SCIENTIFIC OPINION

efsa JOURNAL

ADOPTED: 21 June 2023

doi: 10.2903/j.efsa.2023.8110

Follow-up of the re-evaluation of glycerol esters of wood rosins (E 445) as a food additive

EFSA Panel on Food Additives and Flavourings (FAF),
Maged Younes, Gabriele Aquilina, Gisela Degen, Karl-Heinz Engel, Paul Fowler, Maria Jose Frutos Fernandez, Peter Fürst, Rainer Gürtler, Trine Husøy, Melania Manco, Wim Mennes, Sabina Passamonti, Peter Moldeus, Romina Shah, Ine Waalkens-Berendsen, Matthew Wright, Karlien Cheyns, Reginald Fitzgerald, Manuela Mirat, Alicja Mortensen, Beate Ulbrich, Ruud Woutersen, Marcello Laganaro, Ana Maria Rincon, Laura Ruggeri, Camilla Smeraldi and Ursula Gundert-Remy

Abstract

Glycerol esters of wood rosin (GEWR) (E 445) were re-evaluated in 2018. On the toxicity database and given the absence of reproductive and developmental toxicity data, the acceptable daily intake (ADI) of 12.5 mg/kg body weight (bw) per day for GEWR (E 445) established by the Scientific Committee on Food (SCF) in 1994 was considered temporary. The conclusions of the assessment were restricted to GEWR derived from *Pinus palustris* and *Pinus elliottii* and with a chemical composition in compliance with GEWR used in the toxicological testing. Following a European Commission call for data to submit data to fill the data gaps, the present follow-up opinion assesses data provided by interested business operators (IBOs). Considering the technical data submitted by IBOs, the EFSA Panel on Food Additives and Flavourings (FAF Panel) recommended some modifications of the existing EU specifications for E 445, mainly a revision of the definition of the food additive and lowering the limits for toxic elements. Considering the available toxicological database evaluated during the re-evaluation of E 445 by the ANS Panel in 2018, and the toxicological studies submitted by the IBOs, the Panel established an ADI of 10 mg/kg bw per day based on the no observed adverse effect level (NOAEL) of 976 mg/kg bw per day from the newly available dietary reproduction/developmental toxicity screening study in rats and applying an uncertainty factor of 100. Since GEWR from *P. palustris* and *P. elliottii* were tested in the toxicity studies considered to establish the ADI and in the absence of detailed information on the chemical composition (major constituents) in GEWR generated from other *Pinus* species, thus not allowing read across, the ADI is restricted to the GEWR (E 445) manufactured from *P. palustris* and *P. elliottii*. The Panel concluded that there was no safety concern for the use of GEWR (E 445), at either the maximum permitted levels or at the reported uses and use levels.

Keywords: glycerol esters of wood rosin, GEWR, food additive, E 445

Requestor: European Commission

Question number: EFSA-Q-2021-00564

Correspondence: fip@efsa.europa.eu

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

Declaration of interest: If you wish to access the declaration of interests of any expert contributing to an EFSA scientific assessment, please contact interestmanagement@efsa.europa.eu.

Acknowledgements: The Panel wishes to thank the following for the support provided to this scientific output: Vasantha Palaniappan. The FAF Panel wishes to acknowledge all European competent institutions, Member State bodies and other organisations that provided data for this scientific output.

Suggested citation: EFSA FAF Panel (EFSA Panel on Food Additives and Flavourings), Younes, M., Aquilina, G., Degen, G., Engel, K.-H., Fowler, P., Frutos Fernandez, M. F., Fürst, P., U., Gürtler, R., Husøy, T., Manco, M., Mennes, W., Passamonti, S., Moldeus P., Shah R., Waalkens-Berendsen, I., Wright, M., Cheyns, K., Fitzgerald, R., … Gundert-Remy, U. (2023). Follow-up of the re-evaluation of glycerol esters of wood rosins (E 445) as a food additive. *EFSA Journal, 21*(7), 1–32. https://doi.org/10.2903/j.efsa.2023.8110

ISSN: 1831-4732

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

Summary

GEWR (E 445) were re-evaluated by the EFSA former Panel on Food Additives and Nutrient Sources added to Food (EFSA ANS Panel) in 2018. Based on the toxicity database and given the absence of reproductive and developmental toxicity data at the time of the re-evaluation, the ANS Panel concluded that the ADI of 12.5 mg/kg bw per day for GEWR (E 445) as established by the SCF in 1994 should be temporary pending the provision of such data (EFSA ANS Panel, 2018). The conclusions of the assessment were restricted to GEWR derived from *P. palustris* (longleaf pine) and *P. elliottii* (slash pine) and with a chemical composition in compliance with GEWR used in the toxicological testing.

