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September 16, 2022

Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
5001 Campus Drive
College Park, MD 20740

Re: Generally Recognized as Safe (GRAS) Notice for Polyglycerol Polyricinoleic Acid

Dear Sir/Madam:

We respectfully submit the attached GRAS Notification for polyglycerol polyricinoleic acid (PGPR). PGPR is intended for use as an emulsifier in food categories that have previously been considered in past GRAS Notifications for PGPR with three new use levels for the following categories: chocolate (from 0.30% to 0.50%), chocolate-type products based on vegetable fats other than cocoa butter (from 0.30% to 0.50%), and mayonnaise and spreads (from 0.28% to 0.80%). More detailed information regarding product identification, intended use levels, the manufacturing process, and safety of the ingredient is set forth in the attached GRAS Notification.

Unilever has determined that PGPR is GRAS for its intended uses based on scientific procedures in accordance with 21 C.F.R. § 170.30(b) and in conformance with the guidance issued by the Food and Drug Administration (FDA) under 21 C.F.R. § 170.36, 81 Fed. Reg. 54960 (Aug. 17, 2016). Therefore, the use of the PGPR as described in this GRAS Notification is exempt from the requirement of premarket approval as set forth in the Federal Food, Drug, and Cosmetic Act.

The analytical data, published studies, and information that are the basis for this GRAS Notification will be sent to FDA upon request.

We look forward to the Agency's review of this submission and would be happy to provide Agency officials with any information they may need to complete their assessment. Thank you for your attention to this matter.

Sincerely,

[redacted]

Kristin Spoden
Unilever
Regulatory Affairs Leader

GRAS Notice for Polyglycerol Polyricinoleic Acid (PGPR)

Prepared for: Office of Food Additive Safety (FHS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Campus Dr. College Park, Maryland 20740

Submitted by: Unilever
700 Sylvan Avenue
Englewood Cliffs, NJ 07632
USA

Date: September 16, 2022

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List of Acronyms

ADI	Acceptable Daily Intake
ADME	Absorption, Distribution, Metabolism, And Excretion
CASRN	The Chemical Abstracts Service Registry Number
CEDI	The Cumulative Estimated Daily Intake
CFR	US FDA 21 Code Of Federal Regulations
DHHS	The U.S. Department Of Health And Human Services
EDI	Estimated daily intake
EFSA	European Food Safety Authority
EPA	Environmental Protection Agency
ERS	The U.S. Department Of Agriculture Economic Research Service
FAO	Food and Agriculture Organization
FARE®	Foods And Residue Evaluation Program®
FCC	The Foods Chemicals Codex
FD&C	FD&C Color Additives
FDA	The U.S. Food And Drug Administration
FNDDS	The USDA Food And Nutrient Database For Dietary Studies
FPED	Food Patterns Equivalent Database
GFSA	Codex General Standard For Food Additives
GRAS	Generally recognized as safe
IRIS	EPA's Integrated Risk Information System
JECFA	Joint FAO/WHO Expert Committee On Food Additives
JMPR	Joint FAO/WHO Meeting On Pesticide Residues
NCHS	National Center For Health Statistics
NHANES	National Health and Nutrition Examination Survey
NMT	Not more than
NOAEL	No Observable Adverse Effect Level
NR	Not reported
NTP	The National Toxicology Program
OECD	Organization for Economic Co-operation and Development
QSAR	Quantitative Structure Activity Relationship
USDA	The U.S. Department Of Agriculture
WHO	World Health Organization
WWEIA	What We Eat in America

Part 1. Signed statements and certification

Unilever submits this generally recognized as safe (GRAS) notice in accordance with 21 C.F.R. part 170, subpart E.

Name and address of the notifier

Unilever
700 Sylvan Avenue
Englewood Cliffs, NJ 07632

Name of the notified substance

The substance is commonly known as polyglycerol polyricinoleic acid or by its acronym PGPR.

Applicable conditions of use of the notified substance

Unilever intends to use PGPR as an emulsifier in food categories that have previously been considered in past GRAS Notices with three new use levels for the following categories:

1) Chocolate (from 0.30% to 0.50%),
2) Chocolate-type products based on vegetable fats other than cocoa butter (from 0.30% to 0.50%), and
3) Mayonnaise and spreads (from 0.28% to 0.80%).

Basis for the GRAS determination

Unilever hereby notifies the Agency of its determination that PGPR is GRAS for its intended use, consistent with Section 201(s) of the Federal Food, Drug, and Cosmetic Act (FD&C Act). This GRAS conclusion is based on scientific procedures in accordance with 21 C.F.R. §170.30(a) and (b).

Exclusion from premarket approval

The notified substance is not subject to the premarket approval requirements of the FD&C Act based on our conclusion that the notified substance is GRAS under the conditions of its intended use.

Availability of data and information

The data and information that serve as the basis for this GRAS conclusion will be sent to the FDA upon request or are available for the FDA's review and copying during customary business hours at the office of Unilever, located at 700 Sylvan Ave, Englewood Cliffs, NJ 07632.

Applicability of FOIA Exemptions

Unilever is not claiming any information in Parts 2 through 7 of this document as trade secret, commercial or financial information that is privileged or confidential. Thus, all information and data in this submission are not exempt from the Freedom of Information Act (FOIA), 5 U.S.C. Section 552.

Certification

I hereby certify that, to the best of my knowledge, this GRAS notice is a complete, representative, and balanced submission that includes unfavorable, as well as favorable, information known to me and pertinent to the evaluation of the safety and GRAS status of the intended use of PGPR.

Signature and name and title of the person signing this GRAS notice:

Date: September 16, 2022

Kristin Spoden
Unilever
Regulatory Affairs Leader

Part 2. Identity, method of manufacture, specifications, and physical or technical effect

Identity

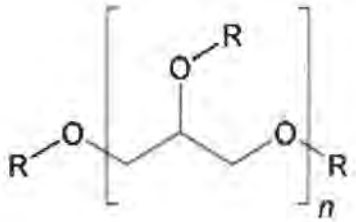
Polyglycerol polyricinoleic acid (PGPR) is a nonionic emulsifier. It is also a class of polyglycerol esters of fatty acid (Bastida-Rodríguez, 2013). PGPR is a product that is formed by the esterification of polyglycerol with condensed castor oil fatty acids, and occurs as a clear, light brown, viscous liquid. PGPR is insoluble in water and alcohol, but soluble in ether, hydrocarbons, and in halogenated hydrocarbons (FCC Vol 12).

PGPR is represented by CASRN 68936-89-0, chemical name: 1,2,3-Propanetriol, homopolymer, (9Z,12R)-12-hydroxy-9-octadecenoate, with a molecular formula of C18H34O3 . x(C3H8O3)n (ChemIDplus, accessed February 2022; EFSA, 2017).[1]

The general structural formula of PGPR is given in **Figure 1.** below.

Chemical Name:	1,2,3-Propanetriol,homopolymer,(9Z,12R)-12-hydroxy-9 octadecenoate
Synonyms:	Polyglycerol Esters of Condensed Castor Oil Fatty Acids Polyglycerol Esters of Interesterified Ricinoleic Acid Polyglycerol Polyricinoleate PGPR
CAS Number:	68936-89-0
Molecular Formula:	C18H34O3 . x(C3H8O3)n

Figure 1. Structure of Polyglycerol Polyricinoleic Acid



n = 2 - 6

R = H or polyricinoleic acid ester

[1] An additional CAS number – 29894-35-7 – has been assigned with the molecular formula (C18H34O3 C3H8O3)n.

Manufacturing

Details of the manufacturing process have been published in the open literature by Wilson et al. (1998) and Bastida-Rodriguez (2013). These manufacturing steps have recently been summarized by EFSA (2017). This four-step process for the production of PGPR is described below:

1. Preparation of the castor oil fatty acids

   Castor oil fatty acids are produced by hydrolyzing 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. This castor oil contains, as its main fatty acids ricinoleic acid (80–90%), oleic acid (3–8%), linoleic acid (3–7%) and stearic acid (0–2%).

2. Condensation of the castor oil fatty acids

   Castor oil fatty acids are condensed by heating at a temperature of 205–210°C under vacuum and a CO2 atmosphere (to prevent oxidation) for ~8 h. This 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) is reached.

3. Preparation of polyglycerols

   The polyglycerol portion can be prepared by three routes: (1) polymerization of glycerol using a strong base as a catalyst, (2) by polymerization of glycidol, leading to linear polyglycerols, or (3) by polymerization of epichlorohydrin, followed by hydrolysis. This leads to linear polyglycerols. The polyglycerols produced by polymerization of epichlorohydrin contain reduced proportions of cyclic components.

4. Partial esterification of the condensed castor oil fatty acids with polyglycerols

   The final stage of the production process involves the esterification of condensed castor oil fatty acids with polyglycerols. The "appropriate" amount of polyglycerol with the polyricinoleic acid is heated. After which, a reaction takes place immediately, and in the same vessel while still hot. The esterification conditions are the same as those for fatty acid condensation. This process will continue until a sample is taken from the reaction mixture and found to have a suitable acid value (i.e., ≤ 6 mg KOH/g) and refractive index (per required specifications).

Analytical Results and Product Specifications

The Foods Chemicals Codex (FCC) states that the acceptance criteria for the polyglycerol moieties of PGPR shall be composed of NLT 75% of di-, tri- and tetraglycerols and shall contain NMT 10% of polyglycerols equal to or higher than heptaglycerol (FCC Vol 12). This PGPR product meets specification requirements for polyglycerol polyricinoleic acid established by the Foods Chemicals Codex (FCC Vol 12). Specifications and analytical data for three consecutive batches of product are given in **Table 1.** below.

Table 1. Product specifications and data for three consecutive batches

Test Parameter	Unit	Specification	Result Batch 1	Result Batch 2	Result Batch 3
Hydroxyl Value	mg KOH/g oil	80-100	85	88	84
Iodine Value		72-103	86	88	86
Refractive Index		1.463-1.467	1.4652	1.4654	1.4650
Saponification Value	mg KOH/g oil	170-210	180	181	181
Acid Value	mg KOH/g oil	NMT 6	0.8	0.9	0.3
Polyglycerols	%	NLT 75% di-, tri- and tetraglycerols; NMT 10% heptaglycerols or higher	91% di-, tri- and tetraglycerols; 0.2% heptaglycerols and higher	91% di-, tri- and tetraglycerols; 0.2% heptaglycerols and higher	91% di-, tri- and tetraglycerols; 0.3% heptaglycerols and higher
Arsenic	mg/Kg	NMT 3	0.1	0.1	0.1
Lead	mg/Kg	NMT 1	0.1	0.1	0.1
Mercury	mg/Kg	NMT 1	0.0	0.0	0.0
Cadmium	mg/Kg	NMT 1	0.0	0.0	0.0

Part 3. Dietary Exposure

The information forming the basis for this GRAS notification was prepared by experts at Exponent, Inc. who are qualified by scientific training and experience to evaluate the safety of substances added to food.

History of Consumption

Polyglycerol esters such as PGPR have a history of use in food in several countries. They have been used as food additives in the United States and Europe since the 1940s. They were first officially approved for use in food in the US in the 1960s (Bastida-Rodríguez 2013). PGPR was first used in chocolate couverture in the United Kingdom in 1952 (Wilson et al., 1998).

US GRAS Notices

Several GRAS notices for PGRP exist, including GRN 9 (Quest International, 1998), GRN 179 (Stepan Company, 2005), GRN 266 (Palsgaard A/S, 2008), GRN 270 (Stepan Company, 2008), and GRN 466 (McCormick & Company, Inc., 2012). The U.S. Food and Drug Administration (FDA) reviewed these GRAS notifications and issued "no-questions" letters for each (FDA 1999, 2006a, 2008, 2009, 2013). The existing food uses of PGPR as an emulsifier includes use in chocolate, chocolate-type products based on vegetable fats other than cocoa butter, margarine, low fat creamers, low fat dairy analogs, condiments and spreads, cheese powder (snacks), flavors, and color additives with use levels ranging from 0.1 to 5%

Polyglycerol esters of fatty acids, up to and including the decaglycerol ester, are allowed for use as an emulsifier in foods in amounts not greater than that required to produce the intended physical or technical effect in accordance with conditions described by 21CFR172.854 (b).

Additional Regulated Uses

JECFA additionally reviewed and approved the use of PGPR as a food additive (JECFA, 1974). Per this evaluation there are many GFSA approved uses of PGPR as an emulsifier. PGPR is approved for use in milk and cream powder analogues, processed cheese, dairy-based desserts, fat spreads, dairy fat spreads and blended spreads, fat emulsions mainly of type oil-in-water, including mixed and/or flavored products based on fat emulsions, fat-based desserts excluding dairy-based dessert products of food category, edible ices, including sherbet and sorbet, fruit-based desserts including fruit-flavored water-based desserts, fruit fillings for pastries, cocoa and chocolate products, imitation chocolate, chocolate substitute products, confectionery including hard and soft candy, nougats (including confectionery other than chocolate, chewing gum, and

bakery decorations), chewing gum, decorations (for fine bakery wares), cocoa mixes (powders) and cocoa mass/cake, pre-cooked pastas and noodles and like products, cereal and starch based desserts (e.g. rice pudding, tapioca pudding), edible casings (e.g. sausage casings), toppings (non-fruit) and sweet sauces, cooked fish and fish products, egg products, egg-based desserts, mixes for sauces and gravies, emulsified sauces and dips (e.g. mayonnaise, salad dressing, onion dip). PGPR is allowed as an emulsifier in these food categories at maximum permitted levels ranging from 500 to 10,000 mg/kg (JCAC, last updated 2019).

In Europe PGPR (E 476) usage levels have been defined in Annex II to Regulation (EC) No. 1333/2008 on food additives, as amended. PGPR is allowed as a food additive in five food categories, including other fat and oil emulsions, spreads, and liquid emulsions; cocoa and chocolate products; other confectionery including breath freshening micro sweets; decorations, coatings and fillings; and sauces as defined by council regulation and as covered by directive 2000/36/EC. PGPR is allowed in these categories at maximum permitted levels ranging from 4,000 to 5,000 mg/kg. PGPR is additionally allowed as an emulsifier in preparations of food colors as defined by Annex III, Part 2 of Regulation (EC) No 1333/2008.

Background Uses

Background dietary intake of PGPR was evaluated based on the existing food uses and use levels of PGPR as described in U.S. GRAS Notices (GRNs) 9 (Quest International, 1998), 179 (Stepan Company, 2005), 266 (Palsgaard A/S, 2008), 270 (Stepan Company, 2008), and 466 (McCormick & Company, Inc., 2012). The U.S. Food and Drug Administration (FDA) reviewed these GRAS notifications and issued no questions letter for each (FDA 1999, 2006a, 2008, 2009, 2013). The existing food uses of PGPR as an emulsifier includes use in chocolate, chocolate-type products based on vegetable fats other than cocoa butter, margarine, low fat creamers, low fat dairy analogs, condiments and spreads, cheese powder (snacks), flavors, and color additives with use levels ranging from 0.1 to 5% as summarized in **Table 2**.

Proposed Use and Level

The use levels of PGPR are proposed to increase from the existing use levels for three food types:

1) Chocolate (from 0.30% to 0.50%),
2) Chocolate-type products based on vegetable fats other than cocoa butter (from 0.30% to 0.50%), and
3) Mayonnaise and spreads (from 0.28% to 0.80%).

These proposed increase in use levels are also summarized in **Table 2.**

Table 2. New Proposed and Existing GRAS uses of PGPR

		Maximum PGPR Use Level, %		
Food Use	Description of Foods Selected for Analysis	Proposed New Uses	Existing GRAS Uses[1]	Combined Existing GRAS and Proposed Uses[2]
Chocolate	All candies within 'candy containing chocolate' WWEIA category and white chocolate	0.50	0.30	0.50
Chocolate-type products based on vegetable fats other than cocoa butter	Chocolate coatings	0.50	0.30	0.50
Margarine	Margarine	NA	1	1
Low fat creamers, liquid	Cream substitutes, liquid	NA	1	1
Low fat dairy analogs	Milk substitutes, all types including soy, almond, coconut and rice milk	NA	1	1
Condiments and spreads				
Condiments	All condiments except mayonnaise and mayonnaise-based spreads, including ketchup, mustard, soy sauce, and enchilada sauce	NA	0.28	0.28
Mayonnaise and spreads	All mayonnaise and mayonnaise-based spreads, including tartar sauce, salad dressing for sandwiches, and vegan mayonnaise	0.80	0.28	0.80
Cheese powders (snacks)[3]	Dry grated and hard parmesan cheese as a surrogate for cheese powders	NA	0.15	0.15
Flavors	NA	NA	0.1	0.1
Color additives	NA	NA	5	5

NA: Not applicable.

[1] Based on current food uses and use levels of PGPR as described in U.S. GRAS Notices (GRNs) 9 (Quest International, 1998), 179 (Stepan Company, 2005), 266 (Palsgaard A/S, 2008), 270 (Stepan Company, 2008), and 466 (McCormick & Company, Inc., 2012).

[2] Maximum use level applied in estimating the cumulative intake from proposed and existing GRAS use.

[3] GRN 466 has "snack" terminology; based on current McCormick's cheese powder products, the cheese powders are assumed to be powder used in making pasta or sprinkled on food/snacks.

Data Source and Methods

The estimated daily intake (EDI) of PGPR from existing (except for flavor and color additive uses) and proposed uses was derived based on food consumption records collected in the What We Eat In America (WWEIA) dietary component of the National Health and Nutrition Examination Survey (NHANES) conducted in 2015-2016 and 2017-2018 (2015-18). This continuous survey uses a complex multistage probability sampling protocol designed to be representative of the civilian United States (U.S.) population (NCHS 2018, 2020). The NHANES datasets provide nationally representative nutrition and health data and prevalence estimates for nutrition and health status measures in the U.S.

As part of the examination, trained dietary interviewers collect detailed information on all foods and beverages consumed by respondents in the previous 24-hour time period (midnight to midnight). A second dietary recall is administered by telephone 3 to 10 days after the first dietary interview, but not on the same day of the week as the first interview. The dietary component of the survey is conducted as a partnership between the U.S. Department of Agriculture (USDA) and the U.S. Department of Health and Human Services (DHHS). DHHS is responsible for the sample design and data collection, and USDA is responsible for the survey's dietary data collection methodology, maintenance of the databases used to code and process the data, and data review and processing. A total of 13,666 individuals in the survey period 2015-2018 provided 2 complete days of dietary recalls.

Food and Nutrient Database for Dietary Studies (FNDDS)

For each food reported in NHANES, the USDA Food and Nutrient Database for Dietary Studies (FNDDS) databases provide information on the amount of energy and approximately 60 nutrients or food constituents per 100 g of each food. Additionally, the FNDDS translates food as reported consumed into its corresponding ingredients (and gram amounts) or recipes. Exponent applied FNDDS version 2017-2018 nutrient composition data and food recipes (corresponding to NHANES 2017-2018) (USDA 2020) to process dietary recall data reported in NHANES 2015-2018 and FNDDS version 2015-2016 recipes (corresponding to NHANES 2015-2016) (USDA 2018) for foods that were only reported consumed in NHANES 2015-2016.

Representative NHANES Foods for the Existing and Proposed Uses of PGPR

The list of all food codes reported consumed in NHANES 2015-2018 was reviewed. With the exception of flavoring and color additive uses, foods corresponding to any of the uses of PGPR as shown in **Table 2.** were identified. The proportion of foods corresponding to a PGPR food use within food mixtures (e.g., mayonnaise ingredient in a tuna salad, chocolate coating component

in an ice cream bar or stick, margarine ingredient in a grilled cheese sandwich, etc.) was identified through USDA's FNDDS. A summary of the descriptions of foods included in the analysis is provided in **Table 2**.

