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Re-evaluation of polyglycerol polyricinoleate (E 476) as a food additive

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Abstract

The Panel on Food Additives and Nutrient Sources added to Food (ANS) provides a scientific opinion re-evaluating the safety of polyglycerol polyricinoleate (PGPR, E 476) used as a food additive. In 1978, the Scientific Committee for Food (SCF) established an acceptable daily intake (ADI) of 7.5 mg/kg body weight (bw) per day for PGPR. PGPR is hydrolysed in the gut resulting in the liberation of free polyglycerols, polyricinoleic acid and ricinoleic acid. Di- and triglycerol are absorbed and excreted unchanged in the urine; long-chain polyglycerols show lower absorption and are mainly excreted unchanged in faeces. Acute oral toxicity of PGPR is low, and short-term and subchronic studies indicate PGPR is tolerated at high doses without adverse effects. PGPR (E 476) is not of concern with regard to genotoxicity or carcinogenicity. The single reproductive toxicity study with PGPR was limited and was not an appropriate study for deriving a health-based guidance value. Human studies with PGPR demonstrated that there is no indication of significant adverse effect. The Panel considered a 2-year combined chronic toxicity/carcinogenicity study for determining a reference point and derived a no observed adverse effect level (NOAEL) for PGPR (E 476) of 2,500 mg/kg bw per day, the only dose tested. Therefore, the Panel concluded that the present data set give reason to revise the ADI of 7.5 mg/kg bw per day allocated by SCF to 25 mg/kg bw per day. Exposure estimates did not exceed the ADI of 25 mg/kg bw per day and a proposed extension of use would not result in an exposure exceeding this ADI. The Panel recommended modification of the EU specifications for PGPR (E 476).

Keywords: polyglycerol polyricinoleate, food additive, E 476, emulsifier, PGPR, CAS Registry Number 68936-89-0

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Summary

Following a request from the European Commission, the Panel on Food Additives and Nutrient Sources added to Food (ANS) was asked to re-evaluate the safety of polyglycerol polyricinoleate (PGPR; E 476) when used as a food additive. In addition, following an application dossier for a modification of the conditions for use of polyglycerol polyricinoleate, the European Commission requested the Panel evaluate the safety of the proposed extension of use.

The Panel was not provided with a newly submitted dossier for the re-evaluation of the food additive and based its assessment on previous evaluations and reviews, additional literature that came available since then and the data available following a public call for data. The Panel noted that not all original studies on which previous evaluations were based were available for re-evaluation by the Panel.

PGPR (E 476) is authorised as a food additive in the European Union (EU) according to Annex II and Annex III of Regulation (EC) No 1333/2008 and specific purity criteria have been defined in the Commission Regulation (EU) No 231/2012.

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) evaluated PGPR in 1969 and in 1974 (JECFA, 1969, 1974) and established on the basis of a reproductive toxicity study in rats an acceptable daily intake (ADI) of 0–7.5 mg/kg body weight (bw) per day.

The Scientific Committee for Food (SCF) established an ADI of 7.5 mg/kg bw per day for PGPR (SCF, 1978). PGPR was also evaluated by the Nordic Council of Ministers (TemaNord, 2002). It was concluded that 'the toxicological data available to JECFA in 1973 or to SCF in 1977 did not include all the data, which are normally required for an ADI to be set for a food additive. However, later data confirm the safety of the substance within the ADI, and taken together with the limited exposure, there seems to be no need for a re-evaluation'.

Since PGPR is a mixture of reaction products formed by the esterification of polyglycerols with condensed castor oil fatty acids, relevant information on existing authorisations and evaluations concerning these moieties have also been examined.

JECFA (1979) considered that castor oil had a long history of use as a laxative and aside from these effects, has been used apparently without harm. JECFA concluded on this ADI on the basis that at levels of exposure that cause laxation, castor oil might be expected to inhibit the absorption of fat soluble nutrients – notably vitamins A and D – and therefore, use of castor oil should be kept well below levels where absorption would be inhibited. JECFA considered that at doses of 4 g in adults (approximately 70 mg/kg bw per day), absorption appears to be complete and may be considered as a 'no-effect level' in man. In the absence of adequate long-term relevant toxicological studies, a conservative margin of safety was applied to derive an ADI of 0–0.7 mg/kg bw per day.

The SCF (2003) allocated an ADI of 0.7 mg/kg bw per day for ricinoleic acid. In its opinion, the SCF endorsed the JECFA ADI of 0–0.7 mg/kg bw per day for castor oil and considered that ricinoleic acid, as the main constituent of castor oil, should be allocated with the same numerical ADI.

The EFSA Panel on Contaminants in the Food Chain (CONTAM) considered the safety of ricinoleic acid as an acceptable previous cargo for edible fats and oils and concluded that it was not of toxicological concern nor was there any concern regarding possible allergenicity. No reaction products of toxicological concern were known or anticipated.

The safety of polyglycerol for use in food contact materials has been reviewed by the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF). The CEF Panel concluded that polyglycerol does not raise a safety concern for the consumer if the substance is only to be used as plasticiser at a maximum use level of 6.5% w/w in polymer blends of aliphatic-aromatic polyesters in contact with all types of food for any time at room temperature and below.

The ANS Panel re-evaluated the safety of glycerol (E 422) when used as a food additive and concluded that there is no need for a numerical ADI and no safety concern regarding the use of glycerol (E 422) as a food additive. However, it also concluded that the manufacturing process of glycerol should not allow the production of glycerol (E 422) that contains genotoxic and carcinogenic residuals at a level which would result in a margin of exposure below 10,000.

From the available *in vivo* absorption, distribution, metabolism and excretion (ADME) studies with oral dosing in rats, the Panel considered that PGPR was hydrolysed in the gut resulting in the liberation of free polyglycerols, polyricinoleic acid and free ricinoleic acid after oral dosing in rats. The absorption of polyglycerols depends on the chain length, e.g. di- and triglycerol were nearly completely absorbed and excreted unchanged in the urine, whereas long-chain polyglycerols like decaglycerol showed lower absorption rates and were mainly excreted unchanged in the faeces. No metabolism and no

accumulation of polyglycerols were observed. The Panel considered that castor oil was hydrolysed in the gastrointestinal tract to glycerol and ricinoleic acid and the ricinoleic acid moiety was absorbed and subjected to similar distribution, metabolism and excretion as orally administered ricinoleic acid. Ricinoleic acid itself was shown to be readily incorporated into the fatty acid pathway, and after oral dosing for up to 30 days, an accumulation of hydroxy acids by 5% of fatty acids in fat tissue was noted. An analysis of adipose tissue indicated an occurrence of shorter chain hydroxyl acids other than ricinoleic acid. No studies were available for polyricinoleic acid.

The Panel considered the acute oral toxicity of PGPR and of polyglycerols is low.

The Panel considered that the increases in liver weight observed in short-term and subchronic toxicity studies were adaptive – not an adverse – response of the liver to the large amount of PGPR administered to the animals (often a very high single dose level, e.g. 16,200 mg/kg bw per day). The Panel further considered that diets containing up to 10% castor oil was not associated with toxicity to any specific organ, organ system, or tissue and identified no observed adverse effect levels (NOAELs) of 20,000 and 9,000 mg/kg bw per day for castor oil in mice and rats, respectively, the highest doses tested. No studies were available for ricinoleic acid, polyricinoleic acid and polyglycerols.

No genotoxicity data on PGPR and polyglycerol were available; however, data were available on castor oil and sodium ricinoleate. Overall, the Panel considered that PGPR (E 476) was not of concern with regard to genotoxicity.

The Panel considered that the increases in liver weight observed in chronic and carcinogenicity studies were an adaptive – not an adverse – response of the liver and that PGPR did not demonstrate any carcinogenic effects in the available studies at doses up to 7,500 and 4,500 mg/kg bw per day in mice and rats, respectively, the only doses tested. No studies were available for ricinoleic acid, castor oil, polyricinoleic acid and polyglycerols. Overall, the Panel considered a combined 2-year combined chronic toxicity/carcinogenicity study as the critical study for determining a reference point because this combination of studies examined the most extensive range of endpoints including histopathological examinations of reproductive organs. The Panel considered that the no observed adverse effect level (NOAEL) for PGPR (E 476) from this study was 2,500 mg/kg bw per day, the only dose tested.

No data on the developmental toxicity of PGPR, ricinoleic acid, castor oil, polyricinoleic acid and polyglycerols were available. The single reproductive toxicity study with PGPR was considered to have limitations in study design (not comparable with a two-generation reproductive toxicity study); in that the number of animals initially pregnant in the test group was low (n = 13); in that there was infection in the animals; in that there was reduced breeding in the second generation and in the limited reporting of the study. The Panel noted that this reproductive study was used by the SCF (and by JECFA) to derive the current ADI of 7.5 mg/kg bw per day. However, the Panel considered that due to the above limitations, this study was not an appropriate study for deriving a health-based guidance value for PGPR.

The Panel considered that human studies with PGPR demonstrated that there is no indication of significant adverse effect after exposure up to 10,000 mg per day (equivalent to 142.8 mg/kg bw per day) over 3 weeks. In clinical studies with castor oil used at a dose of 960 mg/kg bw, only minor gastrointestinal discomfort was reported. No human studies were available for ricinoleic acid, polyricinoleic acid and polyglycerols.

The Panel considered that although the only reproductive toxicity study had limitations and no data were available regarding potential developmental toxicity of PGPR, an additional uncertainty factor was not required because the oral 2-year combined chronic toxicity/carcinogenicity study in rats included histopathology of reproductive organs and no changes were observed. In addition, at markedly higher doses (up to 13,000 mg/kg bw per day in mice and 16,200 mg/kg bw per day in rats), no adverse effects were observed in other chronic studies in rats and a carcinogenicity study in mice. Furthermore, no adverse effects were observed in the limited reproductive toxicity study.

Considering the available toxicological database and based on the absence of adverse effects in an oral 2-year combined chronic toxicity/carcinogenicity study from which a NOAEL of 2,500 mg PGPR/kg bw per day, the highest dose tested, was identified and applying an uncertainty factor of 100, the Panel derived an ADI of 25 mg PGPR/kg bw per day. Therefore, the Panel considered that the available data set give reason to revise the ADI of 7.5 mg/kg bw per day allocated by SCF in 1978, to a new ADI of 25 mg/kg bw per day.

The Panel concluded that PGPR (E 476) as a food additive at the permitted or reported use and use levels would not be of safety concern considering that exposure estimates did not exceed the ADI of 25 mg/kg bw per day in any of the exposure scenarios for any population group both at the mean and the 95th percentile.

The Panel also calculated the exposure to PGPR (E 476) considering an additional use in emulsified sauces, including mayonnaise, using the *regulatory maximum level exposure assessment scenario*. The Panel also concluded that the proposed extension of use of PGPR (E 476) at 4,000 mg/kg in the food category emulsified sauces would not result in an exposure to PGPR (E 476) that exceeds the ADI.

The Panel recommended that:

- the maximum limits for the impurities of toxic elements (lead, mercury and arsenic) in the EC specification for PGPR (E 476) should be revised in order to ensure that PGPR (E 476) as a food additive will not be a significant source of exposure to those toxic elements in food;
- a maximum limit for active ricin should be included in the EU specifications for PGPR (E 476);
- a maximum limit for 3-monochloropropane-1,2-diol (3-MCPD) should be included in the EU specifications for PGPR (E 476);
- given that during the manufacturing processes of glycerol, potential impurities of toxicological concern could be formed, limits for such impurities should be included in the EU specifications for PGPR (E 476);
- given that during the manufacturing processes of polyglycerols, genotoxic impurities – e.g. epichlorohydrin and glycidol – could be present, limits for such impurities should be included in the EU specifications for PGPR (E 476);
- an analytical method for the determination of actual PGPR (E 476) content in food should be developed.

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1. Introduction

The present opinion deals with the re-evaluation of the safety of polyglycerol polyricinoleate (PGPR) (E 476) when used as a food additive.

1.1. Background and Terms of Reference as provided by the European Commission

1.1.1. Background as provided by the European Commission

1.1.1.1. Re-evaluation of polyglycerol polyricinoleate (E 476) as a food additive

Regulation (EC) No 1333/2008[1] of the European Parliament and of the Council on food additives requires that food additives are subject to a safety evaluation by the European Food Safety Authority (EFSA) before they are permitted for use in the European Union (EU). In addition, it is foreseen that food additives must be kept under continuous observation and must be re-evaluated by EFSA.

For this purpose, a programme for the re-evaluation of food additives that were already permitted in the European Union before 20 January 2009 has been set up under Regulation (EU) No 257/2010[2]. This Regulation also foresees that food additives are re-evaluated whenever necessary in the light of changing conditions of use and new scientific information. For efficiency and practical purposes, the re-evaluation should, as far as possible, be conducted by group of food additives according to the main functional class to which they belong.

The order of priorities for the re-evaluation of the currently approved food additives should be set on the basis of the following criteria: the time since the last evaluation of a food additive by the Scientific Committee on Food (SCF) or by EFSA, the availability of new scientific evidence, the extent of use of a food additive in food and the human exposure to the food additive taking also into account the outcome of the Report from the Commission on Dietary Food Additive Intake in the EU[3] of 2001. The report 'Food additives in Europe 2000[4]' submitted by the Nordic Council of Ministers to the Commission, provides additional information for the prioritisation of additives for re-evaluation. As colours were among the first additives to be evaluated, these food additives should be re-evaluated with a highest priority.

In 2003, the Commission already requested EFSA to start a systematic re-evaluation of authorised food additives. However, as a result of adoption of Regulation (EU) 257/2010 the 2003 Terms of References are replaced by those below.

1.1.1.2. Extension of use for polyglycerol polyricinoleate (E 476) in emulsified sauces

The use of food additives is regulated under the European Parliament and Council Regulation (EC) No 1333/2008 on food additives. Only food additives that are included in the Union list, in particular in Annex II to that Regulation, may be placed on the market and used in foods under the conditions of use specified therein. Moreover, food additives should comply with the specifications as referred in Article 14 of that Regulation and laid down in Commission Regulation (EU) No 231/2012[5].

Polyglycerol polyricinoleate (E 476) is an emulsifier, which is authorised for use as a food additive in the Union. Since, polyglycerol polyricinoleate was permitted in the Union before 20 January 2009, it belongs to the group of food additives which will be subject to a new risk assessment by EFSA, in line with the provisions of the Regulation (EU) No 257/2010, which sets up the programme for the re-evaluation of food additives, the re-evaluation of polyglycerol polyricinoleate shall be completed by 31 December 2016.

The European Commission has received an application from the company EMULSAR SARL for a modification of the conditions for use of polyglycerol polyricinoleate. In particular, the applicant requests an extension of use for this additive in emulsified sauces, including mayonnaise (Food

[1] Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. OJ L 354, 31.12.2008.

[2] Commission Regulation (EU) No 257/2010 of 25 March 2010 setting up a programme 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.

[3] COM(2001) 542 final.

[4] Food Additives in Europe 2,000, Status of safety assessments of food additives presently permitted in the EU, Nordic Council of Ministers, TemaNord (2002), p. 560.

[5] 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. OJ L 83, 22.3.2012, p. 1–295.

Category 12.6 of part E of Annex II to Regulation (EC) No 133/2008). The maximum level requested by the applicant is of 4,000 mg/kg. The use of this emulsifier would allow lowering the fat content in emulsified sauces without modifying the organoleptic properties and appeal of the product.

1.1.2. Terms of Reference as provided by the European Commission

1.1.2.1. Re-evaluation of polyglycerol polyricinoleate (E 476) as a food additive

The Commission asks EFSA to re-evaluate the safety of food additives already permitted in the Union before 2009 and to issue scientific opinions on these additives, taking especially into account the priorities, procedures and deadlines that are enshrined in the Regulation (EU) No 257/2010 of 25 March 2010 setting up a programme for the re-evaluation of approved food additives in accordance with the Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives.

1.1.2.2. Extension of use for polyglycerol polyricinoleate (E 476) in emulsified sauces

The European Commission requests EFSA to provide a scientific opinion on the safety of the proposed extension of use for polyglycerol polyricinoleate in accordance with Regulation (EC) No 1331/2008[6] establishing a common authorisation procedure for food additives, food enzymes and food flavourings.

Taking into account that EFSA is currently working on a new risk assessment for polyglycerol polyricinoleate, it is proposed that EFSA incorporates in that risk assessment the assessment of the safety of the proposed extension of use for polyglycerol polyricinoleate. In particular, EFSA is requested to prepare an additional exposure assessment scenario that incorporates the proposed extension of use for this additive in emulsified sauces (food category 12.6 of part E of Annex II to Regulation (EC) No 1333/2008), which a maximum level of 4,000 mg/kg.

1.2. Information on existing evaluations and authorisations

PGPR (E 476) is an authorised food additive in the EU according to Annex II and Annex III of Regulation (EC) No 1333/2008 and specific purity criteria have been defined in the Commission Regulation (EU) No 231/2012[7].

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) evaluated PGPR in 1969 and in 1974 (JECFA, 1969, 1974) and established on the basis of a reproductive toxicity study in rats an acceptable daily intake (ADI) of 0–7.5 mg/kg bw per day.

The Scientific Committee for Food (SCF) established an ADI of 7.5 mg/kg bw per day for PGPR (SCF, 1978). PGPR was also evaluated by the Nordic Council of Ministers (TemaNord, 2002). It was concluded that 'the toxicological data available to JECFA in 1973 or to SCF in 1977 did not include all the data, which are normally required for an ADI to be set for a food additive. However, later data confirm the safety of the substance within the ADI, and taken together with the limited exposure, there seems to be no need for a re-evaluation'.

Since PGPR is a mixture of reaction products formed by the esterification of polyglycerols with condensed castor oil fatty acids, relevant information on existing authorisations and evaluations concerning these moieties has also been examined.

JECFA considered that castor oil had a long history of use as a laxative and aside from these effects, has been used apparently without harm (JECFA, 1979). JECFA considered that at doses of 4 g in adults (approximately 70 mg/kg bw per day), absorption of castor oil appears to be complete and may be considered as a 'no-effect level' in man. In the absence of adequate long-term relevant studies, a conservative margin of safety (MoS) was applied to derive an ADI of 0–0.7 mg/kg bw per day. JECFA concluded on this ADI on the basis that at levels of exposure that cause laxation, castor oil might be expected to inhibit the absorption of fat soluble nutrients – notably vitamins A and D – and therefore, use of castor oil should be kept well below levels where absorption would be inhibited.

The SCF allocated an ADI of 0.7 mg/kg bw per day for ricinoleic acid (SCF, 2003). In its opinion, the SCF endorsed the JECFA ADI of 0–0.7 mg/kg bw per day for castor oil and considered that ricinoleic acid, as the main constituent of castor oil, be allocated with the same numerical ADI.

[6] Regulation (EC) No 1331/2008 of the European Parliament and of the Council of 16 December 2008 establishing a common authorisation procedure for food additives, food enzymes and food flavourings. OJ L 354, 31.12.2008, p. 1–6.

[7] 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. OJ L 83, 22.3.2012, p 1.

The EFSA Panel on Contaminants in the Food Chain (CONTAM) considered the safety of ricinoleic acid as an acceptable previous cargo for edible fats and oils (EFSA CONTAM Panel, 2012a) and concluded that it was not of toxicological concern nor was there any concern regarding possible allergenicity. No reaction products of toxicological concern were known or anticipated.

The safety of polyglycerol for use in food contact materials has been reviewed by the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) (EFSA CEF Panel, 2013). The CEF Panel concluded that polyglycerol does not raise a safety concern for the consumer if the substance is only to be used as plasticiser at a maximum use level of 6.5% w/w in polymer blends of aliphatic-aromatic polyesters in contact with all types of food for any time at room temperature and below.

The EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) re-evaluated the safety of glycerol (E 422) and concluded that there is no need for a numerical ADI and no safety concern regarding the use of glycerol (E 422) as a food additive (EFSA ANS Panel, 2017). However, it was also concluded that the manufacturing process of glycerol should not allow the production of glycerol (E 422) that contains genotoxic and carcinogenic residuals at levels, which result in a MoS below 10,000.

Polyglyceryl-10-polyricinoleate, polyglyceryl-3-polyricinoleate, polyglyceryl-4-polyricinoleate and polyglyceryl-5-polyricinoleate are permitted in cosmetic products in the EU (European Commission database-CosIng[8]).

Castor oil (PM Ref. No 14411 and 42880) is included in the Union list of authorised substances that may be intentionally used in the manufacture of plastic layers in plastic materials and articles (Annex I to Commission Regulation (EU) No 10/2011[9]).

2. Data and methodology

2.1. Data

The Panel on Food Additives and Nutrient Sources added to Food (ANS) was not provided with a newly submitted dossier. EFSA launched public calls for data[10,11] to collect information from interested parties.

The Panel based its assessment on information submitted to EFSA following the public calls for data, information from previous evaluations and additional available literature up to November 2016. Attempts were made at retrieving relevant original study reports on which previous evaluations or reviews were based; however, these were not always available to the Panel.

The EFSA Comprehensive European Food Consumption Database (Comprehensive Database[12]) was used to estimate the dietary exposure.

The Mintel's Global New Products Database (GNPD) is an online resource listing food products and compulsory ingredient information that should be included in labelling. This database was used to verify the use of PGPR (E 476) in food products.

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.

The ANS Panel assessed the safety of polyglycerol polyricinoleate (E 476) as a food additive in line with the principles laid down in Regulation (EU) 257/2010 and in the relevant guidance documents: Guidance on submission for food additive evaluations by the SCF (2001).

When the test substance was administered in the feed or in the drinking water, but doses were not explicitly reported by the authors as mg/kg bw per day based on actual feed or water consumption,

[8] Available online: http://ec.europa.eu/consumers/cosmetics/cosing/index.cfm?fuseaction=search.simple

[9] Commission Regulation (EU) No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food. OJ L 12, 15.1.2011, p. 1–89.

[10] Call for scientific data on food additives permitted in the EU and belonging to the functional classes of emulsifiers, stabilisers and gelling agents. Published: 23 November 2009 and modified 5 February 2010. Available online: http://www.efsa.europa.eu/en/dataclosed/call/ans091123

[11] Call for food additives usage level and/or concentration data in food and beverages intended for human consumption. Published: 12 October 2015. Available online: http://www.efsa.europa.eu/en/data/call/151012

[12] Available online: http://www.efsa.europa.eu/en/datexfoodcdb/datexfooddb.htm

the daily intake was calculated by the Panel using the relevant default values as indicated in the EFSA Scientific Committee guidance document (EFSA Scientific Committee, 2012a) for studies in rodents or, in the case of other animal species, by JECFA (2000). In these cases, the daily intake is defined in the text as 'equivalent to'. 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 polyglycerol polyricinoleate (E 476) from its use as a food additive was estimated combining food consumption data available within the EFSA Comprehensive European Food Consumption Database with the maximum permitted levels and/or reported use levels submitted to EFSA following a call for data. Different scenarios were used to calculate exposure (see Section 3.3.1). Uncertainties on the exposure assessment are identified and discussed.

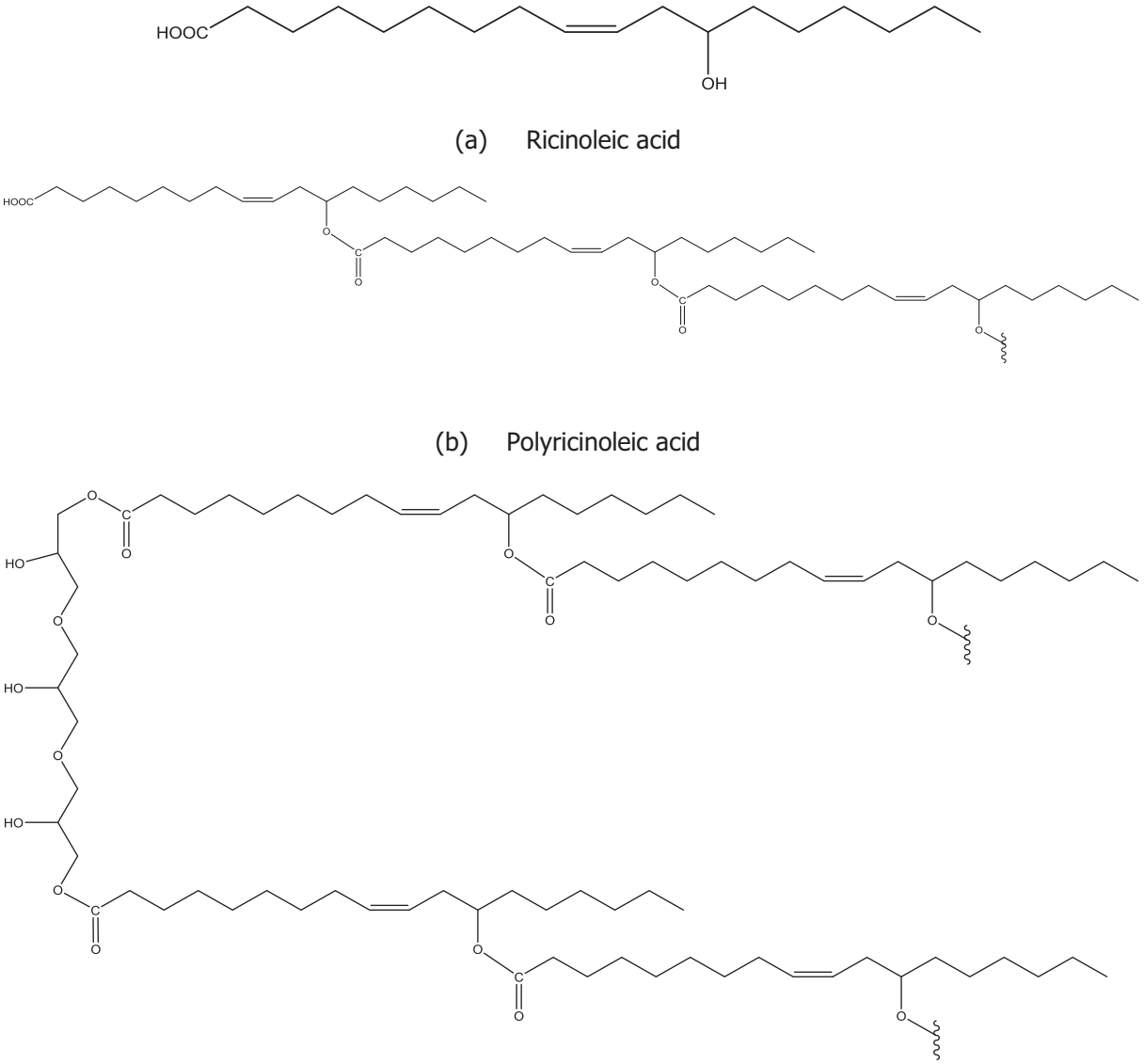
3. Assessment

3.1. Technical data

3.1.1. Identity of the substance

PGPR (E 476) is a mixture of products formed by the esterification of polyglycerols with condensed castor oil fatty acids. According to the Commission Regulation (EU) No 231/2012, the polyglycerol moiety is mainly composed of di-, tri- and tetraglycerol with not more than 10% equal or higher than heptaglycerol. The castor oil fatty acids are mainly composed of ricinoleic acid (80–90%). Other components are oleic acid (3–8%), linoleic acid (3–7%), and stearic acid (0–2%) (Quest International, 1998 [Documentation provided to EFSA n. 13]).

The chemical name of the main component for the CAS Registry number is 68936-89-0 is 1,2,3-propanetriol, homopolymer, (9*Z*,12*R*)-12-hydroxy-9-octadecenoate (Scifinder, online) with a molecular formula of $C_{18}H_{34}O_3 \cdot x(C_3H_8O_3)_n$, an EINECS number has not been assigned. The Panel noted that an additional CAS number – 29894-35-7 – has been assigned with the molecular formula $(C_{18}H_{34}O_3 \cdot C_3H_8O_3)_n$. The general structural formula of PGPR is given in Figure 1:



(a) Ricinoleic acid

(b) Polyricinoleic acid

(c) A polyglycerol polyricinoleate consisting of a polymer of 3 glycerol moieties, with single polyricinoleic acid moieties esterified onto the proximal and distal glycerols

Figure 1: Structural formula of polyglycerol polyricinoleate, adapted from Bastida-Rodriguez, (2013). (Copyright © 2013 Josefa Bastida-Rodríguez, Creative Commons Attribution License CC BY 3.0)

PGPR is also known by the synonyms glycerol esters of condensed castor oil fatty acids, polyglycerol esters of polycondensed fatty acids from castor oil and polyglycerol esters of interesterified ricinoleic acid (Commission Regulation (EU) No 231/2012).

The polyglycerol moieties of PGPR are mainly linear condensation products of glycerol (Behrens and Mieth, 1984). The polyglycerols' primary hydroxyl groups react mostly with polyricinoleate and in a minor proportion with monoricinoleate and other fatty acids (i.e. oleic, linoleic, stearic acid) to obtain PGPR (Orfanakis et al., 2013)

According to the Commission Regulation (EU) No 231/2012, PGPR is a clear, highly viscous liquid, which is insoluble in water and ethanol, but soluble in ether, hydrocarbons and halogenated hydrocarbons. In EFEMA (2009) [Documentation provided to EFSA n. 5], the colour of the liquid is described as light brown and it does not crystallise at 0°C.

3.1.2. Specifications

Specifications for PGPR (E 476) have been defined in Commission Regulation (EU) No 231/2012 and by JECFA (2006). The available specifications are listed in Table 1.

Table 1: Specifications for PGPR (E 476) according to Commission Regulation (EU) No 231/2012 and JECFA (2006)

	Commission Regulation (EU) No 231/2012	**JECFA (2006)**
Definition	Polyglycerol polyricinoleate is prepared by the esterification of polyglycerol with condensed castor oil fatty acids	Prepared by the esterification of polyglycerol with condensed castor oil fatty acids The article of commerce may be specified further as to saponification value, solidification point of the free fatty acids, iodine value, acid value, hydroxyl value, ash content and refractive index
Description	Clear, highly viscous liquid	Highly viscous liquids
Identification		
Solubility	Insoluble in water and in ethanol; soluble in ether, hydrocarbons and halogenated hydrocarbons	Insoluble in water and in ethanol; soluble in ether, hydrocarbons and halogenated hydrocarbons
Test for glycerol	Passes test	Spot 5–20 μL of the aqueous layer obtained in the test for fatty acids under Identification test for functional groups alongside control spots of glycerol on paper such as Whatman No. 3 and develop using descending chromatography for 36 h with isopropanol:water, 90:10. The glycerol spot moves 40 cm and the polyglycerols are revealed in succession below that for glycerol when the paper is sprayed with either permanganate in acetone or ammoniacal silver nitrate
Test for polyglycerol	Passes test	Spot 5–20 μL of the aqueous layer obtained in the test for fatty acids under Identification test for functional groups alongside control spots of glycerol on paper such as Whatman No. 3 and develop using descending chromatography for 36 h with isopropanol:water, 90:10. The glycerol spot moves 40 cm and the polyglycerols are revealed in succession below that for glycerol when the paper is sprayed with either permanganate in acetone or ammoniacal silver nitrate
Test for ricinoleic acid	Passes test	The fatty acids liberated in test for fatty acids Identification tests for functional groups should have a hydroxyl value corresponding to that for castor oil fatty acids (about 150–170)
Refractive index	$[n]_D^{65}$ between 1.4630 and 1.4665	
Tests for fatty acids		Passes tests
Purity		
Polyglycerols	The polyglycerol moiety shall be composed of not less than 75% of di-, tri- and tetraglycerols and shall contain not more than 10% of polyglycerols equal to or higher than heptaglycerol	The polyglycerol moiety shall be composed of not less than 75% of di-, tri- and tetraglycerols and shall contain not more than 10% of polyglycerols equal to or higher than heptaglycerol
Hydroxyl value	Not less than 80 and not more than 100	
Acid value	Not more than 6	
Arsenic	Not more than 3 mg/kg	

	Commission Regulation (EU) No 231/2012	JECFA (2006)
Lead	Not more than 2 mg/kg	Not more than 2 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, 'Instrumental Methods'
Mercury	Not more than 1 mg/kg	–
Cadmium	Not more than 1 mg/kg	–

The Panel noted that according to the EU specifications for PGPR (E 476), impurities of the toxic elements arsenic, lead, mercury and cadmium are accepted up to a concentration of 3, 2, 1 and 1 mg/kg, respectively. Contamination at such levels could have a significant impact on the exposure to these metals, for which the exposure already are close to the health-based guidance values or benchmark doses (lower Confidence Limits) established by EFSA (EFSA CONTAM Panel, 2009a,b, 2010, 2012b–d, 2014).

According to the available information on the manufacturing process (Section 3.3), PGPR (E 476) is manufactured from glycerol and castor oil. The Panel noted that following extraction of castor oil, ricin is left in the press-cake/castor bean meal (also called castor meal, castor residue, castor extract and de-oiled castor cake) (EFSA CONTAM Panel, 2008). The Panel noted limits for active ricin are not included in the EU specifications for PGPR (E 476).

The Panel also noted that a maximum residual level for 3-monochloropropane-1,2-diol (3-MCPD) (not more than 0.1 mg/kg) has been established in EU specifications for glycerol (E 422) (Commission Regulation (EU) No 231/2012); however, there are no limits for 3-MCPD in the EU specifications for PGPR (E 476).

Information on the manufacturing processes of glycerol has been considered by the ANS Panel in the re-evaluation of glycerol (E 422) (EFSA ANS Panel, 2017). The Panel noted that glycerol (E 422) can be produced by a variety of methods and that many of them lead to the presence or formation of contaminants, which are of toxicological concern. The Panel considered that the manufacturing process for PGPR (E 476) should not allow the presence of residuals of genotoxic or/and carcinogenic concern at a level which would result in a margin of exposure (MOE) below 10,000. The Panel considered that maximum limits for potential impurities in glycerol as raw material in the manufacturing process of PGPR should also be established for the EU specifications for PGPR (E 476).

The Panel noted that epichlorohydrin and glycidol may be present in PGPR (E 476) from the manufacturing processes of polyglycerols. The Panel considered that the presence of epichlorohydrin and/or glycidol in PGPR (E 476) would need further assessment as their presence could raise a safety concern.

3.1.3. Manufacturing process

Wilson et al. (1998) and Bastida-Rodriguez (2013) described a process for the manufacture of PGPR, involving the following four steps:

i) Preparation of the castor oil fatty acids

The castor oil fatty acids are produced by hydrolysing castor oil with water and steam at a pressure of approximately 2.8 MPa without a catalyst; the resulting fatty acids are freed from glycerol by water washing. Castor oil contains, as main fatty acids: ricinoleic acid (80–90%), oleic acid (3–8%), linoleic acid (3–7%) and stearic acid (0–2%).

ii) Condensation of the castor oil fatty acids

The castor oil fatty acids are condensed by heating the castor oil fatty acids at a temperature of 205–210°C under vacuum and a CO_2 atmosphere (to prevent oxidation) for approximately 8 h. The reaction is controlled, by monitoring the acid value, until an acid value of 35–40 mg KOH/g (i.e. about 4–5 fatty acid residues per molecule of condensed substance).

iii) Preparation of polyglycerols

According to Bastida-Rodriguez (2013), the polyglycerol portion can be prepared by three routes: (1) by polymerisation of glycerol using a strong base as a catalyst, (2) by polymerisation of glycidol, which leads to linear polyglycerols or (3) by polymerisation of epichlorohydrin, followed by hydrolysis, which also leads to linear polyglycerols. Polyglycerols produced by polymerisation of epichlorohydrin contain reduced proportions of cyclic components.

The Panel noted that epichlorohydrin and glycidol may be present in PGPR (E 476) from the manufacturing processes of polyglycerols. Epichlorohydrin and glycidol are classified as carcinogen 2A according to IARC (1999) and probably carcinogenic to humans (2A) according to IARC (2000). The EFSA CONTAM Panel has characterised glycidol as genotoxic and carcinogenic (EFSA CONTAM Panel, 2016).

iv) Partial esterification of the condensed castor oil fatty acids with polyglycerols

The final stage of the production involves heating of an appropriate amount of polyglycerol with the polyricinoleic acid. The reaction takes place immediately following the preparation of the latter and in the same vessel, while the charge is still hot. The esterification conditions are the same as those for fatty acid condensation. The process is continued until a sample withdrawn from the reaction mixture is found to have a suitable acid value (i.e. $\leq$ 6 mg KOH/g) and refractive index, as required by the specifications.

Tenore (2012) described a new process for the manufacturing of PGPR using as starting materials polyglycerols and castor oil fatty acids obtained as described by Wilson et al. (1998). In this new process, non-polymerised ricinoleic acid is combined with polyglycerols (preferably with a molecular weight in the range of 160–400 g/mol) at a ratio of about 11:1 (w/w). Non-polymerised ricinoleic acid is condensed to polyricinoleate which is then, in a one-step process, reacted with polyglycerol. Water is continuously removed under reduced pressure (about 0.068 MPa). The condensation, the contemporaneous co-polymerisation and the interesterification is conducted at a temperature of 200°C. The condensation and co-polymerisation reactions are maintained until the PGPR in the reaction mixture reaches the characteristics complying with EU specifications. It is indicated by the author that in this new process a catalyst is not necessary, but that the reaction rate can be increased using either a basic catalyst (sodium or potassium hydroxide) or an acidic catalyst (phosphoric or phosphorous acid). It is also indicated that an enzymatic catalyst (lipase approved for food applications; not further specified) can be used. When the enzymatic catalyst is used, the reaction temperature is reduced to 75°C.

Gómez et al. (2011) described the enzymatic biosynthesis of polyglycerol polyricinoleate (E 476) starting from polyglycerol and polyricinoleic acid using *Rhizopus arrhizus* lipase as a catalyst. The reaction takes place in the presence of a very limited amount of aqueous phase. It is stated that no organic solvent was necessary to solubilise the substrates, allowing a reaction medium solely composed of the required substrates. In the process, lipase is immobilised by physical adsorption onto an anion exchange matrix. PGPR produced by this process had an acid value of 16 mg KOH/g which was far above the required EU specification for this parameter (i.e. acid value $<$ 6 mg KOH/g), However, when synthesised under controlled atmosphere in a vacuum reactor with dry nitrogen intake, the PGPR reaction product obtained in this way had an acid value of 4.9 mg KOH/g, complying with the EU specifications. It is stated that the method is a starting point for using the enzymatic procedure in the industrial biosynthesis of PGPR.

In subsequent publications, the same authors (Ortega et al., 2013; Ortega-Requena et al., 2014) described improved methods using lipases from *R. arrhizus*, *Rhizopus oryzae* and *Candida antartica*, resulting in the production of a PGPR with an acid value of 4.91, 5.31 and 1.30 mg KOH/g, respectively.

Information on the manufacture of glycerol is included in the EFSA opinion on the re-evaluation of glycerol (E 422) as a food additive (EFSA ANS Panel, 2017).