GEWR is a complex mixture of glycerol di- and triesters of resin acids from wood rosin, with residual fractions of glycerol monoesters of resin acids, neutrals (non-acidic saponifiable and unsaponifiable substances) and free resin acids. The ANS Panel considered the compounds of these fractions of particular interest, since they may be absorbed or be hydrolysed to other compounds which may be absorbed.

At the request of the European Commission, the EFSA Panel on Food Additives and Flavourings (FAF Panel) provides in this opinion an updated safety assessment of GEWR (E 445). The Panel was also requested to assess the data provided by IBOs in support of an amendment of the EU specifications for this food additive in Commission Regulation (EU) No 231/2012. The present opinion deals with the assessment of the data provided by IBOs as a response to a dedicated European Commission call for data.

The Panel considered the exposure calculations for E 445 as presented in the re-evaluation of the food additive E 445 for which the refined brand-loyal scenario was considered as the most relevant exposure scenario.

New data on toxic elements (lead, mercury, cadmium and arsenic) in samples of GEWR were submitted by IBOs and new limits for the specification of E 445 were proposed. The Panel considered the potential presence of these toxic elements in E 445 at (i) at the current limit in the EU specifications and also; (ii) at the lowest limits proposed by the IBOs as 1 mg/kg for lead, 0.5 mg/kg for mercury, 0.5 mg/kg for cadmium and 1 mg/kg for arsenic. The potential exposure to these impurities from the use of E 445 was evaluated against the available health-based guidance values (HBGVs) and reference points (RPs) showing that for arsenic, in both scenarios mentioned above, the lower end of the range of calculated MOE values was below the target value of 1,000. For the other toxic elements (cadmium, mercury, lead), the limit values proposed by the IBO did not give rise to safety concerns. Considering the results of the estimation calculated by the Panel and the fact that the food additive is not the only potential dietary source of toxic elements, the Panel recommended that the maximum limits be lowered.

The Panel considered that if the glycerol used for the manufacturing process of E 445 complied with the specifications of E 422 in Commission Regulation (EU) No 231/2012, as stated by the IBOs, there was no need for setting a specification limit in Commission Regulation (EU) No 231/2012 for the content of potential impurities in E 445 from the use of glycerol.

Data on the concentrations of the fractions of 'glycerol monoesters', 'free resin acids' and 'neutrals' in GEWR manufactured from *P. palustris* and *P. elliottii* were submitted by two IBOs. The Panel noted a substantial difference among the results of the concentration of glycerol monoesters determined in GEWR by applying different methods for the detection. No quantitative information on the fraction of neutrals was submitted by one of the IBOs and it could not be compared with the data submitted by the other IBO. The concentration of free resin acids reported by both IBOs was in the same range. Therefore, based on the data submitted, the Panel was not able to propose a specific maximum concentration for the fractions of glycerol monoesters and neutrals that can define better the GEWR used as E 445. However, the Panel considered that the constituents of these fractions should be as low as technologically achievable.

The Panel recommended some modifications of the existing EU specifications, mainly a revision of the definition of the food additive E 445 as presented in Table 8.

Detailed information on the chemical composition of GEWR originating from *Pinus halepensis* and *Pinus brutia* (and potentially other pine species), in particular on the concentrations of the toxicologically relevant fractions of glycerol monoesters, free resin acids and neutrals were requested. The response, received from only one IBO, was limited to the total concentrations of the known fractions of toxicological relevance. The data submitted showed that the concentration of the fractions of neutrals and free resin acids were in the same range for GEWR originating from *P. halepensis* and

P. brutia as for GEWR originating from *P. palustris* and *P. elliottii*. However, the concentration reported for the fraction of glycerol monoesters was significantly lower for GEWR originating from *P. halepensis* and *P. brutia*. Because the major constituents of the fractions were not identified, the Panel could not exclude the presence of constituents which might affect the toxicity of GEWR from *P. halepensis* and *P. brutia*. Therefore, the Panel considered that read across of toxicological data from GEWR from *P. palustris* and *P. elliottii* for the assessment of GEWR from *P. halepensis* and *P. brutia* was not possible.

