered the 'amount applied per use day (g)' and the 'frequency of applications (n. per day)'. The applicant included in the calculation a frequency of application of 2 per day, which is not foreseen in the Scientific Committee on Consumer Safety (SCCS) Guidance (SCCS, 2018). Therefore, the daily exposure to toothpaste estimated by the applicant is twice as high as would result from the SCCS Guidance. The daily amount applied (2.75 g) reported in the SCCS Guidance was generated from probabilistic analyses, which encompasses both frequency of and amount per application. According to the SCCS Guidance (2018), the value of 2.75 g per day is the estimated daily amount applied, which multiplied by the retention factor (0.05) results in a daily exposure to toothpaste of only 0.14 g per day.

Consequently, the estimated exposure from toothpaste of 1–3.6 μg/kg bw per day (corresponding to 60–216 μg/person per day), as provided by the applicant, is incorrect. The Panel estimated the exposure to the substances to be in the range from 28 to 112 μg/person per day, or 0.5–1.9 μg/kg bw per day.

According to the applicant, exposure of children through toothpaste does not have to be calculated as the resulting strong cooling sensation is not preferred and the toothpaste would be rejected.

No use in cosmetic formulations on skin is reported by the applicant. However, an estimated exposure has been provided for three potential formulations on skin (see Appendix C).

3.3.3. Chronic dietary exposure

The exposure assessment to be used in the Procedure for the safety evaluation of (*E*)-3-benzo [1,3]dioxol-5-yl-*N*,*N*-diphenyl-2-propenamide is the chronic added portions exposure technique (APET) estimate (EFSA CEF Panel, 2010). The chronic APET for [FL-no: 16.135] has been calculated for adults and children (see Table 4), and these values, expressed per kg body weight (bw), will be used in the Procedure (see Appendices A and B). The chronic APET calculation is based on the proposed normal use levels and the standard portion size (see Appendix B).

Based on the information provided by the applicant, the Panel considered that (*E*)-3-benzo [1,3]dioxol-5-yl-*N*,*N*-diphenyl-2-propenamide is not intended to be used in food category 13.2 (foods for infants and young children).

Subsequent to a request by EFSA for additional information, the applicant responded that, the flavouring substance is not intended to be used in food categories 14.1 and 14.2.1 due to the low solubility in beverage-type applications (see Appendix B and Section 3.1).

Table 4: APET – Chronic Dietary Exposure as calculated by EFSA

Chronic APET	Added as flavouring substance[a]		Other dietary sources[b]		Combined[c]	
	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[d]	13	780	0	0	13	780
Children[e]	32	480	0	0	32	480

APET: added portions exposure technique; bw: body weight.
(a): APET Added is calculated on the basis of the amount of flavouring added to a specific food category.
(b): APET Other Dietary Sources is calculated based on the natural occurrence of the flavouring in a specified food category.
(c): APET Combined is calculated based on the combined amount of added flavour and naturally occurring flavouring in a specified food category.
(d): For the adult APET calculation, a 60-kg person is considered representative.
(e): For the child APET calculation, a 3-year-old child with 15 kg bw is considered representative.

3.3.4. Acute dietary exposure

The acute APET calculation for [FL-no: 16.135] is based on the proposed maximum use levels and large portion size (i.e. three times standard portion size) (EFSA CEF Panel, 2010) (Table 5).

Table 5: APET – Acute Dietary Exposure as calculated by EFSA

Chronic APET	Added as flavouring substance[a]		Other dietary sources[b]		Combined[c]	
	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[d]	375	22,500	0	0	375	22,500
Children[e]	945	14,200	0	0	945	14,200

APET: added portions exposure technique; bw: body weight.
(a): APET Added is calculated on the basis of the maximum amount of flavouring added to a specific food category.
(b): APET Other dietary sources is calculated based on the natural occurrence of the flavouring in a specified food category.
(c): APET Combined is calculated based on the combined amount of added flavouring and naturally occurring flavouring in a specified food category.
(d): For the adult APET calculation, a 60-kg person is considered representative.
(e): For the child APET calculation, a 3-year-old child with 15 kg bw is considered representative.

3.3.5. Cumulative dietary exposure

The Panel considered that there are no flavouring substances with structural similarity to (*E*)-3-benzo[1,3]dioxol-5-yl-*N*,*N*-diphenyl-2-propenamide. Therefore, the calculation of the cumulative exposure is not applicable.

3.4. Biological and toxicological data

3.4.1. Absorption, distribution and elimination

No experimental data were submitted for the flavouring substance [FL-no: 16.135].

3.4.2. Metabolism

Metabolism studies have not been provided for the flavouring substance [FL-no: 16.135].

Taking into account the structure of the substance and in particular the high degree of substitution of the central 3-carbon amide unit, the Panel concluded that the formation of 2-propenamide (acrylamide) as a metabolite can be discounted.

3.4.3. Genotoxicity

3.4.3.1. *In silico* analysis

The flavouring substance has been analysed through the OECD QSAR Toolbox and ToxTree 3.1.0[5] and no structural alerts for genotoxicity or carcinogenicity were identified (data not provided).

3.4.3.2. *In vitro* genotoxicity studies

3.4.3.2.1. Bacterial reverse mutation assay

A bacterial reverse mutation assay was conducted in Salmonella Typhimurium strains TA98, TA100, TA1535, TA1537 and in *Escherichia coli* WP2 uvrA to assess the mutagenicity of (*E*)-3-benzo [1,3]dioxol-5-yl-*N*,*N*-diphenyl-2-propenamide (purity > 95%), both in the absence and in the presence of metabolic activation by phenobarbital/β-naphthoflavone-induced rat liver S9 fraction (S9-mix). Two separate experiments were conducted, the first using the plate incorporation method and the second using the preincubation method (BASF SE, 2009). Study design complies with OECD Test Guideline (TG) 471 (OECD, 1997) and with the GLP principles.

Positive control chemicals and dimethyl sulfoxide (DMSO, as vehicle control) were evaluated concurrently. All tests were evaluated in triplicate plates.

In both experiments, (*E*)-3-benzo[1,3]dioxol-5-yl-*N*,*N*-diphenyl-2-propenamide was tested at concentrations from 22 to 5,500 μg/plate with and without S9-mix. Precipitate was found from 110 μg/plate onward with and without S9-mix.

In the standard plate test, weak bacterial toxicity was occasionally observed at the highest applied concentration, depending on the strain and test conditions. In the preincubation assay, weak bacterial toxicity was occasionally observed at concentrations of about 2,750 μg/plate and above, depending on the strain and test conditions.

All positive control chemicals both with and without S9-mix induced significant increases in revertant colony numbers. Both vehicle controls and positive controls were within the respective historical control ranges.

In both experiments, no increase in the mean number of revertant colonies was observed at any tested concentration in any tester strains in the absence or presence of metabolic activation (BASF SE, 2009).

3.4.3.2.2. In vitro *mammalian cell micronucleus test*

Human peripheral blood lymphocytes from healthy donors were treated with (*E*)-3-benzo [1,3]dioxol-5-yl-*N*,*N*-diphenyl-2-propenamide (purity 97.2%). The *in vitro* micronucleus assay was carried out according to OECD TG 487 (OECD, 2010) and GLP principles. The cytokinesis block micronucleus assay protocol was applied. Positive controls were cyclophosphamide, mitomycin C and vinblastine. DMSO was used as negative control (Covance, 2014).

The highest concentration for cytotoxicity range-finder experiment was 500 μg/mL, selected on the basis of solubility. Concentrations for the micronucleus experiment were selected based on the results of this cytotoxicity range-finder experiment.