3.1.4. Methods of analysis in food

Davies and Harkes (1977 [Documentation provided to EFSA n. 3]) described a method for estimating the levels of PGPR in chocolate. In this method, the lipidic material in the chocolate was extracted with chloroform (Soxhlet method) and the chloroform then evaporated using a rotary evaporator. The residue was hydrolysed with methanolic potassium hydroxide (KOH) in the presence of methyl 2-hydroxydocosanoate as an internal standard and again extracted with chloroform, dried with

magnesium sulfate, filtered and finally evaporated to dryness. This extract was subsequently methylated with diazomethane and the resulting methylated hydroxy fatty acid esters isolated by preparative thin-layer chromatography (TLC) followed by analysis by gas-liquid chromatography (GLC) either directly or after being silylated. For quantitative analysis, standard solutions of methyl ricinoleate and methyl 2-hydroxydocosanoate in toluene were used. The PRGR content in chocolate was estimated on the basis of a known ratio of the ricinolate in the food additive.

The fatty acid components of PGPR were analysed in a chocolate sample after extraction with toluene. The extract was saponified with ethanolic KOH, hydrolysed, silylated and analysed by gas chromatography (GC) (Dick and Miserez, 1976).

The Panel noted that PGPR as such cannot be directly measured in foods and that the methods employed to date involve indirect determination of hydrolysis products, which may also be derived from other food additives. All analytical methods available lead to the detection of ricinoleic acid or its esters in food and cannot be used for quantification of actual PGPR, as the ricinolate ratio in the food additive is not specified in the EU specifications for PGPR (E 476).

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

A stability test of PGPR was performed at 15°C over a period of 32 months. Physical and chemical parameters (acid value, iodine value, saponification value, refractive index, hydroxyl value, peroxide value) were measured. No significant change of these parameters could be observed (Quest International, 1997 [Documentation provided to EFSA n. 12]).

According to Emulsar (2016 [Documentation provided to EFSA n. 7]), the first step in PGPR degradation in food is the hydrolysis of ricinoleic acid moieties from polyglycerol.

Three samples of milk chocolate to which a known amount of PGPR was added, were stored for a period of 12 or 16 months. The content of the ricinolate moiety of PGPR was measured by GC, using the method of Davies and Harkes (1977 [Documentation provided to EFSA n. 3]). The differences in ricinolate content after storage and that measured in the PGPR initially added to each of the chocolates were in all cases within the experimental error indicating a stability of ricinolate. No conclusions were drawn by the authors about the stability of the intact food additive or the degree of hydrolysis needed to yield free ricinoleic acid (Quest International, 1997 [Documentation provided to EFSA n. 12]).

In storage trials of water in oil emulsions at 30°C, it has been demonstrated that the firmness of the emulsions containing PGPR remains high, indicating that the structure of PGPR does not change significantly for the tested period of 3 months (Emulsar, 2016, [Documentation provided to EFSA n. 7]).

3.2. Authorised uses and use levels

Maximum levels of PGPR (E 476) have been defined in Annex II to Regulation (EC) No 1333/2008 on food additives, as amended. In this document, these levels are named maximum permitted levels (MPLs).

Currently, PGPR (E 476) is an authorised food additive in the EU with MPLs ranging from 4,000 to 5,000 mg/kg in five food categories.

Table 2 summarises the food that are permitted to contain PGPR (E 476) and the corresponding MPLs as set by Annex II to Regulation (EC) No 1333/2008.

Table 2: MPLs of PGPR (E 476) in foods according to the Annex II to Regulation (EC) No 1333/2008

Food category number	Food category name	Restrictions/exception	MPL (mg/L or mg/kg as appropriate)
02.2.2	Other fat and oil emulsions including spreads as defined by Council Regulation (EC) No 1234/2007 and liquid emulsions	Only spreadable fats as defined in Article 115 and Annex XV to Regulation (EC) No 1234/2007, having a fat content of 41% or less and similar spreadable products with a fat content of less than 10% fat	4,000
05.1	Cocoa and chocolate products as covered by Directive 2000/36/EC		5,000
05.2	Other confectionery including breath freshening microsweets	Only cocoa-based confectionery	5,000

Food category number	Food category name	Restrictions/exception	MPL (mg/L or mg/kg as appropriate)
05.4	Decorations, coatings and fillings, except fruit-based fillings covered by category 4.2.4	Only cocoa-based confectionery	5,000
12.6	Sauces	Only dressings	4,000

MPL: maximum permitted level.

According to Annex III, Part 2 of Regulation (EC) No 1333/2008, PGPR (E 476) is also authorised as an emulsifier in preparations of food colours E 100 curcumin and E 120 cochineal, carminic acid, carmines and E 163 anthocyanins at the maximum levels of 50,000 mg/kg in the food colour preparations and at 500 mg/kg in the final food only in:

- Surimi and Japanese-type fish products (Kamaboko) (for the food additive E 120 cochineal, carminic acid, carmines);
- meat products, fish pastes and fruit preparations used in flavoured milk products and desserts (for the food additives E 163 anthocyanins, E 100 curcumin and E 120 cochineal, carminic acid, carmines).

3.3. Exposure data

3.3.1. Reported use levels or data on analytical levels of PGPR (E 476)

Most food additives in the EU are authorised at a specific MPL. However, a food additive may be used at a lower level than the MPL. Therefore, information on actual use levels is required for performing a more realistic exposure assessment, especially for those food additives for which no MPL is set and which are authorised according to *quantum satis* (QS).

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 public calls[13,14] for occurrence data (usage level and/or analytical data) on PGPR (E 476). In response to this public call, updated information on the actual use levels of PGPR (E 476) in foods was made available to EFSA by industry. No analytical data on the occurrence of PGPR (E 476) in foods were made available by the Member States.

3.3.1.1. Summarised data on reported use levels in foods provided by industry

Industry provided EFSA with data on use levels (n = 149) of PGPR (E 476) in foods for all the five food categories in which PGPR (E 476) is authorised according to Annex II to Regulation No 1333/2008 (Table 2).

Updated information on the actual use levels of PGPR (E 476) in foods was made available to EFSA by FoodDrinkEurope (FDE), European Food Emulsifiers Manufacturers Association (EFEMA), European Dairy Association (EDA) and Mars.

Data were also provided for food categories in which PGPR (E 476) is not authorised as such but in which chocolate is present as coating. Such data were received for ice-cream (FCS 03) and coated nuts (FCS 15.2).

The Panel noted that EFEMA is not food industry using emulsifiers in its food products but an association of food additive producers. Usage levels reported by food additive producers should not, by default, be considered at the same level as those provided by food industry. Food additive producers might recommend usage levels to the food industry but the final levels used might, ultimately, be different, unless food additive producers confirm that these levels are used by food industry. According to EFEMA, all the submitted data are 'suggested amounts and recommendations by [the] association, based on [their] technological understanding as food emulsifier manufacturers to food industry and on practical experience in model application systems, but not based on actual usage data in final

[13] http://www.efsa.europa.eu/sites/default/files/consultation/ans091123.pdf
[14] http://www.efsa.europa.eu/sites/default/files/consultation/151012.pdf

consumer products'. Therefore, these levels were not used in the exposure assessment of PGPR (E 476).

Appendix A provides data on the use levels of PGPR (E 476) in foods as reported by industry.

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

The Mintel GNPD is an online database, which monitors product introductions in consumer packaged goods markets worldwide. It contains information of over 2 million food and beverage products of which more than 900,000 are or have been available on the European food market. The Mintel GNPD started covering EU's food markets in 1996, currently having 20 out of its 28 member countries and Norway presented in the Mintel GNPD.[15]

For the purpose of this Scientific Opinion, the Mintel GNPD was used for checking the labelling of products containing PGPR (E 476) within the EU's food products as the Mintel GNPD shows the compulsory ingredient information presented in the labelling of products.

According to the Mintel GNPD, PGPR (E 476) is labelled on more than 8,200 products between 2011 and 2016[16] of ice-cream ('Dairy-based frozen products') and chocolates mainly.

Appendix B presents the percentage of the food products labelled with PGPR (E 476) between 2011 and 2016, out of the total number of food products per food subcategories according to the Mintel GNPD food classification.

3.3.3. Food consumption data used for exposure assessment

3.3.3.1. EFSA Comprehensive European Food Consumption Database

Since 2010, the EFSA Comprehensive European Food Consumption Database (Comprehensive Database) has been populated with national data on food consumption at a detailed level. Competent authorities in the European countries provide EFSA with data on the level of food consumption by the individual consumer from the most recent national dietary survey in their country (cf. Guidance of EFSA on the 'Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment' (EFSA, 2011a)). New consumption surveys recently[17] added in the Comprehensive database were also taken into account in this assessment.[18]

The food consumption data gathered by EFSA were collected by different methodologies and thus direct country-to-country comparisons should be interpreted with caution. Depending on the food category and the level of detail used for exposure calculations, uncertainties could be introduced owing to possible subjects' underreporting and/or misreporting of the consumption amounts. Nevertheless, the EFSA Comprehensive Database represents the best available source of food consumption data across Europe at present.

Food consumption data from the following population groups: infants, toddlers, children, adolescents, adults and the elderly were used for the exposure assessment. For the present assessment, food consumption data were available from 33 different dietary surveys carried out in 19 European countries (Table 3).

Table 3: Population groups considered for the exposure estimates of PGPR (E 476)

Population	Age range	Countries with food consumption surveys covering more than 1 day
Infants	From more than 12 weeks up to and including 11 months of age	Bulgaria, Denmark, Finland, Germany, Italy, UK
Toddlers	From 12 months up to and including 35 months of age	Belgium, Bulgaria, Denmark, Finland, Germany, Italy, Netherlands, Spain, UK
Children[(a)]	From 36 months up to and including 9 years of age	Austria, Belgium, Bulgaria, Czech Republic, Denmark, Finland, France, Germany, Greece, Italy, Latvia, Netherlands, Spain, Sweden, UK

[15] Missing Bulgaria, Cyprus, Estonia, Latvia, Lithuania, Luxembourg, Malta and Slovenia.
[16] http://www.gnpd.com/sinatra/home/ accessed on 24/10/2016.
[17] Available online: http://www.efsa.europa.eu/en/press/news/150428.htm
[18] Available online: http://www.efsa.europa.eu/en/datexfoodcdb/datexfooddb.htm

Population	Age range	Countries with food consumption surveys covering more than 1 day
Adolescents	From 10 years up to and including 17 years of age	Austria, Belgium, Cyprus, Czech Republic, Denmark, Finland, France, Germany, Italy, Latvia, Spain, Sweden, UK
Adults	From 18 years up to and including 64 years of age	Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Hungary, Ireland, Italy, Latvia, Netherlands, Romania, Spain, Sweden, UK
The elderly[(a)]	From 65 years of age and older	Austria, Belgium, Denmark, Finland, France, Germany, Hungary, Ireland, Italy, Romania, Sweden, UK

(a): The terms 'children' and 'the elderly' correspond, respectively, to 'other children' and the merge of 'elderly' and 'very elderly' in the Guidance of EFSA on the 'Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment' (EFSA, 2011a).

Consumption records were codified according to the FoodEx classification system (EFSA, 2011b). Nomenclature from the FoodEx classification system has been linked to the food categorisation system (FCS) as presented in Annex II of Regulation (EC) No 1333/2008, part D, to perform exposure estimates. In practice, FoodEx food codes were matched to the FCS food categories.

3.3.3.2. Food categories considered for the exposure assessment of PGPR (E 476)

The food categories in which the use of PGPR (E 476) is authorised were selected from the nomenclature of the EFSA Comprehensive Database (FoodEx classification system), at the most detailed level possible (up to FoodEx Level 4) (EFSA, 2011b).

Some food categories or their restrictions/exceptions are not referenced in the EFSA Comprehensive Database and could therefore not be taken into account in the present estimate. This may have resulted in an underestimation of the exposure. The food category which was not taken into account is described below:

- 05.4 Decorations, coatings and fillings, except fruit-based fillings covered by category 4.2.4.

For the four remaining food categories, the refinements considering the restrictions/exceptions as set in Annex II to Regulation No 1333/2008 were applied.

However, as mentioned above, reported use levels of PGPR (E 476) were also provided for edible ices and processed nuts for which the use is not authorised as such according to Annex II. As the food additive was labelled on many ice-cream products (the main food labelled to contain the food additive according to the Mintel GNPD database) (Appendix B), the Panel decided that exposure via this food category (FC 03) should be taken into account. Therefore, exposure via chocolate from ice-cream products was considered in all exposure scenarios with the assumption that chocolate as an ingredient represents 15% of all ice-cream products. This is very likely an overestimation of the average percentage and is included in the uncertainty analysis (Section 3.4.3). Use levels provided for processed nuts were attributed to coated nuts (belonging to FC 15.2 Processed nuts) selected from the FoodEx nomenclature. The MPL attributed to the coated nuts was the one of the cocoa products (FC 05.1) (Appendix C).

As mentioned above, PGPR (E 476) is also authorised according to Annex III to Regulation (EC) No 1333/2008 with a maximum level of 500 mg/kg in meat products, fish pastes and fruit preparations used in flavoured milk products and desserts. The food categories meat products (FC 08.3) and fish pastes (FC 09.2) were taken into account in the *regulatory maximum level scenario* using the defined level of 500 mg/kg according to Annex III to Regulation (EC) No 1333/2008.

Overall, in the regulatory exposure scenario, eight food categories were included (Appendix C), and in the refined exposure scenario five food categories (no reported use levels available for sauces (FC 12.6) authorised under Annex II to Regulation (EC) No 1333/2008, neither for meat products (FC 08.3) and fish pastes (FC 09.2) authorised under Annex III of the same regulation) were included.

3.4. Exposure estimate

3.4.1. Exposure to PGPR (E 476) from its use as a food additive

The Panel estimated chronic exposure to PGPR (E 476) for the following population groups: infants; toddlers, children, adolescents, adults and the elderly. Dietary exposure to PGPR (E 476) was calculated by multiplying PGPR (E 476) concentrations for each food category (Appendix C) with their

respective consumption amount per kilogram of body weight for each individual in the Comprehensive Database. The exposure per food category was subsequently added to derive an individual total exposure per day. These exposure estimates were averaged over the number of survey days, resulting in an individual average exposure per day for the survey period. Dietary surveys with only 1 day per subject were excluded as they are considered as not adequate to assess repeated exposure.

This was carried out for all individuals per survey and per population group, resulting in distributions of individual exposure per survey and population group (Table 3). On the basis of these distributions, the mean and 95th percentile of exposure were calculated per survey and per population group. The 95th percentile of exposure was only calculated for those population groups where the sample size was sufficiently large to allow this calculation (EFSA, 2011a). Therefore, in the present assessment, the 95th percentile of exposure for infants from Italy and for toddlers from Belgium, Italy and Spain were not included.

Two exposure scenarios were defined and carried out by the ANS Panel regarding the concentration data of PGPR (E 476) used: (1) MPLs as set down 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*). These two scenarios are discussed in detail below.

A possible additional exposure from the use of PGPR (E 476) as a food additive in food additive preparation in accordance with Annex III to Regulation (EC) No 1333/2008 (Part 2) was not considered in the refined exposure assessment scenarios.

3.4.1.1. Regulatory maximum level exposure assessment scenario

The regulatory maximum level exposure assessment scenario is based on the MPLs as set in Annex II and Annex III, Part 2, to Regulation (EC) No 1333/2008 and listed in Table 2. Appendix C summarises the concentration levels of PGPR (E 476) used in this exposure scenario.

The Panel considers the exposure estimates derived following this scenario as the most conservative as it is assumed that the population group will be exposed to PGPR (E 476) present in food at the MPL over a longer period of time.

3.4.1.2. Refined exposure assessment scenario

The refined exposure assessment scenario is based on use levels reported by industry. This exposure scenario can consider only food categories for which these data were available to the Panel.

Appendix C summarises the concentration levels of PGPR (E 476) used in the refined exposure assessment scenario. Based on the available data set, the Panel calculated two refined exposure estimates based on different model populations:

- The brand-loyal consumer scenario: It was assumed that a consumer is exposed long-term to PGPR (E 476) present at the maximum reported use level for one food category. This exposure estimate is calculated as follows:
  - Combining food consumption with the maximum of the reported use levels for the main contributing food category at the individual level.
  - Using the mean of the typical reported use levels for the remaining food categories authorised according Annex II.
- The non-brand-loyal consumer scenario: It was assumed that a consumer is exposed long-term to PGPR (E 476) present at the mean reported use levels in food. This exposure estimate is calculated using the mean of the typical reported use levels for all food categories authorised according Annex II.

3.4.1.3. Dietary exposure to PGPR (E 476)

Table 4 summarises the estimated exposure to PGPR (E 476) from its use as a food additive in six population groups (Table 3) according to the different exposure scenarios (Section 3.4.1). Detailed results per population group and survey are presented in Appendix D.

Table 4: Summary of dietary exposure to PGPR (E 476) from its use as a food additive in the *regulatory maximum level exposure* assessment scenario and in the refined exposure scenarios, in six population groups (minimum–maximum across the dietary surveys in mg/kg bw per day)

	Infants (12 weeks–11 months)	Toddlers (12–35 months)	Children (3–9 years)	Adolescents (10–17 years)	Adults (18–64 years)	The elderly (≥ 65 years)
Regulatory maximum level exposure assessment scenario						
Mean	0.1–1.0	0.8–6.2	2.2–5.9	1.2–3.4	0.5–2.2	0.3–2.1
95th percentile	0.6–4.2	2.9–12.4	5.8–13.3	3.7–8.7	1.6–5.9	1.1–6.0
Refined exposure assessment scenario						
Brand-loyal scenario						
Mean	0.01–0.6	0.3–3.6	0.9–4.0	0.7–2.3	0.2–1.2	0.1–1.5
95th percentile	< 0.01–2.6	1.3–9.2	3.0–12.0	2.9–7.1	1.1–4.1	0.6–5.2
Non-brand-loyal scenario						
Mean	< 0.01–0.2	0.1–2.8	0.3–2.4	0.1–0.9	0.04–0.9	0.02–1.3
95th percentile	< 0.01–0.9	0.7–6.8	1.2–6.0	0.6–2.9	0.2–3.4	0.1–4.7

In the *regulatory maximum level exposure assessment scenario*, mean exposure to PGPR (E 476) from its use as a food additive ranged from 0.1 mg/kg bw per day in infants to 6.2 mg/kg bw per day in toddlers. The 95th percentile of exposure to PGPR (E 476) ranged from 0.6 mg/kg bw per day in infants to 13.3 mg/kg bw per day in children.

In the *refined estimated exposure scenario*, in the *brand-loyal scenario*, mean exposure to PGPR (E 476) from its use as a food additive ranged from 0.01 mg/kg bw per day in infants to 4.0 mg/kg bw per day in children. The high exposure to PGPR (E 476) ranged from < 0.01 mg/kg bw per day in infants to 12.0 mg/kg bw per day in children. In the *non-brand-loyal scenario*, mean exposure to PGPR (E 476) from its use as a food additive ranged from < 0.01 mg/kg bw per day in infants to 2.8 mg/kg bw per day in toddlers. The 95th percentile of exposure to PGPR (E 476) ranged from < 0.01 mg/kg bw per day in infants to 6.8 mg/kg bw per day in toddlers.