GEWR was tested in an oral (dietary) combined repeat dose toxicity study with a reproduction/developmental toxicity screening test on test in accordance with OECD TG 422 and in an oral dietary prenatal developmental toxicity study in rats in accordance with OECD TG 414. No effects were observed. The Panel noted that the actual *Pinus* species used to derive the test item were not known, and therefore could not relate the outcome of the studies to a *Pinus* species.

GEWR from *P. palustris* and *P. elliottii* was tested in a further dietary reproduction/developmental toxicity screening study in rats, which was performed in accordance with OECD TG 421, at doses equal to 0, 414, 976 and 1,842 mg/kg bw per day for males and 0, 532, 1,184 and 2,347 mg/kg bw per day for females. The Panel identified a no observed adverse effect level (NOAEL) of 976 mg/kg bw per day in this study.

Considering the available toxicological database evaluated during the re-evaluation of GEWR (E 445) by the ANS Panel in 2018 and the study performed with GEWR from *P. palustris* and *P. elliottii* submitted by the IBOs following the call for data to address the data gap, the Panel established an ADI of 10 mg/kg bw per day based on the lowest NOAEL of 976 mg/kg bw per day and applying an uncertainty factor of 100. Since GEWR from *P. palustris* and *P. elliottii* were tested in the toxicity study considered to establish the ADI and detailed information on the chemical composition (major constituents) in GEWR generated from other *Pinus* species were not provided, the ADI was restricted to the GEWR (E 445) manufactured from *P. palustris* and *P. elliottii*.

The Panel concluded that there was no safety concern for the use of GEWR (E 445), at either the maximum permitted levels or at the reported uses and use levels.

Table of contents

1. Introduction

The re-evaluation of glycerol esters of wood rosin (GEWR) (E 445) as a food additive was completed by EFSA in 2018 (EFSA ANS Panel, 2018). The EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS Panel) issued several recommendations to amend the specifications of the food additive GEWR (E 445) in Commission Regulation (EU) No 231/2012.

The data gaps and uncertainties identified by the ANS Panel required a follow-up by the European Commission by means of a call for additional data.

The present opinion deals with the assessment of the data provided by interested parties in support of an amendment of the EU specifications for GEWR (E 445).

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

1.1.1. Background

The use of food additives is regulated under the European Parliament and Council Regulation (EC) No 1333/2008 on food additives.[1] Only food additives that are included in the Union list, in particular in Annex II to that Regulation, may be placed on the market and used in foods under the conditions of use specified therein. Moreover, food additives shall comply with the specifications as referred to in Article 14 of that Regulation and laid down in Commission Regulation (EU) No 231/2012[2].

Glycerol esters of wood rosins (E 445) is authorised for use as food additives in the Union. Since glycerol esters of wood rosins (E 445) was permitted in the Union before 20 January 2009, it belongs to the group of food additives which are subject to a new risk assessment by the European Food Safety Authority (EFSA), according to Commission Regulation (EU) No 257/2010[3], and in line with the provisions of Regulation (EC) No 1333/2008.

EFSA completed the re-evaluation of glycerol esters of wood rosins (E 445) as a food additive and published a scientific opinion on 26 July 2018. In that opinion, EFSA concluded that given the absence of reproductive and developmental toxicity data, the current acceptable daily intake (ADI) of 12.5 mg/kg body weight (bw) per day for glycerol esters of wood rosins as established by the Scientific Committee on Food (SCF) in 1994 should be temporary pending the provision of such data. EFSA indicated that this assessment is restricted to glycerol esters of wood rosins derived from *Pinus palustris* (longleaf pine) and *P. elliottii* (slash pine) and with a chemical composition in compliance with the glycerol esters of wood rosins used in the toxicological testing. EFSA concluded that a safety assessment of glycerol esters of wood rosins originating from *P. halepensis* and *P. brutia* could not be performed due to lack of appropriate data.

