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Opinion on the re-evaluation of mono- and diglycerides of fatty acids (E 471) as food additive in foods for infants below 16 weeks of age and follow-up of their re-evaluation as food additives for uses in foods for all population groups

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Abstract

Mono- and diglycerides of fatty acids (E 471) was re-evaluated in 2017 by the former EFSA Panel on Food Additives and Nutrient sources added to Food (ANS). As a follow-up to this assessment, the Panel on Food Additives and Flavouring was requested to assess mono- and diglycerides of fatty acids (E 471) for its use as food additive in food for infants below 16 weeks of age belonging to food categories 13.1.1 (Infant formulae) and 13.1.5.1 (Dietary foods for infants for special medical purposes and special formulae for infants). In addition, the FAF Panel was requested to address the issues already identified during the re-evaluation of the food additive in 2017 when used in food for the general population. The Panel considered that there is no indication of adverse effects from the available animal studies at the highest dose tested and from the post marketing data. A comparison was made between the daily exposure to the sum of mono- and di-acylglycerols from breast milk and that resulting from the use of E 471 in the infant formula. The Panel noted that the resulting exposures are in the same order of magnitude. Overall, the Panel concluded that there is no reason for a safety concern when E 471 used as food additive in FC 13.1.1 and 13.1.5.1 and according to the Annex III to Regulation (EC) No 1333/2008. The risk assessment for toxic elements and impurities clearly indicated the need to lower the current maximum limits for arsenic, lead, cadmium and mercury and to include limits for glycidyl esters, 3-monochloropropane diol and erucic acid in the EU specifications of E 471.

Keywords: mono- and diglycerides of fatty acids, E 471, food additive, infants

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Summary

In accordance with Regulation (EU) No 257/2010, the European Food Safety Authority (EFSA) is currently re-evaluating the safety of food additives already permitted in the Union before 20 January 2009 and issuing scientific opinions on their safety when used in food as per Annexes II and III to Regulation (EC) No 1333/2008. The risk assessment approach followed in the re-evaluation has not covered the use of food additives in food for infants below 12 weeks of age. Additionally, while re-evaluating the safety of food additives referred to above, EFSA identified some concerns, namely (1) Data gaps that have triggered recommendations in the published scientific opinions, and/or (2) data gaps that have increased uncertainties linked to the risk assessment and/or which prevented the Panel from concluding on some aspects of it.

On 31 May 2017, EFSA published a guidance on the risk assessment of substances present in food intended for infants below 16 weeks of age, thus enabling EFSA to assess the safety of food additive used in food for infants below this age. The age up to 16 weeks was selected in the guidance because infants are exposed to formula feeding until this age as the only source of food since complementary feeding is not supposed to be introduced before.

As follow-up of the above, this Opinion addresses the data gaps previously identified during the re-evaluation of mono- and diglycerides of fatty acids (E 471) as food additives and the safety in the special subpopulation of infants below 16 weeks of age.

The process followed involved the publication of a dedicated call for data allowing all interested parties to provide the requested information for completing the assessment and to confirm that the additive is present in food categories 13.1.1 (Infant formulae) and 13.1.5.1 (Dietary foods for infants for special medical purposes and special formulae for infants) and according to the Annex III to Regulation (EC) No 1333/2008. The data submitted in response to the call for data on mono- and diglycerides of fatty acids (E 471) comprised technical information, post marketing surveillance reports and literature data.

According to Commission Regulation (EU) No 231/2012, mono- and diglycerides of fatty acids (E 471) is defined as 'mono- and diglycerides of fatty acids consisting of mixtures of glycerol mono-, di- and tri-esters of fatty acids occurring in food oils and fats'. Specifications for mono- and diglycerides of fatty acids (E 471) have been defined in Commission Regulation (EU) No 231/2012.

In response to the call for data, analytical data on levels of toxic elements (namely arsenic, lead, cadmium, mercury) in commercial samples of mono- and diglycerides of fatty acids (E 471) were provided by one interested business operator. The Panel noted that the measured levels of these toxic elements are substantially lower than the lowest technologically achievable levels proposed by the interested business operator. The Panel performed a risk assessment considering the available data and health-based guidance values/reference points and concluded that the potential exposure to toxic elements resulting from the consumption of E 471 could be substantial. Therefore, the Panel considered feasible to amend the EU specifications based on the information submitted in response to the call for data. This refers to lowering existing limits for toxic elements (arsenic, lead, cadmium, mercury).

Regarding the other impurities, including carry-over and process impurities (acrolein, butanetriol, free and bound 3-monochloropropane diol (3-MCPD) and glycidyl esters (GEs)) fatty acids (*trans* fatty acid and erucic acid) and solvents, the Panel proposed to amend the EU specifications based on the information submitted in response to the call for data. This refers to the inclusion of limits for GEs, 3-MCPD and erucic acid. According to the interested business operator, acrolein and butanetriol were not detected in E 471; therefore, the Panel concluded that there would be no need for including limits in the EU specifications for these impurities. The manufacturing process of E 471 does not involve the use of any solvents, therefore, the Panel concluded that there is no need for including limits in the EU specifications for solvents.

Regarding *trans* fatty acids, the Panel noted that their levels cover a wide range depending on the degree of hydrogenation of the raw material (non, partially or fully hardened). Based on the information from suppliers, only fully hydrogenated oils/fats are used for the production of E 471 in infant formula (13.1.1) and infant FSMPs (13.1.5.1). The levels of *trans* fatty acids referred to as 'fully hardened' ranged between 0.01% and < 1%. In infant formula and follow-on formula, the *trans* fatty acid content is regulated and shall not exceed 3% of the total fat content (Regulation (EU) No 127/2016 supplementing Regulation (EU) No 609/2013). For the general population, E 471 can be manufactured also from 'non-hardened' or 'partially hardened' fats. The content of *trans* fatty acids in these cases ranged between 0.01 and 59.92%. The Panel noted that '*latest national recommendations in the EU, and*

most recent recommendations from medical professional associations in Europe and the US indicate that consumption of TFA should be as low as possible' (EFSA, 2018). On the basis of this advice, a maximum limit of 2 grams of *trans* fat per 100 g fat in food for the final consumer was set by Regulation (EU) No 2019/649 amending Regulation (EC) No 1925/2006).

Dietary exposure to mono- and diglycerides of fatty acids (E 471) for infants below 16 weeks of age from their uses as food additives was assessed based on (1) MPLs set out in the EU legislation (defined as the regulatory maximum level exposure assessment scenario) and (2) the reported use levels (defined as the refined exposure assessment scenario). Both scenarios are based on the recommended consumption levels from the available Scientific Committee Guidance which recommends values of 200 and 260 mL formula/kg bw per day as conservative mean and high-level consumption values for 14- to 27-day-old infants. For infants below 16 weeks of age consuming infant formulae (FC 13.1.1), exposure to mono- and diglycerides of fatty acids (E 471) in the regulatory maximum level exposure assessment scenario was estimated at 800 mg/kg bw per day for mean consumption, while at the high-level consumption was estimated at 1,040 mg/kg bw per day. Exposure estimates are the same in the refined scenario using the maximum use level reported by industry as this maximum equals the MPL. In the refined estimated exposure assessment scenario using the mean of the reported use levels from industry, exposure estimates for mono- and diglycerides of fatty acids (E 471) were of 418 mg/kg bw per day at the mean and 543 mg/kg bw per day at the high level of consumption. For infants below 16 weeks of age consuming special infant formulae (FC 13.1.5.1), exposure to mono- and diglycerides of fatty acids (E 471) in the regulatory maximum level exposure assessment scenario was estimated at 1,000 mg/kg bw per day for mean consumption while at the high-level consumption was estimated at 1,300 mg/kg bw per day. Exposure estimates are the same in the refined scenario using the maximum use level reported by industry as this maximum equals the MPL. In the refined estimated exposure assessment scenario using the mean of the reported use levels from industry, exposure estimates for mono- and diglycerides of fatty acids (E 471) were of 418 mg/kg bw per day at the mean and 543 mg/kg bw per day at the high level of consumption.

The Panel considered the results of the previously available data from the re-evaluation of E 471 and the newly submitted toxicity studies. No study in neonatal animal model has been provided, however, in the ANS Panel opinion a two-generation reproduction toxicity study has been described which provides information on this life period. In this study, no adverse effects have been observed at the highest dose tested. The Panel considered that the information available covers also the neonatal life period and, therefore, studies in neonatal animal model were not considered necessary. No clinical studies were available in which the safety of mono- and diglycerides of fatty acids was investigated. The available post-marketing data had no reports of signs and symptoms of concern. Overall, the Panel considered that there is no indication of adverse effects from the animal studies at the highest dose tested and from the post marketing data.

A comparison was made between the daily exposure to the sum of mono- and di-acylglycerols from breast milk and that resulting from the use of E 471 in the infant formula. The Panel noted that the resulting exposures are in the same order of magnitude.

Overall, the Panel concluded that there is no reason for a safety concern when E 471 used as food additive in FC 13.1.1 and 13.1.5.1 and according to the Annex III to Regulation (EC) No 1333/2008.

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1. Introduction

The present opinion deals with:

- the risk assessment of mono- and diglycerides of fatty acids (E 471) in food for infants below 16 weeks of age in the food categories (FC) 13.1.1 (Infant formulae as defined by Commission Delegated Regulation (EU) 2016/127/EC[1]), 13.1.5.1 (Dietary foods for infants for special medical purposes and special formulae for infants) and according to the Annex III to Regulation (EC) No 1333/2008.
- the follow-up on issues that have been expressed in the conclusions and recommendations of the Scientific Opinion on the re-evaluation of mono- and diglycerides of fatty acids (E 471) as a food additive (EFSA ANS Panel, 2017a).

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

1.1.1. Background

The composition of food intended for infants and young children, as defined by Regulation (EU) No 609/2013[2], is regulated at EU level and such rules include requirements concerning the use of substances as food additives.

The use of food additives is regulated by Regulation (EC) No 1333/2008 on food additives. Only food additives that are included in the Union list, in particular in Annex II and III to that Regulation, may be placed on the market and used in food under the conditions of use specified therein.

In accordance with Regulation (EU) No 257/2010[3], EFSA is currently re-evaluating the safety of food additives already permitted in the Union before 20 January 2009 and issuing scientific opinions on their safety when used in food as per Annexes II and III to Regulation (EC) No 1333/2008. However, the risk assessment approach followed until now has not covered the use of food additives in food for infants below 12 weeks of age. Consequently, EFSA published several scientific opinions on the re-evaluation of the safety of food additives permitted in food category 13.1 but not addressing their use in food for infants below 12 weeks of age.

In addition, in these opinions EFSA identified some concerns, namely (1) Data gaps that have triggered recommendations in the (to be) published scientific opinions, and/or; (2) Data gaps that have increased uncertainties linked to the risk assessment and/or which prevented the EFSA from concluding on some aspects of it.

On 31 May 2017, EFSA published a guidance document (EFSA Scientific Committee, 2017) on the risk assessment of substances present in food intended for infants below 16 weeks of age, thus enabling EFSA to assess the safety of food additives used in food for infants below 12 weeks of age.[4] Now EFSA is expected to launch dedicated calls for data to be able to perform such risk assessments.

The EC considers it is more effective that EFSA, in the context of these dedicated calls for data, also addresses all the issues and data gaps already identified in the relevant (to be) published scientific opinions on the re-evaluation of the safety of food additives permitted in food category 13.1.

In accordance with the current EC approach for the follow-up of EFSA's scientific opinions on the re-evaluation of the safety of permitted food additives for which some concerns have been identified, a specific call for data would be published by the EC on DG SANTE's website[5] on food additives and additional (missing) information would then be provided by interested business operators to the EC.

However, for those scientific opinions on the re-evaluation of the safety of permitted food additives in food category 13.1 for which the risk assessment does not address their uses in food for infants

[1] Commission Delegated Regulation (EU) 2016/127 of 25 September 2015 supplementing Regulation (EU) No 609/2013 of the European Parliament and of the Council as regards the specific compositional and information requirements for infant formula and follow-on formula and as regards requirements on information relating to infant and young child feeding (Text with EEA relevance). OJ L 25, 2.2.2016, p. 1–29.

[2] Regulation (EU) No 609/2013 of the European Parliament and of the Council of 12 June 2013 on food intended for infants and young children, food for special medical purposes, and total diet replacement for weight control and repealing Council Directive 92/52/EEC, Commission Directives 96/8/EC, 1999/21/EC, 2006/125/EC and 2006/141/EC, Directive 2009/39/EC of the European Parliament and of the Council and Commission Regulations (EC) No 41/2009 and (EC) No 953/2009. OJ L 181, 29.6.2013, p. 35–56.

[3] Commission Regulation (EU) No 257/2010 of 25 March 2010 setting up a program for the re-evaluation of approved food additives in accordance with Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives. OJ L 80, 26.3.2010, p. 19–27.

[4] See Section 1.1.3.

[5] https://ec.europa.eu/food/safety/food_improvement_agents/additives/re-evaluation_en

below 12 weeks of age and for which some concerns have been identified by EFSA, the EC considers that for the sake of efficiency it would be appropriate to streamline the approach as described above.

Therefore, the EC requests EFSA to address all the issues and data gaps already identified in the relevant published scientific opinions of those food additives (or groups of additives that can be addressed simultaneously) as part of the upcoming work on the safety assessment of food additives uses in food for infants below 12 weeks of age.

This follow-up aims at completing the re-evaluation of the food additives in question for all food categories, and includes calls for data covering the actual use and usage levels of food additives in food for both infants below 12 or 16 weeks of age as well as for older infants, young children and other groups of the population for which EFSA has already finalised its assessment.

The future evaluations of EFSA should systematically address the safety of use of food additives for all age groups, including the infants below 12 or 16 weeks of age.

1.1.2. Terms of Reference

In accordance with Article 29(1)(a) of Regulation (EC) No 178/2002[6], and as part of EFSA 's work in completing its risk assessments concerning the use of food additives in food for infants below 12 weeks of age[5], covered by the re-evaluation programme and its terms of reference, the European Commission requests the European Food Safety Authority to address all the data gaps specified in the recommendations made in this scientific opinion on the re-evaluation of the safety of food additives permitted in food category 13.1 (food for infants and young children) of annex II to Regulation (EC) No 1333/2008.

1.1.3. Interpretation of Terms of reference

Before the publication of the EFSA Scientific Committee Guidance on the risk assessment of substances present in food intended for infants below 16 weeks of age (EFSA Scientific Committee, 2017), EFSA has taken 12 weeks as a cut off age for the applicability of the safety assessment. However, according to EFSA Scientific Committee (2017), the assessment will include infants up to 16 weeks of age because they are exposed to formula feeding until this age as the only source of food since complementary feeding is not supposed to be introduced before this age (see EFSA Scientific Committee, 2017).

1.2. Previous evaluations of mono- and diglycerides of fatty acids (E 471) for use in foods for infants

Mono- and diglycerides of fatty acids (E 471) as food additive was evaluated by the Scientific Committee on Food (SCF) for its use in infant formulae, follow-on formulae, weaning food and food for special medical purposes (FSMPs) for infants and young children. The use of mono- and diglycerides of fatty acids in nutrient preparations for use in infant formulae and follow-on formulae was considered acceptable within the direct additive limit of 4 g/L and within the direct additive limit of 5 g/kg for use in weaning foods (SCF, 1997). The use in FSMPs for infants and young children was considered acceptable at levels up to 5 g/L (SCF, 1999).

Mono- and diglycerides of fatty acids (E 471) was evaluated by JECFA in 1973 (JECFA, 1974) and an ADI 'not limited' was established.

1.3. Summary of the previous EFSA re-evaluation of mono- and diglycerides of fatty acids (E 471) for uses in food for all population groups except for infants below 12 weeks of age[7]

Under the framework of Regulation (EC) No 257/2010, the EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) has re-evaluated the safety of mono- and diglycerides of fatty acids (E 471) when used as a food additive (EFSA ANS Panel, 2017a).

[6] Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, p. 1–24.

[7] According to the EFSA Scientific Committee Guidance (EFSA Scientific Committee, 2017) this opinion will include infants up to 16 weeks of age.