For the micronucleus experiment, lymphocytes were treated with (*E*)-3-benzo[1,3]dioxol-5-yl-*N*,*N*-diphenyl-2-propenamide at concentrations ranging from 10 to 100 μg/mL in the 3 h treatment in the presence of metabolic activation (from rats treated with Aroclor 1,254), 5–100 μg/mL in the 3 h treatment in the absence of metabolic activation and from 2 to 40 μg/mL in the 24 h treatment in the absence of metabolic activation. Precipitate was observed at 30 μg/mL and above in the 3 h treatment, both in the presence and in the absence of metabolic activation and at 40 μg/mL in the 24 h treatment.

The Replication Index (RI) cytotoxicity data were used to select the concentrations for the micronucleus (MN) analysis.

In the treatment of 3 h + 21 h in the presence of S9-mix, the following concentrations were chosen for MN analysis: 10, 30, 55 and 60 μg/mL (cytotoxicity of 7%, 27%, 57% and 50%, respectively).

In the treatment of 3 h + 21 h in the absence of S9-mix, the following concentrations were chosen for MN analysis: 10, 30 and 60 μg/mL (cytotoxicity of 11%, 27% and 55%, respectively).

[5] https://toxtree.sourceforge.net/download.html#Toxtree_3.1.0

In the treatment of 24 h in the absence of S9-mix, the following concentrations were chosen for MN analysis: 8, 14 and 20 μg/mL (cytotoxicity of 10%, 24% and 55%, respectively).

(*E*)-3-Benzo[1,3]dioxol-5-yl-*N*,*N*-diphenyl-2-propenamide did not increase the frequency of micronucleated cells compared to vehicle (DMSO) controls in any of the testing conditions.

3.4.3.3. *In vivo* genotoxicity studies

No *in vivo* studies were performed due to the absence of genotoxicity effects observed *in vitro*.

3.4.3.4. Conclusion on genotoxicity studies

No indications of mutagenicity were obtained from an adequate bacterial reverse mutation assay, and no indications for clastogenicity or aneugenicity were obtained from an adequate *in vitro* mammalian cell micronucleus test. Therefore, the Panel concluded that flavouring substance (*E*)-3-benzo[1,3]dioxol-5-yl-*N*,*N*-diphenyl-2-propenamide does not raise a concern for genotoxicity.

3.4.4. Toxicity

3.4.4.1. 14-Day Range-Finding toxicity study in rats

A 14-day dose range-finding study (Product Safety Labs, 2013a) was performed to evaluate the palatability and general toxicity of (*E*)-3-benzo[1,3]dioxol-5-yl-*N*,*N*-diphenyl-2- propenamide.

Seven to eight weeks old Crl: Sprague–Dawley® CD® IGS rats (5/sex per group) were fed a diet designed to provide 0, 10, 250 and 1,000 mg/kg bw per day of (*E*)-3-benzo[1,3]dioxol-5-yl-*N*,*N*-diphenyl-2-propenamide (purity 98.6%) for 14 days. The calculated average daily intakes of the test substance were 0, 14.6, 359 and 1,443 mg/kg bw per day and 0, 13.8, 378 and 1,381 mg/kg bw per day for males and females, respectively.

No mortality occurred and no test substance-related clinical observations, body weight, body weight gain or food consumption were adversely affected. Further, no gross pathology findings were observed. It was concluded that the dose level of 1,443 and 1,381 mg/kg bw per day was well tolerated by males and females, respectively.

3.4.4.2. 90-Day toxicity study in rats

(*E*)-3-Benzo[1,3]dioxol-5-yl-*N*,*N*-diphenyl-2-propenamide (purity 98.6%) was tested in a 90-day repeated dose toxicity study in rats (Product Safety Labs, 2013b) with GLP compliance and according to OECD TG 408 (OECD, 1998). Seven to eight weeks old Crl: Sprague–Dawley® CD® IGS rats (10/sex per group) were fed diets with 0, 30, 100 or 500 mg/kg bw per day of (*E*)-3-benzo[1,3]dioxol-5-yl-*N*,*N*-diphenyl-2-propenamide (nominal dosing). The feed was prepared fresh every week. The neat test substance was shown to be stable for 10 days following preparation and was shown to be evenly distributed in the feed. Test substance homogeneity was demonstrated at the beginning of the study.

Ophthalmoscopy was performed at the start and at the end (day 88) of the study. Cage-side observations were performed daily during the study period. Animals were inspected for clinical signs weekly in more detail. Animals were weighed twice during acclimation, on study day 0 and weekly thereafter and prior to sacrifice. Food consumption and efficiency were measured and calculated weekly. A Functional Observational Battery (FOB) and Motor activity (MA) examinations were performed at week 12. Samples for blood biochemistry, haematology and urinalysis were collected at the end of the study. Full necropsy, collection of tissues and measurements of organ weights were performed on all animals. Histological examination was performed on preserved organs form animals from the control and the highest dose groups.

All animals survived until the end of the study. In-life daily and weekly detailed clinical observations did not reveal any treatment-related abnormal signs. Throughout the study, all dose groups (males and females) had a comparable mean body weight compared with the control group. Mean daily body weight gain for female rats in the highest dose group was decreased ($p < 0.05$) on day 0–7 compared with the female controls. Mean daily intake of the test substance for the different exposure groups was calculated to be 29.4, 97.5 and 489.5 mg/kg bw per day for male rats and 29.4, 98.6 and 492.2 mg/kg bw per day for female rats.

There were no differences in FOB and MA parameters between the exposed groups and the controls. For male rats, changes in haematological parameters between exposed and controls included a decrease in the number of basophils (high-dose: 31% decrease, $p < 0.05$) and statistically non-significant decreases in total white blood cells (WBC) count, neutrophils, lymphocytes, eosinophils and large unstained cells in both sexes and in basophiles in females at the highest dose level. For female

rats, statistically significant differences between exposed and controls included a decrease in haemoglobin and haematocrit levels ($p < 0.05$, mid-dose) and a decrease in mean corpuscular haemoglobin concentration ($p < 0.05$, low-dose). Decrease in serum alanine aminotransferase (ALT) activity and an increase in potassium concentration were observed in high-dosed and low-dosed females, respectively. There were no changes in urinalysis parameters between control and exposed animals.

Macroscopic findings included the observation of focal liver fibrosis in one male rat in the mid-dose. For females, macroscopic examination revealed fluid-filled uteri in two females in the low-dose, four females in the mid-dose and high-dose. It was stated that the fluid-filled uteri were attributable to variation in the oestrous cycle in individual animals.

Histopathological examination showed renal tubular cell hyperplasia in one male rat in the high-dose group. Slight to moderate laryngeal inflammation was observed in one male and one female control animal, one male and two female high-dosed animals. Inflammatory cell infiltrates in the prostate gland were observed in four control males and four males in the high dose. Minimal inflammation in the glandular lumens was observed for three males in the highest dose group.

Low-dosed males had decreased brain weight (5% decrease) compared to controls. There was an increase in liver-to-body weight ratio (10% increase) for the males in the high-dose group compared to controls and increased liver-to-body weight ratios (mid-dose: 11% increase; high-dose: 10% increase), increased liver-to-brain weight ratios (low dose: 17% increase; mid-dose: 16% increase; high dose: 14% increase), and kidney-to-brain weight ratios (low dose: 13% increase). These changes were statistically significant, but they were not clearly dose-related, relatively small and occurred without noticeable histopathological changes.

Based on the information above, JECFA (2016a, 2017) derived a NOAEL of 490 mg/kg bw per day from this study, which is the highest dose tested (Appendix F).