3.4.1.4. Main food categories contributing to exposure to PGPR (E 476) using the regulatory maximum level exposure assessment scenario (Tables 5, 6 and 7)

Table 5: Main food categories contributing to exposure to PGPR (E 476) using maximum permitted levels (> 5% to the total mean exposure) and number of surveys in which each food category is contributing

Food category number	Food category name	Infants	Toddlers	Children	Adolescents	Adults	The elderly
		Range of % contribution to the total exposure (number of surveys)[a]					
02.2	Fat and oil emulsions mainly of type water-in-oil	22.8 (1)	5.4–43.5 (5)	5.5–39.6 (7)	5.9–34.1 (5)	5.4–44.4 (6)	6.5–59.0 (7)
03	Edible ices[b]	5.4–12.7 (3)	5.2–19.2 (6)	5.0–23.7 (11)	5.0–24.5 (11)	6.9–22.1 (4)	5.2–20.3 (3)
05.1	Cocoa and Chocolate products as covered by Directive 2000/36/EC	8.3–90.6 (5)	9.3–74.6 (10)	16.8–68.1 (18)	22.0–69.9 (17)	15.6–58.3 (17)	10.9–50.1 (14)
05.2	Other confectionery including breath refreshening microsweets[b]	7.1 (1)	6.5–18.7 (5)	7.9–20.3 (4)	5.0–27.2 (4)	5.7–20.0 (5)	5.1–12.0 (4)
08.3	Meat products[c]	9.4–96.2 (6)	18.3–52.6 (10)	14.4–54.7 (18)	12.5–42.5 (17)	17.2–64.8 (17)	14.2–74.9 (14)
09.2	Processed fish and fishery products including molluscs and crustaceans – only fish paste[c]	5.4–6.0 (2)	5.6–6.1 (3)	6.9 (1)	–	–	–

Food category number	Food category name	Infants	Toddlers	Children	Adolescents	Adults	The elderly
		Range of % contribution to the total exposure (number of surveys)[a]					
12.6	Sauces	6.9 (1)	6.2–7.1 (2)	5.6–16.0 (6)	7.6–18.8 (7)	11.3–23.8 (10)	5.8–30.2 (11)
15.2	Processed nuts	–	6.3 (1)	–	–	–	–

–: Food categories not contributing or contributing less than 5% to the total mean exposure.
(a): The total number of surveys may be greater than the total number of countries as listed in Table 3, as some countries submitted more than one survey for a specific population.
(b): Considering only the chocolate coating or filling representing 15%.
(c): FC authorised according to Annex III to Regulation No 1333/2008.

3.4.1.5. Main food categories contributing to exposure to PGPR (E 476) using the refined exposure assessment scenario

Table 6: Main food categories contributing to exposure to PGPR (E 476) using the brand-loyal refined exposure scenario (> 5% to the total mean exposure) and number of surveys in which each food category is contributing

Food category number	Food category name	Infants	Toddlers	Children	Adolescents	Adults	The elderly
		Range of % contribution to the total exposure (number of surveys)[a]					
02.2	Fat and oil emulsions mainly of type water-in-oil	15.6–55.5 (3)	7.5–72.4 (5)	6.7–61.6 (9)	7.9–54.6 (8)	6.5–67.8 (8)	12.1–79.9 (9)
03	Edible ices[b]	7.3–31.8 (4)	6.0–31.7 (4)	5.8–25.4 (7)	5.3–26.2 (3)	5.1–31.9 (7)	5.0–32.8 (7)
05.1	Cocoa and Chocolate products as covered by Directive 2000/36/EC	16.9–100 (6)	15.7–100 (10)	28.9–98.3 (18)	42.8–98.8 (17)	31.1–97.9 (17)	19.1–95.0 (14)
05.2	Other confectionery including breath refreshening microsweets[b]	10.2–22.8 (2)	9.8–34.7 (5)	5.3–37.5 (6)	6.1–51.1 (5)	8.0–52.2 (7)	5.7–30.2 (5)
15.2	Processed nuts	–	6.1–12.3 (2)	–	–	–	–

–: Food categories not contributing or contributing less than 5% to the total mean exposure.
(a): The total number of surveys may be greater than the total number of countries as listed in Table 3, as some countries submitted more than one survey for a specific population.
(b): Considering only the chocolate coating or filling representing 15%.

Table 7: Main food categories contributing to exposure to PGPR (E 476) using the non-brand-loyal refined exposure scenario (> 5% to the total mean exposure) and number of surveys in which each food category is contributing

Food category number	Food category name	Infants	Toddlers	Children	Adolescents	Adults	The elderly
		Range of % contribution to the total exposure (number of surveys)[a]					
02.2	Fat and oil emulsions mainly of type water-in-oil	43.6–89.1 (3)	24.7–86.2 (5)	4.9–81.1 (12)	7.8–80.5 (10)	21.0–87.3 (8)	28.8–92.6 (9)
03	Edible ices[b]	7.4 (1)	–	5.1 (1)	6.1 (1)	7.8 (1)	7.5 (1)
05.1	Cocoa and Chocolate products as covered by Directive 2000/36/EC	8.2–100 (6)	5.7–100 (10)	12.0–98.4 (18)	19.0–98.8 (17)	12.5–99.0 (17)	7.3–97.8 (14)

Food category number	Food category name	Infants	Toddlers	Children	Adolescents	Adults	The elderly
		Range of % contribution to the total exposure (number of surveys)[a]					
05.2	Other confectionery including breath refreshening microsweets	22.8–33.2 (2)	7.9–49.8 (5)	5.0–56.2 (11)	8.6–69.0 (8)	6.7–66.6 (9)	7.7–47.2 (5)
15.2	Processed nuts	–	10.8–17.4 (2)	–	–	–	–

–: Food categories not contributing or contributing less than 5% to the total mean exposure.
(a): The total number of surveys may be greater than the total number of countries as listed in Table 3, as some countries submitted more than one survey for a specific population.
(b): Considering only the chocolate coating or filling representing 15%.

3.4.2. Exposure to PGPR (E 476) considering the proposed extension of use for this food additive in emulsified sauces (food category 12.6)

A request was made to extend the use of PGPR (E 476) in FCS 12.6, emulsified sauces, including mayonnaise at a maximum level of 4,000 mg/kg. The exposure to PGPR (E 476) according to the *regulatory maximum level exposure assessment* scenario including this proposed use level is presented in Table 8. Detailed results per population group and survey are presented in Appendix E.

Table 8: Summary of dietary exposure to PGPR (E 476) from its use as a food additive in the *regulatory maximum level exposure assessment* scenario including the proposed use level of 4,000 mg/kg for food category 12.6 (emulsified sauces) in six population groups (minimum–maximum across the dietary surveys in mg/kg bw per day)

	Infants (12 weeks–11 months)	Toddlers (12–35 months)	Children (3–9 years)	Adolescents (10–17 years)	Adults (18–64 years)	The elderly (≥ 65 years)
Regulatory maximum level exposure assessment scenario taking into account the new proposed use						
Mean	0.1–2.2	0.9–9.4	2.3–8.4	1.4–5.2	0.5–3.5	0.3–3.0
95th percentile	0.6–10.5	2.9–16.9	6.1–20.8	4.0–12.3	1.8–9.8	1.3–8.2

In the *regulatory maximum level exposure assessment scenario* including the proposed extension of use of PGPR (E 476) to emulsified sauces, mean exposure to PGPR (E 476) ranged from 0.1 mg/kg bw per day in infants to 9.4 mg/kg bw per day in toddlers. The 95th percentile of exposure to PGPR (E 476) ranged from 0.6 mg/kg bw per day in infants to 20.8 mg/kg bw per day in children.

Compared to the exposure estimates taking into account only the current authorised uses, the intakes increased up to a factor 2 for certain population groups and countries.

3.4.2.1. Main food categories contributing to exposure to PGPR (E 476) according to the *regulatory maximum level exposure assessment* scenario including the proposed use level of 4,000 mg/kg for food category 12.6 (emulsified sauces) (Table 9)

Table 9: Main food categories contributing to exposure to PGPR (E 476) using maximum permitted levels considering the proposed extension of use for this food additive in emulsified sauces (> 5% to the total mean exposure) and number of surveys in which each food category is contributing

Food category number	Food category name	Infants	Toddlers	Children	Adolescents	Adults	The elderly
		Range of % contribution to the total exposure (number of surveys)[a]					
02.2	Fat and oil emulsions mainly of type water-in-oil	11.8 (1)	5.3–31.9 (4)	5.6–29.4 (5)	5.0–15.8 (6)	6.2–23.6 (4)	5.1–40.4 (6)
03	Edible ices[b]	10.8–11.5 (2)	8.0–17.9 (4)	5.1–22.7 (10)	5.7–22.1 (5)	5.8–19.7 (4)	8.2–19.0 (2)
05.1	Cocoa and Chocolate products as covered by Directive 2000/36/EC	7.6–90.6 (5)	6.1–63.1 (10)	9.8–60.3 (18)	13.6–60.2 (17)	9.3–48.9 (17)	7.4–43.7 (14)

Food category number	Food category name	Infants	Toddlers	Children	Adolescents	Adults	The elderly
		Range of % contribution to the total exposure (number of surveys)[a]					
05.2	Other confectionery including breath refreshening microsweets[b]	6.4 (1)	5.5–11.7 (4)	5.9–14.8 (4)	8.8–16.7 (3)	6.2–12.0 (4)	5.2–7.0 (2)
08.3	Meat products[c]	9.4–95.4 (6)	11.7–48.9 (10)	9.5–53.1 (18)	8.0–35.7 (17)	9.1–64.2 (17)	9.7–74.9 (14)
09.2	Processed fish and fishery products including molluscs and crustaceans – only fish paste[c]	–	5.4 (1)	6.5 (1)	–	–	–
12.6	Sauces	8.9–54.7 (4)	7.7–46.0 (8)	5.5–42.6 (16)	9.8–59.7 (16)	10.8–52.9 (16)	6.9–48.4 (12)

–: Food categories not contributing or contributing less than 5% to the total mean exposure.
(a): The total number of surveys may be greater than the total number of countries as listed in Table 3, as some countries submitted more than one survey for a specific population.
(b): Considering only the chocolate coating or filling representing 15%.
(c): FC authorised according to Annex III to Regulation No 1333/2008.

With the current authorised uses, sauces (FC 12.6) contributed up to 30% for one survey in the elderly, and in some surveys, it contributed less than 5% to the total mean exposure estimates (e.g. for infants, only in one survey the contribution is above 5%). Taking into account the proposed extension of use to emulsified sauces, the contribution of FC 12.6 to the mean exposure to PGPR (E 476) increased in most of the surveys for all population groups to almost 60% (in one adults survey).

3.4.3. Uncertainty analysis

Uncertainties in the exposure assessment of PGPR (E 476) have been discussed above. In accordance with the guidance provided in the EFSA opinion related to uncertainties in dietary exposure assessment (EFSA, 2007), the following sources of uncertainties have been considered and summarised in Table 10.

Table 10: Qualitative evaluation of influence of uncertainties on the dietary exposure estimate

Sources of uncertainties	Direction[a]
Uncertainties common for all assessments	
Consumption data: different methodologies/representativeness/underreporting/misreporting/no portion size standard	+/–
Use of data from food consumption survey of a few days to estimate long-term (chronic) exposure for high percentiles (95th percentile)	+
Correspondence of reported use levels to the food items in the EFSA Comprehensive Food Consumption Database: uncertainties to which types of food the levels refer to	+/–
Uncertainty in possible national differences in use levels of food categories	+/–
Specific uncertainties for this assessment	
Food categories selected for the exposure assessment: exclusion of food categories due to missing FoodEx linkage (n = 1/5 food categories from Annex II Regulation (EC) No 1333/2008)	–
Concentration data:	
• levels considered applicable for all items within the entire food category	+
Regulatory maximum level exposure assessment scenario:	
• food categories which may contain PGPR (E 476) due to carry-over (according to Annex III to Regulation (EC) No 1333/2008) partially considered	+/–
• food categories authorised at MPL according to Annex II to Regulation (EC) No 1333/2008	+

Sources of uncertainties	Direction[(a)]
Refined exposure assessment scenarios:	
• food categories which may contain PGPR (E 476) due to carry-over (according to Annex III to Regulation (EC) No 1333/2008) not considered	−
• exposure calculations based on the maximum or mean levels (reported use from industries)	+/−
• Food categories selected for the refined exposure assessment: exclusion of food categories due to missing use levels (n = 1/5 food categories from Annex II Regulation (EC) No 1333/2008)	−

(a): +, uncertainty with potential to cause overestimation of exposure; −, uncertainty with potential to cause underestimation of exposure.

The Panel noted that there might be a slight underestimation of the exposure estimate because not all authorised uses of PGPR (E 476) according to Annex III were considered in the *regulatory maximum level exposure assessment* scenario and none of these uses were taken into account in the refined exposure assessment scenario.

Overall, the Panel considered that the uncertainties identified would, in general, result in an overestimation of the exposure to PGPR (E 476) as a food additive in European countries considered in the EFSA European Comprehensive Food Consumption Database for both the regulatory maximum level exposure scenario and the refined exposure scenario.

For the scenario including the extended uses of PGPR (E 476) to emulsified sauces, the Panel also considered that the uncertainties identified would result in an overestimation of the exposure to PGPR (E 476) as this scenario assumes that all emulsified sauces would contain the food additive at the proposed maximum use level of 4,000 mg/kg.

3.4.4. Exposure via other sources

Further uses of PGPR, other than as a food additive, are in drugs, cosmetics and personal care products, textile finishing, oil and water emulsions, and release agents (Austen Business Solutions Ltd, 2011 [Documentation provided to EFSA n. 2]) (EFEMA, 2009 [Documentation provided to EFSA n. 5]). The exposure via these uses is unknown, and was therefore not taken into account in this opinion.

3.5. Biological and toxicological data

This section describes the biological and toxicological data available for PGPR. However, after oral uptake, the Panel considered that PGPR is subjected to hydrolysis by lipases in the gastrointestinal tract to liberate polyricinoleic acid, ricinoleic acid and other fatty acids present as minor component moiety fatty acids of castor oil, polyglycerols and glycerol. Relevant biological and toxicological data concerning ricinoleic acid, castor oil, polyricinoleic acid and polyglycerols have also been described.

The Panel did not consider biological and toxicological data for glycerol in this opinion since its use as a food additive was recently re-evaluated by the Panel (EFSA ANS Panel, 2017). The Panel noted however, that glycerol is liberated from normal lipid dietary constituents (e.g. triglycerides) and is re-esterified at, or soon after absorption (EFSA NDA Panel, 2010).

The Panel noted that the test material used in the Grieco (1974) studies was stated to be an emulsifier widely used in Europe in the manufacturing of various chocolate products. However, it is unknown if this material meets the current EU specifications for PGPR (E 476).

3.5.1. Absorption, distribution, metabolism and excretion

3.5.1.1. PGPR

The metabolic fate of ^{14}C[polyglycerol]PGPR was investigated in six rats by gavage of 1.0 mL per rat as a 50% aqueous emulsion. Expired CO_2 was monitored for 36 h. Urine and faeces were collected at 24 h intervals for 4 days. Animals were provided with food and water *ad libitum*. A total of 70% of the administered dose was recovered after 4 days. Chromatographic analysis of the urine revealed that the radioactivity was primarily lower glycerol polymers, i.e. diglycerol and triglycerol. Analysis of the faeces by TLC revealed that approximately 85% of the ^{14}C recovered was as free polyglycerols. The author considered that the PGPR was broken down in the intestine. The data indicated, however, that approximately 10% of the ^{14}C found in the faeces was either undigested or partially digested PGPR.

The author suggested that the administered emulsion had separated in the stomach and formed globules of PGPR which could not be completely digested (Grieco, 1974 [Documentation provided to EFSA n. 9]).

In another experiment, ^{14}C[polyglycerol]PGPR was administered in an aqueous dietary slurry. Analysis of expired CO_2, urine and faeces revealed radioactive recoveries of 8%, 31% and 54%, respectively (time course not specified). According to the author, chromatographic analysis showed that PGPR was completely broken down in this study to liberate polyglycerols. The author considered that PGPR was digested in the rat liberating polyglycerols, some of which are absorbed and excreted unchanged in the urine (lower polymers) and others, which remained to be excreted in the faeces (higher polymers) (Grieco, 1974 [Documentation provided to EFSA n. 9]).

Twelve adult male Colworth Wistar rats were administered PGPR also containing ricinoleic acid (tritiated at the 12 or 9, 10 positions) by gavage with a dietary slurry. Immediately after dosing, animals were placed in metabolism cages and provided with food and water *ad libitum*. Animals were sacrificed in groups of 3, after 3, 6, 12 and 24 h. For each group, terminal urine, faeces and expired CO_2 were monitored for radioactivity. Excised samples of liver, epididymal fat, brain, heart, blood, gastrointestinal tissue and its contents were monitored for radioactivity. Analysis of the gastrointestinal contents after 24 h revealed that less than 1% of the labelled material was present, and only approximately 5% of the dose was found in the faeces. Examination of the stomach and intestinal tissues after 24 h showed $< 0.5\%$ and $< 0.1\%$ of the administered radioactivity present, respectively. The blood lipid radioactivity level remained low throughout the experiment. Approximately 5% of the administered radioactivity was deposited in the epididymal fat of which only 30% of the radioactivity was as hydroxy fatty acid. According to the author, 10% of the total administered radioactivity appeared in the urine, either as water or metabolites (Grieco, 1974 [Documentation provided to EFSA n. 9]).

3.5.1.2. Ricinoleic acid

The accumulation of hydroxy acids in depot fat of rats dosed orally with ricinoleic acid was studied in two experiments. In the first experiment, adult male rats (no further information available) were fed a 5% emulsion (20 mL) of ricinoleic acid for 7 days, while in a second experiment, the rats were dosed over 27 days. The lipid extraction from the fat tissue was hydrolysed to yield fatty acids which were separated by TLC, eluted, methylated and subjected to GC analysis. The authors reported that the following hydroxy fatty acids with shorter chain lengths than ricinoleic acid were detected, likely resulting from gut bacteria catabolism of ricinoleic acid: 10-hydroxyhexadecenoic acid (experiment 1: 0.6% of total fatty acids; experiment 2: 0.33% of total fatty acids), 8-hydroxytetradecenoic acid (experiment 1: 0.03% of total fatty acids; experiment 2: 0.08% of total fatty acids) and 6-hydroxydodecenoic acid (experiment 2: 0.03% of total fatty acids) (Uchiyama et al., 1963).

In another study, male albino rats (ca 120 g body weight) were dosed via gavage three times per day for up to 30 days with 1,500 mg of ricinoleic acid or an emulsion containing 5% (w/v) ricinoleic acid. Faeces were collected every 24 h until sacrifice and the rats were killed 20 h having received the last dose. At necropsy, subcutaneous adipose tissue was removed and analysed. Treatment with ricinoleic acid resulted in up to 18% of the dose excreted as ricinoleic acid in the faeces with no ricinoleic acid detected in control animal faeces. After dosing for up to 27 days, 4.5% of the fatty acids in fat tissue where determined to be hydroxy acids, of which 3.9% was ricinoleic acid. The levels in untreated animals were not determined (Okui et al., 1964).

Ricinoleic acid was administered via oral gavage to male rats (minimum body weight of 400 g, strain number and dose not stated) with a cannulated thoracic duct (Rao et al., 1969). Lymph was collected for 48 h, and the lipids were then extracted and separated into various lipid classes. Ricinoleic acid was present in the triglyceride, diglyceride, monoglyceride and free fatty acid fractions. Peak absorption of ricinoleic acid occurred within 30 min post administration. Ricinoleic acid was not present in the phospholipid or cholesterol ester fractions of the lymph lipids.