In some cases, USDA FNDDS recipes did not have a complete breakdown of ingredients for foods identified to have PGPR food uses based on the main food description. An alternate approach was taken to identify the proportion of foods with PGPR uses and this proportion was included in the analysis. Specifically, the average proportion of similar foods or foods within the same WWEIA category was assumed to correspond to the PGPR food use for foods without a complete recipe breakdown in USDA FNDDS. For example, several sandwiches with spread lacked recipes and it was assumed that these sandwiches contained the average mayonnaise proportion from burgers with recipe breakdowns. Among foods with incomplete recipes, an average proportion was assumed based on similar foods or foods within the same WWEIA category that had recipe breakdowns for the chocolate coating in ice cream and frozen desserts; mayonnaise in sandwiches and potato salad; chocolate and chocolate coating in cookies, cereal bars, and pretzels; and soy sauce condiment in food mixtures.

It should be noted that although GRN 466 (McCormick & Company, Inc., 2012) indicated that PGPR food use includes its use in cheese powders for snacks, an internet search for "McCormick" and "cheese powder" shows that McCormick cheese powder is marketed for use in pastas (i.e., mac and cheese). Therefore, dry grated and hard parmesan cheese and the proportions in food mixtures was identified as representative surrogate uses of cheese powder. The cheese powder proportion in grain mixtures with cheese sauce made from package mix (e.g., Easy Mac, Hamburger Helper) was also included in the assessment. The cheese powder proportion in these grain mixtures was assumed to be 30% cheese powder mix based on the grain component ratio of uncooked mac and cheese and uncooked pasta per 100 grams.

The PGPR EDI from flavor and color additive uses was derived using food production statistics and published data as described below.

Analysis

Using the NHANES 2015-2018 consumption data, Exponent estimated the 2-day average daily intake of PGPR on a *per capita* and *per user* basis. *Per capita* estimates refer to the intake based on the population of interest whereas *per user* estimates refer to individuals who reported consuming a food use of PGPR on either of the survey days. For each subject with a complete 2-day dietary recall, the 2-day average intake of a food of interest was derived by summing an individual's intake of that food on day 1 and day 2 of the survey, expressed as grams per day (g/day), multiplied by the corresponding maximum PGPR use level (%) (see **Table 2.**) and 1000,

and then dividing that sum by two to express PGPR intake in milligrams (mg) per day. Thus, if a survey participant consumed a food of interest on only one of the survey days, their resulting PGPR intake after accounting for the PGPR use level for that food on that one day was divided by 2 to obtain their 2-day average intake. The mean and 90th percentile of 2-day average PGPR intakes were calculated for the U.S. population 2+ y and subpopulations including children (2-5 y and 6-12 y), adolescents 13-18 y, and adults 19+ y. Estimates of PGPR intake were also derived on a body weight (bw) basis based on each participant's measured body weight in kilograms (kg) collected during the examination. PGPR intake from flavors and color additive uses were separately derived.

Estimates of intake per person were generated using Exponent's Foods Analysis and Residue Evaluation Program (FARE® version 14.06) software. The analysis was limited to individuals who provided two complete and reliable dietary recalls as determined by the National Center for Health Statistics (NCHS). Exponent uses the statistically weighted values from the survey in its analyses. The statistical weights compensate for variable probabilities of selection, adjust for non-response, and provide intake estimates that are representative of the U.S. population.

PGPR uses in Flavors and Color Additives

No exact listing of foods or food groups in which PGPR can be used as an emulsifier in flavors or color additives is available. The potential PGPR intake associated with uses in flavors and color additives was derived largely based on publicly available summary data on the per capita intakes of major food groups and intake estimates of select food, drug, and cosmetic (FD&C) color additives reported by FDA scientists (Doell et al. 2016). This section describes the approach used to estimate the intake of PGPR from flavor and color additive uses.

Flavors Uses

Intake estimates of PGPR associated with the existing GRAS use in flavors was derived by combining estimates of the per capita intakes of major food groups from the USDA Economic Research Service (ERS) with estimates of the fraction of these foods that could be processed, the fraction of the processed foods that could contain flavor, the flavor concentration in processed foods, and the maximum PGPR use level of 0.1% in flavors. The following describes in detail the approach and assumptions made to derive the intake estimate of PGPR from flavor uses.

Step 1: Obtain the estimate of the total food consumption per year by major food group for the total U.S. population based on the USDA ERS Food Availability (Per Capita) Data which serve as proxies for actual consumption at the national level (see **Table 3.**) and convert to grams per day. Since the USDA ERS data no longer reports data for non-alcoholic beverages, the grams per day for this category was calculated by the difference of the total diet intake and the sum of all major food groups including

water. The total diet intake was based on the 2-day average intake of all foods and beverages reported consumed in NHANES 2017-2018. Similarly, water intake was based on the 2-day average intake of bottled water intake from NHANES 2017-2018.

Step 2: Obtain the percent of each major food group that is processed based on 2018-2020 USDA ERS Food Availability Data with the exception for red meat and poultry meats, where it was not possible to get this information from the USDA ERS data and published data was used (see **Table 3.**). Similarly, there was no data on the amount of processed fats/oils, grains, and non-alcoholic beverages and it was conservatively assumed that fats/oils, grains, and non-alcoholic beverages are processed although it is unlikely that all grain intake is from processed forms. Processed fruits and vegetables data were assumed to be 100% processed while none were assumed to be processed for data on fresh and frozen fruits and vegetables and water. The intake of processed food was calculated as the product of the grams per day (Step 1) and percent of major category that is processed.

Step 3: It was assumed that nearly every processed food (i.e., 90%) contains flavor. The intake of processed food that could contain flavor was calculated as the product of the intake per day of processed food (Step 2) and the percent of processed food that could contain flavor (i.e., 90%). The total daily intake of processed foods that could contain flavor was estimated to be 1398 g/day (see **Table 3.**) and calculated from the sum of each individual food group intake that are processed and that could contain flavor.

Step 4: Obtain the concentration for flavors in foods based on recently compiled average maximum use levels of new Flavor Extract Manufacturers Association (FEMA) Generally Recognized as Safe (GRAS) Flavoring Substances in various food categories (Cohen et al., 2020). The average maximum use levels among 65 new FEMA GRAS flavoring substances at the 97.5th percentile of 2500 ppm (or 0.25%) was conservatively assumed as the flavor use level in all processed foods that could contain flavor.

Step 5: Calculate the per capita mean estimated intake of PGPR for the total U.S. population based on the daily intake of foods that could contain flavor (1398 g/day from Step 3), the flavor concentration (0.25% from Step 4), the PGPR existing maximum use level of 0.1% in flavor from GRN 466, and a default average adult bodyweight of 60 kg.

The calculation is as follows:

$$0.0583 \text{ mg/kg-bw/day} = 1398 \frac{\text{g}}{\text{day}} \times 0.25\% \times 0.1\% \times 1000 \div 60 \text{ kgbw}$$

Following the steps and assumptions described above, the per capita mean estimated intake of PGPR for the total U.S. population from flavoring uses was calculated to be 0.0583 mg/kg-bw/day. The pseudo 90th percentile intake of the PGPR from flavoring uses was then estimated as two times the mean intake based on guidance from FDA (2006b), i.e., 2 x 0.0583 mg/kg-bw/day = 0.117 mg/kg-bw/day.

Table 3. Estimated Intake of the Diet that could be Processed and Contain Flavors

	Step 1		*Step 2*		*Step 3*	
Major Food Groups	*Per Capita Intake (lbs./year)*[1]	*Per Capita Intake (g/day)*	*Percent Processed*[2]	*Per Capita Intake of Processed Products (g/day)*	*Percent of Processed Intake that could contain Flavor*	*Per Capita Intake that could contain Flavor (g/day)*
Red meat & poultry	207	257	22%	57	90%	50.9
Fish	16	20	24%	5	90%	4.3
Eggs[3]	32	39	30%	12	90%	10.5
Dairy	240	299	41%	123	90%	110.3
Fats/oils	84	104	100%	104	90%	93.8
Fresh + frozen fruit	145	180	0%	0	0%	0
Processed fruit (canned/dried/juice/other processed)	96	119	100%	119	90%	107.2
Fresh + frozen vegetables + legumes	278	346	0%	0	0%	0
Processed vegetables (canned, dried, potato chips)	123	152	100%	152	90%	137.1
Grains	174	216	100%	216	90%	194.2
Non-alcoholic beverages[4]		766	100%	766	90%	689.7
Water[5]		508	0%	0	0%	0
Total[6]		3007		1554		1398

[1] Based on 2018-2020 USDA ERS data, except for fats\oils based on 2010 (https://www.ers.usda.gov/data-products/food-availability-per-capita-data-system/food-availability-per-capita-data-system/#Food%20Availability)

[2] Data sources for % processed
Red Meat & Poultry: Daniel et al. (2011)
Fish: Canned or cured fish/shellfish in 2018 ERS data
Eggs: Processed eggs in 2019 ERS data
Dairy: All dairy except fluid milk in 2019 ERS data
Fresh/frozen fruits or vegetables: assumed 0 % processed
Processed fruits or vegetables: assumed 100% processed
No data for grains, fats/oils, and non-alcoholic beverages. It was conservatively assumed that all intake of fats/oils, grains, and non-alcoholic beverages are processed although it is unlikely that all grain intake is processed.

[3] ERS data are for number of eggs; assumed large egg (50 g/egg).

[4] Calculated as the difference of the total diet intake and the sum of all major food groups including water.

[5] Based on the 2-day average intake of bottled water intake from NHANES 2017-2018.

[6] Based on the 2-day average intake of all foods and beverages reported consumed in NHANES 2017-2018.

Color Additive Uses

Intake estimates of PGPR associated with the existing GRAS use in color additives was based on the maximum intake estimate reported across seven FD&C color additives by FDA scientists

(Doell et al., 2016). The maximum intake estimate reported across FD&C color additives was derived from analytical data and dietary consumption data from NHANES 2007-2010 under a high exposure scenario representing the highest exposure to each FD&C color additive evaluated (Doell et al., 2016).

The per capita intake estimate of PGPR from color additive uses was calculated for the U.S. 2+ y, children, and adolescent males based on the product of the following data and assumptions:

- Per user maximum color additive exposure in the high exposure scenario from FD&C Red No. 2 for the U.S. population 2+ y as well as for the other populations analyzed (i.e., children 2-5 y and adolescent males 13-18 y) with ≥94% all populations consuming at least one food containing FD&C Red No. 2 (Doell et al., 2016). The per capita estimate can be assumed to be equal to the per user estimate since almost everyone in the population is a consumer.
- Arbitrary factor of 10x applied to intake estimates by Doell et al. (2016) to conservatively account for additional exposure from other colors.
- PGPR existing maximum use level of 5% in color from GRN 270.

Table 44. summarizes the per capita estimated intake of PGPR based on data from Doell et al. (2016).

Table 4. Per Capita Estimated Daily Intake of PGPR from Color Additive Uses

Population	Color Intake[1] mg/kg-bw/day	PGPR Intake from Color Uses[2]
U.S. 2+ y	0.4	0.20
Children 2-5 y	0.9	0.45
Adolescent males 13-18 y	0.5	0.25

[1] Based on the per user maximum color additive exposure across FD&C color additives evaluated using NHANES 2007-10 by Doell et al. (2016). Color intake based on FD&C Red No. 2 with at least 94% within each population consuming at least one food containing the color additive. Therefore, the per capita estimate can be assumed to be equal to the per user estimate.
[2] Product of color intake, factor of 10 to account for additional exposure from other colors, and PGPR existing maximum use level of 5% in color from GRN 270.

Cumulative Estimated Daily Intake (CEDI) of PGPR

The cumulative estimated daily intake (CEDI) of PGPR that account for all uses in foods including uses in flavors and color additives was calculated on a bodyweight basis. The PGPR CEDI was conservatively calculated by adding: (1) per user 90th percentile PGPR intake

estimates from existing GRAS uses (not including uses in flavors and color additives) combined with new proposed uses derived using NHANES 2015-2018 (from **Table 7**), (2) the pseudo 90th percentile intake of PGRP from flavor uses for the total U.S. population of 0.117 mg/kg-bw/day derived from the USDA ERS data, and (3) color additive uses derived from maximum estimates in Doell et al. (2016) (from **Table 4**). Estimates of PGPR from color uses for children 2-5, adolescent males 13-18 y, and US 2+ that were derived from Doell et al. (2016) were used in the PGPR CEDI calculation for children 6-12, all adolescents 13-18 y and adult 19+.

Results

Two-day average intake estimates of PGPR from existing GRAS uses (not including uses in flavors and color additives), new proposed uses[2], and existing GRAS uses (not including uses in flavors and color additives) combined with new proposed uses are shown in **Table 5**, **Table 6**, and **Table 7**, respectively, for the U.S. population 2+ y and subpopulations based on maximum use levels of PGPR (see

Table 2) and dietary consumption data from NHANES 2015-2018. The conservatively derived PGPR CEDI that accounts for existing PGPR uses in flavors and color additives by the U.S. population 2+ y and subpopulations are provided in **Table 8**.

[2] Higher use levels in chocolate (from 0.30% to 0.50%), chocolate-type products based on vegetable fats other than cocoa butter (from 0.30% to 0.50%), and mayonnaise and spreads (from 0.28% to 0.80%).

Table 5. Two-day Average EDI of PGPR from Existing GRAS Uses (not including Flavor and Color Additive Uses) by the U.S. Population 2+ y and Subpopulations

			Per Capita		Per User		Per Capita		Per User	
Population	N*	% User	Mean	90th Percentile	Mean	90th Percentile	Mean	90th Percentile	Mean	90th Percentile
			---- mg/day ----				---- mg/kg-bw/day ----			
U.S. 2+ y	11522	92	214	410	232	449	3.31	5.70	3.59	6.15
Children 2-5 y	898	91	117	127	128	137	7.28	7.53	7.99	8.38
Children 6-12 y	1585	93	114	172	123	176	3.47	5.26	3.75	5.61
Adolescents 13-18 y	1248	88	183	275	209	332	2.95	3.89	3.37	4.69
Adults 19+ y	7791	93	236	470	254	507	3.06	5.83	3.30	6.23

* Un-weighted number of users; % user, *per capita* and *per user* estimates were based on NHANES 2015-2018 and derived using the statistical weights provided by the NCHS.

Table 6. Two-day Average EDI of PGPR from New Proposed Uses* by the U.S. Population 2+ y and Subpopulations

			Per Capita		Per User		Per Capita		Per User	
Population	N**	% User	Mean	90th Percentile	Mean	90th Percentile	Mean	90th Percentile	Mean	90th Percentile
			---- mg/day ----				---- mg/kg-bw/day ----			
U.S. 2+ y	6837	58	61	173	104	228	0.90	2.55	1.55	3.37
Children 2-5 y	474	49	26	82	54	117	1.54	4.87	3.14	6.86
Children 6-12 y	975	60	46	123	77	149	1.38	3.59	2.31	4.85
Adolescents 13-18 y	754	57	54	147	96	201	0.86	2.43	1.51	3.19
Adults 19+ y	4634	59	65	185	111	244	0.81	2.32	1.37	2.98

* New proposed uses of PGPR are proposed to increase from the existing use levels for chocolate (from 0.30% to 0.50%), chocolate-type products based on vegetable fats other than cocoa butter (from 0.30% to 0.50%), and mayonnaise and spreads (from 0.28% to 0.80%).

** Un-weighted number of users; % user, *per capita* and *per user* estimates were based on NHANES 2015-2018 and derived using the statistical weights provided by the NCHS.

Table 7. Two-day Average EDI of PGPR from Existing (not including Flavor and Color Additive Uses) and New Proposed Uses by the U.S. Population 2+ y and Subpopulations

			Per Capita		Per User		Per Capita		Per User	
				90th		90th		90th		90th
		%	Mean	Percentile	Mean	Percentile	Mean	Percentile	Mean	Percentile
Population	N*	User	---- mg/day ----				---- mg/kg-bw/day ----			
U.S. 2+ y	11522	92	246	481	267	523	3.78	6.82	4.10	7.41
Children 2-5 y	898	91	130	177	143	189	8.04	10.27	8.82	11.09
Children 6-12 y	1585	93	136	219	147	228	4.13	6.78	4.46	7.11
Adolescents 13-18 y	1248	88	211	351	241	391	3.39	5.37	3.87	6.04
Adults 19+ y	7791	93	271	545	292	597	3.49	6.66	3.77	7.26

* Un-weighted number of users; % user, *per capita* and *per user* estimates were based on NHANES 2015-2018 and derived using the statistical weights provided by the NCHS.

Table 8. Cumulative Estimated Daily Intake (CEDI) of PGPR from Existing (including Flavor and Color Additive Uses) and New Proposed Uses by the U.S. Population 2+ y and Select Subpopulations

Population	PGPR Estimated Daily Intake (mg/kg-bw/day)			
	A) Per User 90th Percentile EDI from NHANES (Table 6)	**B) Pseudo 90th Percentile Intake from Uses in Flavors**	**C) Maximum and High-End Intake from Uses in Colors (Table 3)**	**Cumulative (A+B+C)**
U.S. 2+ y	7.41	0.117	0.20	7.73
Children 2-5 y	11.09	0.117	0.45	11.66
Children 6-12 y	7.11	0.117	0.45*	7.67
Adolescents 13-18 y	6.04	0.117	0.25	6.41
Adults 19+ y	7.26	0.117	0.20**	7.58

* Based on children 2-5 y; ** Based on U.S. 2+ y

Part 4. Self-limiting Levels of Use

The use of PGPR in foods is considered to be self-limiting for technological reasons, such as product texture and/or flavor profile, either of which could affect consumer acceptance.

Part 5. Experience Based on Common Use in Food Before 1958

The basis of this GRAS assessment is upon scientific procedures. Examples of common use in food before 1958 are provided in Part 3 as supplemental information.

Part 6. Safety Narrative

Introduction

The safety of polyglycerol polyricinoleic (PGPR) (CAS RN 68936-89-0), has been reviewed by several agencies and authoritative bodies. The following resources were searched[3] for information bearing on the safety of PGPR: the Organization for Economic Co-operation and Development (OECD), US FDA 21 Code of Federal Regulations (CFR), Joint FAO/WHO Expert Committee on Food Additives (JECFA), European Food Safety Authority (EFSA)[4], National Toxicology Program (NTP), Environmental Protection Agency (EPA) and EPA's Integrated Risk Information System (IRIS). A literature review through PubMed was also conducted and search terms included polyglycerol polyricinoleic, 29894-35-7, and PGPR, as well as the Chemical Abstracts Service Registry Number (CASRN) where appropriate. The search was performed in August 2021. Extensive safety data for PGPR exist, including a series of toxicology studies, was conducted in the 1950s and 1960s, and these studies have been submitted to and reviewed by public health agencies, and some of the studies and their results have also been published in the open literature.

A full summary of all the PGPR toxicology studies was previously submitted to the US FDA as part of GRAS Notice (GRN 00009) by Quest in 1998 and provided substantiative information on the toxicology of PGPR (henceforth cited as Quest, 1998). There are also key studies, including reproductive, chronic toxicity, carcinogenicity, and human clinical reported in the published literature for PGPR (Howes et al., 1998; Smith et al., 1998; Wilson and Smith, 1998a; Wilson and Smith, 1998b; Wilson et al. 1998). JECFA conducted an evaluation of the unpublished PGPR toxicology studies, derived acceptable daily intake (ADI) of 7.5 mg/kg bw/day (JECFA, 1974). The European Commission Scientific Committee for Food conducted an evaluation of PGPR in 1978 including a review of the unpublished PGPR toxicology studies and derived the same ADI of 7.5 mg/kg bw/day as JEFCA (EC, 1978). EFSA conducted a re-evaluation of PGPR as a food additive in 2017. Based on its review of the published and unpublished literature, EFSA revised the ADI to 25 mg/kg bw/day (EFSA, 2017).