In its scientific opinion, the ANS Panel considered that it is very likely that hydrolysis of mono- and diglycerides of fatty acids in the gastrointestinal tract would occur resulting in the release of glycerol and fatty acids. Glycerol (E 422) and fatty acids (E 570) have been re-evaluated and the Panel concluded that there was no safety concern regarding their use as food additives (EFSA ANS Panel, 2017b,c). In addition, in the available toxicological studies with mono- and diglycerides rich in unsaturated fatty acids, no evidence for adverse effects was reported in short-term, subchronic studies, chronic, reproductive and developmental toxicity studies. The available studies did not raise any concern with regard to genotoxicity and carcinogenicity. The Panel concluded that there was no need for a numerical acceptable daily intake (ADI) and that the food additive mono- and diglycerides of fatty acids (E 471) was of no safety concern at the reported uses and use levels.

The following recommendations relevant for this evaluation were issued by the ANS Panel:

- the European Commission considers lowering the current limits for toxic elements (arsenic, lead, mercury and cadmium) in the EU specifications for mono- and diglycerides of fatty acids (E 471) in order to ensure that the food additive will not be a significant source of exposure to these toxic elements in food
- the European Commission considers revising the EU specifications for mono- and diglycerides of fatty acids (E 471) including maximum limits for impurities currently included in the EU specifications for glycerol (E 422) or recommended by the Panel in the re-evaluation of glycerol (E 422) (EFSA ANS Panel, 2017b,c)
- the European Commission considers revising the EU specifications for mono- and diglycerides of fatty acids (E 471) including maximum limits for residual solvents which can be used when manufacturing mono- and diglycerides of fatty acids (E 471), i.e. *tert*-butanol or *tert*-pentanol
- the European Commission considers revising the EU specifications for mono- and diglycerides of fatty acids (E 471) including maximum limits for *trans* fatty acids because mono- and diglycerides of fatty acids (E 471) can be manufactured by glycerolysis of hydrogenated fats and/or oils, which contain significant amounts of *trans* fatty acids
- the European Commission considers revising the EU specifications for mono- and diglycerides of fatty acids (E 471) including maximum limits for glycidyl esters (GEs) because refined vegetable oil, which can be used for manufacturing of mono- and diglycerides of fatty acids (E 471) is the only identified source of GEs of fatty acids, which are formed during deodorisation
- the European Commission considers revising the EU specifications for mono- and diglycerides of fatty acids (E 471) including maximum limits for erucic acid because erucic acid can be present among the fatty acids in edible oils, which can be used for manufacturing of mono-and diglycerides of fatty acids (E 471)
- more data should be generated to decrease uncertainty arising from the occurrence of compounds of toxicological concern (e.g. 3-monochloropropane diol (3-MCPD) or GEs), which can be produced under certain processing conditions from the food additive mono- and diglycerides of fatty acids (E 471)

2. Data and methodologies

2.1. Data

EFSA launched a public call for data[8] to collect relevant information from interested parties.

The Panel based its assessment on the information submitted to EFSA following the public call for data, the conclusions and recommendations from previous evaluations and the additional available literature up to 8 September 2021.

The Mintel's GNPD is an online database which monitors new introductions of packaged goods in the market worldwide. It contains information of over 3.4 million food and beverage products of which more than 1,300,000 are or have been available on the European food market. Mintel started covering EU's food markets in 1996, currently having 24 out of its 27 member countries, Norway presented in the Mintel GNPD.

[8] Call for technical and toxicological data on mono- and diglycerides of fatty acids (E 471) as a food additive for uses in foods for all population groups including infants below 16 weeks of age. Published: 29 November 2018. Available online: https://www.efsa.europa.eu/sites/default/files/consultation/callsfordata/2018-00953_Call-for-data_mono-and-diglycerides-of-fatty-acids-E471.pdf

2.2. Methodologies

This opinion was formulated following 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 and in particular the EFSA Guidance of the Scientific Committee on the risk assessment of substances present in food intended for infants below 16 weeks of age (EFSA Scientific Committee, 2017).

In order to conclude on the safety of mono- and diglycerides of fatty acids (E 471) for all population groups and to address the data gaps identified during the re-evaluation, the FAF Panel assessed the information provided:

- for the follow-up on issues that have been raised in the conclusions and recommendations of the Scientific Opinion on the re-evaluation of mono- and diglycerides of fatty acids (E 471) as a food additive (EFSA ANS Panel, 2017a); and
- for the risk assessment of mono- and diglycerides of fatty acids (E 471) in food for infants below 16 weeks of age in food categories (FC) 13.1.1 (Infant formulae as defined by Commission Delegated Regulation (EU) 2016/127/EC), 13.1.5.1 (Dietary foods for infants for special medical purposes and special formulae for infants) and according to the Annex III to Regulation (EC) No 1333/2008.

When in animal studies, the test substance was administered in the feed or in drinking water, but doses were not explicitly reported by the authors as mg/kg bw per day based on actual feed or water consumption, the daily intake is calculated by the Panel using the relevant default values. In case of rodents, the values as indicated in the EFSA Scientific Committee Guidance document (EFSA Scientific Committee, 2012a) are applied. In the case of other animal species, the default values by JECFA (2000) are used. In these cases, the dose was expressed as 'equivalent to mg/kg bw per day'. If a concentration in feed or drinking water was reported and the dose in mg/kg bw per day was calculated (by the authors of the study report or the Panel) based on these reported concentrations and on reported consumption data for feed or drinking water, the dose was expressed as 'equal to mg/kg bw per day'. When in human studies in adults (aged above 18 years), the dose of the test substance administered was reported in mg/person per day, the dose in mg/kg bw per day was calculated by the Panel using a body weight of 70 kg as default for the adult population as described in the EFSA Scientific Committee Guidance document (EFSA Scientific Committee, 2012a).

Dietary exposure to mono- and diglycerides of fatty acids (E 471) from its use as a food additive in foods for infants below 16 weeks of age was estimated combining the mean and high level consumption values for infant formulae reported for the period of 14–27 days of life, which corresponds to 200 and 260 mL/kg bw per day (EFSA Scientific Committee, 2017), respectively, with the maximum levels according to Annex II and Annex III, Part 5 Section B to Regulation (EC) No 1333/2008 and reported use levels submitted to EFSA following a call for data. Different scenarios were used to calculate exposure (see Section 3.3). Uncertainties on the exposure assessment were identified and discussed.

3. Assessment

3.1. Technical data

3.1.1. Identity of the substance

According to Commission Regulation (EU) No 231/2012[9], the food additive E 471 is named as mono- and diglycerides of fatty acids (E 471), and defined as '*mono- and diglycerides of fatty acids consisting of mixtures of glycerol mono-, di- and tri-esters of fatty acids occurring in food oils and fats. It may contain small amounts of free fatty acids and glycerol*'. Based on this definition, E 471 is not a discrete chemical substance but a mixture. According to Commission Regulation (EU) No 231/2012, synonyms are: glyceryl monostearate; glyceryl monopalmitate; glyceryl monooleate, etc.; monostearin; monopalmitin; monoolein, etc.; GMS (for glyceryl monostearate). In its re-evaluation opinion, the ANS Panel had already noted that these are not actual synonyms of the food additive E 471 (EFSA ANS Panel, 2017a).

[9] Commission Regulation (EU) No 231/2012 of 9 March 2012 laying down specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council Text with EEA relevance. OJ L 83, 22.3.2012, p. 1–295.

According to one interested business operator (Documentation provided to EFSA n. 1), E 471 is added to the various food categories with different levels of saturation (not saturated, partially saturated, fully saturated) of fatty acids, and with various fractions of monoglycerides of fatty acids (typically between approx. 40 and 90%). These data are in accordance with the data provided by the same interested business operator at the time of the re-evaluation (EFSA ANS Panel, 2017a), see also Table 1.

Table 1: Composition (% by weight) of different commercial grades of mono- and diglycerides of fatty acids (E 471) as provided by the interested business operator (see EFSA ANS Panel, 2017a)

Grade[(a)]	Glycerol[(b)]	Others	Monoglycerides	Diglycerides	Triglycerides
40%	4	2	42	44	8
60%	1	2	60	32	5
90%	1	2	93	4	0

(a): Refers to the monoglyceride content of the E 471.
(b): Free glycerol (i.e. not esterified).

Upon request for additional information, one IBO declared that there is no hardening/ hydrogenation process involved in the production of E 471 (Documentation provided to EFSA n. 2). However, the oils, fats and fatty acids used in the production of E 471 may be non-hardened, partially hardened or fully hardened. Furthermore, mono- and diglycerides of fatty acids E 471, as specified by Regulation (EU) No. 231/2012, cover a wide range of products which all consist of mixtures of glycerol, mono-, di- and tri-esters of fatty acids occurring in food oils and fats.

The food additive E 471 is produced by either interesterification of triacylglycerols (fats and oils) with glycerol or by direct esterification of fatty acids (found in fats and oils) and glycerol. The resulting mixture may be further distilled to obtain high-purity monoglycerides. The 'degree of hardening' of E 471 merely reflects the type of oil used in the production of E 471.

Following a request for clarification from EFSA, another IBO stated that based on the available information from suppliers, only fully hydrogenated oils/fats are used for the production of E 471 intended for use in infant formula (13.1.1) and infant FSMPs (13.1.5.1) (Documentation provided to EFSA n. 4, 5).

For more information on the physical properties and the chemical composition and structure of mono- and diglycerides of fatty acids (E 471), the reader is referred to the ANS Panel opinion (EFSA ANS Panel, 2017a).

3.1.2. Specifications

The specifications for mono- and diglycerides of fatty acids (E 471), as defined in the Commission Regulation (EU) No 231/2012 and as proposed by JECFA (2006), are listed in Table 2.

Table 2: Specifications for mono- and diglycerides of fatty acids (E 471) according to Commission Regulation (EU) No 231/2012 and proposed by JECFA (2006)

	Commission Regulation (EU) No 231/2012[(a)]	JECFA (2006)
Definition	Mono- and diglycerides of fatty acids consist of mixtures of glycerol mono-, di- and tri-esters of fatty acids occurring in food oils and fats. They may contain small amounts of free fatty acids and glycerol Mono- and diglycerides of fatty acids consist of mixtures of glycerol mono-, di-and tri-esters of fatty acids occurring in food oils and fats. They may contain small amounts of free fatty acids and glycerol	A mixture of mono- and diglyceryl esters of long chain, saturated and unsaturated fatty acids that occur in food fats; contain not less than 30% of alpha-monoglycerides and may also contain other isomeric monoglycerides, as well as di- and triglycerides, free glycerol, free fatty acids, soap and moisture; usually manufactured by the glycerolysis of edible fats and oils, but may also be prepared by esterification of fatty acids with glycerol, with or without molecular distillation of the product
Assay	Content of mono- and di-esters: not less than 70%	–

	Commission Regulation (EU) No 231/2012[a]	JECFA (2006)
	Content of mono- and di-esters: not less than 70%	
Description	The product varies from a pale yellow to pale brown oily liquid to a white or slightly off-white hard waxy solid. The solids may be in the form of flakes, powders or small beads The product varies from a pale yellow to pale brown oily liquid to a white or slightly off-white hard waxy solid. The solids may be in the form of flakes, powders or small beads	White or cream-coloured hard fats of waxy appearance, plastic products or viscous liquids
Identification		
Infrared absorption spectrum Infrared absorption spectrum	Characteristic of a partial fatty acid ester of a polyol Characteristic of a partial fatty acid ester of a polyol	The infrared spectrum of the sample is characteristic of a partial fatty acid ester of a polyol The infrared spectrum of the sample is characteristic of a partial fatty acid ester of a polyol
Test for glycerol	Passes test	Passes test
Test for fatty acids	Passes test	Passes test
Solubility	Insoluble in water, soluble in ethanol and toluene at 50°C Insoluble in water, soluble in ethanol and toluene at 50°C	Insoluble in water; soluble in ethanol, chloroform and benzene
Purity		
Water content	Not more than 2% (Karl Fischer method)	Not more than 2% (Karl Fischer method)
Acid value	Not more than 6	Not more than 6
Free glycerol	Not more than 7%	Not more than 7%
Polyglycerols	Not more than 4% diglycerol and not more than 1% higher polyglycerols both based on total glycerol content Not more than 4% diglycerol and not more than 1% higher polyglycerols both based on total glycerol content	–
Arsenic	Not more than 3 mg/kg	–
Lead	Not more than 2 mg/kg	Not more than 2 mg/kg
Mercury	Not more than 1 mg/kg	–
Cadmium	Not more than 1 mg/kg	–
Total glycerol	Not less than 16% and not more than 33% Not less than 16% and not more than 33%	–
Sulfated ash	Not more than 0.5% determined at 800 ± 25°C	–
Soap	–	Not more than 6%, calculated as a sodium oleate Not more than 6%, calculated as a sodium oleate

(a): According to Commission Regulation (EU) No 231/2012, purity criteria apply to the additive free of sodium, potassium and calcium salts of fatty acids; however, these substances may be present up to a maximum level of 6% (expressed as sodium oleate).

According to the available information from EFSA ANS Panel (2017a), mono- and diglycerides of fatty acids (E 471) can be manufactured by direct esterification of glycerol with fatty acids. Additionally, E 471 could also be produced by a transesterification process where natural or hydrogenated fats/oils react with glycerol. The fats/oils can be derived from one single source or may consist of a blend of fats and oils from different sources. Glycerol can be produced by a variety of methods, many of them leading to the possible presence of impurities, some of which are of toxicological concern. For this reason, the ANS Panel had recommended an update of the EU specifications for E 471 to include limit values for free and bound 3-MCPD and other impurities of toxicological concern (e.g. butanetriols, acrolein) which are listed in the EU specifications of the food additive glycerol (E 422) which can be used in the manufacturing process of E 471.

In order to support the revision of the existing specifications, the Panel has assessed the data provided by the IBOs in response to the follow-up re-evaluation opinion.

3.1.3. Analytical data from commercial samples of the food additive

3.1.3.1. Toxic elements

The call for data requested:

- analytical data on current levels of lead, mercury, cadmium and arsenic in commercial samples of the food additive,
- the lowest technologically achievable level for lead, mercury, cadmium and arsenic in order to adequately define their maximum limits in the specifications

Analytical levels for lead (Pb), mercury (Hg), cadmium (Cd) and arsenic (As) in 28 batches of commercial samples of E 471, manufactured between 11/2015 and 11/2019, and analysed between 02/2016 and 01/2020 were submitted by one interested business operator (IBO) (Documentation provided to EFSA n. 1, 2). According to the IBO, the respective data were provided by six out of their seven members. For lead, five results were reported as 0.1 mg/kg (LOQ: 0.05 mg/kg), the remaining batches were all reported as below LOQ, which ranged between 0.01 and 0.1 mg/kg. For mercury, all samples were below LOQs which ranged from 0.005 to 0.05 mg/kg. For cadmium, all results were below LOQs ranging between 0.005 and 0.02 mg/kg. For arsenic, one result was reported as 0.037 mg/kg and the remaining batches were all reported as below LOQs between 0.01 and 0.1 mg/kg. The analyses were performed with methods such as ICP-MS, ICP-OES and cold-vapour AAS.

According to the IBO providing the analytical data, the levels of toxic elements in the food additive E 471 are mainly dependent on their concentrations in the raw materials used in the manufacturing process. The lowest technologically achievable levels for toxic elements in E 471 for all food uses proposed by the IBO are reported in Table 3.

Table 3: Lowest technologically achievable levels for the toxic elements lead, mercury, cadmium and arsenic in commercial E 471 for all food uses, as proposed by one interested business operator (documentation provided to EFSA n. 1, 2)

Lead	Mercury	Cadmium	Arsenic
1 mg/kg	0.5 mg/kg	0.2 mg/kg	1 mg/kg

The levels proposed by the IBO are based on the highest measured value or LOQ, whichever is highest, applying a margin of 10 times this value. Furthermore, in order to accommodate for international trade with countries that do not necessarily have access to ICP-MS, the IBO proposed that the maximum limits for toxic elements in E 471 should not be lower than 0.5 mg/kg.

3.1.3.2. Other impurities

3.1.3.2.1. Carry over and process impurities

The call for data requested:

- analytical data on current levels of impurities of toxicological concern (e.g. butanetriols, acrolein, chlorinated compounds and 3-MCPD) as identified in the EU specifications of the food additive glycerol (E 422) – which can be used in the manufacturing process of E 471 – in commercial samples of the food additive E 471.