However, the Panel noted that in the mid- and high-dose group, statistically significant decreases in thymus weights were observed for female rats (mid-dose: 24% decrease; high-dose: 23% decrease). For female rats, differences in relative organ weight included decrease in thymus-to-body ratios (mid-dose: 24% decrease). However, when submitted to dose–response analysis using EFSA PROAST webtool,[6] no dose-related trend could be established (results not shown). Decreases were also observed in total WBC and the white blood cell subpopulations (see above). This would raise a concern for the immune system, in particular related to bone marrow. White blood cells and the subpopulations originate from the haematopoietic stem cell populations that reside in the bone marrow. In a process called maturation, daughter cells from these stem cells differentiate into the red cell lineage and the thrombocytic lineage (which were both not affected in this study) and in the white cell lineage under the influence of specific cytokines and growth factors. In further steps during the maturation, the various cell types seen in the differential blood count are formed. The Panel noted that in this study, all the cells from the white cell lineage (both myeloid and lymphoid cells) decreased and that differential blood count did not indicate that changes were not limited to a specific cell type. This may indicate that the substance has an effect on the development of the white cell lineage in an early step of the maturation before the differentiation. Therefore, the Panel decided to use the decrease in total WBC count as a proxy to reflect the effect of the substance on the WBC maturation process, since this would encompass all WBC in the differential blood count.

The data for this parameter were submitted to dose response analysis using the EFSA PROAST webtool, in line with the EFSA Scientific Committee Guidance document (EFSA Scientific Committee, 2017). Instead of using the default value of 5% for the BMR, the Panel employed an endpoint-specific benchmark response (BMR), based on the theory developed by Slob (2017). This theory takes better account of the natural variability in the measured parameters, than the default BMRs. This results in biologically more plausible BMRs and subsequently more plausible BMDLs. The endpoint-specific BMR was calculated with the RIVM PROAST webtool[7] and a BMR of 19% (reflecting a decrease in total WBC count) was obtained to represent a minimal effect size. With this BMR, from the study data, BMDL – BMDU 90% confidence intervals around the BMD for decrease in total WBC count of 124–781 and 101–1,470 mg/kg bw per day could be calculated for males and females, respectively.

The reports from the EFSA PROAST dose response modelling tool have been included in Appendix G.

[6] Available through the R4EU platform at https://www.efsa.europa.eu/en/science/tools-and-resources.
[7] https://proastweb.rivm.nl/

3.4.4.2.1. Conclusion on the 90-day toxicity study

There were no dose-dependent and no treatment-related differences between exposed and control animals for histopathological findings. The Panel noted a reduction in thymus weight and decreases in WBC and in the white blood cell subpopulations. This would raise a concern for the immune system. Therefore, the data for decreased thymus weight and decreased WBC (used as a proxy for white blood cell maturation) were submitted to dose response analysis. For the reduction in thymus weight, no dose-related trend could be established. In contrast, for the decrease in total white blood cell count, a dose-related downward trend was identified. Using an endpoint-specific BMR of 19% for decrease WBC, BMDL–BMDU 90% confidence intervals of 124–781 and 101–1,470 mg/kg bw per day could be calculated for males and females, respectively. The Panel considered these confidence intervals acceptable. The lowest BMDL of 101 mg/kg bw per day from the data for the females will be used for the evaluation of the flavouring substance.

3.4.4.3. Prenatal developmental toxicity study in rats

(*E*)-3-Benzo[1,3]dioxol-5-yl-*N*,*N*-diphenyl-2-propenamide (purity 99.7%) was tested in a prenatal developmental toxicity study in CRL Sprague-Dawely CD® IGS rats (Product Safety Labs, 2017) according to OECD TG 414 (OECD, 2001) and GLP principles.

Five groups of animals (20 pregnant females per group) were administered the test substance at 125 (Group 2), 250 (Group 3), 500 (Group 4), 1,000 (Group 5) mg/kg bw per day (corresponding to 25 mg/mL (2.5%), 50 mg/mL (5.0%), 100 (10.0%) or 200 mg/mL (20.0%) w/v mixture in corn oil) or the vehicle control, corn oil (Group 1).

The test substance or vehicle control was administered daily (7 days/week) via oral intubation to each rat during gestation days (GD) 5–19. All animals survived until sacrifice at GD 20.

Incidental clinical signs noted in females included slight alopecia on the abdomen, head or right flank of 1/20 Group 2 and 3/20 Group 4 animals; and a lesion on the nose/snout in 1/10 Group 3 animals.

No changes in body weight and body weight gain were observed compared to control group.

No effects were observed in uterine and reproductive parameters (including early and late resorptions).

One hundred and twenty-six fetuses from 20 litters from Group 1, and 125, 113, 126 and 120 fetuses from 20 litters from Groups 2, 3, 4 and 5, respectively, were evaluated for skeletal malformations and developmental variations. No visceral or skeletal teratogenic effects were observed.

In line with the study authors, the Panel concluded that 1,000 mg/kg bw per day can be considered as a NOAEL.

3.5. Application of the procedure

No structural/metabolic similarity of the flavouring substance to flavouring substances in an existing FGE was identified.

Since (*E*)-3-benzo[1,3]dioxol-5-yl-*N*,*N*-diphenyl-2-propenamide [FL-no: 16.135] does not raise a concern for genotoxicity, it is appropriate to evaluate the use of [FL-no: 16.135] as a flavouring substance following the stepwise evaluation procedure for individual substances as outlined in the 'Guidance on the data required for the risk assessment of flavourings to be used in or on foods' (EFSA CEF Panel, 2010) and Appendix A.

Step 1

The substance (*E*)-3-benzo[1,3]dioxol-5-yl-*N*,*N*-diphenyl-2-propenamide is allocated to structural class III.[8]

Step 2

Since no data on metabolism are available to demonstrate that metabolites are innocuous, the substance is evaluated via the right (B-)side of the Procedure (see Appendix A, Figure A.1).

Step B3–B4

The conditions of use result in APET exposure estimates of 13 and 32 μg/kg bw per day (780 and 480 μg /person per day), for adults and children. These estimates are above the TTC for Cramer Class III (90 μg/person per day), but below 10-fold this TTC (900 μg/person per day). Therefore, a 90-day

[8] Determined with OECD QSAR Toolbox (version 4.5 available at https://qsartoolbox.org/)

toxicity study and a developmental toxicity study have been performed. In the developmental toxicity study, no toxicity was observed. In the 90-day toxicity study, a consistent decrease was observed in the numbers of white blood cells (total cell count as well as subpopulations), indicative of interference of the flavouring substance with white blood cells maturation. For this effect, a BMDL of 101 mg/kg bw per day was calculated based on a BMR of 19% for a decrease in total WBC count.

Using this BMDL at step B4 of the Procedure, adequate Margins of Exposure (MoE) of 7,800 and 3,200 could be calculated for adults and children, respectively, when assessing the intake based on APET.

3.6. Assessment of acute, combined and cumulative exposure

The estimates for acute exposure are approximately 10 times higher than the TTC for structural class III. However, this TTC is related to subchronic rather than acute toxicity. No signs of acute toxicity were observed in a short-term range-finding study with dosing up to 1,440 mg/kg bw per day (actual dose level), in a developmental toxicity study with dose levels up to 1,000 mg/kg bw per day and in a subchronic toxicity study with dose levels up to approximately 500 mg/kg bw per day. Since these dose levels are far above the potential acute exposure in humans, there is no concern for acute toxicity.

Since the substance does not occur naturally in food, no exposure is anticipated from that source, but additional oral exposure to the substance may occur from its use in oral personal care products, in particular in toothpaste. At most this would add 1.9 μg/kg bw per day to the exposure from food in adults. If so, then the MoE for adults would be reduced from 7,800 to 6,800, which is still adequate.

Because no structurally related substances were identified, a safety assessment for cumulative exposure is not included in this FGE.