The key PGPR endpoints that served as the basis for the JECFA (1974) and EFSA (2017) ADI were liver and kidney effects. Therefore, in the current assessment, a weight of evidence evaluation was conducted based on current WHO guidance (JMPR, 2015) as well as other authoritative guidance

[3] Exponent conducted the primary search through the commercial database ToxPlanet ChemEXPERT™ module

[4] Includes older preceding organizations including the European Commission Scientific Committee for Food (SCF) whose responsibilities have been transferred to EFSA

(Sellers et al., 2007; Hall et al., 2012; Palazzi et al., 2016) to fully establish that the observed increase in liver and kidney weights were adaptive effects. Based on all the available data for PGPR, it can be concluded from current WHO guidance and the weight of evidence that the observed increase in liver and kidney weights from the chronic studies were adaptive and are not adverse effects. Therefore, the pivotal PGPR study for ADI derivation is the published chronic toxicity/carcinogenicity study in rats with a no observable adverse effect level (NOAEL) of 2,500 mg/kg bw/day.

The various lines of scientific evidence presented herein individually and collectively demonstrate that PGPR has no toxicological endpoint of concern via dietary route of exposure, and based on a NOAEL of 2,500 mg/kg bw/day from a published chronic toxicity/carcinogenicity study in rats, an ADI for PGPR of 25 mg/kg bw/day can be established.

Safety Information

Absorption, Distribution, Metabolism, and Excretion

A published study of the absorption, distribution, metabolism, and excretion (ADME) of PGPR and its associated moieties is available (Howes et al., 1998). The results from the study indicate complete digestion of PGPR and absorption of the fatty acids.

Briefly, male rats were dosed via oral gavage with various radiolabeled associated PGPR components, including [1-^{14}C]glycerol, [^{14}C]polyglycerol and ([^{14}C]polyglycerol)PGPR, and their urine, feces and expired CO_2 were monitored for ^{14}C. Further, rats were administered a dietary slurry of [^{14}C]PGPR, [1-^{14}C] stearic acid, [12-^{3}H]PGPR or [9, 10-^{3}H]PGPR to evaluate the fate of the other PGPR components via a dietary route.

As reported by the authors, the results from the [1-^{14}C]glycerol treated animals showed extensive metabolism of glycerol. For [^{14}C]polyglycerols, the lower polyglycerols were preferentially absorbed from the intestine and were excreted unchanged in the urine while the higher polyglycerols were found in the feces. After 4 days, 93% of the dose of polyglycerols was recovered, of which some 30% was found in the urine and 60% in the feces. Traces of ^{14}C activity were found in depot fat and liver. The excretory pattern and urinary metabolites from ([^{14}C]polyglycerol) PGPR were very similar to that of [^{14}C]polyglycerol. Analysis of urinary and fecal ^{14}C material indicated that the PGPR polymer was digested to give free polyglycerol and polyricinoleic acid. PGPR was synthesized. For, [^{14}C]PGPR or [1-^{14}C] stearic acid, the results following dietary exposure indicated complete digestion of PGPR and absorption of the fatty acids. The ^{14}C-material absorbed was extensively detected in depot fat and some metabolism to $^{14}CO_2$ was demonstrated. The fate of the stearic acid was similar whether dosed alone or incorporated into the PGPR polymer. An overview of the metabolites is provided below.

Table 9. Metabolites identified in tissues or tissue contents of rats 23±24 hr after an oral dose of [12-3H]PGPR or [9, 10-3H]PGPR

Time after dosing (hr)	Tissue or tissue contents	[12-^{3}H]PGPR metabolites	[9,10-^{3}H]PGPR metabolites
3	Stomach contents	PGPR	PGPR
	Lower small intestinal contents	Polyricinoleic acid and free hydroxy fatty acids (5%)	Polyricinoleic acid
	Liver	--	Hydroxy and non-hydroxy fatty acids
6	Cecal contents	Free hydroxy fatty acids with 5% of non-hydroxy fatty acids and polyricinoleic acid	--
	Liver	Non-hydroxy fatty acids	--
	Epididymal fat	--	Non-hydroxy fatty acids (60%) and hydroxy fatty acids (40%)
24	Epididymal fat	Non-hydroxy fatty acids (70%) and hydroxy fatty acids (30%)	--
	Carcass	Non-hydroxy fatty acids (70%) and hydroxy fatty acids (30%)	--
	Faeces/caecal and rectal contents	--	Polyricinoleic acid and trace of non-hydroxy fatty acids
Table adapted from Howes et al., 1998			

Howes et al. (1998) note that that polyricinoleic acid is further degraded to the monomer fatty acid component, ricinoleic acid, which is absorbed and readily metabolized through the typical physiological pathways of fatty acid metabolism, and there was no indication that PGPR or its sub-polymers were stored/accumulated in tissue.

In vitro digestion of PGPR by porcine pancreatic lipase and rat intestinal fractions was also demonstrated in Howes et al. (1998). The results indicate extensive digestion of the PGPR polymer to polyglycerols and fatty acids. The fatty acids are metabolized extensively. The mono, di- and tri-glycerols are extensively absorbed from the intestinal tract and rapidly excreted in the urine unchanged but the hexa-, penta- and higher-polyglycerols are essentially not absorbed and excreted in the feces unchanged.

Acute Oral Toxicity

Acute oral studies have been conducted for PGPR in multiple species and are summarized in Wilson et al. (1998). The studies indicate very low acute toxicity of PGPR with the LD_{50} range being >20 to >100 ml/kg (~>20,000 to > 100,000 mg/kg).

Table 10. Acute oral toxicity studies of PGPR (Wilson et al., 1998)

Species	LD_{50} (ml/kg)
Rat	>20

Species	LD_{50} (ml/kg)
Mouse	>100
Rabbit	>25
Chicken	>30
Guinea pig	>30

Short-Term Toxicity

Unpublished studies of short-term animal toxicity of PGPR were identified and summarized in JECFA (1974), Wilson et al. (1998), Quest (1998), and EFSA (2017). As no full reports of these studies were publicly available, this current evaluation relied solely on the summaries. Consistent adaptive increased liver weight without effects in clinical chemistry or histopathology is reported.

Table 11. Summary overview of short-term toxicity studies

Study type	Dose levels	NOAEL	Critical effects	Reference
5-day study in rats	0 (control), 10,000 mg/kg	Not determined (limited evaluation[a])	No effects on mortality, food consumption, body weight gain or macroscopic observations	JECFA (1974); Quest (1998); Wilson et al. (1998); EFSA (2017)
14-day study in rats	0 (control), 16,200 mg/kg	16,200 mg/kg	No adverse outcomes. Adaptative increase in liver weights.	JECFA (1974); Quest (1998); Wilson et al. (1998); EFSA (2017)
14-day study in mice	0% (control), 5, 10 or 15% PGPR	Not determined (limited evaluation[a])	Increase in liver weights at all dose levels, which returned to control values during 2-week recovery period	JECFA (1974); Quest (1998); Wilson et al. (1998); EFSA (2017)
14-day study in mice	0% (control), 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7% or 8% PGPR	Not determined (limited evaluation[a])	Increased liver weights noted at ≥3% (~2700 mg/kg bw/day)	JECFA (1974); Quest (1998); EFSA (2017)
13-week study in rats	0% (control), 1%, 2%, 4% or 8% PGPR	Not determined (limited evaluation[a])	Increase in liver weights at ≥4% (~3600 mg/kg bw/day)	JECFA (1974); Quest (1998); Wilson et al. (1998); EFSA (2017)

[a] No histopathology and/or hematology reported

Subacute Toxicity

An unpublished 5-day toxicity study in rats was summarized in JECFA (1974), Wilson et al. (1998), Quest (1998), and EFSA (2017). Weanling Colworth rats (6 male and 6 female) were dosed once daily with PGPR at 10 mL/kg/day (approximately 10,000 mg/kg bw/day) via oral gavage for five consecutive days; the study was terminated after 14 days following dosing. 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 of PGPR caused no adverse effects on mortality, food consumption, body weight gain, or gross pathology.

An unpublished 14-day study in rats was conducted to evaluate liver enlargement and the underlying mode of action, and was summarized in JECFA (1974), Wilson et al. (1998), Quest (1998), and EFSA (2017). Male weanling Colworth albino rats (8 animals/dose group, 21 days old) were fed ad libitum either purified diet or treated diet as described in the table below.

Table 12. 14-day study design

	Week 1	**Week 2**
Group 1	10% groundnut oil[a]	18% PGPR (~16,000 mg/kg bw/day) 2% groundnut oil
Group 2	10% groundnut oil	18% castor oil[b] (~16,000 mg/kg bw/day) 2% groundnut oil
Group 3	10% groundnut oil	20% groundnut oil
Group 4 (controls)	Stock diet	Stock diet

[a] Groundnut oil is also known as peanut oil

[b] Castor oil is 90% ricinoleic acid, a primary PGPR metabolite

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/kg bw/day or castor oil (equivalent to 16,200 mg/kg bw/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. The authors noted that liver enlargement was attributed to hypertrophy the liver parenchymal cells and was not indicative of a hyperplastic (proliferative) change (Wilson et al. 1998).

An unpublished 14-day study in mice was also conducted to evaluate liver enlargement and was summarized in JECFA (1974), Wilson et al. (1998), Quest (1998), and EFSA (2017). In this study, 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/day, respectively) for 14-days. Each group was further subdivided into 10 male and 10 female mice and fed their respective diets either ad libitum or by restricting feeding 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 euthanized, 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/kg bw/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. This enlargement was transient, since liver weights returned to control values when the mice were fed for a further 2 weeks on a non-PGPR diet (Wilson et al., 1998; Quest 1998). 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.

Another unpublished 14-day study in mice was conducted to evaluate liver enlargement at lower dose levels and was summarized in JECFA (1974), Wilson et al. (1998), Quest (1998), and EFSA (2017). In this study, 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/day, respectively) for 14-days. 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. neither PGPR nor groundnut oil treatment had any effect on body weights. In male and female mice dosed with ≥ 2,700 mg/kg bw/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/day and in female mice dosed with ≥ 2,700 mg/kg bw/day compared with mice fed identical levels of groundnut oil. No significant differences were observed for the relative kidney weights.

Subchronic Toxicity

An unpublished 13-week dietary study in rats was conducted to further evaluate liver enlargement and summarized in JECFA (1974), Wilson et al. (1998), Quest (1998), and EFSA

(2017). 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/day, respectively) for 13-weeks. 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 were increased at 4 and 8% (approximately ≥ 3,600 mg/kg bw/day).

Reproductive Toxicity

PGPR is not considered a reproductive toxicant based on data from a published continuous breeding study that evaluated a total of three generations (Wilson and Smith, 1998a). This published study has previously been reviewed by JECFA and EFSA, which have both concluded there were no adverse outcomes observed (JECFA, 1974; EFSA, 2017).

Continuous Breeding Study (Published)

Wilson and Smith (1998a) tested the effects of PGPR in a continuous breeding reproduction study. The first-generation parents were selected from five litters which were assigned randomly into two groups: a control (11 males and 17 females) and a treatment group fed 1.5% PGPR (six males and 13 females). The first-generation parents 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 the control and the PGPR groups were increased to 92 and 44 rats of each sex, respectively.

Measured parameters in each of the three generations included 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.

There was no treatment-related effect on overall growth or weights for males or females (see **Table 13**)

Table 13. Growth of rats from weaning to mating (females) or 65 days (males)

		Females			Males		
Group	**Generation**	**No. of females**	**Average weight at weaning (g)**	**Average weight gain at mating (g)**	**No. of males**	**Average weight at weaning (g)**	**Average weight gain at 65 days (g)**
Control	1	17	40	131	11	43	155
	2	42	38[a]	134[a]	52	39	150
	3	92	35	130	92	35	126
1.5% PGPR	1	13	38	127	6	40	143
	2	32	37	127	32	39	143
	3	44	33	119	44	35	112
Table adapted from Wilson and Smith (1998a)							

There was no treatment-related adverse effect on breeding performance (see **Table 14** and **Table 15**). The authors do note that the control rats had a significantly greater percentage of litters weaned entirely (38%) when compared to the treated group (22%). However, it was noted overall that breeding performance was poor in the second generation and there was no indication of this effect in the 3rd generation in which treated rats had a higher percentage of litters wean entirely. The authors attribute this outcome to a "unknown environmental factor" and there no adverse effect on reproductive capacity or development of the offspring during three generations of continuous exposure.

Table 14. Breeding performance

	1st generation		2nd generation		3rd generation	
Reproductive parameter	**Control**	**PGPR**	**Control**	**PGPR**	**Control**	**PGPR**
No. of females	17	13	52	32	92	44
Females failing to breed (%)	0	0	15	16	17	16
Litters born (average/female)	4.7	4.6	4.4	3.2	3.5	4.0
Females producing five litters (%)	76	85	50	41	52	70
Average size of litters	8.4	7.4	7.2	7.2	7.0	7.4
Rats weaned/rats born (%)	79	79	54	44	70	83
Litters entirely weaned (%)	57	64	38	22	53	67
Average weight of weanling males (g)	37.8	37.3	33.7	33.7	36.8	35.4
Average weight of weanling females (g)	36.1	36.2	32.5	30.8	36.4	33.6
Weanling males/all weanling (%)	48	48	48	57	46	50
Table adapted from Wilson and Smith (1998a)						

Table 15. Proportion of rats born and weaned

Treatment	Generation	No. of breeding	Rats born	Rats weaned	% Weaned
Control	1	17	664	526	79.0
PGPR		13	447	353	78.9
Control	2	52	1312	710	54.0
PGPR		32	749	334	44.0
Control	3	92	2263	1595	70.0
PGPR		44	1325	1098	82.8
Table adapted from Wilson and Smith (1998a)					

The authors concluded that the ingestion of 1.5% PGPR (2,000 mg/kg bw/day as reported by authors) via the diet did not produce any adverse effect on reproductive capacity or development of the offspring during three generations of continuous exposure.

Developmental Toxicity and Teratogenicity

No developmental studies are available for PGPR.

Genotoxicity

There were no available *in vitro* or *in vivo* studies for PGPR. Therefore, an *in silico* approach was conducted on two primary moieties of PGPR, glycerol (CAS RN 56-81-5) and ricinoleic acid (CAS RN 141-22-0) using OECD QSAR Toolbox (v.4.3.1). The genotoxicity QSAR profile modules used included *in vitro* mutagenicity (Ames test) alerts by ISS, DNA alerts for CA and MNT by OASIS, carcinogenicity (genotox and nongenotox) alerts by ISS, DNA alerts for AMES by OASIS, *in vivo* mutagenicity (Micronucleus) alerts by ISS, protein binding alerts for Chromosomal aberration by OASIS.

No structural alerts were found except an alert for 'Hacceptorpath3- Hacceptor' in the *in vivo* micronucleus test for glycerol. This alert is 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 low, ranging from 'none' (34%) to just 63% depending on the database, with a high incidence of false positives (Benigni et al., 2010). In the absence of any other structural alert, PGPR is considered non-genotoxic. Further, the two PGPR carcinogenicity studies conducted in rats and mice (see below) presented no oncogenic outcomes or chronic toxicity.

In summary, based on the weight of evidence, it is concluded that PGPR is not genotoxic.

Chronic Toxicity and Carcinogenicity Studies

There was a combined chronic toxicity and carcinogenicity study in Colworth Wistar rats and a carcinogenicity study in Colworth C57B1 mice identified in the published literature (Smith et al., 1998). There were no adverse outcomes observed; the only treatment-related effects being

adaptive increases in liver and kidney weights without changes in clinical chemistry or histopathology.

An unpublished chronic toxicity study was conducted in the third-generation cohort of the reproductive study and is summarized in JECFA (1974), Quest (1998), and Wilson and Smith (1998a). There was also an unpublished supplemental dietary feeding study conducted over 30 and 45-weeks in Wistar rats that was summarized in JECFA (1974), Quest (1998), Wilson et al. (1998) and EFSA (2017) and is also briefly discussed below.

Table 16. Summary overview of chronic toxicity and carcinogenicity studies

Study type	Dose levels	NOAEL	Critical effects	Reference
Two year combined chronic toxicity and carcinogenicity study rat	0 (control), 5% PGPR	5% PGPR or ~2500 mg/kg bw/day No oncogenic effects	No adverse outcomes observed. Adaptive increase in kidney and liver relative weights.	Smith et al. (1998); EFSA (2017)
Carcinogenicity study in mouse	0 (control), 5% PGPR	5% PGPR or ~2500 mg/kg bw/day No oncogenic effects	No adverse outcomes observed. Adaptive increase in kidney and liver absolute weights.[a]	Smith et al. (1998); EFSA (2017)
Chronic toxicity study in rats (third-generation cohort from reproductive study)	0 (control), 1.5% PGPR	1.5% or ~750 mg/kg bw/day[c]	No indication of systemic effects.[b]	JECFA (1974); Quest (1998); Wilson and Smith (1998a)
Chronic dietary feeding study (30 and 45-weeks) in rats	0 (control), 9% PGPR	9% PGPR or ~4500 mg/kg bw/day[d]	No adverse outcomes observed. Adaptive increase in liver weight but no associated effects on liver function or histology.	JECFA (1974); Quest (1998); Wilson et al. (1998); EFSA (2017)

[a] Authors report the increase in absolute kidney and liver weights as an adverse effect but do not report relative weights and no other associated adverse outcome is noted, e.g. no histopathology. Based on the weight of evidence the mouse findings are considered non-adverse adaptive effects.
[b] There was a noted infection of the liver, *Cysticercus fasciolaris*, (intermediate stage of the cat tape worm) in 14 to 16% of animals in both groups
[c] mg/kg bw/day as reported by JECFA (1974) and EFSA (2017)
[d] mg/kg bw/day as reported by EFSA (2017)

Rat Combined Chronic and Carcinogenicity Assay (Smith et al. 1998)

120 Colworth Wistar rats (60 of each sex) were randomly divided at 32±42 days old into test and control groups, each group consisted of 30 male and 30 female animals. The rats were housed in individual cages. Test animals were fed a diet containing 2% PGPR for the first 10 weeks and

then 5% PGPR for the remainder of the 2-yr period. Control animals were fed the same diet except that PGPR was replaced with groundnut oil.

Animals were observed daily for clinical signs of toxicity or changes in behavior and were weighed weekly. Food consumption was measured three times weekly and evaluated as a weekly amount. Liver function was examined after 84 and 103 weeks using the bromosulfothalein excretion test. Kidney function was assessed at the same times by measuring urine concentration. Blood was collected by cardiac puncture under ether anesthesia after 80 weeks from four rats of each sex fed PGPR or the control diet, and on all surviving animals at study termination. Blood samples were analyzed for erythrocyte and leucocyte counts, hemoglobin concentrations and value, red cell fragility and prothrombin time. Each animal was subjected to gross examination at autopsy. 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.

There were no treatment-related clinical signs or effects on survival were reported. No treatment-related adverse effects were found for body weight and food consumption. Liver function tests (bromosulfothalein excretion) and blood analyses revealed no signs of treatment-related effects. The authors note urinalysis revealed no difference in specific gravity at week 84 but a significantly lower specific gravity for urine from PGPR fed rats at week 103, but all values fell within the historical value range.

Organ weight measurements showed that kidneys from male and female rats and livers from female (but not male) rats fed PGPR were heavier than those fed the control diet (see **Table 17**). These increases are below the threshold of adversity (<15%) for a toxicological effect on relative organ weights (JMPR, 2015) and without associated changes in clinical chemistry or histopathology and therefore are considered adaptive. No effects were noted for heart, spleen, pituitary, thyroid, adrenals and testes organ weights. There were no observed treatment-related histopathological effects on any organ, which further supports the organ weight differences are not reflective of an adverse outcome.