EFSA concluded that the mean and the high exposure levels (P95) of the brand-loyal refined exposure scenario did not exceed the temporary ADI in any of the population groups from the use of glycerol esters of wood rosins (E 445) as a food additive at the reported use levels. EFSA recommended that the EU specifications for glycerol esters of wood rosins (E 445) in Commission Regulation (EU) No 231/2012 are updated to current standards.

Therefore, the European Commission published on 23 November 2018 a call for data[4] addressing the recommendations made by EFSA in the scientific opinion on the re-evaluation of glycerol esters of wood rosins (E 445) as a food additive. Interested business operators (CARAGUM International, Intertek/Pinova Incorporated, Megara Resins and T&R Chemicals) completed the submission of new toxicological and technical data on glycerol esters of wood rosins (E 445) in December 2020.

Consequently, the European Commission has decided to consult EFSA on this matter.

1.1.2. Terms of Reference

In accordance with Article 29(1)(a) of Regulation (EC) No 178/2002[5], the European Commission requests the European Food Safety Authority (EFSA) to provide a scientific opinion on the safety of the food additive glycerol esters of wood rosins (E 445) and its specifications. In particular, EFSA is requested to carry out a scientific evaluation of the new reproductive and developmental toxicity data, to determine whether it would allow EFSA to reconsider the temporary ADI for glycerol esters of wood

[1] OJ L 354, 31.12.2008, p. 16.
[2] OJ L 83, 22.3.2012, p. 1.
[3] OJ L 80, 26.3.2010, p. 19.
[4] https://food.ec.europa.eu/system/files/2019-10/fs_food-improvement-agents_reeval_call_20181123_E445_data.pdf
[5] OJ L 31, 1.2.2002, p. 1.

rosins (E 445) established by EFSA in 2018. EFSA is also requested to determine whether the new technical data on glycerol esters of wood rosins originating from *P. halepensis* and *P. brutia* support a chemical (compositional) equivalence to glycerol esters of wood rosins originating from *P. palustris* and *P. elliottii*, and, consequently, whether read across of toxicological data obtained with glycerol esters of wood rosins originating from *P. palustris* and *P. elliottii* would be possible. Moreover, EFSA is requested to confirm that the technical data provided by interested business operators adequately support an amendment of the specifications of the food additive glycerol esters of wood rosins (E 445) to bring them to current standards, in line with the recommendations made by EFSA during the re-evaluation of the safety of this food additive.

1.2. Background information

GEWR (E 445) was re-evaluated by the EFSA ANS Panel in 2018. On the toxicity database and given the absence of reproductive and developmental toxicity data at the time of the re-evaluation, the ANS Panel concluded that the acceptable daily intake (ADI) of 12.5 mg/kg body weight (bw) per day for GEWR (E 445) as established by the Scientific Committee on Food (SCF) in 1994 should be temporary pending the provision of such data (EFSA ANS Panel, 2018). The conclusions of the assessment were restricted to GEWR derived from *P. palustris* (longleaf pine) and *P. elliottii* (slash pine) and with a chemical composition in compliance with GEWR used in the toxicological testing. The ANS Panel also concluded that the mean and the high exposure levels (P95) of the brand-loyal refined exposure scenario did not exceed the temporary ADI in any of the population groups from the use of GEWR (E 445) as a food additive at the reported use levels.

For GEWR originating from *P. halepensis* and *P. brutia*, the ANS Panel noted that:

- concentrations of the fractions of 'glycerol monoesters', 'free resin acids' and 'neutrals', which are considered to be of particular toxicological relevance, were not known,
- therefore, the evaluation of chemical equivalence with GEWR originating from *P. palustris* (longleaf pine) and *P. elliottii* (slash pine) was not possible,
- no data on stability were available,
- no toxicological data were available.

Therefore, the ANS Panel concluded that a safety assessment of GEWR originating from *P. halepensis* and *P. brutia* could not be performed.