4. Discussion

The European Commission requested EFSA to carry out the safety assessment of the substance (*E*)-3-benzo[1,3]dioxol-5-yl-*N*,*N*-diphenyl-2-propenamide [FL-no: 16.135] (CAS no. 1309389–73-8) as a new flavouring substance in accordance with Regulation (EC) No 1331/2008.

EFSA evaluated (*E*)-3-benzo[1,3]dioxol-5-yl-*N*,*N*-diphenyl-2-propenamide [FL-no: 16.135] in Flavouring Group Evaluation 415 (FGE.415) and used the procedure as referred to in Regulation (EC) No 1334/2008. No other substances with structural similarity to the flavouring substance have been identified in existing FGEs. The substance is not known to occur naturally and is obtained through chemical synthesis.

The provided specifications, which include a 98% purity requirement, are considered adequate. The flavouring substance does not possess chiral centres and exists as *trans*-configured isomer. The information provided on the manufacturing process, the composition and the stability of the flavouring substance was considered sufficient. This information did not raise a safety concern.

For the use of (*E*)-3-benzo[1,3]dioxol-5-yl-*N*,*N*-diphenyl-2-propenamide [FL-no: 16.135] as a flavouring substance, adequate information on uses and use levels has been provided, as specified in Appendix B. The substance is not intended to be used in food for infants and young children. The chronic dietary exposure to the candidate substance has been estimated using the APET method. The chronic APET exposure estimates are 13 and 32 μg/kg bw per day (780 and 480 μg /person per day) for adults and children (15-kg bw; 3-years-old), respectively. The acute APET exposure estimates are 375 and 945 μg /kg bw per day (22,500 and 14,200 μg /person per day, for adults and children respectively).

Based on the available data, the Panel concluded that this substance does not raise a concern for genotoxicity.

No substance-specific information on absorption, distribution, metabolism and elimination (ADME) of [FL-no: 16.135] has been submitted. Therefore, the Panel cannot conclude that the substance will be metabolised to innocuous products, and its evaluation proceeds via the B-side of the Procedure. The substance is allocated to structural class III and the APET exposure estimates are between the TTC (90 μg/person per day) and 10 times the TTC applicable for this class. Based on the applicable guidance document (EFSA CEF Panel, 2010), the applicant submitted a 90-day subchronic toxicity study and a developmental toxicity study in which the substance was given to rats via the diet or via gavage, respectively. No developmental toxicity was observed with dose levels up to 1,000 mg/kg bw per day. In the 90-day study, indications were obtained that the substance interferes with white blood cell maturation. For this endpoint, a BMDL of 101 mg/kg bw per day was calculated from the study

data related to a 19% decrease in total WBC count. With this BMDL adequate margins of exposure were calculated for the use of the substance [FL-no: 16.135] as food flavouring, for the APET exposure estimates based on the proposed uses and use levels as specified in Appendix B. The same result was obtained when exposure from the use as flavouring substance was combined with exposure from oral personal care products (i.e. toothpaste). Exposure from other food or non-food sources is not anticipated.

The Panel noted that no data on acute toxicity are available. However, considering the results from the repeated dose toxicity studies, there is no concern for acute toxicity.

5. Conclusions

Overall, the Panel concluded that there is no safety concern for [FL-no: 16.135], when used as a flavouring substance at the estimated level of dietary exposure calculated using the APET approach, based on the intended uses and use levels as specified in Appendix B. The Panel further concluded that the combined exposure to [FL-no: 16.135] from its use as a food flavouring substance and from its presence in toothpaste is also not of safety concern.

6. Documentation as provided to EFSA

1) Technical Information Submission for a New Flavouring Substance by Symrise AG to the European Food Safety Authority (EFSA) according to the "Common Authorisation Procedure for the application for evaluation of a new flavouring substance" (Regulation (EC) No 1334/2008, Regulation (EC) No 1331/2008, Regulation (EU) No 234/2011). November 2019. Submitted by Symrise AG.
2) Additional information received on 08 October 2021, submitted by Symrise AG in response to a request from EFSA (2 March 2021).
3) Additional information received on 19 January 2022, submitted by Symrise AG in response to a request from EFSA (11 November 2021).
4) BASF SE, 2009. Salmonella Typhimurium/Escherichia coli Reverse mutation assay (Standard plate test and preincubation test). BASF SE, study number 40 M0457/094294. November 2009. Unpublished study report submitted by Symrise AG.
5) Covance, 2014. (*E*)-3-Benzo[1,3]dioxol-5-yl-*N*,*N*-diphenyl-2-propenamide: induction of micronuclei in cultured human peripheral blood lymphocytes. Covance Laboratories Ltd, study number 8288432. February 2014. Unpublished study report submitted by Symrise AG.
6) Product Safety Labs, 2013a. (*E*)-3-Benzo[1,3]dioxol-5-yl-*N*,*N*-diphenyl-2-propenamide: Palatability/Toxicity study: a 14-day dietary study in rats. Product Safety Labs, study number 35137. January 2013. Unpublished study report submitted by Symrise AG.
7) Product Safety Labs, 2013b. (*E*)-3-Benzo[1,3]dioxol-5-yl-*N*,*N*-diphenyl-2-propenamide: a 90-day dietary study in rats. Product Safety Labs, study number 35494. September 2013. Unpublished study report submitted by Symrise AG.
8) Product Safety Labs, 2017. (*E*)-3-Benzo[1,3]dioxol-5-yl-*N*,*N*-diphenyl-2-propenamide: A Prenatal Developmental Toxicity Study In Rats. Product Safety Labs, study number 43149. April 2017. Unpublished study report submitted by Symrise AG.

References

EFSA (European Food Safety Authority), 2012. Proposed template to be used in drafting scientific opinion on flavouring substances (explanatory notes for guidance included). EFSA Journal 2012;10(1):218, 45 pp. https://doi.org/10.2903/sp.efsa.2012.EN-218

EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2010. Guidance on the data required for the risk assessment of flavourings to be used in or on foods. EFSA Journal 2010;8(6):1623, 38 pp. https://doi.org/10.2093/j.efsa.2010.1623

EFSA Scientific Committee, 2009. Guidance of the Scientific Committee on transparency in the scientific aspects of risk assessments carried out by EFSA. Part 2: general principles. EFSA Journal 2009;7(7):1051, 22 pp. https://doi.org/10.2903/j.efsa.2009.1051

EFSA Scientific Committee, 2017. Update: use of the benchmark dose approach in risk assessment. EFSA Journal 2017;15(1):4658, 41 pp. https://doi.org/10.2903/j.efsa.2017.4658.