Table 17. Mean kidney and liver organ weights for rats fed for 104 weeks on control and PGPR diets (mean ± SD)

		Males		Females	
Treatment		**Control**	**5% PGPR**	**Control**	**5% PGPR**
Kidney					
Absolute Weight[a]	g	3.19 ± 0.45	3.59* ± 0.61 ↑13%	2.37 ± 0.34	2.52* ± 0.50 ↑6%
Rel. Body Weight	g/100 g rat body wt	3.38 ± 0.58	3.38 ± 0.58	0.95 ± 0.14	1.08* ± 0.20 ↑14%

		Males		Females	
Treatment		**Control**	**5% PGPR**	**Control**	**5% PGPR**
Liver					
Absolute Weight[a]	g	11.45 ± 1.61	12.18 ± 1.84	8.67 ± 1.72	9.15* ± 2.72 ↑6%
Rel. Body Weight[a]	g/100 g rat body wt	3.24 ± 0.32	3.38 ± 0.58	3.48 ± 0.47	3.92* ± 0.85 ↑13%
*Significantly different from control (p = 0.05)					

There were no treatment related effects on the tumor incidence of rats fed PGPR (see **Table 18** below).

Table 18. Tumor incidence of rats fed PGPR or control diet

	Animals killed after 104 weeks				Animals dying during test			
	Males		Females		Males		Females	
	Control	**5% PGPR**	**Control**	**5% PGPR**	**Control**	**5% PGPR**	**Control**	**5% PGPR**
Total no. of rats examined	23	8	2	8	7	2	9	2
Total primary tumors	9(39. %)	9(50.0%)	(52.4%)	8(44.4%)	2(28.6%)	(8.3%)	2(22.2%)	4(33.3%)
Rats with one tumor	7	6	6	6	2		2	2
Rats with multiple tumors			2		0	0	0	
Uterus								
Adenocarcinoma	-	-	1	0	-	-	0	0
Fibroma	-	-	0	1	-	-	1	0
Thymus								
Thymoma	0	1	0	1	0	0	2	0
Thyroid								
Adenoma	4	5	2	3	0	0	0	0
Pituitary								
Adenoma	3	2	5	6	1	1	1	0
Testis								
Adenoma (Leydig cell)	2	0	-	-	0	0	-	-
Mesentery								
Lipoma	0	1	0	0	0	0	0	0
Stomach								
Squamous cell papilloma	0	0	0	0	0	1	0	0
Tongue								
Sarcoma	0	0	0	0	0	0	0	1
Subcutaneous tissue								
Fibrosarcoma	0	0	0	0	0	0	0	1

The authors concluded that there was no oncogenic potential of PGPR. The authors also concluded that the changes in weight of liver and kidneys in PGPR-fed animals was consistent with those seen in previous studies and considered to be an adaptive effect as a result of metabolic compensation in response to the high level of PGPR ingested.

Mouse Carcinogenicity Assay (Smith et al. 1998)

100 Colworth C57B1 mice (50 of each sex) were randomly divided when 6±8 wk old into two groups each of 25 male and 25 female animals. One group was fed a purified diet containing 5% PGPR, while a second group received the same purified diet but with groundnut oil replacing the PGPR. The animals were caged individually and provided with food and water *ad lib*.

Animals were observed daily for clinical signs of toxicity or changes in behavior and were weighed weekly. Food consumption was measured twice weekly and evaluated as a weekly amount. Blood was collected by cardiac puncture under ether anesthesia on all surviving animals at study termination. The blood was analyzed for erythrocyte and leucocyte counts and hemoglobin concentrations. Each animal was subjected to gross examination at autopsy (at test termination and for those animals dying during the test). The following organs were weighed: heart, kidney, liver and testes. These organs, together with 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.

No treatment-related clinical signs were observed. There were no significant differences in survival between mice fed either PGPR or control purified diet. The growth of mice fed PGPR was similar to those fed the control purified. The amount of food consumed was similar for PGPR treated animals and those eating control purified diet.

No differences in hematological parameters were found between mice fed the purified diet containing PGPR and those animals fed the control purified diet.

Absolute organ weight measurements revealed that livers and kidneys from female mice fed PGPR were heavier than those from mice fed the control purified diet. However, no relative organ weights were reported in the published study. There were no treatment-related effects on spleen, heart, and testes organ weights. There were no treatment-related histopathological effects.

The authors did note that the increase in kidney and liver organs was an adverse effect though they also noted that PGPR had no adverse effect on growth, food consumption, survival, hematology and histological appearance of the tissues. No relative kidney or liver weights are reported in the study.

Table 19. Mean kidney and liver organ weights for rats fed for 104 weeks on control and PGPR diets (mean ± SD)

	Males		Females	
Treatment	**Control**	**5% PGPR**	**Control**	**5% PGPR**
Kidney				

		Males		Females	
Treatment		**Control**	**5% PGPR**	**Control**	**5% PGPR**
Absolute Weight[a]	G	0.68	0.70	0.53	0.66* (↑25%)
Liver					
Absolute Weight[a]	g	2.54	2.81	2.26	3.55* (↑55%)

*Significantly different from control (p = 0.05)

There were no treatment related effects on the tumor incidence of rats fed PGPR (see **Table 20** below).

Table 20. Tumor incidence of mice fed PGPR or control diet

	Animals killed after 80 wk				**Animals dying during test**			
	Males		**Females**		**Males**		**Females**	
	Control	**5% PGPR**	**Control**	**5% PGPR**	**Control**	**5% PGPR**	**Control**	**5% PGPR**
Total no. of mice examined	16	18	15	16	9	7	10	9
Total animals with primary tumors	1 (6.2%)	4 (22.2%)	7 (46.6%)	5 (31.2%)	1 (6.2%)	4 (22.2%)	0	1 (11.1%)
Mice with one tumor	1 (6.2%)	4 (22.2%)	7 (46.6%)	5 (31.2%)	1 (11.1%)	0	0	1 (11.1%)
Mice with multiple tumors	0	0	0	0	0	0	0	0
Total primary tumors	1	4	7	5	0	0	0	1
Total secondary tumors	0	0	0	0	0	0	0	0
Liver								
Hepatoma	0	1	0	1	0	0	0	0
Adenoma	0	2	0	1	0	0	0	0
Secondary tumor	0	0	0	0	0	0	0	0
Lung								
Secondary tumor	0	0	0	0	0	0	0	0
Kidney								
Secondary tumor	0	0	0	0	0	0	0	0
Skin								
Papilloma	0	0	-	-	0	0	-	-
Keratoacanthoma	0	0	0	0	0	0	0	0
Squamous cell carcinoma	0	0	0	0	0	0	0	0
Sarcoma	0	0	0	0	0	0	0	0
Stomach								
Squamous cell carcinoma	0	0	0	0	0	0	0	0
Intestine								
Carcinoma	0	1	0	0	0	0	0	0
Omentum								
Lipoma	0	0	1	1	0	0	0	0
Mammary gland	0	0	1	1	0	0	0	0
Thymus	0	0	0	0	0	0	0	0

	Animals killed after 80 wk				Animals dying during test			
	Males		Females		Males		Females	
	Control	5% PGPR	Control	5% PGPR	Control	5% PGPR	Control	5% PGPR
Thyroid	1	0	0	0	0	0	0	0
Leukaemia	0	0	4	3	0	0	0	1

Chronic toxicity study in rats (third-generation cohort from reproductive study)

The three-generation reproductive study described in the published study, Wilson and Smith (1998a), also included an unpublished chronic toxicity component in the third-generation cohort in which animals were exposed continuously for a year to a control diet or 1.5% PGPR. Wilson and Smith (1998a) only report in their abstract that a histological examination of selected tissues from those rats continued for 1 year failed to show any lesions but do not provide further details on this examination in the methods or results. The details on the chronic toxicity component are primarily described as an unpublished study summarized in Quest (1998) which was previously submitted to the FDA in a GRN 0009. The 1-year of dietary PGPR exposure in the third-generation cohort was followed by gross observation of the viscera at sacrifice and microscopic examination of selected tissues, including the liver, kidney, adrenal, spleen, testis, gastro-duodenal junction and small intestine.

Quest (1998) notes that there was a noted infection of the liver, *Cysticercus fasciolaris*, (intermediate stage of the cat tape worm) in 14 to 16% of animals in both groups. Based on Quest (1998), there was no indication that organ weights were evaluated. The histopathological examination did not indicate any abnormal tissue morphology in the liver, kidney, adrenal, spleen, testis, gastro-duodenal junction and small intestine.

Chronic (30 and 45-weeks) dietary feeding studies in rats (unpublished)

An unpublished chronic feeding study in rats was conducted over 30 and 45-weeks, and is summarized in JECFA (1974), Wilson et al. (1998), Quest (1998), and EFSA (2017). For each duration, 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 behavior; 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, hematology, 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. Given that the effects were confined to the enlargement of the liver, with no accompanying change on liver function or histology, the summaries supported that no significant toxic effect occurred (JECFA, 1974; Wilson et al. 1998; Quest, 1998; EFSA, 2017).

Human Studies

A published controlled human dietary study conducted with PGPR confirmed that the compound has no adverse effect on liver and kidney function (Wilson and Smith, 1998b). Nineteen healthy adults participated in the study and comprised of two females (aged 64 and 66 years old) and eight male and nine female university students (19-24 years old). They were maintained in pairs for 3 weeks (between March 1964 and August 1965) in the metabolic unit of Glasgow Royal Infirmary, which was fully equipped for diet preparation and sample collection. The volunteers were allowed to go out for short periods but were requested not to eat anything except the diets supplied by the hospital and were also required not to pass any feces or urine while they were away since these had to be collected quantitatively for laboratory examination.

PGPR was introduced into three items: (1) soups (5 g/pint); (2) cakes (10 g/cake or 2.5 g/portion); (3) toffee bars (5 g/bar). The soups and cakes were supplied to the hospital in the form of a dry mix which was freshly prepared each day in the hospital kitchens. The toffee bars were prepared at the Unilever Research Laboratory, Colworth House. The amount of PGPR fed to the volunteers was carefully regulated over a 3-wk period as follows: week 1: none; week 2: 5 g PGPR/day; week 3: 10 g PGPR/ day. The diet was designed to maintain constant intakes of fat (150 g/day, excluding PGPR) and protein (75 g/day) with a flexible level of carbohydrates to suit individual requirements.

Blood samples were taken at twice-weekly intervals throughout the study. The following serum clinical chemistry parameters were determined: albumin, globulin, serum electrophoresis, thymol turbidity, bilirubin, cholesterol, alanine aminotransferase, cholinesterase and creatine clearance. Feces and urine samples were collected throughout the study and pooled for individuals as weekly samples. The feces samples were analyzed for fat and fecal nitrogen, while the urine samples were analyzed for creatinine. The means and standard deviations were calculated.

No treatment-related effects were observed for liver and kidney function. The level of fecal fat was determined to investigate whether there was any evidence of interference with fat digestion. Most of the results were within the normal limits. Where there were deviations from normal, these could not be ascribed to the consumption of PGPR. Nitrogen determinations on the feces provide an

indicator of digestion and absorption in the alimentary tract. Analysis of randomly selected samples did not indicate any consistent effect produced by PGPR.

Authoritative Reviews

JECFA (1974)

JECFA originally reviewed all the studies presented herein and published a monograph on these studies in 1974 including the acute toxicity tests, subacute rat and mouse toxicity studies, a rat chronic toxicity/multigeneration reproduction study, rodent metabolism, carcinogenicity testing in rat and mouse and a human clinical evaluation. JECFA's concluding comments were the long-term studies in rats and mice did not show carcinogenic potential, and the enlargement of liver and kidneys observed in the chronic studies was not accompanied by any lesions detectable by histopathology. JECFA also noted that "the rat study shows a no-effect level for liver enlargement", which likely refers to the rat subchronic dietary study that JECFA notes had no liver enlargement at dose levels below 2%.

Further, JECFA determined the level causing no toxicological effect was 1.5% PGPR in the diet which they noted was equivalent to 750 mg/kg bw/day. The exact basis for this level is not fully articulated in the JEFCA (1974) monograph though it could be reasonably inferred that it is based on the lack of adverse outcomes in the reproductive study including no fertility effects or liver histological effects up to 1.5% PGPR (Wilson and Smith, 1998a; JECFA, 1974; Quest, 1998). Based on that designated level, JECFA estimated an acceptable daily intake (ADI) of 7.5 mg/kg bw/day.

European Commission Scientific Committee for Food

In 1978, the European Commission Scientific Committee for Food conducted a toxicological evaluation of PGPR, including a review of the unpublished toxicology studies, for its use as an emulsifier based on the data that is described above. The Committee's toxicological evaluation is very brief, and they do not summarize each study though they also reviewed JECFA's monograph. The Committee noted that development of hepatomegaly observed in rats at PGPR concentrations up to 18% was reversible, and there were no significant histopathological abnormalities of the liver with the liver enlargement not being associated with hyperplasia. The Committee established an ADI of 7.5 mg/kg bw/day.

EFSA

In 2017, EFSA conducted a re-evaluation of PGPR, which is authorized as a food additive in the European Union. The EFSA evaluation included a review of all the PGPR toxicology studies

described above, as well as additional studies on structural analogues including ricinoleic acid, castor oil, polyricinoleic acid, and polyglycerols.

Based on their review of the acute toxicity data, the EFSA Panel concluded that acute oral toxicity of PGPR and its structural analogues was low. In their review of the short-term toxicity data, the Panel noted that the dose levels administered were very high and key target organ/effect was an increase in relative liver weight. Further, the Panel also noted that the lack of associated histopathology and the transient nature of the liver enlargement supported the conclusion that it was an adaptive effect and not an adverse outcome

EFSA (2017) noted there were no genotoxicity studies available for PGPR. EFSA did conduct an *in silico* genotoxicity evaluation on PGPR, which had no structural alerts of concern. They also noted there was no genotoxicity observed in *in vitro* and *in vivo* studies on structural analogues, sodium ricinoleate and castor oil. EFSA (2017) also noted that any potential reactive aldehyde compound formed from metabolism of PGPR would have a bulky aliphatic chain that would prevent interactions with DNA.

The EFSA (2017) review of the chronic toxicity/carcinogenicity study in rats and carcinogenicity study in mice concluded there were no oncogenic effects and the observed increase in liver and kidney weights were a non-adverse adaptive effect.

EFSA (2017) noted that there were no adverse outcomes observed in the PGPR reproductive study though they did also emphasize the study design limitations including low sample size, low breeding success in controls, and the liver infection observed in the third-generation cohort. Due to these limitations, EFSA concluded that the reproductive study was inappropriate to use for deriving health-based guidance value. EFSA noted that there were no developmental studies available for PGPR.

The PGPR human study was also evaluated by EFSA (2017), and they noted that there were no effects of exposure on the biochemical markers of kidney and liver function.

Finally, the EFSA Panel concluded that the combined 2-year chronic toxicity/carcinogenicity study in rats was 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. Further, the EFSA Panel also noted that the long-term studies confirmed that the increases in relative liver and kidney weights were non-adverse adaptive outcomes. Using an uncertainty factor of 100, the EFSA Panel derived an ADI of 25 mg/kg bw/day.

Current Weight-of-Evidence Approach for Adaptive Outcomes

The JEFCA (1974) Monograph reported the ADI as 7.5 mg/kg bw/day based on their no observed effect level for liver enlargement. The more recent evaluation (EFSA, 2017) reports the ADI as 25 mg/kg bw/day based on the absence of adverse effects at the highest dose levels tested in the toxicological database. EFSA (2017) determined the increased liver and kidney weights in the toxicological database to be adaptive effects in the absence of corresponding clinical chemistry or histopathological effects.

Since the JECFA (1974) monograph for PGPR, there has been updates in scientific understanding and guidance for evaluating adaptive vs adverse outcomes in animal toxicological bioassays. Importantly, the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) guidance document for WHO monographers and reviewers specifically addresses the evaluation of liver toxicological effects, which was and is still considered the key endpoint for the PGPR toxicological database. Further supporting guidance on technical evaluations of adaptive responses that supports this weight-of-evidence approach can also be found in authoritative reviews including Sellers et al. (2007), Hall et al. (2012) and Palazzi et al. (2016). In short, increased organ weight and hypertrophic changes are considered adaptive effects in the absence of effects on liver function and other adverse histopathological changes.

As described below, the current JMPR (2015) guidance weight-of-evidence approach supports that a re-evaluation of the PGPR toxicological database would conclude the observed liver effects were adaptive and non-adverse.

1. **Line of Evidence #1:** Does the histological evidence support the hypothesis that the hepatocellular hypertrophy is an adaptive effect?

   As described above, the PGPR toxicological dataset supports that increased liver weights are due to hepatocellular hypertrophy; an adaptive effect observed only at high doses. Increases in hepatocellular hypertrophy in response to xenobiotics is an adaptive effect in response to increased metabolic needs of the liver. Specifically, the presence of xenobiotics induces metabolic enzymes in the organelles of hepatocytes results in the increased size (hypertrophy) of the hepatocytes, which consequently results in increased organ weight. Hepatocellular hypertrophy is reversible upon cessation of exposure. The short-term toxicity studies indicate that hepatocellular hypertrophy at high doses is not accompanied by any other adverse hepatic response, and that it is reversible when exposure ceases (JECFA, 1974; Quest, 1998; Wilson et al., 1998; EFSA, 2017). The chronic toxicity studies in mice and rats indicate, even in the presence of increased liver weights, no histopathological effects on the liver, and no liver-associated effects on clinical chemistry or hematology (Smith et al., 1998).

2. **Line of Evidence #2:** Does the clinical chemistry support the hypothesis that the hepatocellular hypertrophy is an adaptive effect? If there is no evidence of histopathological change, do the clinical chemistry findings exclude a conclusion of hepatotoxicity?

   The clinical chemistry findings exclude a conclusion of hepatotoxicity. In the chronic rat study, liver function was assessed using the bromosulfothalein (BSP) liver function excretion test at 84 and 103 weeks with no indication of an effect (Smith et al. 1998). Similarly, dietary studies of 30 and 45 weeks in rats also conducted BSP assays with no indication of an adverse effect (Quest, 1998). Lastly, the human studies directly evaluated albumin, globulin, serum electrophoresis, thymol turbidity, bilirubin, cholesterol, alanine aminotransferase, cholinesterase and creatine clearance levels from PGPR doses of 5 g/day and 10 g/day (Wilson and Smith, 1998b). No adverse effects on liver function were observed in any of the participants. In summary, the liver function has been sufficiently evaluated in PGPR in both animals and humans, with no indication of hepatotoxicity.

3. **Line of Evidence #3:** Are the liver changes transient or sustained? Is there a progression of the effect?

   The liver changes have been noted as reversible and there is no indication of a progression to more severe effects or effects at lower dose level following chronic exposure. The short-term toxicity studies indicate that hepatocellular hypertrophy at high doses is not accompanied by any other adverse hepatic response, and that it is reversible when exposure ceases (JECFA, 1974; Quest, 1998; Wilson et al., 1998; EFSA, 2017). The chronic toxicity studies in mice and rats indicate that even with long-term exposure to PGPR, the primary treatment-related outcome is restrained to increased liver weights with no histopathological or adverse liver function effects. The effect level for increased liver weight does not decrease over time.

4. **Line of Evidence #4:** Is liver hypertrophy accompanied by the induction of P450 or other xenobiotic metabolizing enzymes? Are there any toxicological effects consequent to that induction?

   Physiologically, liver cell hypertrophy is the result of the demand for increased metabolic capacity of an organ and the result of the induction of metabolic enzymes. This natural increase in cellular capacity as a response to metabolic demand is not alone considered an adverse outcome (Hall et al., 2012). Induction of P450's of other metabolic enzymes was not specifically evaluated in any of the studies.

There were no toxicological effects consequent from enzyme induction. As noted before the only observed treatment-related effects from chronic exposure were an increase in liver organ weights with no associated histopathology (Smith et al., 1998) and no associated effects in clinical chemistry. In short-term studies at very high doses, hepatocellular hypertrophy was not accompanied by any other hepatic response and was shown to be reversible after exposure ceased (JECFA, 1974; Quest, 1998; Wilson et al., 1998; EFSA, 2017). Further, as liver enzyme induction becomes toxicologically adverse, changes in clinical pathology (Hall et al., 2012) will occur, which was not observed in animals or humans exposed to PGPR (Smith et al. 1998; Quest, 1998; Wilson and Smith, 1998b). Lastly, an evaluation of DNA content in the presence of PGPR-induced liver enlargement did not indicate any change suggesting that there was no increase in cell replication (hepatocellular hyperplasia) or increased in RNA (Quest, 1998).