3.5.1.3. Castor oil

Adult rats (number, weights and strain not stated) received a diet containing 48.4% castor oil for 4–6 weeks (Stewart and Sinclair, 1945). Control rats received a control diet. At the end of the feeding period, excised organs/tissues were ground thoroughly and samples of phospholipid fatty acids were obtained from the liver, small intestine and muscle; glyceride fatty acids were obtained from the liver and fat depots. Average percentages of ricinoleic acid in the phospholipid fatty acids were as follows: liver (test: $1.3 \pm 0.6\%$ (9 analyses); controls: $1.7 \pm 1.1\%$ (7 analyses)), small intestine (test:

4.9 ± 1.7% (8 analyses); controls: 6.0 ± 4.4% (4 analyses)) and skeletal muscle (test: 3.6 ± 2.9% (8 analyses); controls: 4.0 ± 1.7% (7 analyses)). Average percentages of ricinoleic acid in glycerides and cholesterol esters: fat depots (test: 6.8 ± 4.2% (11 analyses); controls: 0.5 ± 0.5% (7 analyses)) and liver (test: 7.2 ± 2.4% (8 analyses); controls: 5.6 ± 4.1% (5 analyses)). Faeces were collected from three rats on the castor oil diet. The fatty acids excreted by each of three rats amounted to 2.1%, 2.2% and 3.6% of those ingested. Total body fat in these three animals was also determined, and it was calculated that 1–2% of absorbed ricinoleic acid was deposited in the fat depots. The authors concluded that the feeding of castor oil did not lead to the appearance of significant amounts of ricinoleic acid in phospholipids of the small intestine, liver and skeletal muscle, nor in glycerides of the liver. Additionally, they concluded that ricinoleic acid is a component acid of the glycerides in the fat depots, comprising 7% of the total fatty acids but is also rapidly metabolised.

The digestion, absorption and metabolism of castor oil was studied in eight male 100–200 g body weight Sprague–Dawley rats (Watson and Gordon, 1962). The fatty acid composition of the castor oil was: ricinoleic acid, 90.0%; linoleic acid, 4.7%; oleic acid, 3.2%; stearic acid, 1.0%; palmitic acid, 1.0% and palmitoleic acid, 1%. In the first experiment, four rats were fed rat chow *ad libitum*, and the remaining four were fasted overnight. On the following morning, castor oil (1.0 mL, equivalent to 4.8–9.6 mg/kg bw) was dosed via gavage and chyle was collected over a 24 h period. The mean values for per cent recovery of ricinoleic acid in fasted and fed rats were 6.8% and 24.2%, respectively ($p < 0.01$).

In the second experiment, seven weanling rats were fed a diet consisting of rat chow that had been mixed with castor oil (20% by weight, equivalent to 18,000 mg/kg bw per day). The control group was fed an olive oil-supplemented diet. After 4 and 8 weeks of feeding, an epididymal fat pad was removed from a rat in either group, and fatty acid composition determined using GLC. Mean values for ricinoleic acid in the fat pads of rats fed castor oil after 4 and 8 weeks of feeding were 9.1 ± 1.7% and 9.7 ± 1.0%, respectively. Ricinoleic acid was undetectable in the fat pads of control rats fed olive oil. Random analyses of faeces indicated a high levels of hydroxystearic acid in rats fed castor oil, which was undetectable in the faeces of rats fed a normal diet. The authors suggested that ricinoleic acid is hydrogenated in the gut lumen by intestinal bacteria.

The relationship between dose and absorption of castor oil was studied in the final experiment. Polyethylene cannulae were inserted into the thoracic duct and duodenum of two rats. On the following morning, one rat received 0.2 mL and one rat received 0.6 mL of castor oil (equivalent to 0.96–1.92 and 2.88–5.76 mg/kg bw respectively), and the rats were killed 45 min post-dosing. The high dose induced diarrhoea at 45 min. Intestines (small and large) were excised, homogenised and tissue lipids were extracted. Chyle was also collected and extracted. Total lipid and GLC analysis of fatty acids were performed on the extracts. For the rat dosed with 0.2 mL and 0.6 mL castor oil, the percentages of ricinoleic acid in the chyle was 18.1% and 3.0%, respectively, and the percentage detected in the small bowel lipids was 6.2% and 3.1%, respectively. The authors concluded that there was a close correlation between the dose of castor oil administered and the percentage of ricinoleic acid in the faeces, i.e. greater absorption at the lower dose.

Castor oil (0.5 mL/rat) was administered by gavage to germ-free and conventional Agus rats (Hagenfeldt et al. 1986; no further details stated). Urine was collected at intervals over a 24 h period. In both conventional and germ-free rat urines, 3,6-epoxyoctanedioic acid, 3,6-epoxydecanedioic acid and 3,6-epoxydodecanedioic acid were detected. These acids were not detected prior to dosing with castor oil. The authors concluded that the cyclisation of ricinoleic acid occurs endogenously and does not require the presence of intestinal bacteria.

Two groups of five male Wistar rats (3 weeks old) received 10% castor oil (equivalent to 9,000 mg/kg bw per day) in the diet (cholesterol-enriched and cholesterol-free, respectively) for 20 days (Ihara-Watanabe et al., 1999). In both dietary groups, a very small quantity of ricinoleic acid was present in perirenal adipose tissue, but not in serum or hepatic tissue. The authors noted that the perirenal fatty acid profiles did not reflect those of the dietary fats, either in the absence or presence of dietary cholesterol. The faecal recovery of ricinoleic acid was approximately 0.5% of the total ingested. The authors concluded that castor oil was readily digested, absorbed and metabolised.

3.5.1.4. Polyricinoleic acid

No studies were available.

3.5.1.5. Polyglycerol

Michael and Coots (1971) conducted studies on the metabolic fate of ^{14}C-labelled triglycerol and decaglycerol in male Sprague–Dawley rats (n = 4 per group). The polyglycerols were administered by

oral gavage as 7–8 g of liquid diet containing 1% labelled compound (corresponding to 14–20 mg/kg bw of compound). The cumulative excretion of applied radioactivity was measured in metabolism cages as well as the radioactivity remaining in the carcass and in the gastrointestinal contents at termination, 51 h after administration (see Table 3). The total recovery of radioactivity ranged from 88% to 98% of that fed (no further data).

Table 11: Metabolic fate of ^{14}C-labelled triglycerol and decaglycerol (according to Michael and Coots, 1971)

Labelled compound	% of recovered radioactivity (means)					Absorption in %[(a)]
	CO_2	Urine	Faeces	GI content	Carcass	
Triglycerol	2.1	88.3	5.5	2.9	1.2	91.6
Decaglycerol	4.2	34.1	23.9	35.2	2.5	40.8

GI: gastrointestinal.
(a): total absorption in % of recovered radioactivity (assuming no excretion via the bile; not calculated by the authors): total recovery (100%) minus unabsorbed radioactivity in faeces and GI contents

Nearly complete absorption was shown for the triglycerol. The absorption rate was 91.6% of recovered radioactivity (sum of recovered radioactivity in carcass, urine and exhaled CO_2), whereas a lower rate of 40.8% was measured for decaglycerol (Table 11). However, both polyglycerols were not accumulated in the carcass or metabolised (only minor amounts of radioactivity in the carcass or in exhaled CO_2). The unchanged polyglycerols (shown by TLC) were excreted after oral absorption mainly via urine (Michael and Coots, 1971).

Colworth Wistar Rats (250–300 g body weight, number of animals not specified) were administered 1 mL of a ^{14}C-polyglycerol as a 50% (v/v) solution in water by oral gavage (Grieco, 1974 [Documentation provided to EFSA n. 9]). The animals were provided with food and water *ad libitum* prior to and during the study. Expired CO_2, urine and faeces were evaluated for radioactivity. After 48 h, the animals were sacrificed, and liver and epididymal fat were excised for determination of radioactivity. After 48 h, 52% was recovered in the faeces, 30.5% in the urine and 5.5% in expired CO_2. Chromatographic analyses of urinary and faecal radioactivity revealed that polyglycerols were excreted unchanged. Based on these results, the author concluded that polyglycerols are not metabolised in the rat and that the ether linkage present between glycerol units renders the compound metabolically inert. Residues of ^{14}C activity found in the liver and epididymal fat were too low for chromatographic analysis.

Analogous results were presented by Howes et al. (1998) in studies with male Wistar rats. A mixture of ^{14}C-labelled polyglycerol was fractionated on Sephadex G10 to obtain four overall fractions rich in polyglycerols: fraction 1, lower chain length up to fraction 4, higher chain length. The excretion pattern after oral application of each fraction was determined in metabolism cages over a period of 2 days (n = 1–2 per fraction). TLC analyses showed that polyglycerols were excreted unchanged via urine or faeces. The smaller polyglycerols (di- and triglycerols) were preferentially excreted in the urine (oral absorption), while the larger polyglycerols (tetra-, penta and hexaglycerol) were preferentially excreted in the faeces (no or less absorption). About 85% of the dose was recovered for each fraction in agreement with the percentage excretion of un-fractionated ^{14}C-polyglycerol. No $^{14}CO_2$ was expired by the animals dosed with fractions which were free of monoglycerol.

Overall, from the available *in vivo* absorption, distribution, metabolism and excretion (ADME) studies, the Panel considered that PGPR is hydrolysed in the gut resulting in the liberation of free polyglycerols, polyricinoleic acid and free ricinoleic acid after oral dosing in rats. The absorption of polyglycerol depends on the chain length, e.g. di- and triglycerol were nearly completely absorbed and excreted unchanged in the urine, whereas long-chain polyglycerols like decaglycerol showed lower absorption rates and were mainly excreted unchanged via faeces. No metabolism and no accumulation of polyglycerols were observed. The Panel considered that castor oil is hydrolysed in the gastrointestinal tract to glycerol and ricinoleic acid and that the ricinoleic acid moiety is absorbed and subjected to identical distribution, metabolism and excretion as orally administered ricinoleic acid. The Panel considered that ricinoleic acid itself and shortened hydroxy fatty acids derived therefrom are incorporated into glycerolipids and may be sequestered and stored in adipose tissue.

3.5.2. Acute toxicity

3.5.2.1. PGPR

For rats and mice, oral median lethal dose (LD_{50}) values of > 18,500 mg/kg bw or > 100,000 mg/kg bw have been reported (JECFA, 1974).

The acute oral toxicity of PGPR was studied in 55 weanling male and female (proportion of each sex not stated) Colworth rats dosed once with about 20 mL PGPR/kg bw (approximately 20,000 mg/kg bw) via gavage. 22 weanling male and female (proportion of each sex not stated) rats were treated with 'a similar dosage of groundnut oil' and were defined as the controls. The Panel noted that groundnut oil was often used as a control for many studies with PGPR and considered it a control for the calorific content associated with administration of PGPR. Apart from slight diarrhoea seen in 42 PGPR-dosed rats and 2 groundnut oil dosed rats, no other untoward toxic effects were observed (Grieco, 1974 [Documentation provided to EFSA n. 9]).

3.5.2.2. Ricinoleic acid

A single 0.5 mL dose of ricinoleic acid (100 mg/mL) was administered by oral gavage to fasted, specific pathogen-free mice (strain CD-1, number not stated), the dose (50 mg/mouse) determined by the authors, on a weight basis, to be approximately that used therapeutically in man (Morehouse et al., 1986). Groups of mice were killed at various intervals and light, transmission and scanning electron microscopy were used to identify structural alterations in the gastrointestinal tract. At 2 h post-dosing, the duodenal villi were markedly shortened when compared to control duodenal villi. This erosion of the villi throughout the duodenum caused massive exfoliation of columnar and goblet cells, filling the lumen with cellular debris and mucus. Disruption of the mucosal barrier resulted in continuity between the intestinal lumen and lamina propria of the villi, with the loss of formed blood elements and lamina propria constituents into the intestinal lumen. The mucosal damage was much more localised at 4 h post-dosing, and the erosion of the villi had been largely repaired. Repair was complete at 6 h post-dosing.

3.5.2.3. Castor oil

In a study by Capasso et al. (1994), castor oil (2 mL, equivalent to 10,700–12,000 mg/kg bw) was administered orally to 10 male Wistar rats (160–180 g body weight). The animals were killed up to 9 h after exposure and two segments from standardised regions of the duodenum and jejunum were evaluated for macroscopic damage. Copious diarrhoea was reported for all animals at 3, 5 and 7 h post-dosing. Macroscopic damage, characterised mainly by vasocongestion, was observed throughout the duodenum and jejunum. The injury observed ranged from mild (at 1 h) to severe (at 5 h) and was less severe at 7 h. Injury was not observed at 0.5 or 9 h after dosing. Castor oil-induced mucosal damage was associated with statistically significant intraluminal release of acid phosphatase.

3.5.2.4. Polyricinoleic acid

No studies were available.

3.5.2.5. Polyglycerols

An oral LD_{50} value for polyglycerol of 20,000 mg/kg bw was reported in studies with mice (no further details available; ChemIDplus, online).

In acute oral toxicity studies, the LD_{50} value of diglycerol in rats was > 2,000 mg/kg bw (no further details available; Solvay, 2015a).

In rats gavaged with polyglycerols-3 (43.3% triglycerol, 19% tetraglycerol), the LD50 was > 2,000 mg/kg bw (no further data; Solvay, 2015b). The oral LD50 of polyglycerols-4 (41.2% triglycerol, 35.2% tetraglycerol) in was also > 2,000 mg/kg bw (no further details; Solvay, 2015c).

Overall, the Panel considered the acute oral toxicity of PGPR and of polyglycerols was low and considered that bolus doses (10,700–12,000 mg/kg bw) of castor oil were capable of causing diarrhoea, which is accompanied with reversible gastrointestinal vasocongestion and mucosal injury.

3.5.3. Short-term and subchronic toxicity

3.5.3.1. PGPR

Mice

In a 14-day study (Grieco, 1974 [Documentation provided to EFSA n. 9]), 100 male and 100 female 6–8 weeks old C57BL mice were randomly selected and placed into 18 groups of five males and five females and fed *ad libitum* with a diet supplemented PGPR or groundnut oil at 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7% or 8% (PGPR dose levels equivalent to 450, 900, 1,800, 2,700, 3,600, 4,500, 5,400, 6,300 and 7,200 mg/kg bw per day, respectively). Ten males and 10 females fed stock diet (Spital) were used as controls. Investigated parameters included body weight gain, food consumption and gross examination of the viscera and liver and kidney weights at necropsy. Food consumption was slightly greater for mice fed the 450–3,600 mg PGPR/kg bw per day compared to the groundnut oil-treated groups. However, neither PGPR nor groundnut oil treatment had any effect on body weights. In male and female mice dosed with ≥ 2,700 mg/kg bw per day, the relative liver weights were significantly increased compared with the non-treated control (Spital) group. Significant increased relative liver weights (not further specified) were also observed in male mice dosed with 3,600, 5,400 or 7,200 mg/kg bw per day and in female mice dosed with ≥ 2,700 mg/kg bw per day compared with mice fed identical levels of groundnut oil. Relative weights of kidneys were not significantly affected. The author considered that no significant adverse effects were seen at a dose level of 1,800 mg PGPR/kg bw per day. In the absence of further details (e.g. the extent of increase in liver weights, dose-effect relationship) and in the absence of histopathological examinations, the Panel could not conclude on the significance of the findings.

In another 14-day study (Grieco, 1974 [Documentation provided to EFSA n. 9]), 140 male and 140 female 6–8 weeks old C57BL mice were randomly selected and placed into 7 groups of 20 males and 20 females and fed with a diet supplemented PGPR or groundnut oil at 0, 5, 10 or 15% (equivalent to 0, 4,500, 9,000 and 13,500 mg/kg bw per day, respectively). Each group was further subdivided into 10 male and 10 female mice and fed their respective diets either *ad libitum* or by restricting to 7 h per day. During the study body weights and food consumption were recorded. On day 14 of the study, five male and five female mice from each group were killed, while all remaining animals were fed a diet supplemented with 10% groundnut oil for two further weeks. All mice fed the diets *ad libitum* showed comparable weight gains with the exception of smaller weight gains in male mice fed with 13,500 mg PGPR/kg bw per day. Weight gains in mice maintained on restricted diets were smaller than those observed for animals fed *ad libitum*, but were higher in animals fed groundnut oil compared with mice fed PGPR. Food consumption was comparable within all dose groups. In all PGPR dose groups, the relative liver weights were increased and this effect was more pronounced in mice maintained on the restricted diet. In contrast, the relative kidney weights were not affected at any dose level of PGPR or groundnut oil. In mice fed 10% groundnut oil for the additional two weeks, there was no evidence for a liver enlargement.

Rats

Weanling Colworth rats (6 male and 6 female) were dosed once daily with about 10 mL/kg bw per day (approximately 10,000 mg/kg bw per day) via oral gavage for five consecutive days (Grieco, 1974 [Documentation provided to EFSA n. 9]). Two control groups were included in the study and were dosed similarly by oral gavage with either groundnut oil or physiological saline (number of control animals/group not stated). The dosing caused no adverse effects on mortality, food consumption and body weight gain and also a gross observation of all animals 14 days after the last dosing gave no indications for adverse effect caused by PGPR.

In a 14-day study (Grieco, 1974 [Documentation provided to EFSA n. 9]), 24 male and 24 female Colworth Wistar rats (approx. 6 weeks old) were randomly divided into four groups and fed *ad libitum* with either purified diet containing 2% groundnut oil and 18% PGPR (equivalent to 16,200 mg/kg bw per day) during week one followed by stock diet (Spital) during week two (Group 1); purified diet containing 20% groundnut oil during week one followed by stock diet (Spital) during week two (group 2); stock diet (Spital) during week one followed by purified diet containing 2% groundnut oil and 18% PGPR (equivalent to 16,200 mg/kg bw per day) during week two (Group 3) or stock diet (Spital) during week one followed by purified diet containing 20% groundnut oil during week two Group 4). At the end of the study, all animals were sacrificed and the viscera were examined for gross abnormalities. The livers were excised, weighed and sectioned for microscopic examination. Treatment with 16,200 mg PGPR/kg bw

per day and 2% groundnut oil over 1 week caused significantly increased liver weights (not stated if liver weight was determined relative to body weight) compared to rats treated with groundnut oil alone. Histological examination of the livers indicated that there were no significant pathological changes to hepatic parenchymal cell cytoplasm. In animals fed with 16,200 mg PGPR/kg bw per day during the second week, small quantities of neutral fat in periportal parenchymal liver cells were observed. This was also seen in male rats fed groundnut oil, but there was a different distribution (although not further described by the author), suggested by the author, to be associated with divergent metabolism of the lipids. Since this effect was not seen in rats fed PGPR or groundnut oil in the first week followed by a stock diet during the second, the author considered this hepatic change as reversible and caused by the feeding of a 20% dietary level of fat. The author noted from microscopic examination that hepatic parenchymal cell nuclei did not present any evidence of degenerative changes or increased mitotic activity. Using a nuclear counting technique, the author considered the increase in liver weights correlated with a decrease in the number of nuclei visible per unit area of liver, and therefore that there was an enlargement of the cytoplasm of hepatocytes (hypertrophy). The author considered that there was no hyperplastic change possibly associated with liver cell damage. The Panel noted that in this study there was not a group of animals treated with PGPR alone.

In a 14-day study (Grieco, 1974 [Documentation provided to EFSA n. 9]), 32 male weanling Colworth albino rats (21 days old) were randomly divided into four groups and fed *ad libitum* either purified diet containing 10% groundnut oil during week one followed by purified diet containing 18% PGPR (equivalent to 16,200 mg/kg bw per day) and 2% groundnut oil during week two (Group 1); purified diet containing 10% groundnut oil (equivalent to 8,100 mg/kg bw per day) during week one followed by purified diet containing 18% castor oil (equivalent to 16,200 mg/kg bw per day) and 2% groundnut oil during week two; purified diet containing 10% groundnut oil during week one followed by purified diet containing 20% groundnut oil during week two or stock diet (Spital) during weeks one and two. The rats in groups 1–3 were conditioned to the purified diet during the first week and were then provided with the test diets during the second week. Body weights were recorded initially and after the first and second weeks, and food consumption was calculated twice weekly. At necropsy, liver and kidneys were excised from each animal, weighed, examined and prepared for a histological evaluation. Liver and kidney RNA and DNA contents were determined in addition to total moisture, total solids and total nitrogen. In rats dosed with 16,200 mg PGPR/kg bw per day or castor oil (equivalent to 16,200 mg/kg bw per day), the weight gain was decreased compared with rats dosed with 20% groundnut oil or the Spital control group. In addition, the relative liver weights were increased while relative kidney weights were not affected. Liver enlargement was not accompanied by an increase in DNA content. In rats dosed with PGPR and to a lesser extent also in rats dosed with castor oil, there were increases in liver cell water and nitrogen contents. The RNA/DNA ratios in the liver were increased, but not in the kidneys. The author considered the changes observed in the liver were indicative of an adaptive hypertrophy and not an adverse effect. The Panel agreed with the author's overall conclusion that PGPR treatment resulted in an adaptive hepatocellular hypertrophy.