EFSA Scientific Committee, 2021a. Guidance on technical requirements for regulated food and feed product applications to establish the presence of small particles including nanoparticles. EFSA Journal 2021;19(8):6769, 100 pp. https://doi.org/10.2903/j.efsa.2021.6769

EFSA Scientific Committee, 2021b. Guidance on risk assessment of nanomaterials to be applied in the food and feed chain: human and animal health. EFSA Journal 2021;19(8):6768, 111 pp. https://doi.org/10.2903/j.efsa.2021.6768
FAO/WHO, 2008. Joint FAO/WHO Expert Committee on Food Additives. Sixty-ninth meeting, Rome, Italy, 17–26 June 2008. Summary and conclusions. JECFA/69/SC, 21 pp. Issued 4 July 2008 Available online: https://www.fao.org/documents/card/en/c/c1dfe308-c04e-444d-9885-e2b20ef6bb07/
Hall B, Tozer S, Safford B, Coroama M, Steiling W, Leneveu-Duchemin MC, McNamara C and Gibney M, 2007. European consumer exposure to cosmetic products, a framework for conducting population exposure assessments. Food and Chemical Toxicology 45, 2097–2108. https://doi.org/10.1016/j.fct.2007.06.017
Hall B, Steiling W, Safford B. Coroama M, Tozer S, Firmani C, McNamara C and Gibney M, 2011. European consumer exposure to cosmetic products, a framework for conducting population exposure assessments, Part 2. Food and Chemical Toxicology 49, 408–422. https://doi.org/10.1016/j.fct.2010.11.016
JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1999. Evaluation of certain food additives and contaminants. Forty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series No. 884.
JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2016a. Evaluaton of certain food additives. Eighty-second report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series no. 1000, Geneva, Switzerland.
JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2016b. Compendium of food additives specifications. Eighty-second meeting of the Joint FAO/WHO Expert Committee on Food Additives, FAO JECFA Monographs no. 19.
JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2017. Safety evaluation of certain food additives. Eighty-second meeting of the Joint FAO/WHO Expert Committee on Food Additives. WHO Food Additives Series no. 73, Geneva, Switzerland.
Kroes R, Renwick AG, Feron V, Galli CL, Gibney M, Greim H, Guy RH, Lhuguenot JC and van de Sandt JJ, 2007. Application of the threshold of toxicological concern (TTC) to the safety evaluation of cosmetic ingredients. Food and Chemical Toxicology, 45, 2533–2562. https://doi.org/10.1016/j.fct.2007.06.021
OECD (Organisation for Economic Co-operation and Development), 1995. Test No. 105: Water Solubility. OECD Guidelines for the Testing of Chemicals, Section 1. OECD Publishing, Paris.
OECD (Organisation for Economic Co-operation and Development), 1997. Test No. 471: Bacterial Reverse Mutation Test. OECD Guidelines for the Testing of Chemicals, Section 4. OECD Publishing, Paris.
OECD (Organisation for Economic Co-operation and Development), 1998. Test No. 408: Repeated Dose 90- day Oral Toxicity Study in Rodents. OECD Guidelines for the Testing of Chemicals, Section 4. OECD Publishing, Paris.
OECD (Organisation for Economic Co-operation and Development), 2001 Test No. 414: Prenatal Developmental Toxicity Study. OECD Guidelines for the Testing of Chemicals, Section 4. OECD Publishing, Paris.
OECD (Organisation for Economic Co-operation and Development), 2004 Test No. 117: Partition Coefficient (n-octanol/water), HPLC Method. OECD Guidelines for the Testing of Chemicals, Section 1. OECD Publishing, Paris.
OECD (Organisation for Economic Co-operation and Development), 2010. Test No. 487: In vitro Mammalian Cell Micronucleus Test. OECD Guidelines for the Testing of Chemicals, Section 4. OECD Publishing, Paris.
SCCS (Scientific Committee on Consumer Safety), 2018. The SCCS Notes of Guidance for the testing of cosmetics ingredients and their safety evaluation 10th Revision, 24–25 October 2018.
SCCS (Scientific Committee on Consumer Safety), 2021. The SCCS Notes of Guidance for the testing of cosmetics ingredients and their safety evaluation 11th Revision, 30–31 March 2021.
Shen J, Kromidas L, Schultz T and Bhatia S, 2014. An in silico skin absorption model for fragrance materials. Food and Chemical Toxicology, 74, 164–176. https://doi.org/10.1016/j.fct.2014.09.015
Slob W, 2017. A general theory of effect size, and its consequences for defining the benchmark response (BMR) for continuous endpoints. Critical Reviews in Toxicology, 47, 342–351. https://doi.org/10.1080/10408444.2016.1241756
VCF (Volatile Compounds in Food) online database, 2021. Version 16.8, BeWiDo BV 1992–2021. Available online: https://www.vcf-online.nl/VcfHome.cfm

Abbreviations

ADME	Absorption, Distribution, Metabolism and Elimination
ALT	Alanine aminotransferase
APET	Added Portions Exposure Technique
BMD	Benchmark Dose
BMDL	Benchmark Dose lower boundary of confidence interval (95% single sided)
BMDU	Benchmark Dose upper boundary of confidence interval (95% single sided)
BMR	Benchmark Response
BW	body weight
CAS	Chemical Abstract Service

CEF	Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
DMSO	Dimethyl sulfoxide
FGE	Flavouring Group Evaluation
FLAVIS (FL)	Flavour Information System (database)
FOB	Functional Observational Battery
GLP	Good Laboratory Practice
ID	identity
IR	infrared spectroscopy
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
MA	Motor activity
MoE	Margin of Exposure
mwbc	mean of total white blood cells count per group
NMR	nuclear magnetic resonance
No	number
NOAEL	No observed adverse effect level
OECD	Organisation for Economic Cooperation and Development
sdwbc	standard deviation of total white blood cells count per group
SPET	single-portion exposure technique
TTC	Threshold of Toxicological of Concern
WBC	White Blood Cells
WHO	World Health Organisation

Appendix A – Procedure for the safety evaluation of 'stand-alone' chemically defined flavouring substances

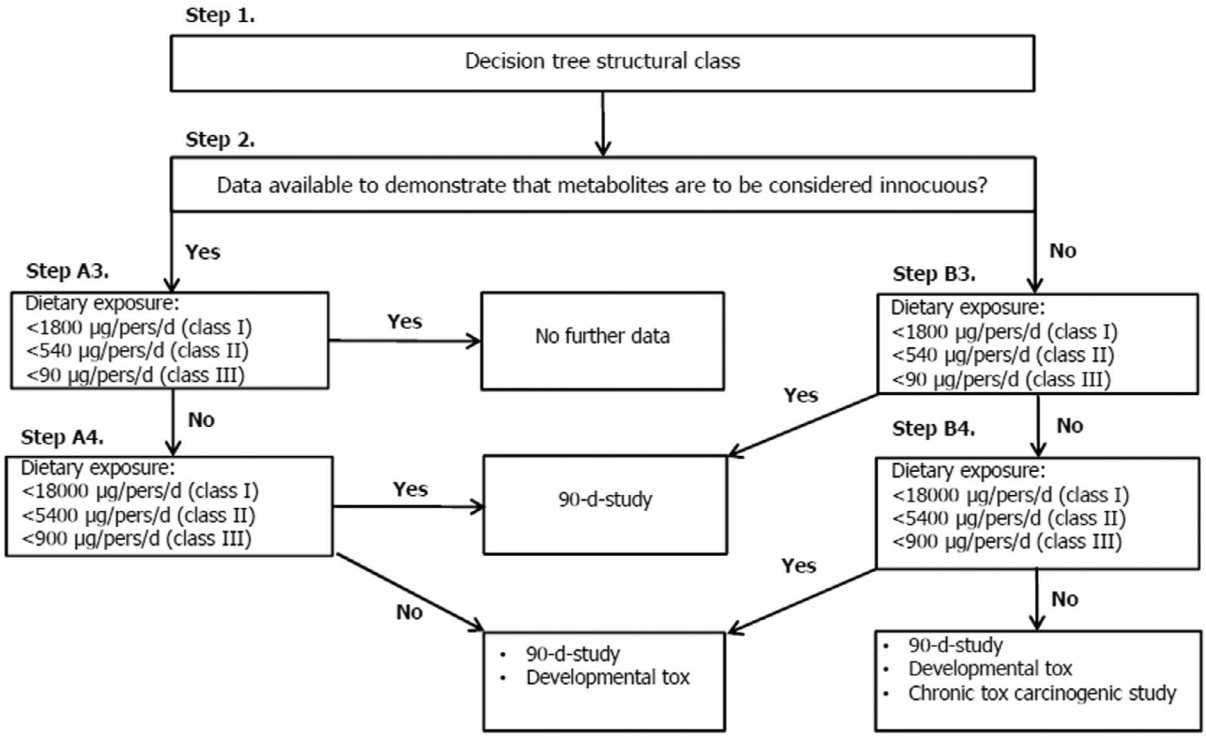


Figure A.1: Procedure applied for the safety evaluation of (*E*)-3-benzo[1,3]dioxol-5-yl-*N*,*N*-diphenyl-2-propenamide according to the data requirements for the risk assessment of flavourings for which no structurally related flavouring substances in existing FGEs can be identified (EFSA CEF Panel, 2010)