In summary, the weight-of-evidence demonstrates that PGPR induces an adaptive hepatic response as stated by the WHO guidance "*[i]n the absence of histopathological damage and relevant clinical chemistry changes, at the dose that induces only hepatocellular hypertrophy and/or liver size/weight changes, hypertrophy should not be identified as an adverse effect or used for establishing health-based guidance values*" (JMPR, 2015).

The livers are consistently presented as the most sensitive target tissue for PGPR though there was also a treatment-related effect on increased kidney weights in the chronic mouse and rat studies. Similar to the liver, this renal effect was considered an adaptive response with no indication of adversity. The JMPR (2015) guidance does not review kidney adaptive responses, however an established fundamental criterion when evaluating any potential effect as adverse is considering whether it impairs the function of the tissue of concern (Palazzi et al., 2016). As such, it should be noted that the increases in kidney weights were not associated with any urinalysis (specific gravity) or histopathological effects. In the chronic rat study, the authors noted urinalysis revealed no difference in specific gravity at week 84 and a significantly lower specific gravity for urine from PGPR fed rats at week 103, but all values fell within the historical value range. Similarly, dietary studies of 30 and 45 weeks in rats also evaluated the specific gravity of urine samples with no indication of an adverse effect (Quest, 1998). Lastly, the human PGPR study evaluated urinary creatinine clearance as a marker of kidney function and no effect was observed (Wilson and Smith, 1998b). In summary, it can be concluded that the increase in kidney weights is an adaptive response based on no associated histopathology or impairment of function.

PGPR Safety Summary

The mammalian and human toxicological properties of PGPR have been well characterized in multiple animal species and humans for a variety of exposure durations and toxicity endpoints.

Studies of sufficient quality for risk assessment have been published that evaluate pharmacology, chronic toxicity, reproductive toxicity and carcinogenic potential. Although these studies have deviations from current guidelines, including many studies using only one-dose and having animal group sizes below guidelines, they still have critical endpoints including bodyweights, fertility, clinical chemistry, hematology, organ weights, and histopathology that are aligned with current practices for determining toxicological risk. Further, there are detailed summaries of unpublished study reports that provide corroborative information on the overall toxicity of PGPR (JECFA, 1974; Wilson et al., 1998; Quest, 1998; EFSA, 2017). Lastly, the data reported on all the studies, published and unpublished, is concordant with a clear target organ/effect of increased liver and kidney weights and substantially supporting evidence indicating that the organ weight effects are non-adverse adaptive effects.

A pharmacology study of PGPR and its associated moieties in rats indicates the compound is completely digested and the fatty acids are absorbed. It should be noted that one of the key metabolites identified, ricinoleic acid, also has supporting toxicology data from the National Toxicology Program (NTP) that supports the key repeat-dose treatment-related effect is a non-adverse adaptive increase in liver weights at high doses (~10% in diet) (NTP, 1992).

The acute studies all indicate low toxicity with LD_{50} range being >20 to >100 ml/kg (~>20,000 to > 100,000 mg/kg). The short-term toxicity studies all indicate no toxicological endpoint of concern with the only consistent treatment-related effect being a non-adverse adaptive increase in liver weight.

A continuous breeding reproductive study in rats indicated no adverse outcomes of PGPR on reproduction or development at the highest dose tested with a NOAEL of 1.5% PGPR or ~750 mg/kg bw/day. No developmental studies were available for PGPR.

It should be noted that in their monograph, JECFA does not clearly articulate their preference for using the reproductive study as the basis for the overall reference dose for their ADI (JECFA, 1974). As such, it is important to consider that the reproductive study has methodological limitations that should preclude its use to derive an ADI, including a low sample size for the first-generation treated animals (six males and 13 females), and potential breeding issues with all the second-generation animals (see **Reproductive Toxicity** section). Secondly, the unpublished data on the chronic toxicity component from the reproductive study, summarized in Quest (1998), indicated that 14 to 16% of the animals in both groups of the third generation had a *Cysticercus fasciolaris* (intermediate stage of the cat tape worm) in the liver. Further, there is no indication that organ weights were measured as part of chronic toxicity component and thus no evaluation of liver enlargement, the key treatment-related endpoint across the PGPR dataset. Given the significant reported deficiencies with the controls in the reproductive study, it should not be relied upon for derivation of the ADI. Lastly there were no adverse effects at the highest dose tested and therefore, it does not actually establish an effective ceiling for the safety of

PGPR. On the other hand, the carcinogenicity and chronic studies (Smith et al., 1998) tested at a higher concentration for a longer period, had an expanded toxicological examination battery including reproductive organs, and noted no adverse outcomes.

No *in vitro* or *in vivo* genotoxicity studies were available for PGPR. An *in silico* QSAR evaluation of PGPR indicated no structural alerts of concern for PGPR's moieties.

The chronic toxicity/carcinogenicity study in rats and the carcinogenicity study in mice indicated no adverse toxicity or oncogenic outcomes at the highest dose tested with a NOAEL of 5% PGPR or ~2,500 mg/kg bw/day (Smith et al., 1998). The only treatment-related effects were a non-adverse adaptive increase in liver and kidney weights. It should be noted the study authors only reported the absolute liver and kidney weights in the mouse carcinogenicity and did not report relative weights. The authors did report the increase in absolute liver and kidneys weights in mice as an adverse outcome. However, conversely, the authors also noted that there was no adverse effect on growth, food consumption, survival, hematology, and histological appearance of the tissues. As the increase in absolute liver and kidney weights are consistent with the known adaptive responses to PGPR, and there was no evidence to indicate any associated toxic effect on other endpoints, the mouse findings are considered adaptive and non-adverse.

A controlled 3-week human study indicated no adverse outcomes on liver or kidney function at PGPR dose levels of 5 – 10 g/day.

Finally, a weight of evidence evaluation was conducted based on current WHO guidance as well as other authoritative guidance to fully establish that the observed increase in liver and kidneys were adaptive effects. The guidance-based approach carefully considered all lines of evidence as it related to impairment of liver and kidney function, which is a key criterion to determine if effects are adaptive or adverse. Based on all the available data for PGPR, it can be concluded that the observed increase in liver and kidney weights from the chronic studies were adaptive and are not adverse effects.

Based on the weight of evidence, the NOAEL of 2,500 mg/kg bw/day, the highest and only test dose in the 2-year chronic toxicity/ carcinogenicity study in rats, can be relied upon to derive an ADI for PGPR.

The EFSA (2017) re-evaluation of PGPR reviewed the same data as presented in this document and came to the same conclusion regarding the basis for deriving the PGPR ADI. Applying uncertainty factors of 100 (10- inter, 10 intra variability) to the NOAEL, an ADI of 25 mg/kg bw/day can be established for PGPR.

Basis for GRAS Determination Regulatory Framework

The regulatory framework for determining whether a substance can be considered Generally Recognized As Safe (GRAS) for its intended use in accordance with section 201(s) (21 U.S.C. § 321(s)) of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. § 301 et. Seq.) ("the Act"), is set forth at 21 CFR §170.30, which states:

(a) General recognition of safety may be based only on the views of experts qualified by scientific training and experience to evaluate the safety of substances directly or indirectly added to food. The basis of such views may be either (1) scientific procedures or (2) in the case of a substance used in food prior to January 1, 1958, through experience based on common use in food. General recognition of safety requires common knowledge about the substance throughout the scientific community knowledgeable about the safety of substances directly or indirectly added to food.

(b) General recognition of safety based upon scientific procedures shall require the same quantity and quality of scientific evidence as is required to obtain approval of a food additive regulation for the ingredient. General recognition of safety through scientific procedures shall ordinarily be based upon published studies, which may be corroborated by unpublished studies and other data information.

These criteria are applied in the analysis below to determine whether the proposed increase in the existing use levels of PGPR in chocolate (from 0.30% to 0.50%), chocolate-type products based on vegetable fats other than cocoa butter (from 0.30% to 0.50%), and mayonnaise and spreads (from 0.28% to 0.80%), is safe and generally recognized as safe (GRAS) by scientific procedures.

Safety Determination

Polyglycerol polyricinoleic acid (PGPR) (CASRN 68936-89-0) is a class of polyglycerol esters of fatty acid, and a nonionic emulsifier (Bastida-Rodríguez, 2013). PGPR is formed by the esterification of polyglycerol with condensed castor oil fatty acids. PGPR is insoluble in water and alcohol, but soluble in ether, hydrocarbons, and in halogenated hydrocarbons (FCC Vol 12). PGPR has a history of use in food in several countries. They have been used as food additives in the United States and Europe since the 1940s (Bastida-Rodríguez 2013).

The use of PGPR as an emulsifier in various foods, including chocolate, chocolate-type products based on vegetable fats other than cocoa butter, margarine, low fat creamers, low fat dairy analogs, condiments and spreads, cheese powder (snacks), flavors, and color additives with use

levels ranging from 0.1 to 5% were previously concluded to be GRAS based on scientific procedures, and for which FDA had no questions (GRNs 9 (Quest International, 1998), 179 (Stepan Company, 2005), 266 (Palsgaard A/S, 2008), 270 (Stepan Company, 2008), and 466 (McCormick & Company, Inc., 2013). The current GRAS dossier examined the proposed increase in the existing GRAS use levels of PGPR as an emulsifier in chocolate (from 0.30% to 0.50%), chocolate-type products based on vegetable fats other than cocoa butter (from 0.30% to 0.50%), and mayonnaise and spreads (from 0.28% to 0.80%).

The estimated daily intake (EDI) of PGPR from existing (except for flavor and color additive uses) and proposed uses was derived based on food consumption records collected in the What We Eat In America (WWEIA) dietary component of the National Health and Nutrition Examination Survey (NHANES) conducted in 2015-2016 and 2017-2018 (2015-18). Intake estimates of PGPR associated with the existing GRAS use in flavors was derived by combining estimates of the per capita intakes of major food groups from the USDA Economic Research Service (ERS) with estimates of the fraction of these foods that could be processed, the fraction of the processed foods that could contain flavor, the flavor concentration in processed foods, and the maximum PGPR use level of 0.1% in flavors. Intake estimates of PGPR associated with the existing GRAS use in color additives was based on the maximum intake estimate across seven FD&C color additives that was derived by the FDA (Doell et al., 2016).

The cumulative estimated daily intake (CEDI) of PGPR that account for all uses in foods including uses in flavors and color additives was conservatively calculated by adding: (1) per user 90th percentile PGPR intake estimates from existing GRAS uses (not including uses in flavors and color additives) combined with new proposed uses derived using NHANES 2015-2018, (2) the pseudo 90th percentile intake of PGRP from flavor uses for the total U.S. population derived from the USDA ERS data, and (3) color additive uses derived from maximum estimates in Doell et al. (2016). Based on this conservative approach, the per user 90th percentile CEDI of PGPR is 7.73 mg/kg bw/day for the US 2+ y, with the children 2-5 y having the highest CEDI of 11.66 mg/kg bw/day.

Extensive publicly available information bearing on the safety of PGPR exist, including a series of toxicology studies, conducted in the 1950s and 1960s, and these studies have been submitted to and reviewed by public health agencies, and some of the studies and their results have also been published in the open literature.

The mammalian and human toxicological properties of PGPR have been well characterized in multiple animal species and humans for a variety of exposure durations and toxicity endpoints. Studies of sufficient quality for risk assessment have been published that evaluate pharmacology, chronic toxicity, reproductive toxicity and carcinogenic potential. Although these studies have

deviations from current guidelines, including many studies using only one-dose and having animal group sizes below guidelines, they still have critical endpoints including bodyweights, fertility, clinical chemistry, hematology, organ weights, and histopathology that are aligned with current practices for determining toxicological risk. Further, there are detailed summaries of unpublished study reports that provide corroborative information on the overall toxicity of PGPR (JECFA, 1974; Wilson et al., 1998; Quest, 1998; EFSA, 2017). Lastly, the data reported on all the studies, published and unpublished, is concordant with a clear target organ/effect of increased liver and kidney weights and substantially supporting evidence indicating that the organ weight effects are non-adverse adaptive effects.

A pharmacology study of PGPR and its associated moieties in rats indicates the compound is completely digested and the fatty acids are absorbed. It should be noted that one of the key metabolites identified, ricinoleic acid, also has supporting toxicology data from the National Toxicology Program (NTP) that supports the key repeat-dose treatment-related effect is a non-adverse adaptive increase in liver weights at high doses (~10% in diet) (NTP, 1992).

The acute studies all indicate low toxicity with LD_{50} range being >20 to >100 ml/kg (~>20,000 to > 100,000 mg/kg). The short-term toxicity studies all indicate no toxicological endpoint of concern with the only consistent treatment-related effect being a non-adverse adaptive increase in liver weight.

A continuous breeding reproductive study in rats indicated no adverse outcomes of PGPR on reproduction or development at the highest dose tested with a NOAEL of 1.5% PGPR or ~750 mg/kg bw/day. No developmental studies were available for PGPR. It is noted that JECFA did not clearly articulate their preference for using the reproductive study as the basis for the overall reference dose for their ADI (JECFA, 1974). As such, it is important to consider that the reproductive study has methodological limitations that should preclude its use to derive an ADI, including a low sample size for the first-generation treated animals (six males and 13 females), and potential breeding issues with all the second-generation animals. Secondly, the unpublished data on the chronic toxicity component from the reproductive study, summarized in Quest (1998), indicated that 14 to 16% of the animals in both groups of the third generation had a *Cysticercus fasciolaris* (intermediate stage of the cat tape worm) in the liver. Further, there is no indication that organ weights were measured as part of chronic toxicity component and thus no evaluation of liver enlargement, the key treatment-related endpoint across the PGPR dataset. Given the significant reported deficiencies with the controls in the reproductive study, it should not be relied upon for derivation of the ADI. Lastly there were no adverse effects at the highest dose tested and therefore, it does not actually establish an effective ceiling for the safety of PGPR.

No *in vitro* or *in vivo* genotoxicity studies were available for PGPR. An *in silico* QSAR evaluation of PGPR indicated no structural alerts of concern for PGPR's moieties.

The chronic toxicity/carcinogenicity study in rats and the carcinogenicity study in mice indicated no adverse toxicity or oncogenic outcomes at the highest dose tested with a NOAEL of 5% PGPR or ~2,500 mg/kg bw/day (Smith et al., 1998). The only treatment-related effects were a non-adverse adaptive increase in liver and kidney weights. It should be noted the study authors only reported the absolute liver and kidney weights in the mouse carcinogenicity and did not report relative weights. The authors did report the increase in absolute liver and kidneys weights in mice as an adverse outcome. However, conversely, the authors also noted that there was no adverse effect on growth, food consumption, survival, hematology, and histological appearance of the tissues. As the increase in absolute liver and kidney weights are consistent with the known adaptive responses to PGPR, and there was no evidence to indicate any associated toxic effect on other endpoints, the mouse findings are considered adaptive and non-adverse.

A controlled 3-week human study indicated no adverse outcomes on liver or kidney function at PGPR dose levels of 5 – 10 g/day.

Finally, a weight of evidence evaluation was conducted based on current WHO guidance as well as other authoritative guidance to fully establish that the observed increase in liver and kidneys were adaptive effects. The guidance-based approach carefully considered all lines of evidence as it related to impairment of liver and kidney function, which is a key criterion to determine if effects are adaptive or adverse. Based on all the available data for PGPR, it can be concluded that the observed increase in liver and kidney weights from the chronic studies were adaptive and are not adverse effects.

Based on the weight of evidence, the NOAEL of 2,500 mg/kg bw/day, the highest and only test dose in the 2-year chronic toxicity/ carcinogenicity study in rats, can be relied upon to derive an ADI for PGPR. The EFSA (2017) re-evaluation of PGPR reviewed the same data as presented in this document and came to the same conclusion regarding the basis for deriving the PGPR ADI. Applying uncertainty factors of 100 (10- inter, 10 intra variability) to the NOAEL, an ADI of 25 mg/kg bw/day can be established for PGPR.

Overall, the various lines of scientific evidence individually and collectively demonstrate that PGPR has no toxicological endpoint of concern via dietary route of exposure, and based on a NOAEL of 2,500 mg/kg bw/day from a published chronic toxicity/carcinogenicity study in rats, an ADI for PGPR of 25 mg/kg bw/day can be established. The conservatively estimated per user 90th percentile CEDI of PGPR is 7.73 mg/kg bw/day for the US 2+ y and the highest CEDI of 11.66 mg/kg bw/day is among the children 2-5 y, both of which are well below the ADI for PGPR.

Safety Conclusion

Collectively, the publicly available safety data for PGPR continues to support the safe use of PGPR as an emulsifier in various foods that were previously concluded to be GRAS (GRN 9, 179, 266 270 and 466), as well as the safety of the proposed increase in the existing GRAS use levels of PGPR in chocolate (from 0.30% to 0.50%), chocolate-type products based on vegetable fats other than cocoa butter (from 0.30% to 0.50%), and mayonnaise and spreads (from 0.28% to 0.80%). It is therefore reasonable to conclude that the proposed increase in use levels of PGPR in chocolate, chocolate-type products based on vegetable fats other than cocoa butter, and mayonnaise and spreads, is safe, and safe within the meaning of the FD&C Act, i.e., the proposed use meets the standard of reasonable certainty of no harm under the conditions of intended use.

General Recognition of Safety

General recognition of safety through scientific procedures requires common knowledge throughout the scientific community knowledgeable about the safety of food ingredients that there is a reasonable certainty that a substance is not harmful under the intended conditions of use in foods. The aforementioned regulatory and scientific reviews related to the consumption and safety of PGRP are published in the scientific literature, and therefore, are generally available and generally known among the community of qualified food ingredient safety experts. There is broad-based and widely disseminated knowledge concerning PGRP. The data and publicly available information supporting the safety of the proposed increase in the existing GRAS use levels of PGPR as an emulsifier in chocolate (from 0.30% to 0.50%), chocolate-type products based on vegetable fats other than cocoa butter (from 0.30% to 0.50%), and mayonnaise and spreads (from 0.28% to 0.80%), are not only widely known and disseminated, but are also commonly accepted among qualified food safety experts.

Discussion of Information Inconsistent with GRAS Determination

No information has been identified that would be inconsistent with a finding that the proposed use of PGRP, meeting appropriate specifications specified herein and used according to cGMP, is safe and GRAS.

Part 7: References

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April 20, 2023

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Re: GRAS Notice No. GRN 1105

Dear Dr. Downey,

Please see the below responses to the United States (U.S.) Food and Drug Administration (FDA)'s email on March 16, 2023 pertaining to information provided within Unilever's Generally Recognized as Safe (GRAS) Notice for the intended use of polyglycerol polyricinoleic acid (PGPR) in food.

Question 1. Please clarify whether your intended uses of polyglycerol polyricinoleic (PGPR) are as an emulsifier in chocolate, chocolate-type products based on vegetable fats other than cocoa butter, and mayonnaise and spreads only or your intended uses also include each of the other food categories and use levels in Table 2 (PDF page 16) of your notice.

Response:

Our intent is to only use PGPR as an emulsifier in chocolate, chocolate-type products based on vegetable fats other than cocoa butter, and mayonnaise and spreads.

Question 2. If your intended uses include use as an emulsifier in flavors and color additives generally, please provide one of the 21 CFR 170.225(c)(11) statements regarding information sharing with the U.S. Department of Agriculture's Food Safety and Inspection Service, if applicable, as these intended uses include products under USDA's jurisdiction.

Response:

Our intended uses do not include use as an emulsifier in flavors and color additives generally.

Question 3. Please provide a statement that all starting materials and processing aids used in the manufacture of PGPR are used in accordance with applicable U.S. regulations, were concluded to be GRAS for their respective uses or are subjects of effective food contact notifications.

Response:

We confirm that all starting materials and processing aids used in the manufacture of PGPR are used in accordance with applicable U.S. regulations or were concluded to be GRAS for their intended uses.