In a 13-week feeding study (Grieco, 1974 [Documentation provided to EFSA n. 9]), groups of 10 male and 10 female rats (unknown strain) were fed a diet supplemented with 0%, 1%, 2%, 4% or 8% of PGPR (equivalent to 0, 900, 1,800, 3,600 and 7,200 mg/kg bw per day, respectively). The following parameters were examined: weekly body weight gain and food consumption, mortality, urine analysis twice weekly during weeks 1 and 4 and once during weeks 2, 5, 6 and 9–13, gross observation of the viscera at necropsy and absolute organ weights for the liver, kidneys and adrenals. None of the test animals died during the study. Body weight gain and food consumption were not affected. While the kidney and adrenal weights were not changed in all dose groups, the liver weights ((not stated if liver weight was determined relative to body weight) were increased predominantly in female rats dosed with $\geq$ 3,600 mg PGPR/kg bw per day. Significantly enlarged livers were also seen in male rats dosed with 3,600 mg PGPR/kg bw per day and to a lesser extent at 7,200 mg PGPR/kg bw per day.

3.5.3.2. Ricinoleic acid

No studies were available.

3.5.3.3. Castor oil

Mice

A subchronic oral toxicity study with castor oil was evaluated using groups of 10 male and 10 female B6C3F1 mice (mean body weights = 22.6–23.0 (males); 17.2–17.7 (females)) (NTP, 1992). Five

groups of test animals received diets containing 0.62%, 1.25%, 2.5%, 5.0% or 10% castor oil (equivalent to 1,240, 2,500, 5,000, 10,0000 and 20,000 mg/kg bw per day, respectively) for 13 weeks. A control group of 20 mice received diets that did not contain castor oil. Compared to controls, liver weights were increased in male and female mice receiving diets containing 5% or 10% castor oil. Increased kidney weights were reported for female mice that received 5% or 10% castor oil in the diet. Microscopically, there was no evidence of morphologic changes that could be associated with the slight differences in organ weights between the groups tested. Additionally, there was no evidence of treatment-related lesions in any of the tissues or organs that were examined (all dietary groups). No treatment-related effects were observed on reproductive parameters (testis weight, epididymal sperm motility, density or testicular spermatid head counts or oestrous cycle). The authors concluded that diets containing up to 10% castor oil to B6C3F1 mice was not associated with toxicity to any specific organ, organ system, or tissue. The Panel agreed with this conclusion and identified a no observed adverse effect level (NOAEL) of 20,000 mg/kg bw per day for castor oil, the highest dose tested.

Rats

Ten male weanling rats (4 weeks old; body weights 42.2 $\pm$ 0.6 g) were fed a diet consisting of 10% castor oil (equivalent to 9,000 mg/kg bw per day) for approximately 5 weeks (Masri et al., 1962). A control group (mean weight = 42.2 $\pm$ 0.5 g) received 10% corn oil in the diet. Castor oil in the diet did not induce overt adverse effects, and growth of these animals was comparable to that observed in the control group. At the end of the experiment, mean body weights for test and control rats were 155.6 $\pm$ 4.6 and 160.5 $\pm$ 4.1 g, respectively. Grossly, there were no abnormalities. Additionally, no abnormalities of the perirenal fat were noted.

A subchronic oral toxicity of castor oil was evaluated using groups of 10 male and 10 female F344/N rats (mean body weights = 126–132 g (males); 107–110 g (females)) (NTP, 1992). Five groups of test animals received diets containing 0.62%, 1.25%, 2.5%, 5.0% or 10% castor oil (equivalent to 558, 1,125, 2,250, 4,500 and 9,000 mg/kg bw per day, respectively) for 13 weeks. A control group of 20 rats received diets that did not contain castor oil. Ten additional rats/sex were included at each dose level for evaluation of haematological and clinical chemistry parameters. On days 5 and 21, these animals were anesthetised with CO_2 and blood samples were obtained. These animals were killed following blood collection on day 21. Blood samples for haematology and clinical chemistry were also collected from core-study rats at the end of the study. All core-study animals were killed and complete necropsies were performed, including complete microscopic examination on tissues, on all test and control animals at 13 weeks. No effect on survival or significant differences in average food consumption were noted in any of the five groups of male and female rats fed castor oil. There were no significant differences in mean body weights between test and control groups. The following haematological effects were reported for male rats: a slight decrease in mean corpuscular haemoglobin concentration (MCHC) (10% castor oil diet), a statistically significant decrease ($p < 0.05$) in mean corpuscular volume (MCV) (10% castor oil diet), a decrease in mean corpuscular haemoglobin (MCH) (5% and 10% castor oil diets), and an increase in platelets (1.25%, 5% and 10% castor oil diets). The only haematological abnormality reported for female rats was a statistically significant ($p < 0.05$) decrease in reticulocyte counts (0.62% or 10% castor oil diets). The authors did not consider the haematological effects to be toxicologically significant. A dose-related increase (treatment-related) in the activity of serum alkaline phosphatase was noted in male and female rats at days 5 and 21, and at the end of the study. Increased heart-to-body weight ratios were reported for male rats that received 0.62%, 2.5% and 10.0% castor oil diets; however, no increase in absolute heart weight was noted. The observed differences in organ-to-body weight ratios were not considered treatment related. Microscopically, no morphological changes were found to be associated with the slight differences in organ weights between the dietary groups. No treatment-related effects were observed on reproductive parameters (testis weight, epididymal sperm motility, density or testicular spermatid head counts or oestrous cycle). The authors concluded that diets containing up to 10% castor oil given to F344/N rats were not associated with toxicity to any specific organ, organ system, or tissue. The Panel agreed with this conclusion and identified a NOAEL of 9,000 mg/kg bw per day for castor oil, the highest dose tested.

3.5.3.4. Polyricinoleic acid

No studies were available.

3.5.3.5. Polyglycerols

No studies were available.

Overall, a common effect reported in short-term and subchronic toxicity studies with PGPR was an increase in relative liver weight. The Panel noted that in these studies, the doses administered were often very high single dose levels (16,200 mg/kg bw per day). When several dose levels were tested, the increase was seen after administration of doses greater than or equal to 2,700 mg/kg bw per day. Histological examination of the liver did not report hyperplastic changes; any evidence of degenerative changes or increased mitotic activity, and the changes were reversible upon cessation of administration. Liver enlargement was not accompanied by an increase in DNA content. Therefore, the Panel considered that the increases in liver weight were adaptive and not an adverse response of the liver to the large amount of test substance administered to the animals.

The Panel further considered that diets containing up to 10% castor oil were not associated with toxicity to any specific organ, organ system, or tissue and derived NOAELs of 20,000 and 9,000 mg/kg bw per day for castor oil in mice and rats respectively, the highest doses tested.

3.5.4. Genotoxicity

No *in vitro* or *in vivo* studies with PGPR, polyricinoleic acid or polyglycerols were available. However, genotoxicity data, both *in vitro* and *in vivo* for castor oil and *in vitro* for sodium ricinoleate were available.

3.5.4.1. Castor oil

In vitro *studies*

In the study by Hachiya (1987), castor oil was assessed for its mutagenicity in the reverse mutation assay using *Salmonella* Typhimurium strains TA1537, TA97, TA98, TA100 and TA102, and the *Escherichia coli* strain WP2uvrA up to a maximum concentration of 5.0 mg/plate in dimethylsulfoxide (DMSO), both in the absence and presence of rat S9 metabolic activation and no mutagenicity was observed. The Panel noted that the strain TA1535 was not employed and that only the liquid pre-incubation method was performed.

In the study by Zeiger et al. (1988), castor oil (United States Pharmacopeia purity grade) was assessed for its mutagenicity in the reverse mutation assay using *S.* Typhimurium strains TA1535, TA1537, TA97, TA98 and TA100 up to a maximum concentration of 10,000 μg/plate in DMSO, both in the absence and presence of rat and Chinese hamster S9 metabolic activation at 10% and 30% and no mutagenicity was observed. The Panel noted that the study complies with current OECD Guideline 471 with the exception that tester strains *S.* Typhimurium TA102 or *E. coli* WP2uvrA bearing AT mutation were not used.

Induction of sister chromatid changes (SCE) by castor oil was evaluated in Chinese hamster ovary (CHO) cells (NTP, 1992). The cells were incubated with castor oil dissolved in DMSO at concentrations up to 5,000 μg/mL both in the absence and presence of rat liver metabolic activation. Castor oil did not induce SCE either with or without metabolic activation. However, the Panel noted that SCE assay does not belong to the *in vitro* tests recommended for regulatory purposes (EFSA Scientific Committee, 2011).

In a chromosomal aberration assay using a CHO cell line, castor oil was assessed for its clastogenicity at concentrations up to 5,000 μg/mL both in the absence and presence of rat liver S9 metabolism for 10 and 2 h, respectively (NTP, 1992). No induction of chromosomal aberration was observed at any treatment point.

In vivo studies

The *in vivo* genotoxic potential of castor oil was evaluated in a micronucleus test in peripheral blood erythrocytes of male and female B6C3F1 mice fed with a diet containing 0.62%, 1.25%, 2.5%, 5.0% and 10% castor oil (equivalent to 1,240, 2,500, 5,000, 10,000 and 20,000 mg/kg bw per day, respectively) for 13 weeks NTP (1992). No induction of micronuclei was observed both in the normochromatic and polychromatic erythrocytes in any animal treatment group.

3.5.4.2. Sodium ricinoleate

In vitro *studies*

In the study by Haworth et al. (1983), sodium ricinoleate was assessed for its mutagenicity in the reverse mutation assay using *S.* Typhimurium strains TA1535, TA1537, TA98 and TA100 up to a

maximum concentration of 10,000 μg/plate in DMSO, both in the absence and presence of rat and Chinese hamster S9 metabolic activation at 10% or 30% and no mutagenicity was observed. The Panel noted that the tester strains *S.* Typhimurium TA102 or *E. coli* WP2uvrA bearing AT mutation were not used.

3.5.4.3. In silico evaluations

Both glycerol and ricinoleic moieties were analysed for potential structural alerts for genotoxicity using the OECD (Q)SAR Toolbox.[19] No structural alerts were found except an alert for 'Hacceptor-path3-Hacceptor' in the *in vivo* micronucleus test. However, the Panel considered this alert not relevant based on the consideration that 'Hacceptor-path3-Hacceptor' refers to non-covalent binding to DNA or proteins as a result of the presence of two bonded atoms connecting two hydrogen bond acceptors and its positive predictivity is quite low, ranging from 'none' (34%) to just 63% depending on the database, with a high incidence of false positives (Benigni et al., 2010, 2012).

No documented metabolites were found in the database. The rat liver S9 metabolism simulator indicated potential formation from both ricinoleic acid and glycerol, of simple aldehydes, which are structural alerts for genotoxicity and genotoxic carcinogenicity (since compounds carrying an aldehyde group can potentially undergo Schiff base formation with a primary amine) (Wang et al., 2000; Speit et al., 2007). These compounds are considered potentially genotoxic, as demonstrated by their ability to react *in vivo* with nucleobases, without metabolic activation, forming adducts, interbase cross-links (both intra and interstrand) and DNA-protein crosslinks (Wang et al., 2000; Speit et al., 2007).

However, carbon chain length for aliphatic aldehydes, and in general molecular size, can strongly modulate the formation of every type of cross-link and accessibility to DNA nucleobases (OECD (Q)SAR Toolbox).

The Panel considered that any aldehydes produced from the ricinoleic acid and glycerol moieties would have a bulky aliphatic carbon chain that would prevent interactions with DNA.

Overall, although no studies on PGPR and polyglycerol were available, data both *in vitro* and *in vivo* for castor oil and *in vitro* for sodium ricinoleate indicated that both compounds did not show a genotoxic potential. In addition, taking into account the information on structure–activity relationships and the observation that glycerol (E 422) as a food additive does not raise concern with respect to genotoxicity following the recent re-evaluation (EFSA ANS Panel, 2017) the Panel considered that PGPR (E 476) was not of concern with regard to genotoxicity.

3.5.5. Chronic toxicity and carcinogenicity

3.5.5.1. PGPR

Mice

In a carcinogenicity study (Grieco, 1974 [Documentation provided to EFSA n. 9]), four groups of Colworth C57BL mice (25 males and 25 females per group) were fed diets for 80 weeks. The groups were fed either a stock diet (non-treated control); 5% groundnut oil; a semipurified diet containing 5% PGPR (equivalent to 7,500 mg/kg bw per day) or the purified diet alone. Animals were observed daily for clinical signs of toxicity or changes in behaviour and were weighed weekly. Food consumption was measured twice weekly. Blood was collected of all surviving animals at study termination. The blood was analysed for erythrocyte and leucocyte counts and haemoglobin concentrations. Each animal was subjected to gross examination at necropsy. The following organs were weighed: heart, kidney, liver and testes. These organs, together with the lung, spleen, adrenals, skin, stomach, intestine, thyroid, thymus, mammary gland and lymph nodes, together with any macroscopic abnormality were removed, fixed and processed for histological examination. The dosing with 7,500 mg PGPR/kg bw per day had no adverse effects on survival, body weight gain and food consumption. Apart from a significantly decreased white blood cell count compared with untreated animals, no other adverse haematological effects were noted. The absolute liver and kidney weights of female mice dosed with PGPR were significantly increased compared with the groundnut oil control mice. In addition, the liver weights of male and female mice fed with PGPR or groundnut oil were significantly increased compared with the non-treated control mice. Based on these findings in combination with the results of the histopathological examination, the author attributed enlargement of the liver to an adaptive hypertrophy. The Panel agreed with this conclusion. The organ weights of

[19] OECD QSAR Toolbox, version 3.3.5.17 (July 2015).

spleen, testes or heart were not significantly affected and histopathological examination of selected tissues showed no adverse effects. No increased incidence in tumours in mice dosed with 7,500 mg/kg bw per day was observed. The Panel noted that the no adverse effects were reported at the only dose tested in this study (7,500 mg/kg bw per day).

Rats

PGPR was tested in two dietary feeding trials with oral dosing over 30 and 45 weeks (Grieco, 1974 [Documentation provided to EFSA n. 9]). In each study, 48 male and female Wistar rat were divided into three groups of eight male and eight females in total. Each of the test groups were maintained on purified diets in which the normal fat content (10% by weight) was replaced with 9% PGPR (equivalent to 4,500 mg PGPR/kg bw per day) and 1% groundnut oil. For comparison, two control diets containing either 1% or 10% groundnut oil were also evaluated. The examined parameters included gross observation of appearance and behaviour; food consumption; body weight gain; bromosulfophthalein liver function test; specific gravity of urine samples; absolute organ weights and gross and microscopic examination of selected tissue samples. After oral dosing with 9% PGPR for 30 and 45 weeks, no adverse effects on growth, food consumption, liver and kidney function, haematology, and gross and microscopic examination of selected tissues including liver, spleen, testes, kidney, adrenal and pituitary were seen. Also, absolute organ weights of kidneys, adrenals, pituitary, spleen or testes were not affected. In several rats, an enlargement of the liver was noted but there was no disturbance in liver function and also no adverse histopathological findings. The dietary treatment of rats with 4,500 mg PGPR/kg bw per day over 30 or 45 weeks was without a significant toxic effect. The Panel noted that no adverse effects were reported at the only dose tested in this study (4,500 mg/kg bw per day).

A 2-year combined chronic toxicity/carcinogenicity study was performed with 30 male and 30 female Colworth Wistar rats per group (aged 32–42 days) and fed diets supplemented with either 5% PGPR (equivalent to 2,500 mg/kg bw per day) or 5% groundnut oil (Grieco, 1974 [Documentation provided to EFSA n. 9]). Animals were observed daily for clinical signs of toxicity or changes in behaviour, and were weighed weekly. Food consumption was measured three times weekly. Liver function was examined after 84 and 103 weeks using the bromosulfophthalein excretion test. Urine specific gravity was also determined at these timepoints. Blood was collected after 80 weeks from four rats of each sex and each group, and on all surviving animals at study termination. Blood samples were analysed for erythrocyte and leucocyte counts; haemoglobin concentrations and value; red cell fragility and prothrombin time. Each animal was subjected to gross examination at necropsy. The following organs were weighed and the organ/body weight ratios determined: adrenals, heart, kidney, spleen, liver, testes, thyroid and pituitary. These organs, together with the lung, ovary, uterus, thymus, stomach, intestine, caecum, bladder, lymph nodes, skin, mammary gland, tongue and any macroscopic abnormality were removed, fixed and processed for histological examination. PGPR treatment had no adverse effects on weekly body weight gain and food consumption, haematological endpoints, liver function and kidney function. The survival rates between both groups were comparable (73.3% vs 60% for PGPR). PGPR treatment resulted in increased relative kidney weights in male and female rats and increased relative liver weights in female rats. The author attributed enlargement of the liver and the kidney to an adaptive hypertrophy. At histopathological examination, no adverse effects were observed and there was no indication for an increased tumour incidence in rats dosed with 2,500 mg PGPR/kg bw per day. Considering the absence of reported adverse effects under histopathological examinations, the Panel agreed with the conclusion of the authors. The Panel noted no adverse effects and no increase in tumour incidences were reported in this study and identified a NOAEL of 2,500 mg/kg bw per day for PGPR, the only dose tested.

3.5.5.2. Ricinoleic acid

No studies were available.

3.5.5.3. Castor oil

No studies were available.

3.5.5.4. Polyricinoleic acid

No studies were available.

3.5.5.5. Polyglycerols

No studies were available.

Overall, similarly to the short-term and subchronic toxicity studies, the most consistent effect reported in chronic and carcinogenicity studies in mice and rats was an increase in liver weight. The Panel noted that in these studies, the animals were administered doses from 2,500 up to 7,500 mg/kg bw per day. Histological examination of the liver did not report any neoplastic changes, fibrosis or inflammation. In addition, as assessed by the bromosulfophthalein test, excretory liver function was not affected. Therefore, the Panel considered that the increases in liver weight were an adaptive – not an adverse – response of the liver. Furthermore, PGPR did not demonstrate any carcinogenic effects in the available studies at doses of 7,500 and 4,500 mg/kg bw per day in mice and rats, respectively, the only doses tested.