- the lowest technologically achievable level for impurities of toxicological concern (e.g. butanetriols, acrolein, chlorinated compounds and 3-MCPD) in order to adequately define their maximum limits in the specifications of E 471.
- analytical data on current levels of any impurity present in glycerol as mentioned in the call for data on glycerol (E 422)[10] – which can be used in the manufacturing process of E 471 – in commercial samples of the food additive E 471.
- the lowest technologically achievable level for any impurity which could be formed during the manufacturing processes of glycerol and be present in E 471, in order to adequately define their maximum limits in the specifications of E 471
- the lowest technologically achievable level of any compound of toxicological concern (e.g. 3-MCPD or GEs), which can be produced under certain processing conditions from the food additive mono- and diglycerides of fatty acids (E 471).

One IBO stated that '*the formation of butanetriols is not expected during the manufacturing process of food emulsifiers. Butanetriols are molecules with four C atoms, while emulsifiers are based on glycerol which is a three C atom* (sic)*. Formation of the C-C bond involves several chemical steps which is unlikely to take place in the esterification reaction that forms emulsifiers*' (Documentation provided to EFSA n.1).

The same IBO further stated that one of its members '*has completed an extensive literature and patent search (…) and concluded that a viable synthetic pathway to produce 1,2,4-butanetriol or 1,2,3-butanetriol from glycerol or glycerol derivatives was not identified. In addition, another EFEMA member has developed a method to quantify butanetriols in E 471 (…) and confirmed that butanetriols were not detected in any samples of mono and diglycerides(…)*' (Documentation provided to EFSA n.1). The Panel noted that a description of the method for quantification has been provided by one IBO claiming that butanetriol was not detected in four samples of E 471.

Additionally, this IBO reported that five commercial samples of E 471 were analysed for acrolein by Static Headspace-GC/MS and reported that acrolein was not detected in the analysed samples. An LOD of 0.4 mg/kg and an LOQ of 1.3 mg/kg were reported.

The IBO also considered that the request for a lowest technologically achievable level for **butanetriol** and **acrolein** is not applicable since these compounds are not found in E 471.

One IBO has submitted data on free and bound **3-MCPD and glycidyl esters** (GEs; expressed as glycidol) in around 80 batches of the additive grouped according to the raw material used in the manufacturing process, level of saturation of the fatty acids and content of monoglycerides in the product (documentation provided to EFSA n. 1, 3).

The concentrations of free and bound 3-MCPD (measured as free 3-MCPD) ranged from < 0.1 to 4.45 mg/kg. All analyses were performed with the modified AOAC Official Method Cd 29b-13 based on GC/MS. According to the IBO, '*a limit for 3-MCPD would not be applicable for E 471, since it is regulated in relevant raw materials used in the production of E 471 and chlorinated compounds are unlikely to be formed in the production process. However, if a limit for 3-MCPD is deemed necessary, EFEMA proposes a maximum limit of 2.5 mg/kg, in order to facilitate international trade of E471 that does have not the same regulatory requirements on the raw materials used*' (Documentation provided to EFSA n. 1). The proposed limit of 2.5 mg/kg for 3-MCPD was based on regulatory specifications for raw materials (edible, vegetable oils) as laid down by Regulation (EC) No 1881/2006.

In contrast to 3-MCPD, GEs (expressed as glycidol) were determined in the same samples at a substantially broader concentration range between < 0.1 and 111.25 mg/kg. The highest GE levels were determined in commercial samples of E 471 sourced from unsaturated rapeseed oil with a monoglyceride content of approximately 40%. The typical use levels of these products would be 2,000–10,000 mg/kg into FC 02.1.3 (Fats and oils essentially free from water (excluding anhydrous milkfat) except virgin oils and olive oils) and 12.6 (Sauces) (Documentation provided to EFSA n. 3).

The same IBO is aware of the considerable variations in the GEs levels and indicates that efficient mitigation measures have been developed and are being implemented across all relevant processing lines. They also propose a lowest technologically achievable level for GEs of 10 mg/kg and commit that they will reach this level for all E 471 products by the end of 2023 (Documentation provided to EFSA n. 1).

The Panel noted the levels of GE up to 111 mg/kg are about 10 times above the proposed lowest technologically achievable level of 10 mg/kg. Referring to an EFSA request for additional information on the mitigation process, the IBO clarified that '*the mitigation method developed by EFEMA members*

[10] https://ec.europa.eu/food/system/files/2019-01/fs_food-improvement agents_reeval_call_20181123_e422_data.pdf

and which is now being implemented across all relevant production lines is based on controlled heat treatment of the reaction mixture after formation of the mono-glyceride product and the associated, un-wanted glycidyl ester by-product. Specifically, the mitigation method consists of applying a holding time of up to 4 h at 150–200°C, which is below the initial reaction temperature (above 200°C) (…). This prolonged time at controlled high temperature allows the reaction equilibrium between formation and transformation of glycidyl ester to reach a level of less than 10 mg/kg for all relevant compositions of the product. (…) As the described holding step at controlled temperature is the only change applied to the production process for mono- and di-glycerides, no further change in composition, contaminants and impurities besides the reduction of glycidyl ester is envisioned or has been documented' (documentation provided to EFSA n. 2).

Regarding the EFSA request to provide analytical data on current levels of any impurity present in glycerol as mentioned in the call for data on glycerol (E 422), see above and Section 3.1.2, one IBO stated that they do not possess any information on any impurities in glycerol being only users of glycerol (documentation provided to EFSA n. 1,2). However, the Panel considered that these carry-over impurities are covered by the analytical data provided by the IBO (e.g. acrolein and 3-MCPD).

3.1.3.2.2. Fatty acids

The call for data requested:

- the lowest technologically achievable level for *trans* fatty acids because mono- and diglycerides of fatty acids (E 471) can be manufactured by glycerolysis of hydrogenated fats and/or oils, which contain significant amounts of *trans* fatty acids.
- the lowest technologically achievable level for erucic acid since erucic acid can be present among the fatty acids in edible oils, which can be used for manufacturing of mono- and diglycerides of fatty acids (E 471).

Analytical data on current levels of ***trans* fatty acids** tested in commercial samples of E 471 were provided by one IBO (documentation provided to EFSA n. 1). The levels of *trans* fatty acids in 15 samples referred to as 'fully hardened' ranged between 0.01% and < 1%. In 12 samples of E 471 noted as 'non-hardened', the content of *trans* fatty acids ranged between 0.34% and 1.59%. Substantially higher concentrations for *trans* fatty acids were reported for seven commercial samples of E 471 noted as 'partially hardened' ranging from 15.88% to 59.92%. All analyses were performed with gas chromatography and flame ionisation detector (GC-FID). The IBO stated that the amount of *trans* fatty acids in E 471 is almost entirely dependent on the *trans* fatty acid content of the fats, oils or fatty acids used in the production of this emulsifier. However, small amounts of *trans* fatty acids may be formed during the production process and production control is used to keep this as low as possible. Since *trans* fat is regulated in food for the final consumer, the IBO was of the opinion that a legal limit for emulsifiers was not applicable, as the consumer safety is already ensured by the existing legal limit. A proposal for the lowest technologically achievable level for *trans* fatty acids was not provided by the IBO.

One IBO provided data on **erucic acid** in 16 samples of E 471 batches derived from fat and oils from the Brassicaceae family as these products are considered the highest risk when it comes to the presence of erucic acid (Documentation provided to EFSA n. 1,2). Four samples had concentrations of erucic acid ranging from 0.03 to 0.29% (w/w). One sample was reported as < 2% (w/w), and the remaining samples as 'nd', 'not quantified', 0.00, < 0.05% and < 0.1% (w/w). The samples were analysed by two methods based on GC-FID after derivatisation of the fatty acids and the reported LOQs were 0.01% and 0.05% (w/w). Since erucic acid is not generated in the manufacturing process of E 471 and is already regulated in its raw materials, the same IBO considered that the request for the lowest achievable level is not applicable. However, in order to facilitate international trade of E 471 that does have not the same regulatory requirements on the raw materials used, the IBO proposed a maximum limit of erucic acid of 20 g/kg.

3.1.3.2.3. Solvents

The call for data requested:

- the lowest technologically achievable level for residual solvents which can be used in the manufacturing process of mono- and diglycerides of fatty acids (E 471), i.e. *tert*-butanol or *tert*-pentanol.

One IBO stated that the manufacturing process of E 471 does not involve the use of any solvents, and therefore neither *tert*-butanol nor *tert*-pentanol are used. Moreover, the same IBO declared that

'although its association mentioned the possible production of certain mono and diglycerides of fatty acids with the use of solvents in the document entitled '*Mono and Diglycerides of Fatty acids E 471 - Specification, Manufacturing methods and Chemistry (EFEMA internal document)' which was sent to EFSA in 2011 in response to the initial call for data on emulsifiers, this manufacturing method was experimental and is no longer implemented by its members*'. Consequently, this IBO was of the opinion that the request for a lowest technologically achievable level for *tert*-butanol and *tert*-pentanol would not be justified (Documentation provided to EFSA n. 1).

3.1.3.3. Information on particular specification requirements in the additive for use in infant formulae

The call for data requested:

- information on particular specification requirements for identity and purity of mono- and diglycerides of fatty acids (E 471) as described in Section A.1.

According to the information provided by the IBOs, fully hydrogenated rapeseed oil and palm oil are the current sources of mono- and diglycerides of fatty acids (E 471) used in infant formulae (13.1.1) and FSMP for infants (13.1.5.1) below 16 weeks of age (Documentation provided to EFSA n. 4, 5).[11]

In addition to the limits in the specifications laid out in Commission Regulation (EU) No 231/2012 for mono- and diglycerides of fatty acids (E 471), additional limits for toxic elements and impurities (e.g. erucic acid, *trans* fatty acid content, glycidyl fatty acid esters, mycotoxins, persistent organic pollutants, pesticides) are defined for finished products for infants and young children by various regulations such as Regulation (EC) No 1881/2006[12].

Based on the information provided by this IBO, the Panel noted that particular specifications for the additive when used in food for infants below 16 weeks of age would not be needed.

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

The call for data requested:

- information on the fate and the reaction products of mono- and diglycerides of fatty acids (E 471) in the infant formulae for use in infants below 16 weeks (food categories 13.1.1 and 13.1.5.1);

In its 2017, re-evaluation of mono- and di-glycerides of fatty acids (E 471) as food additives, the EFSA ANS Panel (EFSA ANS Panel, 2017a) has reviewed data pertaining to the stability and reaction and fate in food and noted that E 471 has the potential to show reactions through rearrangement, inter- and intramolecular acyl group migration, autooxidation and may be sensitive to hydrolysis.

The EFSA ANS Panel (2017a) noted that the production of 3-MCPD and GEs is increased upon heating above 160°C and 200°C, respectively, as demonstrated in various model food heating tests conducted with mono- and diglycerides of fatty acids (EFSA ANS Panel, 2017a). It also noted that GEs may be formed during the dehydration of monoglycerides.

In its response, one IBO noted that concentrations of these reaction products are anticipated to be minimal (i.e. below regulatory limits in the finished product, where applicable) and/or are not of nutritional or toxicological concern. Analytical data were not provided to proof these statements. The IBO stated that analytical testing is conducted to ensure that potential formation of 3-MCPD and GEs does not occur and is within acceptable regulatory limits in the finished product, respectively (Documentation provided to EFSA n. 4). Overall, the IBO stated that the additive mono- and diglycerides of fatty acids (E 471) is stable under the manufacturing conditions of infant formulae (13.1.1) and FSMP for infants (13.1.5.1) below 16 weeks of age (Documentation provided to EFSA n. 4).

Data on 3-MCPD and GEs depending on the raw material and the content of monoglycerides were provided by another IBO representing the primary producers of E 471 used in infant formulae (13.1.1) and FSMP for infants (13.1.5.1) below 16 weeks of age (Documentation provided to EFSA n. 1, 2, 3).

[11] It is noted that in a previous submission one IBO reported also sunflower as source oil not saturated for the manufacturing E 471 to be added in FC 13.1.1. Infant formulae as defined by Commission Directive 2006/141/EC (Documentation provided to EFSA n. 3), this was not reconfirmed in the latest submission (Documentation provided to EFSA n. 5).

[12] Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs (Text with EEA relevance). OJ L 364, 20.12.2006, p. 5–24.

The FAF Panel concluded that there was no substantial new information on the stability in the final product for the consumer, however, noted that there are limits given in the Regulation (Reg. (EC) 1881/2006) for 3-MCPD and GEs, applicable also to FCs 13.1.1 and 13.1.5.1.

3.2. Authorised uses and use levels

Maximum levels of mono- and diglycerides of fatty acids (E 471) in foods for infants below 16 weeks of age are defined in Regulation (EC) No 1333/2008 on food additives, as amended. In this opinion, these levels are termed maximum permitted levels (MPLs).

Currently, according to Annex II to Regulation (EC) No 1333/2008, mono- and diglycerides of fatty acids (E 471) is authorised in 'infant formulae as defined by Commission Delegated Regulation (EU) 2016/127/EC' (FC 13.1.1) at a maximum level of 4,000 mg/kg and in 'dietary foods for infants for special medical purposes and special formulae for infants' (FC 13.1.5.1) at a maximum level of 5,000 mg/kg (Table 4).

According to Annex III, Part 5 section B, to Regulation (EC) No 1333/2008, mono- and diglycerides of fatty acids (E 471) is authorised for uses in all nutrient preparations under the condition that the maximum level in foods mentioned in point 13.1 of Part E of Annex II is not exceeded and the conditions of use specified therein are respected (Table 5).

Table 4: MPLs of mono- and diglycerides of fatty acids (E 471) in foods for infants below 16 weeks of age according to the Annex II to Regulation (EC) No 1333/2008

Food category number	Food category name	E-number	Restrictions/exception	MPL (mg/L or mg/kg as appropriate)
13.1.1	Infant formulae as defined by Commission Delegated Regulation (EU)2016/127/EC	E 471		4,000[(a)]
13.1.5.1	Dietary foods for infants for special medical purposes and special formulae for infants	E 471		4,000[(a)]
13.1.5.1	Dietary foods for infants for special medical purposes and special formulae for infants	E 471	From birth onwards in specialised diets, particularly those devoid of proteins	5,000

MPL: maximum permitted level.

(a): If more than one of the substances E 322, E 471, E 472c and E 473 are added to a foodstuff, the maximum level established for that foodstuff for each of those substances is lowered with that relative part as is present of the other substances together in that foodstuff.

Table 5: Authorisation of mono- and diglycerides of fatty acids (E 471) in foods for infants below 16 weeks of age according to the Annex III to Regulation (EC) No 1333/2008

Authorisation according to	E-number	Maximum level	Nutrient to which the food additive may be added	Food category
Annex III, Part 5 Section B	E 471	For uses in nutrient preparations under the condition that the maximum level in foods mentioned in point 13.1 of Part E of Annex II is not exceeded and the conditions of use specified therein are respected	All nutrients	Foods for infants and young children

3.3. Exposure data

Some food additives are authorised in the EU in infants' formulae as defined by Commission Delegated Regulation (EU) 2016/127/EC (FC 13.1.1) and in dietary foods for infants for special medical purposes and special formulae for infants (FC 13.1.5.1) at a specific MPL. However, a food additive may be used at a lower level than the MPL. Therefore, actual use levels are required for performing a more realistic exposure assessment.

In the framework of Regulation (EC) No 1333/2008 on food additives and of Commission Regulation (EU) No 257/2010 regarding the re-evaluation of approved food additives, EFSA issued a public call[8] for technical and toxicological data on mono- and diglycerides of fatty acids (E 471) as food additives for uses in foods for all population groups including infants below 16 weeks of age. In response to this public call, information on the actual use levels of mono- and diglycerides of fatty acids (E 471) in foods was made available to EFSA by industry. No analytical data on the concentration of mono- and diglycerides of fatty acids (E 471) in foods were made available by the Member States.

3.3.1. Reported use levels in food category 13.1.1 and 13.1.5.1

Industry provided EFSA with 4 use levels of mono- and diglycerides of fatty acids (E 471); these levels were provided by one interested business operator (documentation provided to EFSA n. 4). According to the same interested business operator, mono- and diglycerides of fatty acids (E 471) is typically used at levels up to 2,089 mg/L and at a maximum level of 4,000 mg/L in infant formulae (FC 13.1.1) for infants below 16 weeks of age. For FC 13.1.5.1, mono- and diglycerides of fatty acids (E 471) is typically used at levels up to 2,088 mg/L and at a maximum level of 5,000 mg/L.