Appendix B – Food categories and use levels provided for (*E*)-3-benzo[1,3]dioxol-5-yl-*N*,*N*-diphenyl-2-propenamide

Table B.1: Food categories and use levels (only these food categories are included for which use levels were provided). Portion sizes are according to the EFSA Guidance on the data required for the risk assessment of flavourings to be used in or on foods (EFSA CEF Panel, 2010) and deviate occasionally from those specified by the applicant

CODEX code	Food categories[a]	Standard portions [b] (g)	Intended use level as flavouring substance (mg/kg)		Occurrence level from other sources (mg/kg)		Combined occurrence level from all sources (mg/kg)	
			Normal	Maximum	Normal	Maximum	Normal	Maximum
05.2	Confectionery, including hard and soft candy, nougats, etc., other than 05.1, 05.3 and 05.4	30	25	250	–	–	25	250
05.3	Chewing gum	3	150	500	–	–	150	500
05.4	Decorations (e.g. for fine bakery wares), toppings (non-fruit) and sweet sauces	35	10	150	–	–	10	150
14.1[c]	Non-alcoholic ('soft') beverages	300	–	–	–	–	–	–
14.2.1[c]	Beer and malt beverages	300	–	–	–	–	–	–

(a): Most of the categories reported are the subcategories of Codex GSFA (General Standard for Food Additives, available at https://www.codexalimentarius.net/gsfaonline/CXS_192e.pdf) used by the JECFA in the SPET technique (FAO/WHO, 2008).

(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): These food categories have been proposed by the applicant in the original dossier, but were withdrawn in the development of this opinion (Documentation provided to EFSA No. 2, see Section 3.1.6 on stability).

Appendix C – Non-food sources of exposure

Currently, (*E*)-3-benzo[1,3]dioxol-5-yl-*N*,*N*-diphenyl-2-propenamide is used in oral care formulations, i.e. toothpaste. No use in cosmetic formulations in skin is reported. However, the applicant provided an estimated exposure for three potential formulations (Table C.1).

Table C.1: Estimates of exposure to (*E*)-3-benzo[1,3]dioxol-5-yl-*N*,*N*-diphenyl-2-propenamide from potential cosmetic formulations on skin, as provided by the applicant

Application	Levels of (*E*)-3-benzo[1,3]dioxol-5-yl-*N*,*N*-diphenyl-2-propenamide in product	Exposure to product (g/day)	Exposure (µg/kg bw per day)
Bathing (e.g. Shower Gel)	2000–5,000 ppm	0.19[(a)]	6.3–16 (0.6–1.6)[(b)]
Skin Care (e.g. Body Lotion)	50–120 ppm	7.82[(a)]	6.5–16 (0.7–1.6)[(b)]
Deodorant	300–600 ppm	1.50[(a)]	7.5–15 (0.8–1.5)[(b)]

(a): The SCCS Notes of Guidance for the testing of cosmetic ingredients and their safety evaluation 10th revision Table 2A (Hall et al., 2007, 2011).
(b): Penetration through intact skin estimated to be 10%.

The penetration of (*E*)-3-benzo[1,3]dioxol-5-yl-*N*,*N*-diphenyl-2-propenamide through the skin barrier has been calculated by the applicant using a QSAR model according to Kroes et al. (2007) and Shen et al. (2014). This approach deviates from the SCCS Guidance (2021).

Appendix D – Genotoxicity studies

Table D.1: Summary of *in vitro* genotoxicity data for (*E*)-3-benzo[1,3]dioxol-5-yl-*N*,*N*-diphenyl-2-propenamide [FL-no: 16.135]

Test system *in vitro*	Test object	Concentrations of substance and test conditions	Result	Reference	Comments
Bacterial Reverse Mutation test	*S*. Typhimurium TA98, TA98, TA100, TA1535 and TA1537 *Escherichia coli* WP2 uvrA	22–5,500 µg/plate[(a),(b)]	Negative	BASF SE (2009)	Reliable without restrictions. Study performed in accordance with OECD TG 471 and in compliance with GLP
Micronucleus test	Human peripheral blood lymphocytes	10–60 µg/mL[(c)] 10–60 µg/mL[(d)] 8–20 µg/mL[(e)]	Negative	Covance (2014)	Reliable without restrictions. Study performed in accordance with OECD TG 487 and in compliance with GLP; the given concentrations are those for the cultures that were scored for micronuclei

(a): With and without metabolic activation.
(b): Two experiments, one performed using the plate incorporation method and one the preincubation method.
(c): 3 h incubation with 21-h recovery period, with metabolic activation.
(d): 3 h incubation with 21-h recovery period, without metabolic activation.
(e): 24 h incubation with no recovery period, without metabolic activation.

Appendix E – Toxicity studies

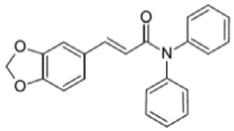
Table E.1: Summary of toxicity studies for (*E*)-3-benzo[1,3]dioxol-5-yl-*N*,*N*-diphenyl-2-propenamide [FL-no: 16.135]

Species; sex No./group	Route of administration	Dose levels (mg/kg bw per day)	Duration (days)	Results	Reference	Comments
Repeated dose toxicity studies						
Sprague–Dawley rats; M and F 5/group	Oral (feed)	0, 10, 250, 1,000	14	No toxicity observed	Product Safety Labs (2013a)	Dose range-finding study
Sprague–Dawley rats; M and F 10/group	Oral (feed)	0, 30, 100, 500	90	BMDL of 101 mg/kg bw per day based on decrease in WBC	Product Safety Labs (2013b)	Study performed in accordance with OECD TG 408 (1998) and in compliance with GLP. Endpoint specific BMR of −19%. The study authors proposed the highest dose (500 mg/kg bw per day) as NOAEL.
Prenatal developmental toxicity study						
Sprague–Dawley rats; F 20/group	Oral gavage	0, 125, 250, 500, 1,000	21	No treatment-related effects were observed	Product Safety Labs (2017)	Study performed in accordance with OECD TG 414 (2001) and in compliance with GLP. The study authors proposed the highest dose (1,000 mg/kg bw per day) as NOAEL for maternal and fetal developmental toxicity

BMDL: benchmark dose lower boundary of confidence interval (95% single sided); BMR: benchmark response; bw: body weight; FL-No: FLAVIS number; GLP: Good Laboratory Practice; NOAEL: no observed adverse effect level; OECD: Organization for Economic Cooperation and Development; TG: test guideline; WBC: white blood cells.