3.5.6. Reproductive and developmental toxicity

3.5.6.1. Reproductive toxicity

PGPR

Colworth Wistar rats randomly selected from five litters were fed (6 males and 13 females) either a commercial pelleted stock diet supplemented with 1.5% PGPR (equivalent to 750 mg/kg bw per day) or fed the commercial pelleted stock diet only as control (11 males and 17 females) (Grieco, 1974 [Documentation provided to EFSA n. 9]; Wilson and Smith, 1998). The rats were weaned at 23 days and mated at 121 days. Breeding was continuous and the males were only separated from the females when it was apparent that the female was pregnant. Each pair occupied a single cage and they were maintained until the female had produced five litters or until such time as it became evident that breeding had ceased. In all instances, the first litters were discarded after weaning and second-generation breeders were randomly selected (two males and two females) from each of the second and fourth litters. By selecting from two first-generation litters, the number of animals was increased to 52 of each sex in the control and 32 of each sex in the PGPR group. The third-generation breeders were selected in a similar manner, by which time the control and the PGPR groups were increased to 92 and 44 rats of each sex, respectively. The main focus of the study design was to observe any effect on breeding and the measured parameters in each of the three generations included the number of litters per dam, average litter size, average weaning weights of males and females, litters per group showing 100% survival and total survival at day 21. The only significant change in breeding performance was a reduction in the percentage of animals weaned in the second generation (rats weaned/rats born: 44%; litters entirely weaned: 22%), but as this occurred in the control group to a similar extent (54 and 38%, respectively), the author of this study concluded that this was due to an unknown environmental factor and was not treatment related. The third generation breeders (83 control and 23 PGPR-treated rats) maintained on their respective diets for more than one year were sacrificed and examined to investigate potential adverse effects. The author considered that the treatment with 750 mg PGPR/kg bw per day did not result in adverse effects related to reproductive capacity or development of the offspring during three generations of continuous exposure and that there was no evidence for any significant morphological effect. However, the Panel considered that there are limitations in the study, in design (not comparable with a two-generation reproductive toxicity study), number of animals initially pregnant in the test group was low (n = 13), infection of the animals, reduced breeding in the second generation and the limited reporting of the study. The Panel noted that due to these limitations this study could not be used as the pivotal study for hazard identification and characterisation.

Ricinoleic acid

No studies were available.

Castor oil

No studies were available.

Polyricinoleic acid

No studies were available.

Polyglycerols

No studies were available.

3.5.6.2. Developmental toxicity

No data on the developmental toxicity of PGPR, ricinoleic acid, castor oil, polyricinoleic acid and polyglycerols were available.

Overall, the Panel considered that the only reproductive toxicity study with PGPR available to the Panel had limitations in its design (as it was not comparable with a two-generation reproductive toxicity study) and in the number of animals initially pregnant in the test group was low (n = 13). The Panel further noted that there was infection in the animals; only one dose tested; reduced breeding in the second generation and limitations in reporting of the study. The Panel noted that this reproductive study was used by the SCF (and by JECFA) to derive the current ADI of 7.5 mg/kg bw per day. However, the Panel considered that due to the above limitations, this study was not appropriate for deriving a health-based guidance value for PGPR.

3.5.7. Other studies

3.5.7.1. Studies in humans

PGPR

In a study with 19 healthy volunteers (8 males and 11 females aged 19–66 years), PGPR was orally administered via soups (ca 2,500 mg/L), cakes (10,000 mg/cake) and mint-flavoured toffee bars (5,000 mg/bar) (Grieco, 1974 [Documentation provided to EFSA n.9]). Endogenous fat excretion was established during a control period (1 week) and then during dietary administration of PGPR at 5,000 and 10,000 mg per day (equivalent to 71.4 and 142.8 mg/kg bw per day, respectively) during the second and third week, respectively. Blood samples were taken twice per week, and urine samples were collected during each of the three weeks. Results obtained by serum protein electrophoresis were considered normal and there was no indication for an increase in serum globulin. No significant effects were observed on serum bilirubin and total and free cholesterol levels; on serum glutamate-pyruvate transaminase and cholinesterase enzyme activities. Creatinine clearance was not affected and total faecal fat and nitrogen were within the normal range.

Ricinoleic acid

Few cases of contact allergy to ricinoleic acid were reported in the literature, when used as one of the component of lipsticks or toothpastes (Lim and Goh, 2000).

Castor oil

Castor oil is used as a laxative and as an alternative medicine for induction of labour in pregnant women. The effects of castor oil were mediated by ricinoleic acid released from castor oil by intestinal lipases. Ricinoleic acid activates intestinal and uterine smooth muscle cells via EP(3) prostanoid receptors which is the mechanism by which the pharmacological effects were mediated (Tunaru et al., 2012).

To elicit uterine contractions, castor oil has been used at a standard oral dose of 60 mL. There were three case reports on severe side effects (two with rupture of the uterus during labours) which have not been clearly related to the administration of castor oil (Schoenes and Stammschulte, 2013). In clinical studies, three prospective (Garry et al., 2000; Azhari et al., 2006; Gilad et al., 2012), one retrospective (Boel et al., 2009), the efficacy and safety of castor oil has been evaluated.

Table 12: Clinical studies on the use of castor oil

Reference	Dose (mL)	Number of participating women	Side effects observed
Azhari et al. (2006)	60[(a)]	24	Nausea (11/24)
Boel et al. (2009)	60[(a)]	205	None reported
Garry et al. (2000)	60[(a)]	52	Nausea (52/52)
Gilad et al. (2012)	60[(a)]	37	None reported

(a): Equivalent to 960 mg/kg bw.

In all studies, the standard dose has been given. No side effects were reported from the study of Boel et al. (2009) and Gilad et al. (2012), whereas all the patients in the study of Garry et al. (2000) and 11 out of 24 patients in the study of Azhari et al. (2006) complained of nausea.

In a review (Schiller, 2001) on the therapy of constipation, it is mentioned that doses of 30–60 mL castor oil produce effective catharsis.

Polyricinoleic acid

No studies were available.

Polyglycerols

No studies were available.

Overall, the Panel considered that human studies with PGPR demonstrated that there is no indication of significant adverse effect after oral intake up to 10,000 mg per day (equivalent to 142.8 mg/kg bw per day) over 3 weeks (Grieco, 1974 [Documentation provided to EFSA n. 9]). In clinical studies with castor oil used at a bolus dose of 960 mg/kg bw, only minor gastrointestinal discomfort was reported.

3.5.8. Studies with other emulsifiers

According to information provided by industry, PGPR is used as a viscosity modifier and it is also used to maintain stable emulsions of oil and water systems at high water content (EFEMA, 2009 [Documentation provided to EFSA n. 6]).

In several recent studies, some other emulsifiers have been reported to alter the gut microbiota, to promote gut inflammation, obesity and to impair glycaemic control (Swidsinski et al., 2009a,b; Renz et al., 2012; Merga et al., 2014; Cani and Everard, 2015; Chassaing et al., 2015; Romano-Keeler and Weitkamp, 2015; Lecomte et al 2016). The Panel noted that, even though some of these effects are not systematically studied in toxicity studies performed according to toxicity testing guidelines, they would be investigated on a case by case basis if indicated by the results of the general toxicity testing as recommended in the Guidance for submission of food additives (EFSA ANS Panel, 2012). The Panel considered that additional studies will be needed to show the relevance of the effects seen in mice for human health.

3.6. Discussion

The Panel noted that PGPR (E 476) is manufactured from glycerol and castor oil and that following extraction of castor oil, ricin is left in the press-cake/castor bean meal (also called castor meal, castor residue, castor extract and de-oiled castor cake) (EFSA CONTAM Panel, 2008). Because active ricin has serious toxic effects even at very low concentration, the Panel considered that a maximum limit for active ricin should be included in the EU specifications for PGPR (E 476).

The Panel noted that according to Commission Regulation (EU) No 231/2012, glycerol (E 422) may be contaminated with 3-MCPD. In addition, information on the manufacturing processes of glycerol has been considered by the ANS Panel in the re-evaluation of glycerol (E 422) (EFSA ANS Panel, 2017). The Panel noted that glycerol (E 422) can be produced by a variety of methods and that many of them lead to the presence or formation of contaminants, which are of toxicological concern. The Panel considered that the manufacturing process for PGPR (E 476) should not allow the presence of residuals of genotoxic and carcinogenic concern at a level which would result in a MOE below 10,000 (EFSA Scientific Committee, 2012b). The Panel considered that maximum limits for potential impurities – e.g. 3-MCPD – in glycerol as a raw material in the manufacturing process of PGPR should also be established for the EU specifications of PGPR (E 476).

The Panel noted that epichlorohydrin and glycidol may be present in PGPR (E 476) from the manufacturing processes of polyglycerols. Epichlorohydrin and glycidol are classified as a carcinogen (2A) according to IARC (1999) and probably carcinogenic to humans (2A) according to IARC (2000), respectively. The EFSA CONTAM Panel has characterised glycidol as genotoxic and carcinogenic (EFSA CONTAM Panel, 2016). The Panel considered that the presence of epichlorohydrin and/or glycidol in PGPR (E 476) would need further assessment as their presence could raise a safety concern.

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 PGPR and effects on the microbiota itself.

From the available *in vivo* ADME studies with oral dosing in rats, the Panel considered that PGPR was hydrolysed in the gut resulting in the liberation of free polyglycerols, polyricinoleic acid and free ricinoleic acid after oral dosing in rats. The absorption of polyglycerols depends on the chain length, e.g. di- and triglycerol are nearly completely absorbed and excreted unchanged in the urine, whereas long-chain polyglycerols like decaglycerol show lower absorption rates and were mainly excreted unchanged via faeces. No metabolism and no accumulation of polyglycerols were observed. The Panel considered that castor oil was hydrolysed in the gastrointestinal tract to glycerol and ricinoleic acid and the ricinoleic acid moiety is absorbed and subjected to identical distribution, metabolism and excretion as orally administered ricinoleic acid. Ricinoleic acid itself was shown to be readily incorporated into the fatty acid pathway, and after oral dosing for up to 30 days, an accumulation of hydroxy acids by 5% of fatty acids in fat tissue was noted. An analysis of adipose tissue indicated an occurrence of shorter chain hydroxyl acids other than ricinoleic acid. No studies were available for polyricinoleic acid.

The Panel considered the acute oral toxicity of PGPR and of polyglycerols is low. No mortality occurred in the single species examined at a single high dose level of PGPR (20,000 mg/kg bw). The Panel considered that bolus doses of castor oil are capable of causing diarrhoea which is accompanied with reversible gastrointestinal vasocongestion and mucosal injury. No studies were available for polyricinoleic acid.

The Panel considered that a common effect reported in short-term and subchronic toxicity studies with PGPR was an increase in relative liver weight. The Panel noted that in these studies, the doses administered were often very high single dose levels (e.g. 16,200 mg/kg bw per day). When several dose levels were tested, the increase was seen after administration of doses greater than or equal to 2,700 mg/kg bw per day. Histological examination of the liver did not report hyperplastic changes; any evidence of degenerative changes or increased mitotic activity and the changes were reversible upon cessation of administration. Liver enlargement was not accompanied by an increase in DNA content. Therefore, overall, the Panel considered that the increases in liver weight were adaptive – not an adverse – response of the liver to the large amount of test substance administered to the animals. The Panel further considered that diets containing up to 10% castor oil was not associated with toxicity to any specific organ, organ system or tissue and derived NOAELs of 20,000 and 9,000 mg/kg bw per day, the highest doses tested, for castor oil in mice and rats, respectively. No studies were available for ricinoleic acid, polyricinoleic acid and polyglycerols.

Although no genotoxicity studies on PGPR and polyglycerol were available, data both *in vitro* and *in vivo* for castor oil and *in vitro* for sodium ricinoleate indicated that both compounds did not show a genotoxic potential. In addition, taking into account the information on structure–activity relationships and the observation that glycerol (E 422) as a food additive does not raise concern with respect to genotoxicity following the recent re-evaluation (EFSA ANS Panel, 2017), the Panel considered that PGPR (E 476) was not of concern with regard to genotoxicity

The Panel considered the most consistent effect reported in chronic and carcinogenicity studies was an increase in liver weight. The Panel noted that in these studies, the animals were administered doses from 2,500 up to 7,500 mg/kg bw per day. Histological examination of the liver did not report any neoplastic changes, fibrosis or inflammation. In addition, as assessed by the bromosulfophthalein test, excretory liver function was not affected. Therefore, the Panel considered that the increases in liver weight were an adaptive – not an adverse – response of the liver. Furthermore, PGPR did not demonstrate any carcinogenic effects in the available studies at doses up to 7,500 and 4,500 mg/kg bw per day in mice and rats, respectively, the only doses tested. Overall, the Panel considered a combined 2-year chronic toxicity/carcinogenicity study in rats as the critical study for determining a reference point because the combination of studies examined the most extensive range of endpoints including histopathological examinations of reproductive organs. In addition, the Panel noted that these long-term studies confirmed that the changes in the relative weight of liver and kidney were adaptive rather than adverse.

No chronic and carcinogenicity studies were available for ricinoleic acid, castor oil, polyricinoleic acid and polyglycerols.

No data on the developmental toxicity of PGPR, ricinoleic acid, castor oil, polyricinoleic acid and polyglycerols were available. The only available reproductive toxicity study with PGPR had limitations in its design (as it was not comparable with a two-generation reproductive toxicity study) and in the number of animals initially pregnant in the test group was low (n = 13). The Panel further noted that there was infection in the animals; only one dose tested; reduced breeding in the second generation and limitations in reporting of the study. The Panel noted that this reproductive study was used by the

SCF (and by JECFA) to derive the current ADI of 7.5 mg/kg bw per day. However, the Panel considered that due to the above limitations, this study was not an appropriate study for deriving a health-based guidance value for PGPR.

The Panel considered that human studies with PGPR demonstrated that there is no indication of adverse effect after oral exposure up to 10,000 mg per day (equivalent to 142.8 mg/kg bw per day) over 3 weeks (Grieco, 1974 [Documentation provided to EFSA n. 9]). In clinical studies with castor oil used at a bolus oral dose of 960 mg/kg bw, only minor gastrointestinal discomfort was reported. No human studies were available for ricinoleic acid, polyricinoleic acid and polyglycerols.

The Panel considered that PGPR was tolerated at high doses (up to 13,000 mg/kg bw per day in mice; 16,200 mg/kg bw per day in rats) in short-term and subchronic studies; at high doses (up to 13,000 mg/kg bw per day in mice; 16,200 mg/kg bw per day in rats) in chronic and carcinogenicity and in human studies at a dose of 142.8 mg/kg bw per day over 3 weeks.

The Panel considered that although the only reproductive toxicity study had limitations and no data were available regarding potential developmental toxicity of PGPR, an additional uncertainty factor was not required because the oral two-year combined chronic toxicity/carcinogenicity study in rats included histopathology of reproductive organs and no changes were observed. In addition, at markedly higher doses (up to 13,000 mg/kg bw per day in mice; 16,200 mg/kg bw per day in rats) no adverse effects were observed in the other chronic studies in rats and a carcinogenicity study in mice. Furthermore, no adverse effects were observed in the limited reproductive toxicity study.

Considering all the available toxicological database and based on the absence of adverse effects in an oral 2-year combined chronic toxicity/carcinogenicity study in rats from which a NOAEL of 2,500 mg PGPR/kg bw per day, the highest dose tested, was identified and applying an uncertainty factor of 100, the Panel derived an ADI of 25 mg PGPR/kg bw per day.

The Panel considered that the available data set gives reason to revise the ADI of 7.5 mg/kg bw per day, allocated by SCF in 1978, to a new ADI of 25 mg/kg bw per day.

To assess the dietary exposure to PGPR (E 476) from its use as a food additive, the exposure was calculated 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*).

The Panel noted that the exposure estimates did not exceed the ADI of 25 mg/kg bw per day in any of the exposure scenarios considered (Section 3.4.1) for any population group both at the mean and the 95th percentile of exposure (Table 4).

The Panel noted that the proposed extension of the use of PGPR (E 476) in emulsified sauces, including mayonnaise (FC12.6) did not result in exposure levels that exceeded the ADI in any of the population groups according to the *regulatory maximum level exposure assessment scenario* (Table 8).

4. Conclusions

Based on the absence of adverse effects in an oral 2-year combined chronic toxicity/carcinogenicity study from which a NOAEL of 2,500 mg PGPR/kg bw per day, the only dose tested, was identified and applying an uncertainty factor of 100, the Panel derived an ADI of 25 mg PGPR/kg bw per day.

Considering that exposure estimates did not exceed the ADI of 25 mg/kg bw per day in any of the exposure scenarios for any population group both at the mean and the 95th percentile, the Panel concluded that PGPR (E 476) as a food additive at the permitted or reported use and use levels would not be of safety concern

The Panel also concluded that the additional use of PGPR (E 476) at 4,000 mg/kg in the food category emulsified sauces would not result in a total exposure to PGPR (E 476) that exceeds the ADI.

5. Recommendations

The Panel recommended that:

- the maximum limits for the impurities of toxic elements (lead, mercury and arsenic) in the EU specification for PGPR (E 476) should be revised in order to ensure that PGPR (E 476) as a food additive will not be a significant source of exposure to those toxic elements in food;
- a maximum limit for active ricin should be included in the EU specifications for PGPR (E 476);
- a maximum limit for 3-MCPD should be included in the EU specifications for PGPR (E 476);

- given that during the manufacturing processes of glycerol, potential impurities of toxicological concern could be formed, limits for such impurities should be included in the EU specifications for PGPR (E 476);
- given that during the manufacturing processes of polyglycerols, genotoxic impurities – e.g. epichlorohydrin and glycidol – could be present, limits for such impurities should be included in the EU specifications for PGPR (E 476);
- an analytical method for the determination of actual PGPR (E 476) content in food should be developed.

Documentation provided to EFSA

1) Application for a modification of the conditions of use of an already authorised food additive. Submitted by EMULSAR Sarl, on 3 June 2016; updated on 7 July 2016.
2) Austen Business Solutions Ltd 2011. Re-evaluation of food emulsifiers. Use of supplementary information collected via REACH. 20.1.2011. Submitted to EFEMA (European Food Emulsifier Manufacturer's Association), P10039-1. Submitted by EFEMA, January 2011.
3) Davies AP and Harkes A 1977. A Procedure for Determination of Polyglycerol Polyricinoleate (PGPR) in Chocolate. Unilever Research Laboratory. England. Submitted by EFEMA, January 2011.
4) EDA (European Dairy Association), 2016. Data on usage levels of Polyglycerol polyricinoleate (E 476) in foods in response to the EFSA call for food additives usage level and/or concentration data in food and beverages intended for human consumption (Batch 4). Submitted to EFSA on 30 May 2016.
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Abbreviations

3-MCPD	3-monochloropropane-1,2-diol
ADI	acceptable daily intake
ADME	absorption, distribution, metabolism and excretion
ANS	EFSA Scientific Panel on Food Additives and Nutrient Sources added to Food
bw	body weight
CAS	Chemical Abstracts Service

CEF	EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CHO	Chinese hamster ovary
CONTAM	EFSA Panel on Contaminants in Food Chain
DMSO	dimethylsulfoxide
EDA	European Dairy Association
EFEMA	European Food Emulsifiers Manufacturers Association
EINECS	European Inventory of Existing Chemical Substances
FAO	Food and Agriculture Organization of the United Nations
FCS	food categorisation system
FDE	FoodDrinkEurope
GC	gas chromatography
GLC	gas-liquid chromatography
GNPD	Global New Products Database
IARC	International Agency for Research on Cancer
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LD_{50}	median lethal dose
MCHC	mean corpuscular haemoglobin concentration
MCV	mean corpuscular haemoglobin
MOE	margin of exposure
MPL	maximum permitted level
NDA	EFSA Panel on Dietetic Products, Nutrition and Allergies
NMR	nuclear magnetic resonance
NOAEL	no observed adverse effect level
NTP	National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
PGPR	polyglycerol polyricinoleate
QS	*quantum satis*
SCE	sister chromatid changes
SCF	Scientific Committee on Food
TemaNord	is a publishing series for results of the often research-based work that working groups or projects under Nordic Council of Ministers have put in motion
TLC	thin-layer chromatography
WHO	World Health Organization

Appendix A – Summary of the reported use levels (mg/kg or mg/L as appropriate) of PGPR (E 476) provided by industry

Food category number	Food category name	N	Typical usage level			Maximum usage level	Information provided by[b]
			Mean	Range (min–max)			
02.2.2	Other fat and oil emulsions	1	3,999.0			4,000.0	EDA
02.2.2	Other fat and oil emulsions	24	1,791.7	500.0	4,000.0	4,000.0	EFEMA
02.2.2	Other fat and oil emulsions	2	3,950.0	3,900.0	4,000.0	4,000.0	FDE
03	Edible ices[a]	45	21.1	0.3	525.0	525.0	FDE
05.1	Cocoa and Chocolate products as covered by Directive 2000/36/EC	10	2,900.0	2,000.0	5,000.0	5,000.0	EFEMA
05.1	Cocoa and Chocolate products as covered by Directive 2000/36/EC	44	1,161.3	43.7	5,000.0	5,000.0	FDE
05.1	Cocoa and Chocolate products as covered by Directive 2000/36/EC	1	2,500			2,500	Mars
05.2	Other confectionery including breath freshening microsweets	10	2,600.0	2,000.0	4,000.0	5,000.0	EFEMA
05.2	Other confectionery including breath freshening microsweets	1	150.0			150.0	FDE
05.4	Decorations, coatings and fillings, except fruit-based fillings covered by category 4.2.4	4	2,250.0	1,500.0	3,000.0	5,000.0	EFEMA
05.4	Decorations, coatings and fillings, except fruit-based fillings covered by category 4.2.4	2	1,357.5	1,000.0	1,715.0	3,000.0	FDE
12.6	Sauces	4	4,000.0	4,000.0	4,000.0	4,000.0	EFEMA
15.2	Processed nuts[a]	1	2,309.0			4,500.0	FDE

(a): FCs in which the additive is not authorised according to Annex II; however, the food additive could be present in the coating.
(b): All data were received in 2016 through the batch 4 call for data except data from Mars which were received in 2009 following the 2009–2010 call for data.