3.3.2. Summarised data extracted from the Mintel's Global New Products Database

The Mintel's GNPD is an online database which monitors new introductions of packaged goods in the market worldwide. It contains information of over 3.8 million food and beverage products of which more than 1,400,000 are or have been available on the European food market. Mintel started covering EU's food markets in 1996, currently having 24 of its 27 member countries, Norway presented in the Mintel GNPD.[13]

For the purpose of this Scientific Opinion, Mintel's GNPD[14] was used for checking the labelling of food and beverage products and food supplements for mono- and diglycerides of fatty acids (E 471) within the EU's food market as the database contains the compulsory ingredient information on the label.

According to Mintel's GNPD, mono- and diglycerides of fatty acids (E 471) was labelled on 15 products of 'Baby formula (0-6 months)' between January 2016 and September 2021, which represent 5% of the total number of food products belonging to this subcategory. It is noted that for the uses authorised according to Annex III (uses in nutrient preparations), the labelling is not mandatory.

3.4. Exposure estimates for infants below 16 weeks

Exposure to mono- and diglycerides of fatty acids (E 471) from its uses as a food additive in formulae for infants below 16 weeks was estimated. This scenario is based on the recommended consumption levels from SC Guidance (EFSA Scientific Committee, 2017). This guidance 'recommends values of 200 and 260 mL formula[15] /kg bw per day as conservative mean and high level consumption values to be used for performing the risk assessments of substances which do not accumulate in the body present in food intended for infants below 16 weeks of age'. These recommended consumption levels correspond to 14- to 27-day-old infants consumption. For the regulatory maximum level exposure assessment scenario, MPLs for infant formulae and FSMP (4,000 mg/kg for FC 13.1.1 and 5,000 for FC 13.1.5.1) were used as well as for the refined scenario.

3.4.1. Dietary exposure to mono- and diglycerides of fatty acids (E 471) from infant formulae

Table 6 summarises the estimated exposure to mono- and diglycerides of fatty acids (E 471) from its use as a food additive in FC 13.1.1 for infants below 16 weeks of age.

[13] Missing Cyprus, Luxembourg and Malta.
[14] http://www.gnpd.com/sinatra/home/ Accessed: 22/9/2021.
[15] The term 'formula' has been added.

Table 6: Dietary exposure to mono- and diglycerides of fatty acids (E 471) in infant formulae (FC 13.1.1) for infants below 16 weeks of age according to Annex II to Regulation (EC) No 1333/2008 (in mg/kg bw per day)

	Infants (< 16 weeks of age)
Regulatory maximum level exposure assessment scenario (4,000 mg/kg)	
• Mean consumption (200 mL/kg bw per day)	800
• High-level consumption (95th percentile, 260 mL/kg bw per day)	1,040
Refined estimated exposure assessment scenario	
Scenario using maximum use level reported by industry (4,000 mg/kg)	
• Mean consumption (200 mL/kg bw per day)	800
• High-level consumption (95th percentile, 260 mL/kg bw per day)	1,040
Scenario using mean of use levels reported by industry (2,089 mg/kg)	
• Mean consumption (200 mL/kg bw per day)	418
• High-level consumption (95th percentile, 260 mL/kg bw per day)	543

bw: body weight.

3.4.2. Dietary exposure to mono- and diglycerides of fatty acids (E 471) from FSMP infant formulae (FC 13.1.5.1)

Table 7 summarises the estimated exposure to mono- and diglycerides of fatty acids (E 471) from its use as a food additive in FC 13.1.5.1 with the restriction to specialised diets, particularly those devoid of proteins, for infants below 16 weeks of age.

Table 7: Dietary exposure to mono- and diglycerides of fatty acids (E 471) in special formulae for infants with the restriction to specialised diets, particularly those devoid of proteins (FC 13.1.5.1) below 16 weeks of age according to Annex II to Regulation (EC) No 1333/2008 (in mg/kg bw per day)

	Infants (< 16 weeks of age)
Regulatory maximum level exposure assessment scenario (5,000 mg/kg)	
• Mean consumption (200 mL/kg bw per day)	1,000
• High level consumption (95th percentile, 260 mL/kg bw per day)	1,300
Refined estimated exposure assessment scenario	
Scenario using maximum use level reported by industry (5,000 mg/kg)	
• Mean consumption (200 mL/kg bw per day)	1,000
• High-level consumption (95^{th} percentile, 260 mL/kg bw per day)	1,300
Scenario using mean of use levels reported by industry (2,088 mg/kg)	
• Mean consumption (200 mL/kg bw per day)	418
• High-level consumption (95^{th} percentile, 260 mL/kg bw per day)	543

bw: body weight.

The maximum occurrence scenario was used in the assessment, the mean occurrence scenario is reported and indicates that there are products on the market showing lower exposure levels.

Dietary exposure to FC 13.1.5.1 (with no restriction) for infants below 16 weeks of age equals the exposure calculated for the FC 13.1.1 at the MPL. Mean use levels reported by industry for FC 13.1.1 or for FC 13.1.5.1 are very similar and mean exposure to FC 13.1.5.1 (with no restriction) is, therefore, anticipated to also be similar.

Exposure from the use of mono- and diglycerides of fatty acids (E 471) according to Annex III, i.e. from carry-over from nutrient preparations, is covered in these exposure estimates from authorisation according to Annex II.

3.4.3. Uncertainty analysis

In accordance with the guidance provided in the EFSA opinion related to uncertainties in dietary exposure assessment (EFSA, 2007), the following sources of uncertainty have been considered and summarised in Table 8.

Table 8: Qualitative evaluation of influence of uncertainties on the dietary exposure estimate

Sources of uncertainties	Direction[(a)]
Consumption data: one reference point only to estimate exposure during the period of up to 16 weeks of age	+/–
Regulatory maximum level exposure assessment scenario: – exposure calculations based on the MPL according to Annex II and Annex III to Regulation (EC) No 1333/2008	+
Refined exposure assessment scenarios: – exposure calculations based on the maximum or typical levels (reported use from industries)	+/–

MPL: maximum permitted level.
(a): +, uncertainty with potential to cause overestimation of exposure; –, uncertainty with potential to cause underestimation of exposure.

Mono- and diglycerides of fatty acids (E 471) is authorised in food categories 13.1.1 and 13.1.5.1 according to Annex II and III to Regulation (EC) No 1333/2008. There is uncertainty around the consumption values used and the typical and maximum levels reported by industry and used in the calculations, since the actual formula consumption and related additive use levels in that formula could be higher or lower than the fixed values used in the scenario(s). This gives rise to the potential both for possible over- and underestimation of exposure (+/– in Table 8). In contrast, the levels used in the MPL scenario cannot legally be exceeded and this means that the uncertainty is one sided. The actual use levels cannot legally be higher (so there is no uncertainty in that direction that could give rise to a potential for overestimation of exposure) but actual use levels (and so exposure) could be lower. The consequence of this one-sided uncertainty is that assuming use levels are all at the MPL has the potential to overestimate exposure (+, Table 8). Based on the assumption that carers of children would be brand-loyal to an infant formula (FC 13.1.1) or infant formulae for special medical purposes (FC 13.1.5.1), the Panel considers that the exposure assessment scenarios (Tables 6 and 7) would in general result in reliable estimates of exposure.

3.5. Proposed revision to existing EU Specifications for mono- and diglycerides of fatty acids (E 471)

3.5.1. Toxic elements

The Panel noted that the occurrence data on toxic elements submitted by the interested business operator are substantially lower than the current limits in the EU specifications (documentation provided to EFSA n. 1, 2). The Panel considered that the maximum limits in the EU specifications for toxic elements should be established based on actual levels in the commercial food additive. If the European Commission decides to revise the current limits in the EU specifications, the estimates of toxic elements intake as below could be considered.

For lead, five results were reported by one IBO (Documentation provided to EFSA n. 1,2) as 0.1 mg/kg (LOQ: 0.05), the remaining batches were below LOQ ranging between 0.01 and 0.1 mg/kg. For mercury, all samples were below LOQs which ranged from 0.005 to 0.05 mg/kg. For cadmium, all results were below LOQs ranging between 0.005 and 0.02 mg/kg. For arsenic, one result was reported as 0.037 mg/kg and the remaining batches were all reported as below LOQ, which ranged between 0.01 and 0.1 mg/kg. Based on these analytical data, the IBO proposed lowest technologically achievable levels for Pb (1 mg/kg), Hg (0.5 mg/kg), Cd (0.2 mg/kg) and As (1 mg/kg). The IBO assessed these levels by choosing the highest measured value or LOQ, whichever is highest, and applying a factor of 10 times this value. This approach is similar to that applied by the FAF Panel in earlier risk assessments. The Panel emphasised that the choice of the factor as well as other considerations, such as on multiple sources of exposure to conclude on the maximum limits for toxic elements in the specifications is in the remit of risk management. The numbers used here are merely taken to support the risk assessment of these toxic elements as presented below.

The potential exposure to the toxic elements from the use of the food additives E 471 can be calculated by assuming contamination of the additive may be up to the specification limit values and then by calculation pro-rata to the estimates of exposure to the food additive itself. With regard to the dietary exposure to the food additive for infants below 16 weeks of age, the Panel considered values of 200 and 260 mL formula/kg bw per day as conservative mean and high-level consumption values, and the scenarios based on the regulatory maximum level exposure assessment, the reported use levels (refined scenario, Tables 6 and 7 and Table 7 from EFSA ANS Panel, 2017a) and the mean use levels reported by industry. The group of infants above 16 weeks of age to 11 months of age has the highest exposure level among all population groups above 16 weeks.

The above-mentioned lowest technologically achievable levels combined with the estimated intakes of E 471 could result in an exposure which can be compared with the following reference points or health-based guidance values for the four elements; a BMDL01 of 0.5 μg/kg bw per day for lead (EFSA CONTAM Panel, 2010), a TWI of 4 μg/kg bw for mercury (EFSA CONTAM Panel, 2012), a TWI of 2.5 μg/kg bw per day for cadmium (EFSA CONTAM Panel 2009a) and a BMDL01 of 0.3–8 μg/kg bw per day for arsenic (EFSA CONTAM Panel, 2009b).

The outcome of the risk assessment of the FAF Panel (Table 9) illustrates the health impact that would result if these toxic elements would be present in the food additive at the lowest technologically achievable levels proposed by the IBO.

Table 9: Risk assessment for toxic elements based on the lowest technologically achievable levels in E 471 for use in food for all age groups as proposed by the interested business operator (documentation provided to EFSA n. 1, 2)

Exposure to E 471 (mg/kg bw per day)[(a),(e)]	MOS/MOE for Pb at 1 mg/kg	% of the TWI for Hg at 0.5 mg/kg	% of the TWI for Cd at 0.2 mg/kg	MOS/MOE for As at 1 mg/kg
800[(b)]	0.63	70	45	0.38–10
1,040[(b)]	0.48	91	58	0.29–7.7
418[(c)]	1.20	37	23	0.72–19
543[(c)]	0.92	48	30	0.55–15
1,000[(d)]	0.50	88	56	0.30–8.0
1,300[(d)]	0.38	114	73	0.23–6.2
247[(e)]	2.02	21	14	1.2–32
620[(e)]	0.81	54	35	0.48–13

(a): Data from Tables 6 and 7 (Sections 3.4.1 and 3.4.2).
(b): Regulatory maximum level exposure assessment scenario and refined estimated exposure assessment scenario – Scenario using maximum use level reported by industry (4,000 mg/kg) in infant formulae (FC 13.1.1) for infants below 16 weeks of age.
(c): Refined estimated exposure assessment scenario for E 471 in infant formulae (FC 13.1.1) and in special formulae (FC 13.1.5.1) – Scenario using mean use level reported by industry (2,089 mg/kg).
(d): Regulatory maximum level exposure assessment scenario and refined estimated exposure assessment scenario - Scenario using maximum use level reported by industry (5,000 mg/kg) in infant formulae (FC 13.1.5.1) for infants below 16 weeks of age.
(e): Highest exposure level for the population above 16 weeks of age (Refined Brand-Loyal Scenario – Toddlers – mean and 95th percentile (data from Table 7, Section 3.4.4, EFSA ANS Panel, 2017a).

The resulting figures show that the exposure to toxic elements from the consumption of E 471 is substantial. The Panel noted that the MOS/MOE for lead and arsenic are very low.

For arsenic, the reference points are $BMDL_{01}$ values (lower and upper bound) of 0.3 and 8 μg/kg bw per day from human epidemiological studies (EFSA CONTAM Panel, 2009b). The reference points are based on carcinogenicity and so the MOS/MOE should be at least 10,000 (EFSA Scientific Committee, 2005, 2012b). Considering that the human studies were the basis to derive the BMDL, an interspecies extrapolation factor may not be needed.

For lead, the reference point is based on a study demonstrating perturbation of intellectual development in children with the critical response size of 1 point reduction in IQ. In the opinion on lead (EFSA CONTAM Panel, 2010), it is mentioned that a 1 point reduction in IQ is related to a 4.5% increase in the risk of failure to graduate from high school and that a 1 point reduction in IQ in children can be associated with a decrease of later productivity of about 2%. If the exposure exceeds the BMDL01, the resulting risk has to be considered (MOS/MOE lower than 1).

The MOS/MOE is calculated by dividing the reference point (i.e. BMDL or NOAEL) through the exposure estimate. The assessment of the uncertainty in the exposure showed no potential for overestimation of exposure for the different scenarios with the exception of the regulatory maximum level exposure assessment scenario (see Table 8). Hence, the MOS/MOE estimates are not underestimated with the exception of that based on the MPLs. Even considering that the MOS/MOE might be underestimated using the exposure estimates at the MPL, the order of magnitude between the MOS/MOE for arsenic and for lead compared to the MOS/MOE requested (see above) exceeds by far the possibility of being explained by an overestimation of the exposure.

Using the existing specifications for As (3 mg/kg), Pb (2 mg/kg), Cd (1 mg/kg) and Hg (1 mg/kg) in E 471, the exposure to toxic elements would be considerably higher and thus the resulting MOS/MOE for As and Pb explicitly lower. The existing specifications for Hg and Cd would certainly lead to exceedance of the TWIs for these element, as illustrated in Table 10.

Table 10: Risk assessment for toxic elements based on the current EU specifications limits for toxic elements in E 471 for use in food for all age groups (Documentation provided to EFSA n. 1)

Exposure to E 471 (mg/kg bw per day)[a],[e]	MOS/MOE for Pb at 2 mg/kg	% of the TWI for Hg at 1 mg/kg	% of the TWI for Cd at 1 mg/kg	MOS/MOE for As at 3 mg/kg
800[b]	0.31	140	224	0.13–3.3
1,040[b]	0.24	182	291	0.10–2.6
418[c]	0.60	73	117	0.24–6.4
543[c]	0.46	95	152	0.18–4.9
1,000[d]	0.25	175	280	0.10–2.7
1,300[d]	0.19	228	364	0.08–2.1
247[e]	1.0	43	69	0.41–11
620[e]	0.40	109	174	0.16–4.3

(a): Data from Tables 6 and 7 (Sections 3.4.1 and 3.4.2).
(b): Regulatory maximum level exposure assessment scenario and refined estimated exposure assessment scenario – Scenario using maximum use level reported by industry (4,000 mg/kg) in infant formulae (FC 13.1.1) for infants below 16 weeks of age.
(c): Refined estimated exposure assessment scenario for E 471 in infant formulae (FC 13.1.1) and in special formulae (FC 13.1.5.1) – Scenario using mean use level reported by industry (2,089 mg/kg).
(d): Regulatory maximum level exposure assessment scenario and refined estimated exposure assessment scenario - Scenario using maximum use level reported by industry (5,000 mg/kg) in infant formulae (FC 13.1.5.1) for infants below 16 weeks of age.
(e): Highest exposure level for the population above 16 weeks of age (Refined Brand-Loyal Scenario – Toddlers – mean and 95th percentile (data from Table 7, Section 3.4.4, EFSA ANS Panel, 2017a).

The Panel noted that the calculations clearly indicate the need to decrease the current maximum limits for lead, mercury, cadmium and arsenic in E 471, considering also other sources of exposure to these toxic elements. Furthermore, the Panel noted that maximum levels for lead and cadmium in infant formula are set by Reg. (EC) No 1881/2006; therefore, the Panel calculated the impact of the level of the toxic elements lead and cadmium in the food additive on the final product and compared that with the legal limits for elements in the final formula (see Appendix B). Considering the results of these estimations and the fact that the food additive is not the only potential source of toxic elements, the Panel emphasises the need to reduce the specification limit values for lead and cadmium in Regulation (EU) no 231/2012.