Question 4. For the administrative record, please confirm that PGPR is manufactured using current good manufacturing practices.

Response:

We confirm that PGPR is manufactured using current good manufacturing practices.

Question 5. You provided the results of the analyses of three consecutive batches of PGPR. Please provide results from the analyses of a minimum of 3 non-consecutive batches.

Response:

Revised Table 1 is provided below with the results of the analyses of three non-consecutive batches of PGPR.

Revised Table 1: Product specifications and data for three non-consecutive batches

Test Parameter	Unit	Specification	Result Batch No. 13970	Result Batch No. 15157	Result Batch No. 16526
Hydroxyl Value	mg KOH/g oil	80-100	89	88	80
Iodine Value		72-103	88.2	88.3	87.5
Refractive Index		1.463-1.467	1.4654	1.4656	1.4657
Saponification Value	mg KOH/g oil	170-210	180.6	184.8	182.9
Acid Value	mg KOH/g oil	NMT 6	1.8	2.6	2.3
Polyglycerols	%	NLT 75% di-, tri- and tetraglycerols; NMT 10% heptaglycerols or higher	77.3% di-, tri- and tetraglycerols; 7.7% heptaglycerols and higher	81% di-, tri- and tetraglycerols; 4.9% heptaglycerols and higher	79.2% di-, tri- and tetraglycerols; 5.1% heptaglycerols and higher
Arsenic	mg/Kg	NMT 3	<0.04	<0.04	<0.04
Lead	mg/Kg	NMT 1	<0.015	<0.015	<0.015
Mercury	mg/Kg	NMT 1	0.02	<0.01	<0.01
Cadmium	mg/Kg	NMT 1	<0.010	<0.010	<0.010

Question 6. Please state the analytical methods used for establishing the specifications for PGPR and confirm that they have been validated for their intended purpose.

Response:

The analytical methods used for establishing the specifications for PGPR are listed in the table below. These methods have been validated for their intended purpose.

Test Parameter	Analytical Method
Hydroxyl Value	AOCS Cd 4-40
Iodine Value	AOCS Cd 1b-87 (modified)
Refractive Index	Refractometer
Saponification Value	AOCS Cd 3-25, Reapproved 2017
Acid Value	Metrohm Application Bulletin No. 80/3 e
Polyglycerols	FAO JECFA monographs No. 1, Vol. 4, mod.
Arsenic	DIN EN 15763, mod.
Lead	DIN EN 15763, mod.
Mercury	DIN EN 15763, mod.
Cadmium	DIN EN 15763, mod.

Question 7. Please consider reducing the specifications for arsenic, cadmium, mercury and lead to reflect the results from the batch analyses presented in the notice.

Response:

We plan to align the heavy metal specifications (arsenic, cadmium, mercury, and lead) to the European draft regulation amending Annex II to Regulation (EC) No 1333/2008 of the European Parliament and of the Council as regards the use of polyglycerol polyricinoleate (E 476). All other specifications remain the same.

Question 8. In table 1, the cadmium and mercury batch analyses results are indicated as 0.0 mg/kg. Please provide the limit of quantification (LOQ) for these analyses and confirm that the analytical results expressed as "0.0 mg/kg" represent the levels below the corresponding LOQ.

Response:

For the non-consecutive batches shown in Revised Table 1, the limit of quantification (LOQ) for arsenic, lead, mercury, and cadmium are listed below.

Test Parameter	Limit of Quantification (LOQ)
Arsenic	0.04 mg/Kg
Lead	0.015 mg/Kg
Mercury	0.01 mg/Kg
Cadmium	0.010 mg/Kg

Question 9. On page 14, the notifier states that they used the estimated dietary intake for FD&C Red No. 2 from the study by Doell et al 2016. FDA notes that FD&C Red No. 2 was banned in the US in 1976. Please clarify whether you meant to use the results presented for FD&C Red 40 in the above stated study. Please discuss the reason for using the dietary exposure estimate for the mean high exposure scenario for the color additive instead of the 90th percentile high dietary exposure presented in Doell et al 2016.

Response:

The reference to FD&C Red No. 2 on page 14 of the GRN was a typographical error. Estimates by Doell et al. (2016) for FD&C Red No. 40 was in fact used to calculate the per capita intake estimate of PGPR from color additive uses. The per capita estimated daily intake of PGPR from color additive uses presented in Table 4 on page 14 of the GRN were based on FD&C Red no. 40.

As discussed in the notice, the maximum color additive exposure across colors and the high exposure scenario estimates for the U.S. 2+ y and subpopulations reported in Doell et al, 2016 was used. According to Doell et al. (2016), the high exposure scenario represents the absolute highest exposures presuming that an individual always consumes products containing the highest levels of the given color additive. Further, we applied an additional 10x factor to the maximum and high exposure scenario estimates to account for additional exposure from other colors. Due to these highly conservative assumptions and the fact that intake from color additive uses was added to the 90th percentile EDI from food uses, it is reasonable to use the mean intake rather than the 90th percentile of intake from color additive uses.

However, since the intake of PGPR from color additive uses are very minor in comparison to food uses, we have revised the intake of PGPR from color additive uses based on the 90th percentile color intake from Doell et al. (2016). The revised Table 4 and Table 8 are provided below.

Revised Table 1. 90th Percentile Estimated Daily Intake of PGPR from Color Additive Uses

	Color Intake[1]	PGPR Intake from Color Uses[2]
Population	mg/kg-bw/day	
U.S. 2+ y	0.9	0.45
Children 2-5 y	2.2	1.10
Adolescent males 13-18 y	1.1	0.55

[1] Based on the per user maximum color additive exposure at the 90th percentile of intake across FD&C color additives evaluated using NHANES 2007-10 by Doell et al. (2016). Color intake based on FD&C Red No. 40 with at least 94% within each population consuming at least one food containing the color additive. Therefore, the per capita estimate can be assumed to be equal to the per user estimate.

[2] Product of color intake, factor of 10 to account for additional exposure from other colors, and PGPR existing maximum use level of 5% in color from GRN 270.

Question 10. The notifier discusses on pg. 23 of the notice (ppt. 31) that PGPR is degraded to the monomer ricinoleic acid. We note that ricinoleic acid is relevant to safety of PGPR due to its ability to cause ultrastructural alterations in the villi of the intestinal mucosa, which is not discussed in the current GRAS notice. Does the notifier anticipate exposure to ricinoleic acid through PGPR at its intended use level will result in adverse GI effects in consumers?

Response:

The noted ability of ricinoleic acid to cause ultrastructural alterations in the villi of intestinal mucosa has been reported in the literature (Gaginella and Phillips 1976; Cline et al. 1976; Gaginella et al. 1977). The route of exposure to ricinoleic acid across these studies is intestinal perfusion, which is not applicable to standard dietary exposure and as such, these tests are investigational only and not a basis for a safety determination. In addition, exposure levels in these references were not biologically relevant. Specifically, in the studies by Gaginella and Philips (1976) and Gaginella et al. (1977) isolated rabbit ileum was perfused with 10mM[1]of sodium ricinoleate. The method of exposure used by Cline et al. (1976) was small bowel *in vivo* perfusion of male Syrian golden hamsters at 2 mM and 8 mM sodium ricinoleate at a volume of 0.75-0.8 mL/min for 40 minutes[2] and, the Cline et al. (1976) authors note "*most likely explanation for the mucosal injury with ricinoleate is related to the detergent properties of the molecule*" (i.e., the property of a concentrated soap) which is not a relevant application for dietary exposure. Lastly, in support of the irrelevancy of these literature findings, the NTP (1992) chronic dietary studies on castor oil (87% ricinoleic acid), which is cited in the GRAS notice, did not reveal any adverse intestinal mucosa pathology or associated effect at doses up to 10% administration in the diet. In summary, the route of administration and concentrations used in these limited studies are not biologically relevant to dietary exposure.

As summarized in the GRAS notice, ricinoleic acid is a monomer fatty acid degradant of PGPR that is absorbed and readily metabolized through the typical physiological pathways of fatty acid metabolism and is not stored or accumulated in tissue (Howes et al., 1998). Studies in humans show that ricinoleic acid (as castor oil)[3] is readily absorbed at low doses and exhibits decreased absorption with increasing dose (Watson et al. 1963; JEFCA, 1979). The absorption of a 50 g dose of castor oil (containing 89-92% of ricinoleic acid) in humans was limited, with nearly 64% of the dose excreted in feces (Watson et al., 1963; JECFA, 1979).

Both Watson et al (1963) and Burdock et al (2006) reported laxative effects in humans at bolus doses 10-15 g castor oil (equivalent to 167 mg/kg bw/day for a 60 kg human). Burdock et al. (2006) further stated

> *"The mechanistic basis for these purgative actions likely includes the membrane-disruptive effects of detergent-like molecules, such as sodium ricinoleate (a 'soap'). These effects have been shown to be dose-related and to exhibit a threshold below which no laxative response was evident, in both animals and in humans. Moreover, admixture of castor oil with food has been shown to mitigate, if not eliminate the cathartic action of ricinoleate on the gastrointestinal tract."*

Burdock et al (2006) reported the acceptable daily intake (ADI) for castor oil set by JECFA of 42 mg/day (0.7 mg/kg in a 60 kg person) was "very conservative". Although chronic toxicity and

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[1] Equivalent to 4.00 mg sodium ricinoleate per minute or a total of 80 mg sodium ricinoleate over the 20-minute exposure time. (10mM) * 320.4 g.mol. Assuming a body weight of a white rabbit of 2.75 kg = 29 mg/kg directly exposed to the intestinal lining.

[2] Equivalent to 2.05 mg sodium ricinoleate per minute or a total of 32 mg sodium ricinoleate over 40 minutes. The hamsters were reported to weigh 80-130 g; therefore, exposure was 246 mg/kg direct to the small intestine.

[3] Ricinoleic acid is the main constituent of castor oil; castor oil contains approximate 90% ricinoleic acid (Watson et al (1963).

carcinogenicity studies with castor oil (or ricinoleic acid) are not available, NTP (1992) conducted a 90-day dietary toxicity study in rats and mice with reported NOAELs of 5,000 mg/kg bw/day in rats and 7,500 mg/kg bw/day in mice. Applying an uncertainty factor of 100 to the 5,000 mg/kg bw/day NOAEL, the ADI for castor oil is 50 mg/kg, or 3000 mg of castor oil per day in an average 60 kg person. Burdock et al. (2006) adjusted the castor oil ADI for ricinoleic acid using the approach developed by Watson et al (1963), which considers the weight fraction of glycerol present, and the distribution of the other fatty acids commonly found in castor oil. On this basis, Burdock et al. (2006) estimated the ADI of ricinoleic acid to be 2,400 mg/person[4], equivalent to 40 mg/kg bw/day in humans (60 kg). The 40 mg/kg bw/day ADI proposed by Burdock et al. (2006) is protective of the laxative effect in humans reported at dose levels of 167 mg/kg bw/day (60 kg person).

No adverse GI effects were reported in the toxicological database of PGPR. Ricinoleic acid and castor oil are GRAS and approved for use in food up to 500 ppm (21CFR172.876). Ricinoleic acid-induced GI effects including laxation are not anticipated to occur at the intended use level of PGPR based on current food uses and use levels of PGPR as described in U.S. GRAS Notices 9 (Quest International, 1998), 179 (Stepan Company, 2005), 266 (Palsgaard A/S, 2008), 270 (Stepan Company, 2008), and 466 (McCormick & Company, Inc., 2012).

The GRAS Notice 466 (McCormick & Company, Inc., 2012) concluded that the intake of ricinoleic acid that result in laxative effects in humans exceeds anticipated dietary intake of PGPR, a source of ricinoleic acid, by at least three orders of magnitude. Other GRAS Notices (GRAS Notices 9 (Quest International, 1998), 179 (Stepan Company, 2005), 266 (Palsgaard A/S, 2008), 270 (Stepan Company, 2008)) addressed the safety of ricinoleic acid potential gastrointestinal effects of ricinoleic acid by inferring ricinoleic acid from the metabolism of PGPR following ingestion is addressed in the toxicological database for PGPR. The PGPR ADI provided in the GRAS notices is based directly on dietary toxicology studies with PGPR, which would inherently be protective of any potential PGPR metabolites, including ricinoleic acid, that could occur downstream after ingestion.

The conservatively estimated per user 90th percentile cumulative estimated dietary intake (CEDI) of PGPR in this current GRAS notice by Unilever is 7.73 mg/kg bw/day for the US 2+ y and the highest (90^{th} percentile) CEDI is 11.66 mg/kg bw/day among the children 2-5 y. Adjusting the highest (90^{th} percentile) CEDI of 11.66 mg/kg for PGPR for ricinoleic acid, results in a CEDI for ricinoleic acid of 8.9 mg/kg bw/day[5]. The ADI of ricinoleic acid in humans is 40 mg/kg bw/day (derived from Burdock et al. 2006). The ADI for ricinoleic acid is approximately 3.4 orders of magnitude higher the highest 90^{th} percentile CEDI of PGPR of 11.66 mg/kg bw/day in children 2-

[4] Burdock et al (2006). Feeding studies with castor oil in rodents provide a basis for a no observable adverse effect level (NOAEL) estimate of 7,500 mg/kg/day and 5,000 mg/kg/day in mice and rats, respectively. Applying an uncertainty factor of 100 to the lesser of these NOAELs, one can thus estimate an acceptable daily intake (ADI) in man to be 50 mg/kg, or 3,000 mg of castor oil per day in an average 60 kg person. As ricinoleic acid constitutes approximately 90% of castor oil, applying this calculation to the 3,000 mg/day estimated ADI in humans for castor oil (given the rapid hydrolysis of castor oil glyceride in the gastrointestinal tract), the acceptable daily intake of ricinoleic acid may be as high as 2,400 mg/person.

[5] The PGPR ADI of 11.66 mg/kg bw/day was adjusted on a molecular weight basis to obtain the ADI for ricinoleic acid. The molecular weight of PGPR is 390.555 and the molecular weight of ricinoleic acid is 198.4608.

5 years old. Based on the information presented, ricinoleic acid is not anticipated to cause adverse GI effects at its intended use level.

Question 11. On pg. 30 if the notice (ppt. 38) the notifier discusses the published 2-year combined chronic toxicity and carcinogenicity study in rats using PGPR in the diet, but it's unclear if the test article used in this study came from a batch of the notified substance. Please clarify if the test article from this study came from a specified batch of the notified substance, or, if not, briefly describe how the test article used in this study compares in manufacturing and/or composition to the subject of this GRAS notice.

Response:

The test materials used in the published studies were manufactured via the same process as that documented in the GRAS notice. The manufacturing methodology of the test material used in the published literature was described in Wilson et al. (1998). Smith et al. (1998) directly refers to the Wilson et al. (1998) methodology. The other published literature (Wilson and Smith 1998a; Wilson and Smith 1998b) refer to the esterification of condensed castor oil fatty acids (primarily ricinoleic acid (>80%) with polyglycerol). The table below compares the methods between that described in the GRAS notice and that available in Wilson et al. (1998). Based on the same preparation of the castor oil fatty acids, condensation of the castor oil fatty acids, preparation of polyglycerols, partial esterification of the condensed castor oil fatty acids with polyglycerols and the similar analytical specifications, the test materials used in the published literature are comparable to manufacturing and/or composition to the subject of this GRAS notice.

Manufacturing Process	**GRAS Notice**	**Wilson et al. (1998)**
Preparation of the castor oil fatty acids	Castor oil fatty acids are produced by hydrolyzing 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. This castor oil contains, as its main fatty acids **ricinoleic acid (80–90%)**, **oleic acid (3–8%)**, **linoleic acid** (**3–7%**) and **stearic acid (0–2%**).	The castor oil fatty acids are prepared by hydro-lysing castor oil with water and steam at 400 psi pressure without any added catalyst after which the resulting fatty acids are freed from glycerol by water washing. Castor oil contains as its main fatty acid component **ricinoleic acid (80±90%**), and it is this fatty acid which is important in the condensation reaction. Other fatty acids present are **oleic acid (3±8%)**, **linoleic acid** (**3±7%**) and **stearic acid (0±2%)**.
Condensation of the castor oil fatty acids	Castor oil fatty acids are condensed by heating at a temperature of 205–210°C under vacuum and a CO_2 atmosphere (to prevent oxidation)	Fatty acid condensation is brought about by heating the castor oil fatty acids at elevated temperatures under vacuum and

Manufacturing Process	**GRAS Notice**	**Wilson et al. (1998)**
	for ~8 h. This 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) is reached.	in an atmosphere of carbon dioxide to prevent oxidation. Samples are taken at regular intervals and tested for their free fatty acid content until an acid value of **35.0** is achieved. This acid value is equivalent to an average of about five fatty acid residues per molecule of the condensed product. During the condensation phase, ricinoleic acid may react in a number of ways. Simple linear esterification is the desired reaction but cyclic esterification, which is a chain terminating process, is theoretically possible. However, no evidence was found for the presence of this type of cyclic material in the condensed castor oil fatty acids. Dehydration is also possible, but occurs to only a small extent.
Preparation of polyglycerols	The polyglycerol portion can be prepared by three routes: (1) polymerization of glycerol using a strong base as a catalyst, (2) by polymerization of glycidol, leading to linear polyglycerols, or (3) by polymerization of epichlorohydrin, followed by hydrolysis. This leads to linear polyglycerols. The polyglycerols produced by polymerization of epichlorohydrin contain reduced proportions of cyclic components.	The preparation of the polyglycerol is achieved by heating glycerol to temperatures above 200 °C in the presence of a small amount of alkali (potassium hydroxide). In this step, two or more molecules of glycerol condense with a loss of water and the formation of an ether linkage between the glycerol molecules. Carbon dioxide is bubbled through the reaction vessel to prevent oxidation, and unchanged glycerol is removed by distillation at the end of the reaction. The process is controlled by monitoring the rise in the refractive index. The result

Manufacturing Process	GRAS Notice	Wilson et al. (1998)
		is a mixture of polyglycerols containing varying numbers of glycerol residues. As the 1- and 3-hydroxy groups of glycerol are more reactive than the 2-hydroxy group, the polyglycerols formed are predominantly straight-chain according to the overall reaction. In addition, small amounts of cyclic by-products may be formed in the reaction mixture as a result of condensation between the 1-hydroxy group of one glycerol molecule and the 2-hydroxy group of another. The cyclic diglycerol product is a solid (m.p. 96°C), and is present at 4% in the polyglycerol or 0.4% in PGPR.
Partial esterification of the condensed castor oil fatty acids with polyglycerols	The final stage of the production process involves the esterification of condensed castor oil fatty acids with polyglycerols. The "appropriate" amount of polyglycerol with the polyricinoleic acid is heated. After which, a reaction takes place immediately, and in the same vessel while still hot. The esterification conditions are the same as those for fatty acid condensation. This process will continue until a sample is taken from the reaction mixture and found to have a suitable acid value (i.e., ≤ 6 mg KOH/g) and refractive index (per required specifications).	The final stage of the preparation involves heating an appropriate amount of polyglycerol with the condensed castor oil fatty acids. 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. The average value of n is about 3. R1, R2 and R3 each may be hydrogen or a linear condensation product of ricinoleic acid with itself, with n being on average between 5 and 8.