The ANS Panel (EFSA ANS Panel, 2018) recommended the European Commission to consider:

- an update of the definition of GEWR (E 445) in the EU specifications. It should be indicated that GEWR (E 445) (i) contain, besides the mentioned glycerol di- and triesters, a residual fraction of glycerol monoesters and (ii) contain residual free resin acids and neutrals (non-acidic other saponifiable and unsaponifiable substances).
- setting limits for glycerol monoesters of resin acids and neutrals in the EU specifications, in accordance with the analytical data provided for GEWR (E 445) from *P. palustris* (longleaf pine) and *P. elliottii* (slash pine), being in compliance with the test material used in the toxicological tests.
- setting a maximum level for free neoabietic acid of 0.05 wt. % (limit of quantitation) in the EU specifications, while the concentration of the total of free resin acids in GEWR being limited already by the acid value of the existing specifications.
- revising the limits for the toxic elements lead, mercury, cadmium and arsenic in the EU specification to ensure that GEWR (E 445) as a food additive will not be a significant source of exposure to these toxic elements in food.
- setting maximum levels for impurities, such as butanetriols, acrolein, chlorinated compounds and 3-monochloropropane-1,2-diol, in the EU specifications, for which limits are defined in the food additive glycerol (E 422).
- requesting the provision of a reproductive and developmental toxicity study, in accordance with the applicable OECD test guidelines, using a test material which is representative of the food additive present on the market and taking into account the above recommendations for the update of the specifications.

2. Data and Methodologies

2.1. Data

The Panel based its assessment on the information submitted in response to the public call for data issued by the European Commission (Documentation provided to EFSA No 1–6) and additional information submitted during the assessment process by interested business operators (IBOs) in response to follow-up requests from EFSA (Documentation provided to EFSA No 7–12).

Following the request for additional data sent by EFSA 11 February 2022, an IBO (Intertek) requested a clarification teleconference, which was held on 11 March 2022.

2.2. Methodologies

This opinion was formulated following the principles described in the EFSA Guidance on transparency with regard to scientific aspects of risk assessment (EFSA Scientific Committee, 2009) and following the relevant existing guidance documents from the EFSA Scientific Committee.

The FAF Panel assessed the safety of GEWR (E 445) as a food additive in line with the Guidance for submission for food additive evaluations in 2012 (EFSA ANS Panel, 2012).

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

3. Assessment

3.1. Identity and specifications of E 445

GEWR (E 445) is a 'complex mixture of tri- and diglycerol esters of resin acids from wood rosin',[6] obtained by the solvent extraction of aged pine stumps followed by a liquid–liquid solvent refining process. Excluded from the additive's definition are substances derived from gum rosin, an exudate of living pine trees and substances derived from tall oil rosin, a by-product of kraft (paper) pulp processing. The source material,[7] wood rosin, is composed of ~ 90% resin acids and 10% neutrals (non-acidic compounds). Its resin acid fraction is a complex mixture of isomeric diterpenoid monocarboxylic acids having the empirical molecular formula of $C_{20}H_{30}O_2$, chiefly abietic acid. The esterified product is purified by steam stripping or by counter current steam distillation.

Specifications for GEWR (E 445) have been defined in Commission Regulation (EU) No 231/2012 as described in Table 1.

[6] The Panel noted that at the time of the re-evaluation (EFSA ANS Panel, 2018), it was recommended to replace the terms 'tri- and diglycerol esters of resin acids 'in the EU specifications, which are not chemically correct, to the terms 'glycerol di- and triesters of resin acids'.

[7] The Commission Regulation (EU) No 231/1012 is using the term 'final product' instead of the term 'the source material' which may be misinterpreted.

Table 1: Specifications for GEWR (E 445) according to Commission Regulation (EU) No 231/2012 and the JECFA (2013)