3.5.2. Other impurities

3.5.2.1. Carry over and process impurities

3.5.2.1.1. Acrolein and butanetriol

According to one IBO, acrolein and butanetriol were not detected in E 471 (see Section 3.1.3.2); therefore, the Panel concluded that there would be no need for including limits in the EU specifications for these process impurities. The Panel acknowledged that a numerical limit for acrolein in glycerol (E 422) is currently being considered as part of the follow-up of the re-evaluation of this food additive.[16]

[16] EFSA-Q-2019-00815.

3.5.2.1.2. Free and bound 3-MCPD

The Panel performed a risk assessment for free and bound 3-MCPD (calculated as free 3-MCPD) estimating the exposure to this impurity from the use of E 471 and considering its presence in E 471 at the level proposed by the IBO of 2.5 mg/kg based on regulatory specifications for raw materials (edible, vegetable oils) as laid down by Regulation (EC) No 1881/2006. The approach is the same as for the toxic elements. The TDI of 2 μg/kg bw per day for 3-MCPD and its fatty acid esters established by the CONTAM Panel in 2018 (EFSA CONTAM Panel, 2018) was used as the health based guidance value, see Section 3.6.3.

The outcome of the risk assessment of the FAF Panel (Table 11) illustrates the health impact that would result if 3-MCPD and its fatty acid esters would be present in the food additive at the lowest technologically achievable levels proposed by the IBO.

Table 11: Risk assessment for 3-MCPD and its fatty acid esters based on the lowest technologically achievable level of E 471 as proposed by the business operator (documentation provided to EFSA n. 1-3)

Exposure to E 471 (mg/kg bw per day)[(a),(e)]	% of the TDI for 3-MCPD and esters at 2.5 mg/kg
800[(b)]	100
1,040[(b)]	130
418[(c)]	52
543[(c)]	68
1,000[(d)]	125
1,300[(d)]	163
247[(e)]	31
620[(e)]	78

(a): Data from Tables 6 and 7 (Sections 3.4.1 and 3.4.2).
(b): Regulatory maximum level exposure assessment scenario and refined estimated exposure assessment scenario – Scenario using maximum use level reported by industry (4,000 mg/kg) in infant formulae (FC 13.1.1) for infants below 16 weeks of age.
(c): Refined estimated exposure assessment scenario for E 471 in infant formulae (FC 13.1.1) and in special formulae (FC 13.1.5.1) – Scenario using mean use level reported by industry (2,089 mg/kg).
(d): Regulatory maximum level exposure assessment scenario and refined estimated exposure assessment scenario – Scenario using maximum use level reported by industry (5,000 mg/kg) in infant formulae (FC 13.1.5.1) for infants below 16 weeks of age.
(e): Highest exposure level for the population above 16 weeks of age (Refined Brand-Loyal Scenario -Toddlers – mean and 95th percentile (data from Table 7, Section 3.4.4, EFSA ANS Panel, 2017a).

The Panel noted that the exposure levels estimated by considering the lowest technologically achievable levels proposed by the IBO would result in the exceedance of the TDI (Table 11). Therefore, the Panel considered that a limit value for 3-MCPD (free and bound), lower than the one proposed by the IBO, should be introduced in the current EU specifications for E 471.

Furthermore, the Panel noted that maximum levels for 3-MCPD are set by Reg. (EC) No 1881/2006; the Panel calculated the impact of the level of these impurities in the food additive on the final product and compared that with the legal limits for elements in the final infant formula (see Appendix B). Considering the results of these estimations, and the fact that the food additive is not the only potential source of 3-MCPD, the Panel emphasised the need to set specification limit values for these impurities in Regulation (EU) no 231/2012.

3.5.1.2.3. Glycidyl esters

The FAF Panel performed a risk assessment for GEs (expressed as glycidol) estimating the exposure level by considering the lowest technologically achievable levels proposed by the IBO i.e. 10 mg/kg and by considering the reference point (T25) for glycidol as derived by the CONTAM Panel (EFSA CONTAM Panel, 2016a), see Section 3.6.2.

The outcome of the risk assessment of the FAF Panel (Table 12) illustrates the health impact that would result if GEs would be present in the food additive at the lowest technologically achievable levels proposed by the IBO.

Table 12: Risk assessment for GEs (expressed as glycidol) based on the lowest technologically achievable level in E 471 as proposed by the business operator (Documentation provide to EFSA n. 1-3)

Exposure to E 471 (mg/kg bw per day)[(a),(e)]	MOS/MOE for GEs at 10 mg/kg
800[(b)]	1,275
1,040[(b)]	981
418[(c)]	2,440
543[(c)]	1,878
1,000[(d)]	1,020
1,300[(d)]	785
247[(e)]	4,130
620[(e)]	1,645

(a): Data from Tables 6 and 7 (Sections 3.4.1 and 3.4.2).
(b): Regulatory maximum level exposure assessment scenario and refined estimated exposure assessment scenario – Scenario using maximum use level reported by industry (4,000 mg/kg) in infant formulae (FC 13.1.1) for infants below 16 weeks of age.
(c): Refined estimated exposure assessment scenario for E 471 in infant formulae (FC 13.1.1) and in special formulae (FC 13.1.5.1) – Scenario using mean use level reported by industry (2,089 mg/kg).
(d): Regulatory maximum level exposure assessment scenario and refined estimated exposure assessment scenario – Scenario using maximum use level reported by industry (5,000 mg/kg) in infant formulae (FC 13.1.5.1) for infants below 16 weeks of age.
(e): Highest exposure level for the population above 16 weeks of age (Refined Brand-Loyal Scenario – Toddlers – mean and 95th percentile (data from Table 7, Section 3.4.4, EFSA ANS Panel, 2017a).

The Panel noted that, by applying the lowest technologically achievable levels proposed by the IBO to estimate the exposure, the resulting MOS/MOE would be substantially lower than the MOS/MOE of 25,000 proposed by the CONTAM Panel (Table 12). Therefore, the Panel considered that a limit value for GEs (expressed as glycidol), lower than the one proposed by the IBO, should be introduced in the current EU specifications for E 471.

Furthermore, the Panel noted that maximum levels for GEs (expressed as glycidol) are set by Reg. (EC) No 1881/2006; therefore, the Panel calculated the impact of the levels of the these impurities in the food additive on the final product and compared that with the legal limits for elements in the final formula (see Appendix B). Considering the results of these estimations, and the fact that the food additive is not the only potential source of GEs, the Panel emphasised the need to set specification limit values for these impurity in Regulation (EU) no 231/2012.

3.5.2.2. Fatty acids

3.5.2.2.1. Trans fatty acids

Analytical data on current levels of *trans* fatty acids tested in commercial samples of E 471 were provided by one IBO (documentation provided to EFSA n. 1,2). The Panel noted that the levels of *trans* fatty acids cover a wide range depending on the degree of hydrogenation of the fat/oil raw material (non, partially or fully hardened). Based on the information from one IBO, only fully hydrogenated oils/fats are used for the production of E 471 in infant formula (13.1.1) and infant FSMPs (13.1.5.1) (Documentation provided to EFSA n. 4,5).[11] The levels of *trans* fatty acids in 15 samples referred to as 'fully hardened' range between 0.01% and < 1%. In infant formula and follow-on formula, the *trans* fatty acid content is regulated and shall not exceed 3% of the total fat content (Regulation (EU) 127/2016 supplementing Regulation (EU) No 609/2013).

For the general population, E 471 can be manufactured also from 'non-hardened' or 'partially hardened' fats. The content of *trans* fatty acids is higher ranging between 0.34% and 1.59% and 15.88–59.92%, respectively (see Section 3.1.3.2.1). The Panel noted that '*latest national recommendations in the EU, and most recent recommendations from medical professional associations in Europe and the US indicate that consumption of TFA* [*trans* fatty acids] *should be as low as possible*' (EFSA, 2018). On the basis of this advice, a maximum limit of 2 grams of *trans* fat per 100 g fat in food for the final consumer was set by Regulation (EU) No 2019/649 amending Regulation (EC) No 1925/2006.

3.5.2.2.2. Erucic acid

The FAF Panel also performed a risk assessment for erucic acid estimating the exposure level by considering the lowest technologically achievable levels of 20 g/kg in E 471 for use in food for all age groups as proposed by the interested business operator. The approach is the same as for the toxic elements and carry over and process impurities. The tolerable daily intake (TDI) of 7 mg erucic acid/kg bw established by the CONTAM Panel (EFSA CONTAM Panel, 2016b) was used as the health-based guidance value (see Section 3.6.2).

The outcome of such an exercise (Table 13) illustrates the health impact that would result if erucic acid would be present in E 471 at the lowest technologically achievable level proposed by the IBO.

Table 13: Risk assessment for erucic acid based on the lowest technologically achievable level in E 471 for use in food for all age groups as proposed by the interested business operator (Documentation provided to EFSA n. 1, 2)

Exposure to E 471 (mg/kg bw per day)[(a),(e)]	% of the TDI for Erucic acid at 20 g/kg
800[(b)]	229
1,040[(b)]	297
418[(c)]	119
543[(c)]	155
1,000[(d)]	286
1,300[(d)]	371
247[(e)]	71
620[(e)]	177

(a): Data from Tables 6 and 7 (Sections 3.4.1 and 3.4.2).
(b): Regulatory maximum level exposure assessment scenario and refined estimated exposure assessment scenario – Scenario using maximum use level reported by industry (4,000 mg/kg) in infant formulae (FC 13.1.1) for infants below 16 weeks of age.
(c): Refined estimated exposure assessment scenario for E 471 in infant formulae (FC 13.1.1) and in special formulae (FC 13.1.5.1) – Scenario using mean use level reported by industry (2,089 mg/kg).
(d): Regulatory maximum level exposure assessment scenario and refined estimated exposure assessment scenario - Scenario using maximum use level reported by industry (5,000 mg/kg) in infant formulae (FC 13.1.5.1) for infants below 16 weeks of age.
(e): Highest exposure level for the population above 16 weeks of age (Refined Brand-Loyal Scenario -Toddlers – mean and 95th percentile (data from Table 7, Section 3.4.4, EFSA ANS Panel, 2017a).

The calculations clearly indicate that the lowest technologically achievable level for erucic acid in E 471 as proposed by the interested business operator would lead to a considerable exceedance of the TDI. Therefore, the Panel considered that a limit value for erucic acid, lower than the one proposed by the IBO, should be introduced in the current EU specifications for E 471.

3.5.2.3. Solvents

According to the received information, the manufacturing process of E 471 does not involve the use of any solvents (see Section 3.1.3.2); therefore, there is no need to amend the EU specifications for E 471 to include a maximum limit for any solvent.

3.5.3. Proposed revisions to the EU specifications

Overall, based on the analytical data provided by the interested business operators in response to the EFSA call for data (documentation provided to EFSA n. 1-3) and the above considerations, the Panel recommends the following revisions of the existing EU specifications for mono- and diglycerides of fatty acids (E 471) as listed in Table 14.

Table 14: Proposal for a revised version of the existing EU Specifications for mono- and diglycerides of fatty acids E 471

	Commission Regulation (EU) No 231/2012	Comment/justification for revision
Definition	See Table 2	Unchanged
Assay	See Table 2	Unchanged
Description	See Table 2	Unchanged
Identification	See Table 2	Unchanged
Infrared absorption spectrum Infrared absorption spectrum	See Table 2	Unchanged
Test for glycerol	See Table 2	Unchanged
Test for fatty acids	See Table 2	Unchanged
Solubility	See Table 2	Unchanged
Purity	See Table 2	Unchanged
Water content	See Table 2	Unchanged
Acid value	See Table 2	Unchanged
Free glycerol	See Table 2	Unchanged
Polyglycerols	See Table 2	Unchanged
Lead	Not more than 2 mg/kg	Maximum limit to be lowered on the basis of the information provided and on the considerations of the Panel
Cadmium	Not more than 1 mg/kg	Maximum limit to be lowered on the basis of the information provided and on the considerations of the Panel
Mercury	Not more than 1 mg/kg	Maximum limit to be lowered on the basis of the information provided and on the considerations of the Panel
Arsenic	Not more than 3 mg/kg	Maximum limit to be lowered on the basis of the information provided and on the considerations of the Panel
3-MCPD (free and bound, calculated as free 3-MCPD)	Not presently specified	Maximum limit to be included on the basis of the information provided and the considerations of the Panel
Glycidyl esters	Not presently specified	Maximum limit to be included on the basis of the information provided and the considerations of the Panel
Erucic acid	Not presently specified	Maximum limit to be included on the basis of the information provided and the considerations of the Panel
Total glycerol	See Table 2	Unchanged
Sulfated ash	See Table 2	Unchanged
Soap	See Table 2	Unchanged

3.6. Biological and Toxicological data

3.6.1. Previous evaluation by ANS Panel (2017)

The following text (in italics) is from the opinion published in 2017 (EFSA ANS Panel, 2017a). New information and assessments related to the specific age group below 16 weeks of age are added in the following paragraphs.

The [ANS] *Panel noted that the diacylglycerol (diglyceride) used in several of the toxicity studies described below was intended to be used for nutritional purposes (as an edible oil substitute) and it had a composition rich in unsaturated fatty acids (> 95%). The* [ANS] *Panel further noted that its composition made this material acceptable with regard to the specifications of E 471. The Panel considered that the results of the toxicological studies with these diacylglycerols can be used for the assessment of E 471.*

Absorption, metabolism and excretion

Hydrolysis of the diglyceride of fatty acids (fatty acid composition: oleic acid 80.5%, total saturated fatty acids 7.9%, total polyunsaturated fatty acids 4.9%, monounsaturated fatty acids other than oleic

acid 6.6%) was examined in a model digestive system in vitro (Martin et al.*, 2014) by using a model of simulated bile secretion. After 60 min at 37°C, 1,3-diolein was shown to be nearly completely hydrolysed to free fatty acids with only low levels of monoglycerides present.*

Ames et al. *(1951) estimated the absorption of naturally produced cottonseed oil and monoglycerides of the fatty esters contained in cottonseed oil in eight male albino rats per group. Fat intake was 25% cottonseed oil or monoglycerides. Intake and fat content in the faeces were determined for 1 week. The absorption was determined by measuring the digestibility factor (e.g. the percentage of ingested fat that was not excreted in the faeces). Naturally produced cottonseed oil was absorbed to 96.8 ± 0.5% and monoglycerides of the fatty esters contained in cottonseed oil were absorbed to 97.8 ± 0.4%.*

Absorption of diacylgycerol was examined in nine 5-week-old male Sprague–Dawley rats (Chen et al.*, 2013). The tested diacylglycerol consisted of 87% diacylglycerols but contained also monoacylglycerols (2%) and triacylglycerols (11%). The total fatty acid composition was primarily C18:2, C18:1, C18:3 (n–3) and C16:0 present at 53.6%, 32.9%, 8.8% and 3%, respectively.*

Diacylglycerol was administered by oral gavage at 2.5 mL diacylglycerol/kg bw plus 7.5 mL/kg bw in distilled water with blood samples collected at 0, 6 and 24 h. The serum fatty acid concentrations was not different at the time points examined compared to the concentration at time 0. Faeces were collected from the last study day. The digestibility coefficient of the diacylglcerol was calculated by the authors to be 58.8 ± 14.33% and equals the absorption.

Overall, in rats, only traces of cottonseed oil monoglycerides were found in the faeces, indicating that after hydrolysis the components were well absorbed (97.8 ± 0.4%). In another study, the absorption of hydrolysis products diglycerides of fatty acids was calculated to be 58.8 ± 14.3%. In a study to elucidate the mechanism of absorption of diglycerides, the hydrolysis of the diglyceride 1,3-diolein was examined in a model digestive system in vitro. 1,3-diolein was shown to be nearly completely hydrolysed to free fatty acids with low levels of monoglycerides present.

Acute, subchronic, genotoxicity, chronic, developmental and reproductivity studies

No study was available for the acute toxicity of E 471.

No evidence for adverse effects were reported in short-term and subchronic studies in rats and hamsters even at the highest dose tested of 2,500 mg diacylglycerol/kg bw per day in the rats and 7,500 mg glyceryl stearate/kg bw per day in hamsters.