Manufacturing Process	**GRAS Notice**	**Wilson et al. (1998)**
Product Specifications	The Foods Chemicals Codex (FCC) states that the acceptance criteria for the polyglycerol moieties of PGPR shall be composed of NLT **75% of di-, tri- and tetraglycerols** and shall contain **NMT 10% of polyglycerols equal to or higher than heptaglycero**l (FCC Vol 12). This PGPR product meets specification requirements for polyglycerol polyricinoleic acid established by the Foods Chemicals Codex (FCC Vol 12).	The JECFA specification for PGPR states that ``the polyglycerol moiety shall be composed of not less than **75 percent of the di-, tri- and tetraglycerols** and shall contain no more than **10 percent of polyglycerols equal to or higher than heptaglycerol''** (FAO, 1992). PGPR is specified further by the following: Hydroxyl value 85-100 Acid value 2.0 max. Iodine value 80±90 Refractive index at 658C
Hydroxyl value	80-100	85-100
Iodine value	72-103	80-90
Refractive index	1.463-1.467	1.4635±1.4665
Saponification value	170-210	
Acid value	Not more than (NMT) 6	NMT 2%
Polyglycerols	Not lower than (NLT) 75% di-, tri- and tetraglycerols; NMT 10% heptaglycerols or higher	Not lower than (NLT) 75% di-, tri- and tetraglycerols; NMT 10% heptaglycerols or higher

Question 12. We note that JECFA and the European Commission Scientific Committee for Food used the reproductive toxicity study with PGPR as the basis for determining their acceptable daily intake level (ADI) of 7.5 mg/kg bw/d (Wilson and Smith, 1998a); whereas it appears the notifiers considered the 2-year combined chronic toxicity and carcinogenicity study in rats (Smith et al., 1998) to be the most sensitive endpoints for ADI derivation. Please provide a short narrative or rationale as to your justification for this approach.

Response:

As stated in the GRAS Notice, it should be noted that in their monograph, JECFA does not clearly articulate their preference for using the reproductive study as the basis for the overall reference dose for their ADI (JECFA, 1974). As such, it is important to consider that the reproductive study has methodological limitations that should preclude its use to derive an ADI, including a low sample size for the first-generation treated animals (six males and 13 females), and potential breeding issues with all the second-generation animals (see Reproductive Toxicity section of the GRAS Notice). Secondly, the unpublished data on the chronic toxicity component from the reproductive study, summarized in Quest (1998), indicated that 14 to 16% of the animals in both groups of the third generation had a *Cysticercus fasciolaris* (intermediate stage of the cat tape worm) in the liver. Further, there is no indication that organ weights were measured as part of chronic toxicity component and thus no evaluation of liver enlargement, the key treatment-related endpoint across the PGPR dataset. Given the significant reported deficiencies with the controls in the reproductive study, it should not be relied upon for derivation of the ADI. Lastly there were no adverse effects at the highest dose tested and therefore, it does not actually establish an effective ceiling for the safety of PGPR. On the other hand, the carcinogenicity, and chronic studies (Smith et al., 1998) tested at a higher concentration for a longer period, had an expanded toxicological examination battery including reproductive organs, and noted no adverse outcomes.

The GRAS notice conclusion agrees with the EFSA (2017) conclusion. In the 2017 Re-evaluation of PGPR, EFSA concluded that the endpoint from the chronic toxicity and carcinogenicity study in rats was the key study with a NOAEL of 2,500 mg/kg bw/day. EFSA (2017) states the following:

> *"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."*

Based on this information, the chronic toxicity and carcinogenicity study in rats (Smith et al. 1998) is the key study for the safety assessment.

Question 13. The notifier states that, as part of a weight-of-evidence approach to address the genotoxicity of PGPR, a published 2-year chronic toxicity and carcinogenicity study was used

to conclude that PGPR is not genotoxic (Smith et al., 1998). Yet, the notifier also states on pg. 29 of the notice (ppt. 37) that an in-silico assessment was used to evaluate PGPR's genotoxicity since "there were no available in vitro or in vivo studies". For the administrative record, please confirm that the data from the published 2-year rat chronic toxicity and carcinogenicity study was the pivotal publicly available information used to make your GRAS conclusion, and that the data generated using the OECD QSAR Toolbox provides supportive evidence for your conclusion.

Response:

The key data for the lack of genotoxicity are the two PGPR carcinogenicity studies conducted in rats and mice, which presented no oncogenic outcomes or chronic toxicity (Smith et al., 1998). The QSAR analyses conducted to provide supportive evidence to corroborate the conclusion that PGPR is non-genotoxic.

Question 14. Please perform an update to the literature search described in the safety narrative from August 2021 to present and discuss if any new data were found that would contradict the current GRAS conclusion.

Response:

An updated safety literature search was conducted for the period of August 1, 2021 to present. The search terms included polyglycerol polyricinoleic, polyglycerol polyricinoleate, 68936-89-0, 29894-35-7, and PGPR. The literature search was conducted through the commercial database ToxPlanet ChemEXPERT™ and through PubMed. In addition, the following resources were included in the search for information bearing on the safety of PGPR: the Organization for Economic Co-operation and Development (OECD), US FDA 21 Code of Federal Regulations (CFR), Joint FAO/WHO Expert Committee on Food Additives (JECFA), European Food Safety Authority (EFSA), National Toxicology Program (NTP), Environmental Protection Agency (EPA) and EPA's Integrated Risk Information System (IRIS).

Fourteen documents on polyglycerol polyricinoleate were reviewed from the results of the ToxPlanet™ search from sources including General Standard for Food Additives, EPA CompTox Chemicals Dashboard, Goodscents, Joint Substance Data Pool of the German Federal Government and the German Federal states, New Zealand Inventory of Chemicals, Substances in Preparation in Nordic Countries, and German Environment Agency. There was no new information or updates to data with bearing on the safety of PGPR.

The PubMed search produced one result on a paper regarding new methods for the quantitative determination of PGPR in foods, which did not provide new safety data.

There were no new data that would contradict the current GRAS conclusion.

Question 15. In your narrative, you described several unpublished studies concerning PGPR. GRAS conclusions must be based on generally available and generally accepted data and information. Please provide an explanation of how there could be a basis for a conclusion of GRAS status if qualified experts do not have access to these unpublished studies (21 CFR 170.250(e)).

Response:

The pivotal key toxicological study, and the basis of the safety endpoint, is available in the peer-reviewed publicly available literature (Smith et al., 1998). The other pivotal data used in the GRAS Notice are listed below.

Howes et al., 1998	Absorption, distribution, metabolism, and excretion
Wilson et al. 1998	Acute toxicity Short-term toxicity Chronic toxicity
Wilson and Smith, 1998a	Reproductive toxicity
Smith et al., 1998	Combined chronic toxicity and carcinogenicity study in rats Carcinogenicity study in mice
Wilson and Smith, 1998b	Controlled human dietary study conducted with PGPR

Unpublished toxicological studies and *in silico* QSAR modeling results were published in part in the EFSA authoritative review of PGPR (EFSA, 2017) and were included in the GRAS notice as supportive data to corroborate the safety of PGPR. While the underlying data in the EFSA review may not be fully publicly available, a summary of those data and the authoritative review of the studies, including acceptability, has been prepared by EFSA's qualified experts. Based on their review, there are no data or information in the unpublished literature that appears inconsistent with the pivotal data and information that is available in the public literature described above.

We hope this information adequately addresses the Agency's questions on GRN 1105, and if there is any additional information or further clarification that is required, we will be happy to provide such information upon request.

Sincerely,

Kristin Spoden
Unilever
Regulatory Affairs Leader

References:

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From: Spoden, Kristin
To: Downey, Jason
Subject: RE: [External] - RE: GRN 1105 - Unilever"s PGPR - Question to the Notifier
Date: Friday, April 28, 2023 12:07:52 PM

Hi Jason,

Yes, we confirm for the record that the heavy metal specification is set at 0.1 mg/kg for arsenic, cadmium, mercury, and lead in Unilever's PGPR GRAS Notice - GRN 1105.

Thank you,
Kristin

From: Downey, Jason <Jason.Downey@fda.hhs.gov>
Sent: Friday, April 28, 2023 10:47 AM
To: Spoden, Kristin <Kristin.Spoden@unilever.com>
Subject: [External] - RE: GRN 1105 - Unilever's PGPR - Question to the Notifier

Hi Kristin,

I apologize for the multiple emails.

To add to the question below, please also confirm for the record that the heavy metal specification in the draft regulation referred to in your April 20, 2023, amendment is 0.1 mg/kg for arsenic, cadmium, mercury, and lead. Additionally, in accordance with FDA's Closer to Zero action plan, we note that specifications for heavy metals should reflect the amounts determined in the analyses of representative batches and be kept as low as possible.

Thank you!

Jason

From: Downey, Jason
Sent: Friday, April 28, 2023 10:00 AM
To: Spoden, Kristin <Kristin.Spoden@unilever.com>
Subject: GRN 1105 - Unilever's PGPR - Question to the Notifier

Hi Kristin,

While evaluating GRN 001105, we identified one point in your April 20, 2023, amendment that

needs clarification. That request is listed below. Please provide a response to the request below within **5 business days**. If you foresee any issue with this timeline or you have any other questions, please feel free to contact me.

Thank you for your attention to our comments.

Jason

Question from FDA:
In your April 20, 2023, amendment in response to FDA's question 7, you state that you "plan to align the heavy metal specifications (arsenic, cadmium, mercury, and lead) to the European draft regulation amending Annex II to regulation (EC) 1333/2008 of the European Parliament and of the Council as regards the use of polyglycerol polyricinoleate (E 476)". Please confirm that these specifications have been implemented in GRN 1105 to support the GRAS conclusion of PGPR for the specified intended uses and use levels.

Jason Downey, Ph.D. (he/him/his)
Regulatory Review Scientist

Division of Food Ingredients
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
jason.downey@fda.hhs.gov

From:	Spoden, Kristin
To:	Downey, Jason
Subject:	RE: [External] - RE: GRN 1105 - Unilever"s PGPR - Question to the Notifier
Date:	Monday, May 8, 2023 11:45:03 AM
Attachments:	Revised GRN 1105 - Unilever Response to FDA Questions.docx

Hello Jason,

Thank you for pointing out that Revised Table 8 was missing from our response. I have added it to our response to question #9 in the document attached. I also noticed that when I saved the Word document as a PDF, the tables were renumbered. The attached response no longer has the error of revised Table 4 being labeled as "Revised Table 1…".

Thank you,
Kristin Spoden

From: Downey, Jason <Jason.Downey@fda.hhs.gov>
Sent: Monday, May 08, 2023 7:18 AM
To: Spoden, Kristin <Kristin.Spoden@unilever.com>
Subject: [External] - RE: GRN 1105 - Unilever's PGPR - Question to the Notifier

Good morning, Kristin,

While evaluating GRN 001105 and its amendments, we identified one point in your April 20, 2023, amendment that needs follow-up. Your response to our Question #9 (PDF page 4) states that revised copies of Tables 4 and 8 are included in the amendment. We found a revised Table 4, labeled "Revised Table 1. 90th Percentile Estimated Daily Intake of PGPR from Color Additive Uses," but we do not find a revised version of Table 8. Please provide the revised Table 8 referred to in your response to our Question #9.

Thank you!

Jason

Jason Downey, Ph.D. (he/him/his)
Regulatory Review Scientist

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May 8, 2023

Jason Downey, Ph.D.
Regulatory Review Scientist
Division of Food Ingredients
Office of Food Additive Safety
Center for Food Safety & Applied Nutrition
U.S. Food and Drug Administration
5001 Campus Drive
College Park, MD 20740

Re: GRAS Notice No. GRN 1105

Dear Dr. Downey,

Please see the below responses to the United States (U.S.) Food and Drug Administration (FDA)'s email on March 16, 2023 pertaining to information provided within Unilever's Generally Recognized as Safe (GRAS) Notice for the intended use of polyglycerol polyricinoleic acid (PGPR) in food.

Question 1. Please clarify whether your intended uses of polyglycerol polyricinoleic (PGPR) are as an emulsifier in chocolate, chocolate-type products based on vegetable fats other than cocoa butter, and mayonnaise and spreads only or your intended uses also include each of the other food categories and use levels in Table 2 (PDF page 16) of your notice.

Response:

Our intent is to only use PGPR as an emulsifier in chocolate, chocolate-type products based on vegetable fats other than cocoa butter, and mayonnaise and spreads.

Question 2. If your intended uses include use as an emulsifier in flavors and color additives generally, please provide one of the 21 CFR 170.225(c)(11) statements regarding information sharing with the U.S. Department of Agriculture's Food Safety and Inspection Service, if applicable, as these intended uses include products under USDA's jurisdiction.

Response:

Our intended uses do not include use as an emulsifier in flavors and color additives generally.

Question 3. Please provide a statement that all starting materials and processing aids used in the manufacture of PGPR are used in accordance with applicable U.S. regulations, were concluded to be GRAS for their respective uses or are subjects of effective food contact notifications.

Response:

We confirm that all starting materials and processing aids used in the manufacture of PGPR are used in accordance with applicable U.S. regulations or were concluded to be GRAS for their intended uses.

Question 4. For the administrative record, please confirm that PGPR is manufactured using current good manufacturing practices.

Response:

We confirm that PGPR is manufactured using current good manufacturing practices.

Question 5. You provided the results of the analyses of three consecutive batches of PGPR. Please provide results from the analyses of a minimum of 3 non-consecutive batches.

Response:

Revised Table 1 is provided below with the results of the analyses of three non-consecutive batches of PGPR.

Revised Table 1: Product specifications and data for three non-consecutive batches

Test Parameter	Unit	Specification	Result Batch No. 13970	Result Batch No. 15157	Result Batch No. 16526
Hydroxyl Value	mg KOH/g oil	80-100	89	88	80
Iodine Value		72-103	88.2	88.3	87.5
Refractive Index		1.463-1.467	1.4654	1.4656	1.4657
Saponification Value	mg KOH/g oil	170-210	180.6	184.8	182.9
Acid Value	mg KOH/g oil	NMT 6	1.8	2.6	2.3
Polyglycerols	%	NLT 75% di-, tri- and tetraglycerols; NMT 10% heptaglycerols or higher	77.3% di-, tri- and tetraglycerols; 7.7% heptaglycerols and higher	81% di-, tri- and tetraglycerols; 4.9% heptaglycerols and higher	79.2% di-, tri- and tetraglycerols; 5.1% heptaglycerols and higher
Arsenic	mg/Kg	NMT 3	<0.04	<0.04	<0.04
Lead	mg/Kg	NMT 1	<0.015	<0.015	<0.015
Mercury	mg/Kg	NMT 1	0.02	<0.01	<0.01
Cadmium	mg/Kg	NMT 1	<0.010	<0.010	<0.010

Question 6. Please state the analytical methods used for establishing the specifications for PGPR and confirm that they have been validated for their intended purpose.

Response:

The analytical methods used for establishing the specifications for PGPR are listed in the table below. These methods have been validated for their intended purpose.

Test Parameter	Analytical Method
Hydroxyl Value	AOCS Cd 4-40
Iodine Value	AOCS Cd 1b-87 (modified)
Refractive Index	Refractometer
Saponification Value	AOCS Cd 3-25, Reapproved 2017
Acid Value	Metrohm Application Bulletin No. 80/3 e
Polyglycerols	FAO JECFA monographs No. 1, Vol. 4, mod.
Arsenic	DIN EN 15763, mod.
Lead	DIN EN 15763, mod.
Mercury	DIN EN 15763, mod.
Cadmium	DIN EN 15763, mod.

Question 7. Please consider reducing the specifications for arsenic, cadmium, mercury and lead to reflect the results from the batch analyses presented in the notice.

Response:

We plan to align the heavy metal specifications (arsenic, cadmium, mercury, and lead) to the European draft regulation amending Annex II to Regulation (EC) No 1333/2008 of the European Parliament and of the Council as regards the use of polyglycerol polyricinoleate (E 476). All other specifications remain the same.

Question 8. In table 1, the cadmium and mercury batch analyses results are indicated as 0.0 mg/kg. Please provide the limit of quantification (LOQ) for these analyses and confirm that the analytical results expressed as "0.0 mg/kg" represent the levels below the corresponding LOQ.

Response:

For the non-consecutive batches shown in Revised Table 1, the limit of quantification (LOQ) for arsenic, lead, mercury, and cadmium are listed below.

Test Parameter	Limit of Quantification (LOQ)
Arsenic	0.04 mg/Kg
Lead	0.015 mg/Kg
Mercury	0.01 mg/Kg
Cadmium	0.010 mg/Kg

Question 9. On page 14, the notifier states that they used the estimated dietary intake for FD&C Red No. 2 from the study by Doell et al 2016. FDA notes that FD&C Red No. 2 was banned in the US in 1976. Please clarify whether you meant to use the results presented for FD&C Red 40 in the above stated study. Please discuss the reason for using the dietary exposure estimate for the mean high exposure scenario for the color additive instead of the 90th percentile high dietary exposure presented in Doell et al 2016.

Response:

The reference to FD&C Red No. 2 on page 14 of the GRN was a typographical error. Estimates by Doell et al. (2016) for FD&C Red No. 40 was in fact used to calculate the per capita intake estimate of PGPR from color additive uses. The per capita estimated daily intake of PGPR from color additive uses presented in Table 4 on page 14 of the GRN were based on FD&C Red no. 40.

As discussed in the notice, the maximum color additive exposure across colors and the high exposure scenario estimates for the U.S. 2+ y and subpopulations reported in Doell et al, 2016 was used. According to Doell et al. (2016), the high exposure scenario represents the absolute highest exposures presuming that an individual always consumes products containing the highest levels of the given color additive. Further, we applied an additional 10x factor to the maximum and high exposure scenario estimates to account for additional exposure from other colors. Due to these highly conservative assumptions and the fact that intake from color additive uses was added to the 90th percentile EDI from food uses, it is reasonable to use the mean intake rather than the 90th percentile of intake from color additive uses.

However, since the intake of PGPR from color additive uses are very minor in comparison to food uses, we have revised the intake of PGPR from color additive uses based on the 90th percentile color intake from Doell et al. (2016). The revised Table 4 and Table 8 are provided below.

Revised Table 4. 90th Percentile Estimated Daily Intake of PGPR from Color Additive Uses

	Color Intake[1]	PGPR Intake from Color Uses[2]
Population	mg/kg-bw/day	
U.S. 2+ y	0.9	0.45
Children 2-5 y	2.2	1.10
Adolescent males 13-18 y	1.1	0.55

[1] Based on the per user maximum color additive exposure at the 90th percentile of intake across FD&C color additives evaluated using NHANES 2007-10 by Doell et al. (2016). Color intake based on FD&C Red No. 40 with at least 94% within each population consuming at least one food containing the color additive. Therefore, the per capita estimate can be assumed to be equal to the per user estimate.

[2] Product of color intake, factor of 10 to account for additional exposure from other colors, and PGPR existing maximum use level of 5% in color from GRN 270.

Revised Table 8. Cumulative Estimated Daily Intake (CEDI) of PGPR from Existing (including Flavor and Color Additive Uses) and New Proposed Uses by the U.S. Population 2+ y and Select Subpopulations

Population	PGPR Estimated Daily Intake (mg/kg-bw/day)			
	A) Per User 90th Percentile EDI from NHANES (Table 7)	**B) Pseudo 90th Percentile Intake from Uses in Flavors**	**C) 90th Percentile EDI from Uses in Colors (Table 4)**	**Cumulative (A+B+C)**
U.S. 2+ y	7.41	0.117	0.45	7.98
Children 2-5 y	11.09	0.117	1.10	12.31
Children 6-12 y	7.11	0.117	1.10*	8.33
Adolescents 13-18 y	6.04	0.117	0.55	6.71
Adults 19+ y	7.26	0.117	0.45**	7.83

* Based on children 2-5 y; ** Based on U.S. 2+ y

Question 10. The notifier discusses on pg. 23 of the notice (ppt. 31) that PGPR is degraded to the monomer ricinoleic acid. We note that ricinoleic acid is relevant to safety of PGPR due to its ability to cause ultrastructural alterations in the villi of the intestinal mucosa, which is not discussed in the current GRAS notice. Does the notifier anticipate exposure to ricinoleic acid through PGPR at its intended use level will result in adverse GI effects in consumers?