Appendix F – Summary of JECFA evaluation

Table F.1: Summary of JECFA evaluation (JECFA 2016a, 2017)

Flavouring agent	JECFA No.	CAS No. and structure	*Step B3*(a) Does estimated dietary exposure exceed the threshold of concern?	*Follow-on from step B3* (b) Are additional data available for the flavouring agent with an estimated dietary exposure exceeding the threshold of concern?	Comments on predicted metabolism	Related structure name (No.) and structure (if applicable)	Conclusion based on current estimated dietary exposure
(*E*)-3-Benzo [1,3]dioxol-5-yl-*N*, *N*-diphenyl-2-propenamide	2228	1309389–73-8 	Yes, SPET: 100	Yes. No. 2228 is non-genotoxic in bacteria, and the NOAEL of 490 mg/kg bw per day (the highest dose tested) in a 90-day study in rats is 245,000 times the estimated dietary exposure to No. 2228 when used as a flavouring agent.	Note 1	–	No safety concern

bw: body weight; CAS: Chemical Abstracts Service; No.: number; NOAEL: no-observed-adverse-effect level; SPET: single-portion exposure technique.
(a): The threshold for human dietary exposure for structural class III is 90 μg/day. All dietary exposure values are expressed in μg/day. The dietary exposure value listed represent the highest daily dietary exposure calculated by either the SPET or the MSDI method. The SPET gave the highest estimated dietary exposure in this case.
(b): The MOE was calculated based on the estimated dietary exposure calculated by the SPET. In cases where the resulting MOE was relatively low, a comparison with the MSDI was also made.
Note 1: Amides are expected to undergo limited hydrolysis and/or oxidation and enter into known pathways of metabolism and excretion.

Appendix G – Benchmark Dose response modelling on total WBC count

G.1. Data description

The endpoint to be analysed is the total white blood cell count. The analysis is based on summary data (group mean (mwbc) and standard deviation (sdwbc) on 10 animals per sex per group), rather than the individual data. Sex was used as a co-variate.

Data used for analysis:

Dose	mwbc	sdwbc	*N*	Sex
0.0	8.73	1.67	10	f
29.4	9.14	2.07	10	f
98.6	7.69	1.98	10	f
492.2	7.01	1.85	10	f
0.0	12.03	2.25	10	m
29.4	12.24	1.91	10	m
97.5	11.42	2.02	10	m
489.5	9.96	2.21	10	m

mwbc: mean of total white blood cells count per group; sdwbc: standard deviation of total white blood cells count per group; N: number of animals; m: males; f: females.

The dose response modelling makes use of the average actual dose levels per group.

G.2. Selection of the BMR

The BMR (benchmark response) used is a 19% change (i.e. a decrease) in mean response compared to the controls. The BMD (benchmark dose) is the dose corresponding with the BMR of interest. The BMR was set based on the Endpoint-specific BMR theory by Slob (2017) and estimated using the RIVM PROAST webtool (https://proastweb.rivm.nl/).

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

G.3. Software used

Results are obtained using the EFSA web tool for BMD analysis, which uses the R-package PROAST, version 70.0, for the underlying calculations.

G.4. Specification of deviations from default assumptions

General assumptions

No deviations from default assumptions

Dose–response models

No other than the default models were used

Default set of fitted models:

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

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

Procedure for selection of BMDL

Default procedure has been followed (Figure G.1).

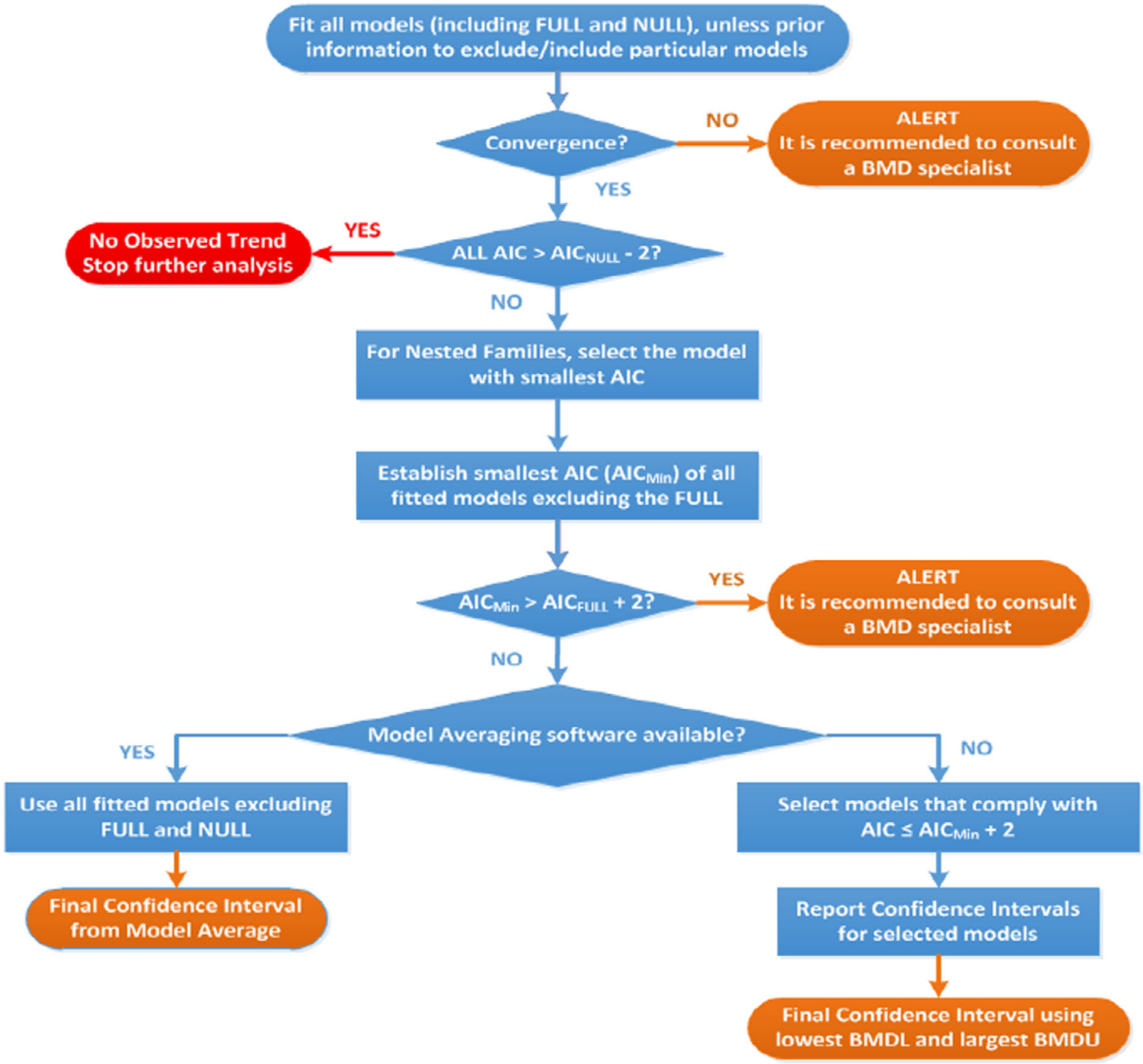


Figure G.1: Flowchart for selection of BMDL

G.5. Results

G.5.1. Response variable: mwbc

G.5.1.1. Fitted models

Model	Converged	loglik	npar	AIC
full model	Yes	15.33	9	−12.66
full-v	Yes	16.37	10	−12.74

Model	Converged	loglik	npar	AIC
null model-v	Yes	−10.98	3	27.96
null model-a-v	Yes	7.77	4	−7.54
Expon. m3-v	Yes	−6.16	5	22.32
Expon. m3-av	Yes	15.19	6	−18.38
Expon. m3-abv	Yes	15.25	7	−16.50
Expon. m5-av	Yes	15.64	7	−17.28
Expon. m5-abv	Yes	15.90	8	−15.80
Hill m3-av	Yes	15.19	6	−18.38
Hill m3-abv	Yes	15.25	7	−16.50
Hill m5-av	Yes	15.67	7	−17.34
Hill m5-abv	Yes	15.98	8	−15.96
Inv.Expon. m3-av	Yes	15.29	6	−18.58
Inv.Expon. m3-abv	Yes	15.37	7	−16.74
Inv.Expon. m5-av	Yes	15.56	7	−17.12
Inv.Expon. m5-abv	Yes	15.75	8	−15.50
LN m3-av	Yes	15.25	6	−18.50
LN m3-abv	Yes	15.32	7	−16.64
LN m5-av	Yes	15.67	7	−17.34
LN m5-abv	Yes	15.95	8	−15.90