The Panel considered that the available studies did not raise any concern with regard to genotoxicity.

No adverse effects were reported in chronic toxicity studies at doses as high as 7,800 and 2,000 mg diacylglycerol/kg bw per day in mice and rats, respectively. In mice and rats, diacylglycerol did neither show carcinogenic potential nor a promotion effect in initiation/promotion studies.

No parental, reproductive or developmental toxicity was observed in a two-generation reproduction toxicity study and a prenatal developmental toxicity study in rats with diacylglycerol oil-rich in unsaturated fatty acids (> 95%) up to the highest dose tested (4,630 mg diacylglycerol/kg bw per day).

The Panel noted that recent studies with other emulsifiers had demonstrated effects on the microbiota, which might also be relevant to emulsifiers in general; however, there were no specific studies on mono- and diglycerides of fatty acids and effects on the microbiota itself.

3.6.2. Glycidyl esters

In 2016, the EFSA CONTAM Panel assessed the risk for human health related to the presence of 3- and 2-monochloropropandiol (MCPD) and their fatty acid esters, and glycidyl fatty acids esters in food (EFSA CONTAM Panel, 2016a). The risk assessment of glycidyl fatty acids was based on animal toxicity studies with glycidol assuming the complete hydrolysis of the esters to free glycidol following ingestion as no in vivo data were identified in a literature search for GEs.

For glycidol, a T25 was taken as reference point from a carcinogenicity study by the National Toxicology Program (NTP, 1990). The value was 10.2 mg glycidol/kg bw per day for the relevant endpoint peritoneal mesotheliomas in male rats. In its opinion, the CONTAM Panel concluded that a MOE of 25,000 and more would be of low concern for glycidol from fatty acid esters.

The FAF Panel agreed with the reference point and the conclusion that an MOE of 25,000 or more would be of low concern.

3.6.3. 3-MCPD

In 2018, an update was published by the EFSA CONTAM Panel concerning the TDI for 3-monochloropropane diol and its fatty acid esters (EFSA CONTAM Panel, 2018). For 3-MCPD, the Benchmark dose modelling of renal tubular hypertrophy reported in the study of Cho et al. (2008) in male mice, resulted in a $BMDL_{10}$ of 0.2 mg/kg bw per day. Applying a default uncertainty factor of 100, a TDI of 2 μg/kg bw per day was derived.

The FAF Panel agreed with the reference point and the derivation of the TDI.

3.6.4. Erucic acid

In 2016, the EFSA CONTAM Panel proposed a tolerable daily intake (TDI) of 7 mg for erucic acid/kg bw (EFSA CONTAM Panel, 2016b). This TDI was based on an overall NOAEL for lipidosis of 0.7 g/kg bw per day, observed in a 7-day feeding study in young rats and in a 2-week feeding study in new-born piglets, using the default uncertainty factor of 100 to account for intra- and interspecies differences.

The FAF Panel agreed with the reference point and the derivation of the TDI.

3.6.5. Newly available data

In response to the call for data, the interested business operators provided literature searches relevant for the safety evaluation of mono- and diglycerides of fatty acids (E 471) in all population groups including infants below 16 weeks of age (documentation provided to EFSA n. 1, 2, 4).

The Panel noted that the diacylglycerol (diglyceride), used in several of the toxicity studies from the literature described below, was intended to be used for nutritional purposes (as an edible oil substitute) and it had a composition rich in unsaturated fatty acids (> 95%). The Panel further noted that its composition made this material acceptable with regard to the specifications of E 471. Consistently with EFSA ANS Panel (2017a), the Panel considered that the results of the toxicological studies with these diacylglycerols can be used for the assessment of E 471.

3.6.5.1. Absorption, distribution, metabolism and excretion

No new relevant studies were submitted by the interested business operators or found in the provided literature search (documentation provided to EFSA n. 1, 2, 4). The Panel considered that the results of the study in 5-week-old male Sprague Dawley rats (Chen et al., 2013) (see above) are relevant for risk assessment in infants below 16 weeks of age.

3.6.5.2. Toxicological data

A 90-day dietary study with Sprague Dawley rats (Bushita et al., 2018a; documentation provided to EFSA n. 1, 2, 4) was performed with alpha linoleic acid (ALA)-enriched diacylglycerol (DAG) oil (prepared using flax seed oil) according to GLP and OECD TG 408 (1998). The soybean oil in the commercial diet (AIN-93 G) was replaced by 1.375%, 2.75% or 5.5% ALA-DAG (corresponding to 737.9, 1,460.5, and 2,915.8 mg ALA-DAG/kg bw per day in males and 846.0, 1,702.7 and 3,326.2 mg/kg bw per day in females and to 613.2, 1,213.7 and 2,423.0 mg DAG/kg bw per day in males and 703.0, 1,414.9, and 2,764.1 mg DAG/kg bw per day in females). As controls rapeseed oil (control 1) or ALA-triacylglycerol (ALA-TAG: control 2) were used. No adverse effects were reported for survival or body weight and food intake of the rats. Single changes in the outcomes of the functional observational battery were not dose-related and not considered treatment-related. No relevant ophthalmic lesions were found. No treatment-related adverse effects in haematology, clinical chemistry and urinalysis were observed except for some changes which were mainly minor and/or not dose-related. No corresponding histopathological findings in bone marrow, liver and kidney were observed. The decreases in total cholesterol and triglycerides were not considered of toxicological relevance. The reported changes in organ weights were not dose-related and therefore not considered treatment-related. The histopathological examination revealed a relatively high number of minimal fatty degeneration in the rapeseed oil controls which was not seen with ALA-DAG in highest dose groups of females and males at the end of the treatment period and was reduced at the end of the recovery period compared to rapeseed recovery. The authors concluded that there was no evidence of adverse effects of ALA-DAG oil at the highest doses tested. The Panel agreed.

A 1-year study in Beagle dogs was performed according to GLP with DAG (composition not specified) (Chengelis et al., 2006; documentation provided to EFSA n.4). Dietary concentrations were 1.5% DAG (+8.0% TAG), 5.5% DAG (+4.0% TAG) and 9.5% DAG (in WIL Certified Canine diet). Two

control groups were fed either with 9.5% TAG (in WIL Certified Canine diet) or a standard basal diet (PMI 5007 with a fat content of 9.5%). The treatment was started in pre-juvenile dogs (2.5 months of age) and the average DAG consumption was calculated to be 348, 1,487 or 2,300 mg/kg bw per day in females and 326, 1,227, or 2,541 mg/kg bw per day in males (4/sex per group). No treatment-related effects were reported for clinical observations, mortality, body weights and body weight gains. Food consumption in the DAG groups was comparable to that in the TAG controls but the consumption in both DAG and TAG groups was lower than that in control dogs fed the basal diet. Similarly, some changes in haematology (neutrophile, lymphocyte and platelet counts) mostly without dose-response were observed in males when compared to the basal diet controls. Statistically significant increases in serum chemistry parameters, i.e. alkaline phosphatase, glucose, calcium, were dose dependent in males while other increases in total protein, total cholesterol and triglycerides were without dose response or not statistically significant compared with the basal diet control. No toxicologically relevant changes of these parameters were observed compared to the TAG control which was considered the appropriate control group. The outcomes of ophthalmic, ECG and macroscopic examinations were not considered treatment-related. Some increases in vacuolisation of minimal severity were observed in the adrenal cortex (females, males), kidney (males) and liver (females) as well as inflammation in lungs (males) compared to the controls fed the basal diet. These microscopic findings were considered spontaneous and/or incidental. The authors concluded that there was no effect of DAG treatment on growth and development of the dogs. Considering that there were no relevant changes between the DAG- or TAG-treated dogs and the effects compared to the controls fed with the basal diet were of no toxicological relevance, the Panel agreed that there was no effect up to the highest dose.

A prenatal developmental toxicity study (Bushita et al., 2018b; documentation provided to EFSA n.1, 2, 4) in Sprague Dawley rats was performed with ALA-DAG (identical to the test item in Bushita et al., 2018a) according to GLP and OECD TG 414 (2001). Female rats (24/group) were treated via gavage with ALA-DAG from GD6 to GD19 at doses of 0, 1,149, 2,325 or 4,715 mg/kg body weight per day (in rapeseed oil) corresponding to doses of 0, 955, 1,932 or 3,918 mg DAG/kg body weight per day. Rats in two control groups (23/group) received either rapeseed oil or ALA-TAG oil. A Caesarean section was performed on GD 20. No treatment-related clinical findings or changes in feed consumption, maternal body weight and body weight gain were reported. No treatment-related adverse findings were reported for intrauterine growth, survival and number of implantations, fetal external, visceral or skeletal abnormalities. The authors considered that the NOAEL in this study was at the highest dose, i.e. at 4,715 mg/kg body weight per day. However, the Panel noted that the post-implantation loss in the high dose group was slightly higher than in the two control groups (10.47% vs. 7.74% in the rapeseed control and 4.12% in the ALA-TAG oil group). In addition, the authors stated that one female in the ALA-DAG oil group, which had 100% implantation loss, was excluded for evaluation. It was not clear to which ALA-DAG oil dose group this female belonged and from which calculation it was excluded. The limitations in reporting and study design prohibit the use of the study for hazard characterisation.

Additional studies

Two subchronic dietary studies in C57BL/6 mice addressed the impact of glycerol monolaurate (GML) on gut microbiota and its potential beneficial effects on inflammation and metabolic syndrome (documentation provided to EFSA n.4). Mo et al. (2019) reported that in a 4-month study at the highest dose of GML (1,600 mg/kg bw per day), the abundance of microbiota considered as 'favourable' by the authors increased and the ratio of plasma LDL to HDL cholesterol decreased. In the second study (Zhao et al., 2019), male mice were fed a high fat diet (HFD) supplemented with GML (0, 150, 300 and 450 mg GML/kg bw per day). GML (at 300 and 450 mg GML/kg bw per day) enhanced in the gut the presence of microbiota considered as favourable according to the authors and attenuated metabolic effects induced by the HDF diet compared to controls.

Two studies on tumour promotion in several organs with ALA-DAG (Honda et al., 2017; documentation provided to EFSA n.1, 2, 4) or DAG oil (Ichihara et al., 2008; documentation provided to EFSA n.4) were available.

In the Honda et al. (2017) study, rats were treated with five genotoxic carcinogens (N-nitrosodiethylamine, N-butyl-N-(4-hydroxybutyl)nitrosamine, 1,2-dimethylhydrazine dihydrochloride, N-nitroso-N-methylurea and diisopropanolnitrosamine) to induce multi-organ tumorigenesis to investigate whether ALA-DAG oil promotes tumorigenesis in the tongue and gastrointestinal tract. More in details, rats were treated until week 4 with the genotoxic carcinogens. After a recovery period (1 week) rats were fed with a semi-synthetic diet (AIN-93G) containing ALA-DAG oil at 0, 13,750,

27,500, and 55,000 mg/kg (equivalent to 616, 1,223 and 2,397 mg/kg bw per day, respectively) (reference control group: AIN-93G containing 55,000 mg/kg ALA triacylglycerol; negative control group: standard basal diet). At week 30, animals were euthanised. No effects on survival, general condition, body weight, food consumption or organ weight were observed in the ALA-DAG oil groups. In the stomachs of the two highest treatment, ALA-DAG groups more discoloured spots than in the control groups were observed. However, there were no differences in the frequency of histopathological findings across groups and no toxicologically relevant increases in the incidence of pre-neoplastic and neoplastic lesions in the tongue and gastrointestinal tract across the groups. The authors, therefore, concluded that ALA-DAG oil does not promote tumour development in the digestive system. The Panel agreed with this conclusion.

Similarly to the Honda et al. (2017) study, Ichihara et al. (2008) investigated the tumour-promoting potential of diacylglycerol (DAG) oil. Six-week old male F344 rats (20/group) were treated first with five carcinogens for 4 weeks (N-nitrosodiethylamine, N-butyl-N-(4-hydroxybutyl)nitrosamine, 1,2-dimethylhydrazine dihydrochloride, N-nitroso-N-methylurea and diisopropanolnitrosamine; DMBDD treatment) and then administered a diet containing DAG oil at 0%, 1.375%, 2.75% or 5.5%. Controls received a diet with 5.5% TAG or standard diet. All animals were euthanised at week 28. No treatment-related changes were observed in survival, general conditions, body weights, food consumption and organ weights in the DAG oil treatment groups. DAG oil was not found to have any effects upon quantitative analysis of glutathione S-transferase placental form (GST-P) positive foci of the liver. The incidence of colon adenomas was significantly increased in rats given 1.375% DAG oil but not at the two highest dose groups when compared to the controls. Due to the absence of dose dependence, the increase at the lowest dose was not considered treatment related. The incidences of hyperplasia and adenoma and/or adenocarcinoma were comparable across all DAG oil-treatment groups. Preneoplastic and neoplastic lesions in the DMBDD treatment group in various organs other than the large intestine were comparable in all cases. The results of this study indicate that DAG oil does not exert modifying potential on tumour development in the colon or any other organ. Regarding risk assessment, treatment with DAG oil did not demonstrate adverse effects. The Panel agreed with this conclusion.

Further rat studies also reported on potential beneficial effects of DAG-rich oils which are not taken into consideration for this risk assessment (Dhara et al., 2012; Kim et al., 2007; Anikisetty et al., 2018; Li et al., 2018, Documentation provided to EFSA n. 4).

Overall, the additional rodent studies and the two studies in calves – Wieland et al., 2015; Ragionieri et al., 2016; documentation provided to EFSA n. 4- do not provide information relevant for the risk assessment of mono- and diglycerides of fatty acids – E 471 as food additive. Similarly, the additional literature studies from Bedford et al. (2018) and Mori et al. (2017) (Documentation provided to EFSA n. 1, 2, 4) do not provide additional information relevant for the risk assessment of mono- and -diglycerides of fatty acids – E 471 as food additive.

A review (Morita and Soni, 2009) on the safety of diacylglycerol oil was also retrieved from the literature (Documentation provided to EFSA n. 1, 2). The Panel noted that the studies relevant for the current assessment summarised in this review were already considered in this opinion or during the re-evaluation (EFSA ANS Panel, 2017a). Therefore, this review does not provide additional references relevant for the risk assessment of mono- and -diglycerides of fatty acids – E 471 as food additive.

3.6.5.3. Clinical data

No clinical data were provided and available.

A literature study on the use of UX007 (triheptanoin, medium odd-chain fatty acid) in the treatment of long-chain fatty acid oxidation disorders in children and adults was provided (Vockley et al. 2017; documentation provided to EFSA n. 1, 2), however, this study does not provide information relevant for the risk assessment of mono- and diglycerides of fatty acids – E 471 as food additive. Similarly, the additionally provided literature studies from Ando et al. (2017), Lee et al. (2017) and Saito et al. (2017) (documentation provided to EFSA n. 1, 2, 4) do not provide additional information relevant for the risk assessment of mono- and diglycerides of fatty acids – E 471 as food additive.

3.6.5.4. Post-marketing surveillance data

Post-marketing data were submitted for the period 1 January 2019 through 31 December 2019 (Documentation provided to EFSA n. 4). In this one year, 65 reports were sent to one IBO in total while roughly 2 million units were placed on the market for the products containing mono- and diglycerides of fatty acids, according to the information of the IBO. Among the 65 reports, no report

indicated a serious adverse reaction. The type of reaction was unspecific. A causal relationship of the reactions to the ingestion of E 471 was not demonstrated.

3.6.5.5. Comparative data on the content of mono- and diglycerides of fatty acids in human milk and in infant formula

The daily exposure via infant formula of mono- and diglycerides of fatty acids for infants below 16 weeks of age according to FC 13.1.1 is 418 (mean) to 543 (high) mg/kg bw per day for the scenario using the mean use levels reported by industry and 800 (mean consumption) to 1,040 (high-level consumption) mg/kg bw per day for the maximum use level. The daily exposure via special medical purposes and special formulas for infants below 16 weeks of age according to FC 13.1.5.1 is 418 (mean consumption) to 542 (high-level consumption) mg/kg bw per day for the scenario using the mean use levels reported by industry and 1,000 (mean consumption) to 1,300 (high-level consumption) mg/kg bw per day for the maximum use level.

Highly variable concentrations have been reported for the lipid content of breast milk, in particular for mono- and diacylglycerides (Bracco et al., 1972; Terai, 1979; Bitman et al., 1986).