Response:

The noted ability of ricinoleic acid to cause ultrastructural alterations in the villi of intestinal mucosa has been reported in the literature (Gaginella and Phillips 1976; Cline et al. 1976; Gaginella et al. 1977). The route of exposure to ricinoleic acid across these studies is intestinal perfusion, which is not applicable to standard dietary exposure and as such, these tests are investigational only and not a basis for a safety determination. In addition, exposure levels in these references were not biologically relevant. Specifically, in the studies by Gaginella and Philips (1976) and Gaginella et al. (1977) isolated rabbit ileum was perfused with 10mM[1]of sodium ricinoleate. The method of exposure used by Cline et al. (1976) was small bowel *in vivo* perfusion of male Syrian golden hamsters at 2 mM and 8 mM sodium ricinoleate at a volume of 0.75-0.8 mL/min for 40 minutes[2] and, the Cline et al. (1976) authors note "*most likely explanation for the mucosal injury with ricinoleate is related to the detergent properties of the molecule*" (i.e., the property of a concentrated soap) which is not a relevant application for dietary exposure. Lastly, in support of the irrelevancy of these literature findings, the NTP (1992) chronic dietary studies on castor oil (87% ricinoleic acid), which is cited in the GRAS notice, did not reveal any adverse intestinal mucosa pathology or associated effect at doses up to 10% administration in the diet. In

[1] Equivalent to 4.00 mg sodium ricinoleate per minute or a total of 80 mg sodium ricinoleate over the 20-minute exposure time. (10mM) * 320.4 g.mol. Assuming a body weight of a white rabbit of 2.75 kg = 29 mg/kg directly exposed to the intestinal lining.

[2] Equivalent to 2.05 mg sodium ricinoleate per minute or a total of 32 mg sodium ricinoleate over 40 minutes. The hamsters were reported to weigh 80-130 g; therefore, exposure was 246 mg/kg direct to the small intestine.

summary, the route of administration and concentrations used in these limited studies are not biologically relevant to dietary exposure.

As summarized in the GRAS notice, ricinoleic acid is a monomer fatty acid degradant of PGPR that is absorbed and readily metabolized through the typical physiological pathways of fatty acid metabolism and is not stored or accumulated in tissue (Howes et al., 1998). Studies in humans show that ricinoleic acid (as castor oil)[3] is readily absorbed at low doses and exhibits decreased absorption with increasing dose (Watson et al. 1963; JEFCA, 1979). The absorption of a 50 g dose of castor oil (containing 89-92% of ricinoleic acid) in humans was limited, with nearly 64% of the dose excreted in feces (Watson et al., 1963; JECFA, 1979).

Both Watson et al (1963) and Burdock et al (2006) reported laxative effects in humans at bolus doses 10-15 g castor oil (equivalent to 167 mg/kg bw/day for a 60 kg human). Burdock et al. (2006) further stated

> *"The mechanistic basis for these purgative actions likely includes the membrane-disruptive effects of detergent-like molecules, such as sodium ricinoleate (a 'soap'). These effects have been shown to be dose-related and to exhibit a threshold below which no laxative response was evident, in both animals and in humans. Moreover, admixture of castor oil with food has been shown to mitigate, if not eliminate the cathartic action of ricinoleate on the gastrointestinal tract."*

Burdock et al (2006) reported the acceptable daily intake (ADI) for castor oil set by JECFA of 42 mg/day (0.7 mg/kg in a 60 kg person) was "very conservative". Although chronic toxicity and carcinogenicity studies with castor oil (or ricinoleic acid) are not available, NTP (1992) conducted a 90-day dietary toxicity study in rats and mice with reported NOAELs of 5,000 mg/kg bw/day in rats and 7,500 mg/kg bw/day in mice. Applying an uncertainty factor of 100 to the 5,000 mg/kg bw/day NOAEL, the ADI for castor oil is 50 mg/kg, or 3000 mg of castor oil per day in an average 60 kg person. Burdock et al. (2006) adjusted the castor oil ADI for ricinoleic acid using the approach developed by Watson et al (1963), which considers the weight fraction of glycerol present, and the distribution of the other fatty acids commonly found in castor oil. On this basis, Burdock et al. (2006) estimated the ADI of ricinoleic acid to be 2,400 mg/person[4], equivalent to 40 mg/kg bw/day in humans (60 kg). The 40 mg/kg bw/day ADI proposed by Burdock et al. (2006) is protective of the laxative effect in humans reported at dose levels of 167 mg/kg bw/day (60 kg person).

No adverse GI effects were reported in the toxicological database of PGPR. Ricinoleic acid and castor oil are GRAS and approved for use in food up to 500 ppm (21CFR172.876). Ricinoleic

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[3] Ricinoleic acid is the main constituent of castor oil; castor oil contains approximate 90% ricinoleic acid (Watson et al (1963).

[4] Burdock et al (2006). Feeding studies with castor oil in rodents provide a basis for a no observable adverse effect level (NOAEL) estimate of 7,500 mg/kg/day and 5,000 mg/kg/day in mice and rats, respectively. Applying an uncertainty factor of 100 to the lesser of these NOAELs, one can thus estimate an acceptable daily intake (ADI) in man to be 50 mg/kg, or 3,000 mg of castor oil per day in an average 60 kg person. As ricinoleic acid constitutes approximately 90% of castor oil, applying this calculation to the 3,000 mg/day estimated ADI in humans for castor oil (given the rapid hydrolysis of castor oil glyceride in the gastrointestinal tract), the acceptable daily intake of ricinoleic acid may be as high as 2,400 mg/person.

acid-induced GI effects including laxation are not anticipated to occur at the intended use level of PGPR based on current food uses and use levels of PGPR as described in U.S. GRAS Notices 9 (Quest International, 1998), 179 (Stepan Company, 2005), 266 (Palsgaard A/S, 2008), 270 (Stepan Company, 2008), and 466 (McCormick & Company, Inc., 2012).

The GRAS Notice 466 (McCormick & Company, Inc., 2012) concluded that the intake of ricinoleic acid that result in laxative effects in humans exceeds anticipated dietary intake of PGPR, a source of ricinoleic acid, by at least three orders of magnitude. Other GRAS Notices (GRAS Notices 9 (Quest International, 1998), 179 (Stepan Company, 2005), 266 (Palsgaard A/S, 2008), 270 (Stepan Company, 2008)) addressed the safety of ricinoleic acid potential gastrointestinal effects of ricinoleic acid by inferring ricinoleic acid from the metabolism of PGPR following ingestion is addressed in the toxicological database for PGPR. The PGPR ADI provided in the GRAS notices is based directly on dietary toxicology studies with PGPR, which would inherently be protective of any potential PGPR metabolites, including ricinoleic acid, that could occur downstream after ingestion.

The conservatively estimated per user 90th percentile cumulative estimated dietary intake (CEDI) of PGPR in this current GRAS notice by Unilever is 7.73 mg/kg bw/day for the US 2+ y and the highest (90^{th} percentile) CEDI is 11.66 mg/kg bw/day among the children 2-5 y. Adjusting the highest (90^{th} percentile) CEDI of 11.66 mg/kg for PGPR for ricinoleic acid, results in a CEDI for ricinoleic acid of 8.9 mg/kg bw/day[5]. The ADI of ricinoleic acid in humans is 40 mg/kg bw/day (derived from Burdock et al. 2006). The ADI for ricinoleic acid is approximately 3.4 orders of magnitude higher the highest 90^{th} percentile CEDI of PGPR of 11.66 mg/kg bw/day in children 2-5 years old. Based on the information presented, ricinoleic acid is not anticipated to cause adverse GI effects at its intended use level.

Question 11. On pg. 30 if the notice (ppt. 38) the notifier discusses the published 2-year combined chronic toxicity and carcinogenicity study in rats using PGPR in the diet, but it's unclear if the test article used in this study came from a batch of the notified substance. Please clarify if the test article from this study came from a specified batch of the notified substance, or, if not, briefly describe how the test article used in this study compares in manufacturing and/or composition to the subject of this GRAS notice.

Response:

The test materials used in the published studies were manufactured via the same process as that documented in the GRAS notice. The manufacturing methodology of the test material used in the published literature was described in Wilson et al. (1998). Smith et al. (1998) directly refers to the Wilson et al. (1998) methodology. The other published literature (Wilson and Smith 1998a; Wilson and Smith 1998b) refer to the esterification of condensed castor oil fatty acids (primarily ricinoleic acid (>80%) with polyglycerol). The table below compares the methods between that described in the GRAS notice and that available in Wilson et al. (1998). Based on the same preparation of the castor oil fatty acids, condensation of the castor oil fatty acids, preparation of polyglycerols, partial esterification of the condensed castor oil fatty acids with polyglycerols and the similar analytical

[5] The PGPR ADI of 11.66 mg/kg bw/day was adjusted on a molecular weight basis to obtain the ADI for ricinoleic acid. The molecular weight of PGPR is 390.555 and the molecular weight of ricinoleic acid is 198.4608.

specifications, the test materials used in the published literature are comparable to manufacturing and/or composition to the subject of this GRAS notice.

Manufacturing Process	**GRAS Notice**	**Wilson et al. (1998)**
Preparation of the castor oil fatty acids	Castor oil fatty acids are produced by hydrolyzing 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. This castor oil contains, as its main fatty acids **ricinoleic acid (80–90%)**, **oleic acid (3–8%)**, **linoleic acid** (**3–7%**) and **stearic acid (0–2%**).	The castor oil fatty acids are prepared by hydro-lysing castor oil with water and steam at 400 psi pressure without any added catalyst after which the resulting fatty acids are freed from glycerol by water washing. Castor oil contains as its main fatty acid component **ricinoleic acid (80±90%**), and it is this fatty acid which is important in the condensation reaction. Other fatty acids present are **oleic acid (3±8%)**, **linoleic acid** (**3±7%**) and **stearic acid (0±2%)**.
Condensation of the castor oil fatty acids	Castor oil fatty acids are condensed by heating at a temperature of 205–210°C under vacuum and a CO_2 atmosphere (to prevent oxidation) for ~8 h. This 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) is reached.	Fatty acid condensation is brought about by heating the castor oil fatty acids at elevated temperatures under vacuum and in an atmosphere of carbon dioxide to prevent oxidation. Samples are taken at regular intervals and tested for their free fatty acid content until an acid value of **35.0** is achieved. This acid value is equivalent to an average of about five fatty acid residues per molecule of the condensed product. During the condensation phase, ricinoleic acid may react in a number of ways. Simple linear esterification is the desired reaction but cyclic esterification, which is a chain terminating process, is theoretically possible. However, no evidence was found for the presence of this type of cyclic material in the condensed castor oil fatty acids.

Manufacturing Process	GRAS Notice	Wilson et al. (1998)
		Dehydration is also possible, but occurs to only a small extent.
Preparation of polyglycerols	The polyglycerol portion can be prepared by three routes: (1) polymerization of glycerol using a strong base as a catalyst, (2) by polymerization of glycidol, leading to linear polyglycerols, or (3) by polymerization of epichlorohydrin, followed by hydrolysis. This leads to linear polyglycerols. The polyglycerols produced by polymerization of epichlorohydrin contain reduced proportions of cyclic components.	The preparation of the polyglycerol is achieved by heating glycerol to temperatures above 200 °C in the presence of a small amount of alkali (potassium hydroxide). In this step, two or more molecules of glycerol condense with a loss of water and the formation of an ether linkage between the glycerol molecules. Carbon dioxide is bubbled through the reaction vessel to prevent oxidation, and unchanged glycerol is removed by distillation at the end of the reaction. The process is controlled by monitoring the rise in the refractive index. The result is a mixture of polyglycerols containing varying numbers of glycerol residues. As the 1- and 3-hydroxy groups of glycerol are more reactive than the 2-hydroxy group, the polyglycerols formed are predominantly straight-chain according to the overall reaction. In addition, small amounts of cyclic by-products may be formed in the reaction mixture as a result of condensation between the 1-hydroxy group of one glycerol molecule and the 2-hydroxy group of another. The cyclic diglycerol product is a solid (m.p. 96 °C), and is present at 4% in the polyglycerol or 0.4% in PGPR.

Manufacturing Process	**GRAS Notice**	**Wilson et al. (1998)**
Partial esterification of the condensed castor oil fatty acids with polyglycerols	The final stage of the production process involves the esterification of condensed castor oil fatty acids with polyglycerols. The "appropriate" amount of polyglycerol with the polyricinoleic acid is heated. After which, a reaction takes place immediately, and in the same vessel while still hot. The esterification conditions are the same as those for fatty acid condensation. This process will continue until a sample is taken from the reaction mixture and found to have a suitable acid value (i.e., ≤ 6 mg KOH/g) and refractive index (per required specifications).	The final stage of the preparation involves heating an appropriate amount of polyglycerol with the condensed castor oil fatty acids. 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. The average value of n is about 3. R1, R2 and R3 each may be hydrogen or a linear condensation product of ricinoleic acid with itself, with n being on average between 5 and 8.
Product Specifications	The Foods Chemicals Codex (FCC) states that the acceptance criteria for the polyglycerol moieties of PGPR shall be composed of NLT **75% of di-, tri- and tetraglycerols** and shall contain **NMT 10% of polyglycerols equal to or higher than heptaglycero**l (FCC Vol 12). This PGPR product meets specification requirements for polyglycerol polyricinoleic acid established by the Foods Chemicals Codex (FCC Vol 12).	The JECFA specification for PGPR states that ``the polyglycerol moiety shall be composed of not less than **75 percent of the di-, tri- and tetraglycerols** and shall contain no more than **10 percent of polyglycerols equal to or higher than heptaglycerol''** (FAO, 1992). PGPR is specified further by the following: Hydroxyl value 85-100 Acid value 2.0 max. Iodine value 80±90 Refractive index at 658C

Manufacturing Process	GRAS Notice	Wilson et al. (1998)
Hydroxyl value	80-100	85-100
Iodine value	72-103	80-90
Refractive index	1.463-1.467	1.4635±1.4665
Saponification value	170-210	
Acid value	Not more than (NMT) 6	NMT 2%
Polyglycerols	Not lower than (NLT) 75% di-, tri- and tetraglycerols; NMT 10% heptaglycerols or higher	Not lower than (NLT) 75% di-, tri- and tetraglycerols; NMT 10% heptaglycerols or higher

Question 12. We note that JECFA and the European Commission Scientific Committee for Food used the reproductive toxicity study with PGPR as the basis for determining their acceptable daily intake level (ADI) of 7.5 mg/kg bw/d (Wilson and Smith, 1998a); whereas it appears the notifiers considered the 2-year combined chronic toxicity and carcinogenicity study in rats (Smith et al., 1998) to be the most sensitive endpoints for ADI derivation. Please provide a short narrative or rationale as to your justification for this approach.

Response:

As stated in the GRAS Notice, it should be noted that in their monograph, JECFA does not clearly articulate their preference for using the reproductive study as the basis for the overall reference dose for their ADI (JECFA, 1974). As such, it is important to consider that the reproductive study has methodological limitations that should preclude its use to derive an ADI, including a low sample size for the first-generation treated animals (six males and 13 females), and potential breeding issues with all the second-generation animals (see Reproductive Toxicity section of the GRAS Notice). Secondly, the unpublished data on the chronic toxicity component from the reproductive study, summarized in Quest (1998), indicated that 14 to 16% of the animals in both groups of the third generation had a *Cysticercus fasciolaris* (intermediate stage of the cat tape worm) in the liver. Further, there is no indication that organ weights were measured as part of chronic toxicity component and thus no evaluation of liver enlargement, the key treatment-related endpoint across the PGPR dataset. Given the significant reported deficiencies with the controls in the reproductive study, it should not be relied upon for derivation of the ADI. Lastly there were no adverse effects at the highest dose tested and therefore, it does not actually establish an effective ceiling for the safety of PGPR. On the other hand, the carcinogenicity, and chronic studies (Smith et al., 1998) tested at a higher concentration for a longer period, had an expanded toxicological examination battery including reproductive organs, and noted no adverse outcomes.

The GRAS notice conclusion agrees with the EFSA (2017) conclusion. In the 2017 Re-evaluation of PGPR, EFSA concluded that the endpoint from the chronic toxicity and carcinogenicity study in rats was the key study with a NOAEL of 2,500 mg/kg bw/day. EFSA (2017) states the following:

> *"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."*

Based on this information, the chronic toxicity and carcinogenicity study in rats (Smith et al. 1998) is the key study for the safety assessment.

Question 13. The notifier states that, as part of a weight-of-evidence approach to address the genotoxicity of PGPR, a published 2-year chronic toxicity and carcinogenicity study was used to conclude that PGPR is not genotoxic (Smith et al., 1998). Yet, the notifier also states on pg. 29 of the notice (ppt. 37) that an in-silico assessment was used to evaluate PGPR's genotoxicity since "there were no available in vitro or in vivo studies". For the administrative record, please confirm that the data from the published 2-year rat chronic toxicity and carcinogenicity study was the pivotal publicly available information used to make your GRAS conclusion, and that the data generated using the OECD QSAR Toolbox provides supportive evidence for your conclusion.

Response:

The key data for the lack of genotoxicity are the two PGPR carcinogenicity studies conducted in rats and mice, which presented no oncogenic outcomes or chronic toxicity (Smith et al., 1998). The QSAR analyses conducted to provide supportive evidence to corroborate the conclusion that PGPR is non-genotoxic.

Question 14. Please perform an update to the literature search described in the safety narrative from August 2021 to present and discuss if any new data were found that would contradict the current GRAS conclusion.

Response:

An updated safety literature search was conducted for the period of August 1, 2021 to present. The search terms included polyglycerol polyricinoleic, polyglycerol polyricinoleate, 68936-89-0, 29894-35-7, and PGPR. The literature search was conducted through the commercial database ToxPlanet ChemEXPERT™ and through PubMed. In addition, the following resources were included in the search for information bearing on the safety of PGPR: the Organization for Economic Co-operation and Development (OECD), US FDA 21 Code of Federal Regulations (CFR), Joint FAO/WHO Expert Committee on Food Additives (JECFA), European Food Safety Authority (EFSA), National Toxicology Program (NTP), Environmental Protection Agency (EPA) and EPA's Integrated Risk Information System (IRIS).

Fourteen documents on polyglycerol polyricinoleate were reviewed from the results of the ToxPlanet™ search from sources including General Standard for Food Additives, EPA CompTox Chemicals Dashboard, Goodscents, Joint Substance Data Pool of the German Federal Government and the German Federal states, New Zealand Inventory of Chemicals, Substances in Preparation in Nordic Countries, and German Environment Agency. There was no new information or updates to data with bearing on the safety of PGPR.

The PubMed search produced one result on a paper regarding new methods for the quantitative determination of PGPR in foods, which did not provide new safety data.

There were no new data that would contradict the current GRAS conclusion.

Question 15. In your narrative, you described several unpublished studies concerning PGPR. GRAS conclusions must be based on generally available and generally accepted data and information. Please provide an explanation of how there could be a basis for a conclusion of GRAS status if qualified experts do not have access to these unpublished studies (21 CFR 170.250(e)).

Response:

The pivotal key toxicological study, and the basis of the safety endpoint, is available in the peer-reviewed publicly available literature (Smith et al., 1998). The other pivotal data used in the GRAS Notice are listed below.

Howes et al., 1998	Absorption, distribution, metabolism, and excretion
Wilson et al. 1998	Acute toxicity Short-term toxicity Chronic toxicity
Wilson and Smith, 1998a	Reproductive toxicity

Smith et al., 1998	Combined chronic toxicity and carcinogenicity study in rats Carcinogenicity study in mice
Wilson and Smith, 1998b	Controlled human dietary study conducted with PGPR

Unpublished toxicological studies and *in silico* QSAR modeling results were published in part in the EFSA authoritative review of PGPR (EFSA, 2017) and were included in the GRAS notice as supportive data to corroborate the safety of PGPR. While the underlying data in the EFSA review may not be fully publicly available, a summary of those data and the authoritative review of the studies, including acceptability, has been prepared by EFSA's qualified experts. Based on their review, there are no data or information in the unpublished literature that appears inconsistent with the pivotal data and information that is available in the public literature described above.

We hope this information adequately addresses the Agency's questions on GRN 1105, and if there is any additional information or further clarification that is required, we will be happy to provide such information upon request.

Sincerely,

Kristin Spoden
Unilever
Regulatory Affairs Leader

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