G.5.1.2. Estimated model parameters

EXP
estimate for var-f: 0.05132
estimate for var-m: 0.03125
estimate for a-f: 8.56
estimate for a-m: 12.14
estimate for CED-: 434.8
estimate for d-: 0.7384
HILL
estimate for var-f: 0.05132
estimate for var-m: 0.03125
estimate for a-f: 8.56
estimate for a-m: 12.14
estimate for CED-: 434.7
estimate for d-: 0.7394
INVEXP
estimate for var-f: 0.05114
estimate for var-m: 0.03121
estimate for a-f: 8.557
estimate for a-m: 12.14
estimate for CED-: 427.4
estimate for d-: 0.1255
LOGN
estimate for var-f: 0.05122
estimate for var-m: 0.03123
estimate for a-f: 8.559
estimate for a-m: 12.14
estimate for CED-: 430.5
estimate for d-: 0.24

G.5.1.3. Weights for model averaging

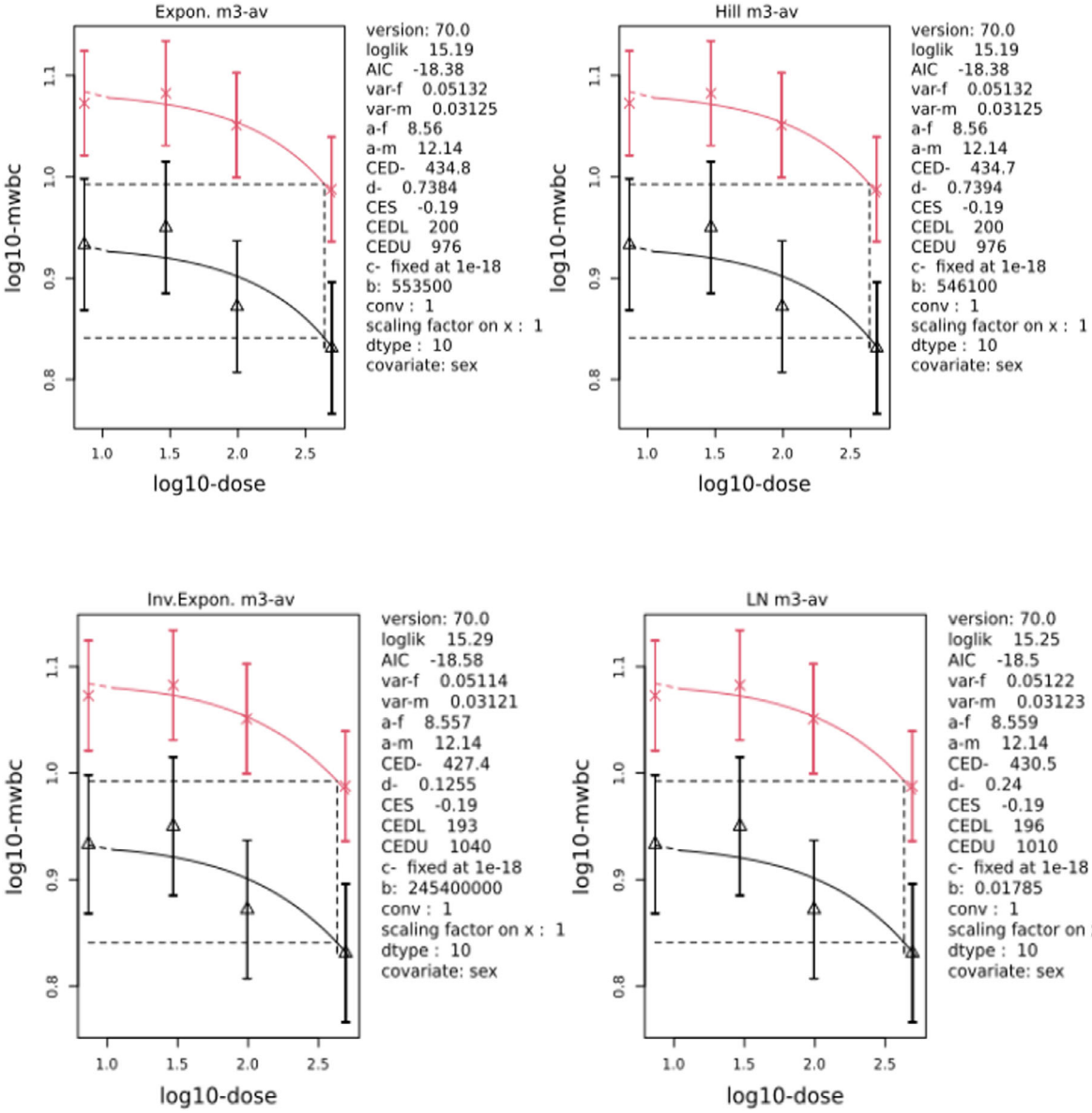
EXP	HILL	INVEXP	LOGN
0.24	0.24	0.27	0.25

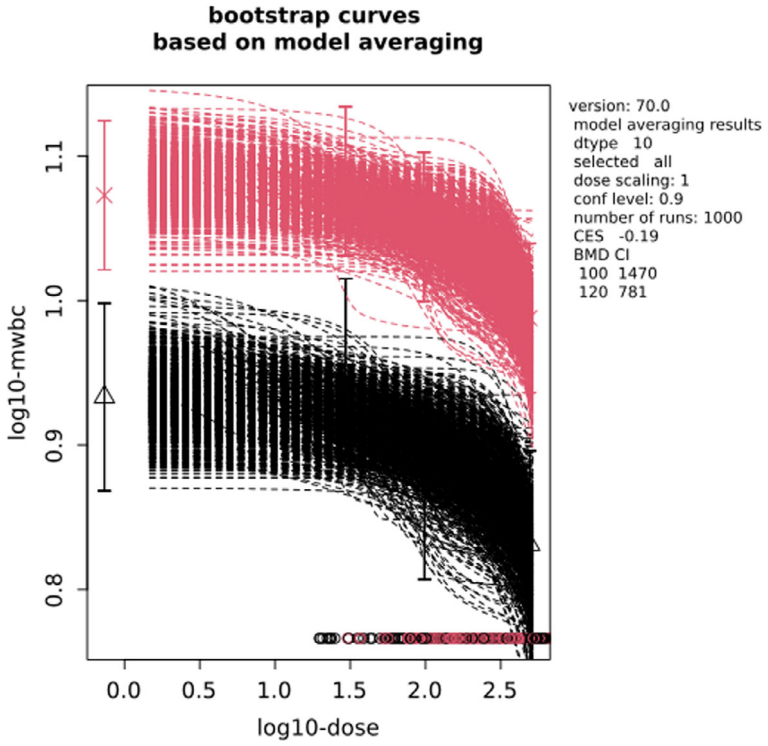
G.5.1.4. Final BMD values

Endpoint	Subgroup	BMDL	BMDU
mwbc	f	101	1,470
mwbc	m	124	781

Confidence intervals for the BMD are based on 1,000 bootstrap data sets.

G.5.1.5. Visualisation





G.6. Conclusions

Using an endpoint-specific BMR of 19% for decrease in total WBC count, the dose response analysis for total WBC count resulted in BMDL–BMDU 90% confidence intervals of 124–781 and 101–1,470 mg/kg bw per day for males and females, respectively.