In a review of Delplanque et al. (2015), the lipid content of breast milk amounts to 3.5–4.5 g/100 g milk. The main component is triacylglycerol (98.1–98.8%) whereas diacylglycerols (0.01–0.7%) and monoacylglycerols (trace amounts) are a minor part.

According to a scientific opinion of the NDA Panel (EFSA NDA Panel, 2014), the total average fat content of breast milk ranges from 24 to 59 g/L. The diglyceride content calculated by applying the percentage in Bracco et al. (1972), Terai et al. (1979) and Bitman et al. (1986) between 0.7% and 8.1% could vary from 168 to 4,779 mg/L which would correspond to 33.6–955.8 mg/kg bw per day for the mean consumption and 43.7–1,242.5 mg/kg bw per day for high-level consumption.

The Panel noted that E 471 can be composed of monoglycerides from 42% to 93%, diglycerides from 4% to 44% and triglycerides from 0% to 8%. Triglyceride exposure from the additive is far below the exposure to triglycerides from breast milk in which triglycerides are the major glyceride fraction. Thus, the focus of the comparison is on the exposure with mono- and diglycerides of fatty acids in human milk and in infant formula. Comparing the daily exposure to the sum of mono- and di-acylglycerols from breast milk and that resulting from the use of E 471 in the infant formula the resulting exposures are in the same order of magnitude for the infants below 16 weeks of age.

3.7. Discussion

The current assessment addresses data gaps previously identified during the re-evaluation of mono- and diglycerides of fatty acids (E 471) and the safety of E 471 when used in food for infants below 16 weeks of age in the food categories (FC) 13.1.1 (Infant formulae as defined by Commission Delegated Regulation (EU) 2016/127/EC) and 13.1.5.1 (Dietary foods for infants for special medical purposes and special formulae for infants) (EFSA ANS Panel, 2017a).

In response to the call for data, analytical data on levels of toxic elements (namely arsenic, lead, cadmium, mercury) in commercial samples of mono- and diglycerides of fatty acids (E 471) were provided by IBO. The Panel noted that the measured levels of these toxic elements are substantially lower than the lowest technologically achievable levels proposed by the IBO. The Panel performed a risk assessment considering the available data and available health based guidance values/reference points and concluded that the potential exposure to toxic elements resulting from the consumption of E 471 could be substantial.

The Panel noted that the MOS/MOE for arsenic and lead are very low. For arsenic the reference point is based on carcinogenicity for which the MOS/MOE should be at least 10,000 (EFSA Scientific Committee, 2005, 2012b). Considering that the human studies were the basis to derive the BMDL, an interspecies extrapolation factor may not be needed.

For lead, the reference point is based on a study demonstrating perturbation of intellectual development in children with the critical response size of 1 point reduction in IQ. In the scientific opinion on lead (EFSA CONTAM Panel, 2010), it is mentioned that a 1 point reduction in IQ is related to a 4.5% increase in the risk of failure to graduate from high school and that a decrease of 1 IQ in children can be associated with a decrease of later productivity of about 2%. If the exposure exceeds the BMDL01, the resulting risk has to be considered (MOS/MOE lower than 1).

Overall, on the basis of the data assessed, the Panel considered feasible to amend the EU specifications to lower existing maximum limits for toxic elements (arsenic, lead, cadmium, mercury) (see Table 14 and Appendix B).

Analytical data on the levels of acrolein and butanetriol were requested in line with the recommendations from the re-evaluation (EFSA ANS Panel, 2017a). According to the IBO, these two impurities were not detected in E 471 (see Section 3.1.3.2); therefore, the Panel concluded that there would be no need for including limits in the EU specifications for these carry over and process impurities. The Panel acknowledged that a numerical limit for acrolein in glycerol (E 422) is currently being considered as part of the follow-up of the re-evaluation of this food additive.[16] If a numerical limit for acrolein will be set in the EU specifications of glycerol (E 422), further consideration on the possible consequences for E 471 might be needed.

With respect to 3-MCPD, the Panel noted that the exposure level from the use of E 471 estimated by considering its presence in E 471 at the lowest technologically achievable level of 2.5 mg/kg proposed by the IBO would result in the exceedance of the TDI (Table 11). Therefore, the Panel considered that a limit value for 3-MCPD (free and bound), lower than the one proposed by the IBO, should be introduced in the current EU specifications for E 471. Maximum levels for 3-MCPD are set by Reg. (EC) No 1881/2006 and were used by the Panel to estimate the fraction of the level of 3-MCPD in E 471 with respect to the regulatory maximum levels in the final product (see Appendix B). Considering the results of these estimations, and the fact that the food additive is not the only potential source of 3-MCPD, the Panel emphasised the need to set specification limit values for this impurity in Regulation (EU) no 231/2012.

For GEs (expressed as glycidol), the Panel noted that, by applying the lowest technologically achievable levels proposed by the IBO to estimate the exposure, the resulting MOS/MOE would be substantially lower than the MOS/MOE of 25,000 proposed by the CONTAM Panel (Table 12). Therefore, the Panel considered that a limit value for GEs (expressed as glycidol), lower than the one proposed by the IBO, should be introduced in the current EU specifications for E 471. Maximum levels for GEs (expressed as glycidol) are set by Reg. (EC) No 1881/2006 and were used by the Panel to estimate the fraction of the level of GEs (expressed as glycidol) in E 471 with respect to the regulatory maximum levels in the final product (see Appendix B). Considering the results of these estimations, and the fact that the food additive is not the only potential source of GEs, the Panel emphasised the need to set specification limit values for these impurity in Regulation (EU) no 231/2012.

The Panel noted that the levels of *trans* fatty acids cover a wide range depending on the degree of hydrogenation of the raw material (non, partially or fully hardened). Based on the information from suppliers, only fully hydrogenated oils/fats are used for the production of E 471 in infant formula (13.1.1) and infant FSMPs (13.1.5.1).[11] The levels of *trans* fatty acids referred to as 'fully hardened' range between 0.01% and < 1%. In infant formula and follow-on formula, the *trans* fatty acid content is regulated and shall not exceed 3% of the total fat content (Regulation (EU) No 127/2016 supplementing Regulation (EU) No 609/2013).

For the general population, E 471 can be manufactured also from 'non-hardened' or 'partially hardened' fats. The content of *trans* fatty acids in these cases ranged between 0.01% and 59.92%. The Panel noted that '*latest national recommendations in the EU, and most recent recommendations from medical professional associations in Europe and the US indicate that consumption of TFA should be as low as possible*' (EFSA, 2018). On the basis of this advice, a maximum limit of 2 g of *trans* fat per 100 g fat in food for the final consumer was set by Regulation (EU) No 2019/649 amending Regulation (EC) No1925/2006).

The Panel performed a risk assessment for erucic acid (see Table 13). The calculations clearly indicate that the lowest technologically achievable level for erucic acid in E 471 as proposed by the interested business operator would lead to a considerable exceedance of the TDI. Therefore, the Panel considered that a limit value for erucic acid, lower than the one proposed by the IBO, should be introduced in the current EU specifications for E 471. The conclusion of the Panel is supported by a recent risk assessment of the German Federal Institute for Risk Assessment (BfR, 2021) which concluded that the regulatory limit (Delegated Regulation (EU) 2019/828) for erucic acid in infant formula and follow-on formula (0.4% of the total fat content) may not be protective for the total dietary exposure towards erucic acid.

According to the received information following the call for data, the manufacturing process of E 471 does not involve the use of any solvents; therefore, the Panel concluded that there is no need for including limits in the EU specifications for solvents.

Overall, the Panel considered it feasible to amend the EU specifications based on the information submitted in response to the call for data. This refers to lowering existing limits for toxic elements (arsenic, lead, cadmium, mercury) and to include limits for GEs, 3-MCPD and erucic acid (see Table 14).

No new information was provided concerning ADME and acute toxicity. The Panel considered that the results of the previously available (see EFSA ANS Panel, 2017a) study in 5-week-old male Sprague Dawley rats (Chen et al., 2013) are representative for the infants below 16 weeks of age. The newly submitted 90-day toxicity study does not show adverse effect up to the highest dose tested (5.5%) and supports the finding reported in the re-evaluation (EFSA ANS Panel, 2017a). Limitations in the reporting and study design prohibit the use of the newly available prenatal developmental toxicity study for hazard characterisation. No study in a neonatal animal model has been provided. However, in the ANS Panel opinion (2017a), a two-generation reproduction toxicity study has been described which provides information on this life period (Morita et al., 2008). In this study, no adverse effects have been observed at the highest dose tested (4,630 mg diacylglycerol/kg bw per day). The Panel considered that the information available covers also the neonatal life period and, therefore, studies in neonatal animal model are not requested (EFSA Scientific Committee, 2017). No clinical studies were available in which the safety of mono and diglycerides of fatty acids was investigated. Post-marketing data provided by the IBO, no report indicated a serious adverse reaction. The type of reaction was unspecific. A causal relationship of the reactions to the ingestion of E 471 was not demonstrated.

Overall, the Panel considered that there are no indications of adverse effects from the animal studies at the highest dose tested and from the post marketing data.

A comparison was made between the daily exposure to the sum of mono- and di-acylglycerols from breast milk and that resulting from the use of E 471 in the infant formula. The Panel noted that the resulting exposures are in the same order of magnitude.

Dietary exposure to mono- and diglycerides of fatty acids (E 471) for infants below 16 weeks of age from their uses as food additives was assessed based on (1) MPLs set out in the EU legislation (defined as the regulatory maximum level exposure assessment scenario) and (2) the reported use levels (defined as the refined exposure assessment scenario). Both scenarios are based on the recommended consumption levels from Scientific Committee Guidance (EFSA Scientific Committee, 2017) which recommends values of 200 and 260 mL formula/kg bw per day as conservative mean and high-level consumption values for 14- to 27-day-old infants.

For infants below 16 weeks of age consuming infant formulae (FC 13.1.1), exposure to mono- and diglycerides of fatty acids (E 471) in the regulatory maximum level exposure assessment scenario was estimated at 800 mg/kg bw per day for mean consumption, while at the high-level consumption was estimated at 1,040 mg/kg bw per day. Exposure estimates are the same in the refined scenario using the maximum use level reported by industry as this maximum equals the MPL. In the refined estimated exposure assessment scenario using the mean of the reported use levels from industry, exposure estimates for mono- and diglycerides of fatty acids (E 471) were of 418 mg/kg bw per day at the mean and 543 mg/kg bw per day at the high level of consumption.

For infants below 16 weeks of age consuming special infant formulae (FC 13.1.5.1), exposure to mono- and diglycerides of fatty acids (E 471) in the regulatory maximum level exposure assessment scenario was estimated at 1,000 mg/kg bw per day for mean consumption while at the high-level consumption was estimated at 1,300 mg/kg bw per day. Exposure estimates are the same in the refined scenario using the maximum use level reported by industry as this maximum equals the MPL. In the refined estimated exposure assessment scenario using the mean of the reported use levels from industry, exposure estimates for mono- and diglycerides of fatty acids (E 471) were of 418 mg/kg bw per day at the mean and 543 mg/kg bw per day at the high level of consumption.

4. Conclusions

The Panel concluded that there are no indications of adverse effects from the animal studies at the highest dose tested and from the post marketing data and based its approach to assess the safety of mono and diglycerides of fatty acids (E 471) for infants below 16 weeks of age on a comparison of the daily intake of mono-and diacylglycerides (and the fatty acids released from them) from human milk and from infant formula containing E 471. After reviewing the available data, the Panel concluded that the exposures are in the same order of magnitude. Overall, the Panel concluded that there is no reason for a safety concern when E 471 is used as food additive in FC 13.1.1 and 13.1.5.1 and according to the Annex III to Regulation (EC) No 1333/2008.

The Panel concluded that the risk assessment for toxic elements clearly indicates the need to lower the current maximum limits for arsenic, lead, cadmium and mercury set for E 471.

The estimates of the exposure to impurities indicate the exceedance of the respective reference values for 3-MCPD for infants below 16 weeks of age and for GEs and erucic acid for all population groups.

5. Recommendations

The Panel recommends the European Commission to consider deleting the synonyms given for E 471 in Commission Regulation (EU) No 231/2012 since they are not actually synonyms of the food additive E 471.

The Panel recommends the European Commission to consider revising the current specifications for the food additive E 471 in line with the proposals made on the basis of the information provided and based on the considerations of the Panel, this means:

- To lower the limits of lead, cadmium, mercury and arsenic
- To include limits for 3-MCPD, GEs (expressed as glycidol) and erucic acid

6. Documentation as provided to EFSA

1) European Food Emulsifiers Manufacturers Association (EFEMA), 2020. Submission of data in response to the call for technical and toxicological data on mono- and diglycerides of fatty acids (E 471) for uses as a food additive in foods for all population groups including infants below 16 weeks of age. Submitted by EFEMA on 17 December 2020.
2) European Food Emulsifiers Manufacturers Association (EFEMA), 2021. Clarification on the data submitted in response to the call for technical and toxicological data on mono- and diglycerides of fatty acids (E 471) for uses as a food additive in foods for all population groups including infants below 16 weeks of age. Submitted by EFEMA on 11 June 2021.
3) European Food Emulsifiers Manufacturers Association (EFEMA), 2021. MCPDs and GEs levels and typical use levels (30.3.2021). Submitted by European Commission to EFSA on behalf of EFEMA on 9 April 2021.
4) Specialised Nutrition Europe (SNE), 2020. Submission of data in response to the call for technical and toxicological data on mono- and diglycerides of fatty acids (E 471) for uses as a food additive in foods for all population groups including infants below 16 weeks of age. Submitted by SNE on 18 December 2020.
5) Specialised Nutrition Europe (SNE), 2021. Clarification on the data submitted in response to the call for technical and toxicological data on mono- and diglycerides of fatty acids (E 471) for uses as a food additive in foods for all population groups including infants below 16 weeks of age. Submitted by SNE on 4 June 2021.

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Abbreviations

3-MCPD	3-monochloropropane diol
ADI	acceptable daily intake
ADME	absorption, distribution, metabolism, excretion
ALA-DAG	oil alpha linoleic-enriched diacylglycerol oil
ANS Panel	EFSA Panel on Food Additives and Nutrient Sources added to Food
BMDL	benchmark dose (lower confidence limit)
bw	body weight
CAS	Chemical Abstract Service
FAF Panel	Panel on Food Additives and Flavourings
FAO/WHO	Food and Drug Organisation/World Health Organisation
FC	Food category
FSMP	Food for special medical purposes
GEs	Glycidyl esters
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LOAEL	lowest-observed-adverse-effect level
Mintel	GNPD Mintel's Global New Products Database
MOE	margin of exposure
MOS	margin of safety
MPL	maximum permitted levels
NOAEL	no-observed-adverse-effect level
NOEL	no-observed-effect level
PND	postnatal day
SC	Scientific Committee of EFSA
SCF	Scientific Committee on Food
T25	the chronic dose rate in mg/kg bw per day, which will give 25% of the animal tumours at a specific tissue site, after specific correction for the spontaneous incidence within the standard life time of that species
TDI	Tolerable Daily Intake
WG	Working Group

Appendix A – Data requested in the call for data (Call for technical and toxicological data on mono- and diglycerides of fatty acids (E 471) for uses as food additive in foods for all population groups including infants below 16 weeks of age.[17]

Kind of data	Data requested in the call for data	Responses from interested parties	Comment
A. Information regarding the follow-up of the conclusions and the recommendations of the EFSA ANS Panel opinion on the safety of mono- and diglycerides of fatty acids (E 471) as food additives			
1. Technical data	• analytical data on current levels of lead, mercury, cadmium and arsenic in commercial samples of the food additive; • the lowest technologically achievable level for lead, mercury, cadmium, and arsenic in order to adequately define their maximum limits in the specifications; • analytical data on current levels of impurities of toxicological concern (e.g. butanetriols, acrolein, chlorinated compounds and 3-monochloropropane-1,2-diol) as identified in the EU specifications of the food additive glycerol (E 422)- which can be used in the manufacturing process of E 471- in commercial samples of the food additive E 471; • the lowest technologically achievable level for impurities of toxicological concern (e.g. butanetriols, acrolein, chlorinated compounds and 3-monochloropropane-1,2-diol) in order to adequately define their maximum limits in the specifications of E 471; • analytical data on current levels of any impurity present in glycerol as mentioned in the call for data on glycerol (E 422)-which can be used in the manufacturing process of E 471- in commercial samples of the food additive E 471; • the lowest technologically achievable level for any impurity which could be formed during the manufacturing processes of glycerol and be present in E 471, in order to adequately define their maximum limits in the specifications of E 471; • the lowest technologically achievable level for residual solvents which can be used in the manufacturing process of mono- and diglycerides of fatty acids (E 471), i.e. tert-butanol or tert-pentanol. • the lowest technologically achievable level for *trans* fatty acids because mono- and diglycerides of fatty acids (E 471) can be manufactured by glycerolysis of hydrogenated fats and/or oils, which contain significant amounts of *trans* fatty acids. • the lowest technologically achievable level for erucic acid since erucic acid can be	Received	Assessed, no further follow up.