Appendix B – Number and percentage of food products labelled with PGPR (E 476) out of the total number of food products present in Mintel GNPD per food subcategory between 2011 and 2016

Mintel subcategory[a]	Total number of products	Products labelled with PGPR (E 476)	
		Number	%
Chocolate Countlines	2,472	372	15.0
Dairy-Based Frozen Products	8,333	1,043	12.5
Margarine & Other Blends	1,056	124	11.7
Individually Wrapped Chocolate Pieces	2,676	246	9.2
Non-Individually Wrapped Chocolate Pieces	5,511	464	8.4
Seasonal Chocolate	5,756	478	8.3
Chocolate Tablets	8,842	724	8.2
Sweet Biscuits/Cookies	18,532	1,007	5.4
Other Chocolate Confectionery	303	14	4.6
Snack/Cereal/Energy Bars	5,365	224	4.2
Cakes, Pastries & Sweet Goods	13,930	425	3.1
Mixed Assortments	332	7	2.1
Toffees, Caramels & Nougat	1,990	39	2.0
Chocolate Spreads	1,219	19	1.6
Butter	1,503	23	1.5
Other Frozen Desserts	1,682	25	1.5
Soft Cheese Desserts	1,608	23	1.4
Nut Spreads	837	9	1.1
Rice Snacks	424	4	0.9
Baking Ingredients & Mixes	9,545	82	0.9
Flavoured Milk	1,497	11	0.7
Other Snacks	150	1	0.7
Dessert Toppings	669	4	0.6
Chilled Desserts	6,558	36	0.5
Shelf-Stable Desserts	3,198	14	0.4
Malt & Other Hot Beverages	1,072	4	0.4
Wheat & Other Grain-Based Snacks	2,074	7	0.3
Caramel & Cream Spreads	309	1	0.3
Cold Cereals	6,685	20	0.3
Other Sugar Confectionery	1,158	3	0.3
Snack Mixes	1,562	4	0.3
Sandwiches/Wraps	2,765	7	0.3
Bread & Bread Products	10,701	24	0.2
Corn-Based Snacks	2,369	5	0.2
Spoonable Yogurt	10,396	21	0.2
Popcorn	1,207	2	0.2
Sports Drinks	847	1	0.1
Beverage Mixes	884	1	0.1
Hors d'oeuvres/Canapes	4,253	4	0.1
Dressings & Vinegar	3,442	3	0.1
Cream	1,711	1	0.1
Honey	1,849	1	0.1
Poultry Products	6,917	3	0.0
Potato Products	3,317	1	0.0

Mintel subcategory[a]	Total number of products	Products labelled with PGPR (E 476)	
		Number	%
Pizzas	4,605	1	0.0
Nuts	4,963	1	0.0
Savoury Biscuits/Crackers	5,036	1	0.0
Table Sauces	6,312	1	0.0
Total sample	**188,422**	**5,535**	**2.9**[b]

PGPR: polyglycerol polyricinoleate; GNPD: Global New Products Database.
(a): According to the Mintel GNPD food categorisation.
(b): In total, around 2.9% of the foods available on the Mintel GNPD are labelled with PGPR (E 476) between 2011 and 2016.

Appendix C – Concentration levels of PGPR (E 476) used in the maximum level exposure assessment scenario and the refined exposure scenarios (mg/kg or mL/kg as appropriate)

Food category number	Food category name	Restrictions/exception	MPL	Concentration levels used in the refined exposure assessment scenario (only reported use levels)		Comments
				Mean	Maximum	
02.2.2	Other fat and oil emulsions including spreads as defined by Council Regulation (EC) No 1234/2007 and liquid emulsions	Only spreadable fats as defined in Article 115 and Annex XV to Regulation (EC) No 1234/2007, having a fat content of 41% or less and similar spreadable products with a fat content of less than 10% fat	4,000	3,950	4,000	Reported use levels
03	Edible ices		750[(a)]	21	525	Reported use levels
05.1	Cocoa and Chocolate products as covered by Directive 2000/36/EC		5,000	1,160	5,000	Reported use levels
05.2	Other confectionery including breath freshening microsweets	Only cocoa-based confectionery	5,000	150	150	Reported use levels
05.4	Decorations, coatings and fillings, except fruit-based fillings covered by category 4.2.4	Only cocoa-based confectionery	–	–	–	Not taken into account (no FoodEx code)
08.3	Meat products[(b)]		500			Not taken into account in the refined scenario (no reported level)
09.2	Processed fish and fishery products including molluscs and crustacean[(b)]	Fish paste	500			Not taken into account in the refined scenario (no reported level)
12.6	Sauces	Only dressings	4,000	–	–	Not taken into account in the refined scenario (no reported level)
15.2	Processed nuts		5,000[(c)]	2,309	4,500	Reported use levels

PGPR: polyglycerol polyricinoleate; MPL: maximum permitted level.
(a): Assuming 15% of chocolate in edible ices. MPL of cocoa products (FC 05.1) was used (5,000 mg/kg) applying a factor of 15%. Therefore, the level of 750 mg/kg was used for the FC 03. Edible ices.
(b): Authorised according to Annex III to Regulation No 1333/2008.
(c): MPL of cocoa products (FC 05.1).

Appendix D – Summary of total estimated exposure of PGPR (E 476) from its use as a food additive for the regulatory maximum level exposure scenario and the refined exposure assessment scenarios per population group and survey: mean and 95th percentile (mg/kg bw per day)

	Number of subjects	MPL scenario		Brand-loyal scenario		Non-brand-loyal scenario	
		Mean	P95	Mean	P95	Mean	P95
Infants							
Bulgaria (NUTRICHILD)	659	0.1	0.6	0.0	0.0	0.0	0.0
Germany (VELS)	159	0.6	2.9	0.3	2.5	0.2	0.9
Denmark (IAT 2006 07)	826	0.7	2.8	0.2	1.0	0.0	0.2
Finland (DIPP 2001 2009)	500	0.2	0.9	0.0	0.0	0.0	0.0
United Kingdom (DNSIYC 2011)	1,369	1.0	4.2	0.4	2.6	0.1	0.7
Italy (INRAN SCAI 2005 06)	12	0.7	–	0.6	–	0.1	–
Toddlers							
Belgium (Regional Flanders)	36	6.2	–	2.8	–	2.3	–
Bulgaria (NUTRICHILD)	428	1.1	5.3	0.6	3.6	0.2	1.2
Germany (VELS)	348	4.2	9.9	3.0	7.8	0.9	2.3
Denmark (IAT 2006 07)	917	3.4	8.7	1.8	6.5	0.4	1.5
Spain (enKid)	17	4.4	–	3.3	–	0.8	–
Finland (DIPP 2001 2009)	500	0.8	2.9	0.3	1.3	0.1	0.7
United Kingdom (NDNS-RollingProgrammeYears1-3)	185	4.1	10.4	1.8	6.1	0.7	2.7
United Kingdom (DNSIYC 2011)	1,314	2.9	8.7	1.3	6.0	0.4	2.1
Italy (INRAN SCAI 2005 06)	36	1.5	–	0.9	–	0.2	–
Netherlands (VCP kids)	322	5.4	12.4	3.6	9.2	2.8	6.8
Children							
Austria (ASNS Children)	128	3.7	9.0	2.2	6.9	0.8	2.8
Belgium (Regional Flanders)	625	5.9	12.6	3.0	7.9	2.2	5.8
Bulgaria (NUTRICHILD)	433	2.2	8.2	1.2	6.9	0.3	1.7
Czech Republic (SISP04)	389	4.0	13.3	3.0	12.0	1.0	4.0
Germany (EsKiMo)	835	4.4	10.4	3.0	8.5	1.1	3.2
Germany (VELS)	293	5.4	12.1	4.0	10.5	1.2	2.8
Denmark (DANSDA 2005-08)	298	4.3	9.3	2.8	7.3	0.6	1.7
Spain (enKid)	156	4.7	12.5	3.1	8.4	0.8	3.0
Spain (NUT INK05)	399	3.8	8.1	1.9	5.7	0.5	1.5
Finland (DIPP 2001 2009)	750	3.7	9.2	2.3	7.3	0.9	2.3
France (INCA2)	482	5.9	11.6	3.0	8.6	0.8	2.3
United Kingdom (NDNS-RollingProgrammeYears1-3)	651	4.2	9.7	1.8	5.7	0.7	2.4
Greece (Regional Crete)	838	2.5	5.8	0.9	3.0	0.3	1.2
Italy (INRAN SCAI 2005 06)	193	2.3	8.1	1.5	6.4	0.3	1.5
Latvia (EFSA TEST)	187	3.8	11.8	2.4	8.8	0.5	2.1
Netherlands (VCP kids)	957	4.9	11.0	3.2	7.5	2.4	6.0
Netherlands (VCPBasis AVL2007 2010)	447	4.2	10.1	1.9	6.1	0.6	2.3
Sweden (NFA)	1,473	4.9	11.2	2.2	7.0	1.3	4.1
Adolescents							
Austria (ASNS Children)	237	1.7	5.0	0.8	3.5	0.3	1.5
Belgium (Diet National 2004)	576	2.0	5.7	1.2	4.1	0.8	2.9

	Number of subjects	MPL scenario		Brand-loyal scenario		Non-brand-loyal scenario	
		Mean	P95	Mean	P95	Mean	P95
Cyprus (Childhealth)	303	2.0	5.0	1.2	4.0	0.5	1.5
Czech Republic (SISP04)	298	2.4	8.7	1.4	6.6	0.5	2.4
Germany (National Nutrition Survey II)	1,011	1.8	5.5	0.9	4.2	0.4	1.7
Germany (EsKiMo)	393	3.4	8.5	2.3	7.1	0.9	2.6
Denmark (DANSDA 2005-08)	377	2.4	6.1	1.7	5.3	0.4	1.2
Spain (AESAN FIAB)	86	1.5	4.8	0.9	4.3	0.2	1.0
Spain (enKid)	209	2.3	5.9	1.3	4.6	0.3	1.2
Spain (NUT INK05)	651	2.4	5.9	1.2	4.7	0.3	1.2
Finland (NWSSP07 08)	306	1.7	5.1	1.1	3.7	0.4	1.0
France (INCA2)	973	3.2	7.0	1.5	4.9	0.4	1.4
United Kingdom (NDNS-RollingProgrammeYears1-3)	666	2.6	6.3	1.2	4.0	0.5	1.9
Italy (INRAN SCAI 2005 06)	247	1.2	3.7	0.7	2.9	0.1	0.6
Latvia (EFSA TEST)	453	2.6	8.3	1.5	6.9	0.3	1.7
Netherlands (VCPBasis AVL2007 2010)	1,142	2.8	6.6	1.2	4.0	0.4	1.4
Sweden (NFA)	1,018	3.4	7.6	1.6	5.2	0.9	2.8
Adults							
Austria (ASNS Adults)	308	2.2	5.9	0.8	3.2	0.4	1.3
Belgium (Diet National 2004)	1,292	1.8	5.2	1.2	4.1	0.9	3.4
Czech Republic (SISP04)	1,666	0.9	2.6	0.3	1.5	0.1	0.8
Germany (National Nutrition Survey II)	10,419	1.7	4.9	0.8	3.3	0.4	1.7
Denmark (DANSDA 2005-08)	1,739	1.2	3.2	0.7	2.5	0.2	0.6
Spain (AESAN)	410	1.0	3.2	0.6	2.6	0.1	0.6
Spain (AESAN FIAB)	981	1.0	3.0	0.5	2.4	0.1	0.6
Finland (FINDIET2012)	1,295	1.3	3.7	0.5	2.3	0.2	0.8
France (INCA2)	2,276	1.8	4.0	0.5	2.1	0.1	0.7
United Kingdom (NDNS-RollingProgrammeYears1-3)	1,266	1.6	3.9	0.5	2.2	0.2	1.0
Hungary (National Repr Surv)	1,074	0.9	2.9	0.4	2.2	0.1	0.7
Ireland (NANS 2012)	1,274	1.8	4.2	0.5	2.2	0.2	0.9
Italy (INRAN SCAI 2005 06)	2,313	0.5	1.6	0.2	1.1	0.0	0.2
Latvia (EFSA TEST)	1,271	1.4	4.4	0.5	3.2	0.1	0.7
Netherlands (VCPBasis AVL2007 2010)	2,057	1.8	4.7	0.6	2.5	0.2	1.0
Romania (Dieta Pilot Adults)	1,254	0.9	2.3	0.3	1.4	0.1	0.4
Sweden (Riksmaten 2010)	1,430	2.2	5.6	0.7	3.0	0.3	1.3
The elderly							
Austria (ASNS Adults)	92	1.5	3.6	0.5	1.2	0.2	0.7
Belgium (Diet National 2004)	1,215	2.1	6.0	1.5	5.2	1.3	4.7
Germany (National Nutrition Survey II)	2,496	1.4	3.5	0.6	2.3	0.4	1.6
Denmark (DANSDA 2005-08)	286	0.8	2.0	0.4	1.7	0.1	0.4
Finland (FINDIET2012)	413	0.8	2.4	0.2	1.5	0.1	1.0
France (INCA2)	348	1.5	3.2	0.2	1.2	0.1	0.5
United Kingdom (NDNS-RollingProgrammeYears1-3)	305	1.2	2.9	0.4	1.7	0.2	0.9

	Number of subjects	MPL scenario		Brand-loyal scenario		Non-brand-loyal scenario	
		Mean	P95	Mean	P95	Mean	P95
Hungary (National Repr Surv)	286	0.6	1.7	0.2	1.0	0.1	0.5
Ireland (NANS 2012)	226	1.2	2.7	0.2	1.0	0.1	0.4
Italy (INRAN SCAI 2005 06)	518	0.3	1.1	0.1	0.7	0.0	0.1
Netherlands (VCPBasis AVL2007 2010)	173	1.4	3.3	0.5	1.8	0.2	0.6
Netherlands (VCP-Elderly)	739	1.4	3.3	0.6	2.0	0.2	0.8
Romania (Dieta_Pilot_Adults)	128	0.6	1.4	0.1	0.6	0.0	0.2
Sweden (Riksmaten 2010)	367	1.5	3.9	0.4	1.9	0.1	0.6

MPL: maximum permitted level; P95: 95th percentile.
–: P95 of exposure was only calculated for those population groups where the sample size was sufficiently large to allow this calculation (EFSA, 2011a).

Appendix E – Summary of total estimated exposure of PGPR (E 476) from its use as a food additive and considering the proposed extension of use for this food additive in emulsified sauces (food category 12.6) for the regulatory maximum level exposure scenario per population group and survey: mean and 95th percentile (mg/kg bw per day)

	Number of subjects	MPL scenario	
		Mean	p95
Infants			
Bulgaria (NUTRICHILD)	659	0.1	0.6
Germany (VELS)	159	1.1	6.2
Denmark (IAT 2006_07)	826	0.8	3.2
Finland (DIPP_2001_2009)	500	0.2	0.9
United Kingdom (DNSIYC_2011)	1,369	2.2	10.5
Italy (INRAN_SCAI_2005_06)	12	0.7	–
Toddlers			
Belgium (Regional_Flanders)	36	9.4	–
Bulgaria (NUTRICHILD)	428	1.1	5.3
Germany (VELS)	348	6.9	14.7
Denmark (IAT 2006_07)	917	4.3	10.9
Spain (enKid)	17	5.2	–
Finland (DIPP_2001_2009)	500	0.9	2.9
United Kingdom (NDNS-RollingProgrammeYears1-3)	185	6.6	16.9
United Kingdom (DNSIYC_2011)	1,314	5.2	16.0
Italy (INRAN_SCAI_2005_06)	36	1.6	–
Netherlands (VCP_kids)	322	7.3	16.5
Children			
Austria (ASNS_Children)	128	4.5	11.0
Belgium (Regional_Flanders)	625	7.7	15.5
Bulgaria (NUTRICHILD)	433	2.3	8.9
Czech Republic (SISP04)	389	4.5	13.7
Germany (EsKiMo)	835	5.0	11.9
Germany (VELS)	293	8.3	18.4
Denmark (DANSDA 2005-08)	298	5.2	11.2
Spain (enKid)	156	6.7	20.8
Spain (NUT_INK05)	399	6.5	15.6
Finland (DIPP_2001_2009)	750	4.8	11.1
France (INCA2)	482	7.1	14.4
United Kingdom (NDNS-RollingProgrammeYears1-3)	651	6.7	16.2
Greece (Regional_Crete)	838	2.6	6.1
Italy (INRAN_SCAI_2005_06)	193	2.5	8.3
Latvia (EFSA_TEST)	187	6.0	16.6
Netherlands (VCP_kids)	957	6.6	14.6
Netherlands (VCPBasis_AVL2007_2010)	447	6.1	13.9
Sweden (NFA)	1,473	8.4	19.4
Adolescents			
Austria (ASNS_Children)	237	1.9	5.8
Belgium (Diet_National_2004)	576	4.3	11.7
Cyprus (Childhealth)	303	2.1	5.1
Czech Republic (SISP04)	298	2.9	9.5

	Number of subjects	MPL scenario	
		Mean	p95
Germany (National_Nutrition_Survey_II)	1,011	3.9	10.6
Germany (EsKiMo)	393	4.0	10.0
Denmark (DANSDA 2005-08)	377	2.7	7.3
Spain (AESAN_FIAB)	86	1.7	5.1
Spain (enKid)	209	4.0	9.9
Spain (NUT_INK05)	651	4.3	9.9
Finland (NWSSP07_08)	306	2.1	5.6
France (INCA2)	973	4.0	8.5
United Kingdom (NDNS-RollingProgrammeYears1-3)	666	4.3	9.9
Italy (INRAN_SCAI_2005_06)	247	1.4	4.0
Latvia (EFSA_TEST)	453	4.3	11.8
Netherlands (VCPBasis_AVL2007_2010)	1,142	4.4	10.5
Sweden (NFA)	1,018	5.2	12.3
Adults			
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Netherlands (VCP-Elderly)	739	2.4	5.6
Romania (Dieta_Pilot_Adults)	128	0.6	1.4
Sweden (Riksmaten 2010)	367	2.6	6.4

MPL: maximum permitted level; P95: 95th percentile.
–: p95 of exposure was only calculated for those population groups where the sample size was sufficiently large to allow this calculation (EFSA, 2011a).