[17] Available online: https://www.efsa.europa.eu/sites/default/files/consultation/callsfordata/2018-00953_Call-for-data_mono-and-di-glycerides-of-fatty-acids-E471.pdf and responses from interested parties

Kind of data	Data requested in the call for data	Responses from interested parties	Comment
	present among the fatty acids in edible oils, which can be used for manufacturing of mono and diglycerides of fatty acids (E 471). • the lowest technologically achievable level of any compound of toxicological concern (e.g. 3- MCPD or GEs), which can be produced under certain processing conditions from the food additive mono- and diglycerides of fatty acids (E 471).		
2. Toxicological data	The risk characterisation at the lowest technologically achievable level of any compound of toxicological concern (e.g. 3-MCPD or glycidyl esters) from the use of mono- and diglycerides of fatty acids (E 471) as food additive.	Assessed	Assessed, no further follow up.
3. Literature searches	Literature searches should be conducted relevant for the safety evaluation of mono- and diglycerides of fatty acids (E 471) for all uses in foods for all population groups from 01/09/2017 up to the date of the data submission, as described in the Guidance for submission for food additive evaluations (section 5.3).	Received	Assessed, no further follow up.
B. Information required for the risk assessment of mono- and diglycerides of fatty acids (E 471) as food additive for use in foods for infants below 16 weeks of age			
1. Technical data	For the uses of mono- and diglycerides of fatty acids (E 471), in the infant formulae for use in infants below 16 weeks (food categories 13.1.1 and 13.1.5.1) EFSA seeks: • information on the levels of use of mono- and diglycerides of fatty acids (E 471), alone or in combination with food additives E 322, E 472c and E 473; • information on the fate and the reaction products of mono- and diglycerides of fatty acids (E 471); • information on particular specification requirements for identity and purity of mono and diglycerides of fatty acids (E 471) as described in section A.1.	Received	Assessed, no further follow up.
2. Toxicological data	Within the frame of the EFSA Guidance of the Scientific Committee on the risk assessment of substances present in food intended for infants below 16 weeks of age the following information on the toxicological properties of mono- and diglycerides of fatty acids (E 471) and its adverse effects relevant for their use in formulae and foods for special medical purposes (FSMP) for infants below 16 weeks is required: • post-marketing surveillance reports on undesired and adverse reactions, indicating the ages and other relevant data of the exposed infants and young children and the use levels in the marketed products • published and unpublished case reports (e.g. available nutrivigilance data) on undesired and adverse effects,	Received	Assessed, no further follow up.

Kind of data	Data requested in the call for data	Responses from interested parties	Comment
	associated with the oral administration of mono- and diglycerides of fatty acids, to infants and young children.		
3. Literature searches	Literature searches relevant for the safety evaluation of mono- and diglycerides of fatty acids (E 471) when used in foods for infants below 16 weeks of age, should be conducted as described in the Guidance for submission for food additive evaluations (section 5.3).	Received	Assessed, no further follow up.

Appendix B – Estimation of the fraction of the levels of toxic elements and other impurities in E 471 with respect to the regulatory maximum levels in the final food product for which the additive is used

B.1. Toxic Elements

The Panel estimated the fraction (%) of the levels of the toxic elements **lead** and **cadmium** in E 471 with respect to the regulatory maximum levels in the final product (formulae) as sold as laid down in Regulation (EC) No 1881/2006[23] considering:

- The current specification for lead and cadmium for E 471 according to Regulation (EU) No 231/2012, 2 and 1 mg/kg, respectively.
- The lowest technically achievable levels of lead and cadmium in commercial E 471 products, 1 and 0.2 mg/kg, respectively, as proposed by one interested business operator (Documentation provided to EFSA n. 1, 2), see also Section 3.5.
- The maximum permitted use level of E 471 in the final food of 4,000 mg/kg in FC 13.1.1, and 5,000 mg/kg in FC 13.1.5.1, and the mean use level reported by industry (2,089 mg/kg) for the uses in food for infants below 16 weeks of age, see Section 3.3.1
- The range of maximum levels (ML) for lead (0.01–0.02 mg/kg) and cadmium (0.005–0.02 mg/kg) in formulae for infants as laid down in Regulation (EC) No 1881/2006

The results of the calculations can be found in Tables B.1 and B.2 for lead and Tables B.3 and B.4 for cadmium.

Table B.1: Estimation of the fraction of the levels of lead in E 471 with respect to the regulatory maximum levels in the final product (liquid formulae for infants below 16 weeks of age)

Specification for toxic elements status	Lead (mg/kg)	Use level of food additive in final product (mg/kg)	Concentration of toxic element in final product (mg/kg)	Maximum level in Reg. 1881/2006 (mg/kg)	Fraction of toxic element from FA on ML of final product ML (%)
Current EU specification	2.0	4,000	0.0080	0.010	80.0
Current EU specification	2.0	2,089	0.0042	0.010	41.8
Current EU specification	2.0	5,000	0.0100	0.010	100.0
Proposal IBO	1.0	4,000	0.0040	0.010	40.0
Proposal IBO	1.0	2,089	0.0021	0.010	20.9
Proposal IBO	1.0	5,000	0.0050	0.010	50.0

Table B.2: Estimation of the fraction of the levels of lead in E 471 with respect to the regulatory maximum levels in the final product (powder formulae for infants below 16 weeks of age)

Specification for toxic elements status	Lead (mg/kg)	Use level of food additive in final product (mg/kg) as reconstituted[(a)]	Use level considering the dilution[(b)]	Concentration of toxic element in final product (mg/kg)	Maximum level in Reg. 1881/2006 (mg/kg) as sold	Fraction of toxic element from FA on ML of final product ML (%)
Current EU specification	2.0	4,000	32,000	0.0640	0.020	320
Current EU specification	2.0	2,089	16,712	0.0334	0.020	167
Current EU specification	2.0	5,000	40,000	0.0800	0.020	400
Proposal IBO	1.0	4,000	32,000	0.0320	0.020	160
Proposal IBO	1.0	2,089	16,712	0.0167	0.020	84

Specification for toxic elements status	Lead (mg/kg)	Use level of food additive in final product (mg/kg) as reconstituted[(a)]	Use level considering the dilution[(b)]	Concentration of toxic element in final product (mg/kg)	Maximum level in Reg. 1881/2006 (mg/kg) as sold	Fraction of toxic element from FA on ML of final product ML (%)
Proposal IBO	1.0	5,000	40,000	0.0400	0.020	200

(a): The maximum levels of food additives set out in Annex II shall apply to the food as marketed, unless otherwise stated. By way of derogation from this principle, for dried and/or concentrated foods which need to be reconstituted the maximum levels shall apply to the food as reconstituted according to the instructions on the label taking into account the minimum dilution factor.

(b): Internal report on the harmonisation of dilution factors to be used in the assessment of dietary exposure, EFSA, 2018, available online https://zenodo.org/record/1256085#.X89vU9hKiUk.

Table B.3: Estimation of the fraction of the levels of cadmium in E 471 with respect to the regulatory maximum levels in the final product (liquid formulae for infants below 16 weeks of age, marketed as powder and manufactured from cow's milk proteins or from cow's milk protein hydrolysates)

Specification for toxic elements status	Cadmium (mg/kg)	Use level of food additive in final product (mg/kg) as reconstituted[(a)]	Concentration of toxic element in final product (mg/kg)[(b)]	Maximum level in Reg. 1881/2006 (mg/kg)	Fraction of toxic element from FA on ML of final product ML (%)
Current EU specification	1.0	4,000	0.004	0.005	80.0
Current EU specification	1.0	2,089	0.002	0.005	41.8
Current EU specification	1.0	5,000	0.005	0.005	100.0
Proposal IBO	0.2	4,000	0.001	0.005	16.0
Proposal IBO	0.2	2,089	0.0004	0.005	8.4
Proposal IBO	0.2	5,000	0.001	0.005	20.0

(a): The maximum levels of food additives set out in Annex II shall apply to the food as marketed, unless otherwise stated. By way of derogation from this principle, for dried and/or concentrated foods which need to be reconstituted the maximum levels shall apply to the food as reconstituted according to the instructions on the label taking into account the minimum dilution factor.

(b): Internal report on the harmonisation of dilution factors to be used in the assessment of dietary exposure, EFSA, 2018, available at https://zenodo.org/record/1256085#.X89vU9hKiUk.

Considering the maximum level of 0.01 mg/kg for infant formulae 'marketed as powder and manufactured from soya protein isolates, alone or in a mixture with cow's milk proteins protein', the fraction of toxic elements from the food additive on the ML of the final product would be half of the respective value in the last column.

Table B.4: Estimation of the fraction of the levels of cadmium in E 471 with respect to the regulatory maximum levels in the final product (powder formulae for infants below 16 weeks of age, marketed as powder and manufactured from cow's milk proteins or from cow's milk protein hydrolysates)

Specification for toxic elements status	Cadmium (mg/kg)	Use level of food additive in final product (mg/kg)[(a)]	Use level considering the dilution[(b)]	Concentration of toxic element in final product (mg/kg)	Maximum level in Reg. 1881/2006 (mg/kg)	Fraction of toxic element from FA on ML of final product ML (%)
Current EU specification	1.0	4,000	32,000	0.032	0.010	320
Current EU specification	1.0	2,089	16,712	0.017	0.010	167
Current EU specification	1.0	5,000	40,000	0.040	0.010	400

Specification for toxic elements status	Cadmium (mg/kg)	Use level of food additive in final product (mg/kg)[(a)]	Use level considering the dilution[(b)]	Concentration of toxic element in final product (mg/kg)	Maximum level in Reg. 1881/2006 (mg/kg)	Fraction of toxic element from FA on ML of final product ML (%)
Proposal IBO	0.2	4,000	32,000	0.006	0.010	64
Proposal IBO	0.2	2,089	16,712	0.003	0.010	33
Proposal IBO	0.2	5,000	40,000	0.008	0.010	80

(a): The maximum levels of food additives set out in Annex II shall apply to the food as marketed, unless otherwise stated. By way of derogation from this principle, for dried and/or concentrated foods which need to be reconstituted the maximum levels shall apply to the food as reconstituted according to the instructions on the label taking into account the minimum dilution factor.

(b): Internal report on the harmonisation of dilution factors to be used in the assessment of dietary exposure, EFSA, 2018, available at https://zenodo.org/record/1256085#.X89vU9hKiUk.

Considering the maximum level of 0.02 mg/kg for 'infant formulae marketed as powder and manufactured from soya protein isolates, alone or in a mixture with cow's milk proteins protein', the fraction of toxic elements from the food additive on the ML of the final product would be half of the respective value in the last column.

Considering the results of the above estimations and the fact that the food additive is not the only potential source of toxic elements, the Panel emphasises the need to reduce the specification limit values for lead and cadmium in Regulation (EU) no 231/2012.

B.2. Other impurities

The Panel estimated the fraction (%) of the levels of the **3-MCPD and GEs** in E 471 with respect to the regulatory maximum levels in the final product (formulae) as sold as laid down in Regulation (EC) No 1881/2006[23] considering:

- The lowest technically achievable levels of 3-MCPD and GEs in commercial E 471 products, 2.5 and 10 mg/kg, respectively, as proposed by one interested business operator (Documentation provided to EFSA n. 1, 2, 3), see also Section 3.5.
- The maximum permitted use level of E 471 in the final food of 4,000 mg/kg in FC 13.1.1, and 5,000 mg/kg in FC 13.1.5.1, and the mean use level reported by industry (2,089 mg/kg) for the uses in food for infants below 16 weeks of age, see Section 3.3.1.
- The range of maximum levels (ML) for 3-MCPD and GEs in formulae for infants as laid down in Regulation (EC) No 1881/2006.

The results of the calculations can be found in Tables B.5 and B.6 for 3-MCPD and Tables B.7 and B.8 for GEs.

Table B.5: Estimation of the fraction of the levels of 3-MCPD in E 471 with respect to the regulatory maximum levels in the final product (liquid formulae for infants below 16 weeks of age)

Specification for total 3-MCPD status	Total 3-MCPD (mg/kg)	Use level of food additive in final product (mg/kg)	Concentration of total 3-MCPD in final product (mg/kg)	Maximum level in Reg. 1881/2006 (mg/kg)	Fraction of total 3-MCPD from FA on ML of final product ML (%)
Proposal IBO	2.5	4,000	0.0100	0.015	67
Proposal IBO	2.5	2,089	0.0052	0.015	35
Proposal IBO	2.5	5,000	0.0125	0.015	83

Table B.6: Estimation of the fraction of the levels of 3-MCPD in E 471 with respect to the regulatory maximum levels in the final product (powder formulae for infants below 16 weeks of age)

Specification for total 3-MCPD status	Total 3-MCPD (mg/kg)	Use level of food additive in final product as reconstituted (mg/kg)[a]	Use level considering the dilution[b]	Concentration of total 3-MCPD in final product (mg/kg)	Maximum level in Reg. 1881/2006 (mg/kg)	Fraction of total 3-MCPD from FA on ML of final product ML (%)
Proposal IBO	2.5	4,000	32,000	0.0800	0.125	64
Proposal IBO	2.5	2,089	16,712	0.0418	0.125	33
Proposal IBO	2.5	5,000	40,000	0.1000	0.125	80

(a): The maximum levels of food additives set out in Annex II shall apply to the food as marketed, unless otherwise stated. By way of derogation from this principle, for dried and/or concentrated foods which need to be reconstituted the maximum levels shall apply to the food as reconstituted according to the instructions on the label taking into account the minimum dilution factor.
(b): Internal report on the harmonisation of dilution factors to be used in the assessment of dietary exposure, EFSA, 2018, available at https://zenodo.org/record/1256085#.X89vU9hKiUk.

Table B.7: Estimation of the fraction of the levels of GE in E 471 with respect to the regulatory maximum levels in the final product (liquid formulae for infants below 16 weeks of age)

Specification for GEs	GEs (mg/kg)	Use level of food additive in final product (mg/kg)	Concentration of GEs in final product (mg/kg)	Maximum level in Reg. 1881/2006 (mg/kg)	Fraction of GEs from FA on ML of final product ML (%)
Proposal IBO	10.0	4,000	0.040	0.006	667
Proposal IBO	10.0	2,089	0.021	0.006	348
Proposal IBO	10.0	5,000	0.050	0.006	833

Table B.8: Estimation of the fraction of the levels of GEs in E 471 with respect to the regulatory maximum levels in the final product (powder formulae for infants below 16 weeks of age)

Specification for GEs status	GEs (mg/kg)	Use level of food additive in final product as reconstituted (mg/kg)[a]	Use level considering the dilution[b]	Concentration of GEs in final product (mg/kg)	Maximum level in Reg. 1881/2006 (mg/kg)	Fraction of GEs from FA on ML of final product ML (%)
Proposal IBO	10	4,000	32,000	0.320	0.05	640
Proposal IBO	10	2,089	16,712	0.167	0.05	334
Proposal IBO	10	5,000	40,000	0.400	0.05	800

(a): The maximum levels of food additives set out in Annex II shall apply to the food as marketed, unless otherwise stated. By way of derogation from this principle, for dried and/or concentrated foods which need to be reconstituted the maximum levels shall apply to the food as reconstituted according to the instructions on the label taking into account the minimum dilution factor.
(b): Internal report on the harmonisation of dilution factors to be used in the assessment of dietary exposure, EFSA, 2018, available at https://zenodo.org/record/1256085#.X89vU9hKiUk.

Considering the results of the above estimations and the fact that the food additive is not the only potential source of 3-MCPD and GEs, the Panel emphasises the need to set specification limit values for these impurities in Regulation (EU) no 231/2012.