	Commission Regulation (EU) No 231/2012 E 445	**JECFA (2018) INS No. 445(iii)**
Synonyms	Ester gum	
Definition	A complex mixture of tri- and diglycerol esters of resin acids from wood rosin. The rosin is obtained by the solvent extraction of aged pine stumps followed by a liquid–liquid solvent refining process. Excluded from these specifications are substances derived from gum rosin, and exudate of living pine trees, and substances derived from tall oil rosin, a by-product of kraft (paper) pulp processing. The final product is composed of ~ 90% resin acids and 10% neutrals (non-acidic compounds). The resin acid fraction is a complex mixture of isomeric diterpenoid monocarboxylic acids having the empirical molecular formula of $C_{20}H_{30}O_2$, chiefly abietic acid. The substance is purified by steam stripping or by counter current steam distillation	Glycerol ester of wood rosin is a complex mixture of glycerol di- and triesters of resin acids from wood rosin, with a residual fraction of glycerol monoesters. In addition, neutrals (non-acidic saponifiable and unsaponifiable substances) and residual free resin acids are present. Wood rosin is obtained by the solvent extraction of aged pine stumps, followed by a liquid–liquid solvent refining process. Refined wood rosin is composed of 90% resin acids and 10% neutrals. The resin–acid fraction is a complex mixture of isomeric diterpenoid monocarboxylic acids having the typical empirical formula $C_{20}H_{30}O_2$, of which the main components are dehydroabietic and abietic acid. GEWR is produced by esterifying the resin acids with food grade glycerol. The product is then purified by steam stripping or by countercurrent steam distillation. These specifications do not cover substances derived from gum rosin, an exudate of living pine trees, and substances derived from tall oil rosin, a by-product of kraft (paper) pulp processing
Description	Hard, yellow to pale, amber-coloured solid	Hard, yellow to pale, amber-coloured solid
Identification		
Solubility	Insoluble in water, soluble in acetone	Insoluble in water, soluble in acetone
Infrared absorption spectrum	Characteristic of the compound	The infrared spectrum of a thin film of the sample (potassium bromide disc) corresponds with the typical infrared spectrum below
Sulfur test	–	Negative Weigh 40–50 mg of sample into a test tube and add 1–2 drops of a 20% (w/v) solution of sodium formate. Place a strip of lead acetate test paper over the mouth of the test tube. Heat the tube until fumes are formed that contact the test paper. Continue heating for 2–5 min. The formation of a black spot of lead sulfide indicates the presence of sulfur-containing compounds. (Detection Limit: 50 mg/kg sulfur)
Gas chromatography of resin alcohols and glycerol	–	Passes test
Purity		
Specific gravity of solution	$[d]^{20}_{25}$ not less than 0,935 when determined in a 50% solution in d-limonene (97%, boiling point 175,5–176°C, d^{20}_4: 0,84)	d_{20}^{25}: Not less than 0.935 (50% solution in d-limonene)
Ring and ball softening range	Between 82°C and 90°C	Not less than 82

	Commission Regulation (EU) No 231/2012 E 445	JECFA (2018) INS No. 445(iii)
Acid value	Not less than 3 and not more than 9	Between 3 and 9
Hydroxyl value	Not less than 15 and not more than 45	–
Arsenic	Not more than 3 mg/kg	–
Lead	Not more than 2 mg/kg	Not more than 1 mg/kg
Mercury	Not more than 1 mg/kg	–
Cadmium	Not more than 1 mg/kg	–
Test for absence of tall oil rosin (sulfur test)	When sulfur-containing organic compounds are heated in the presence of sodium formate, the sulfur is converted to hydrogen sulfide which can readily be detected by the use of lead acetate paper. A positive test indicates the use of tall oil rosin instead of wood rosin	–

AAS: atomic absorption spectroscopy; ICP-AES: inductively coupled plasma atomic emission spectroscopy.

3.2. Technical data submitted

3.2.1. Toxic elements

The following was requested in the European Commission call for data[4]:

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

Analytical levels in 19 commercial samples of GEWR originating from *P. palustris* and *P. elliottii* for lead, mercury, cadmium and arsenic were submitted by one IBO (Documentation provided to EFSA No 1, 7). The analyses were performed by inductively coupled plasma optical emission spectroscopy (ICP-OES), with an LOD of 0.5 mg/kg and LOQ of 1 mg/kg for arsenic, lead and cadmium, and 2 mg/kg for Hg. The Panel noted that most of the reported levels were 'less than' a value lower than the indicated LOQ. No response to the EFSA clarifications on the data submitted were provided and, therefore, these data could not be considered for the assessment.

Initially, analytical levels in five commercial samples of GEWR originating from *P. palustris* and *P. elliottii* for lead, mercury, cadmium and arsenic were submitted by a second IBO (Documentation provided to EFSA No 2 and 10). The method of analysis used was inductively coupled plasma mass spectrometry (ICP-MS) for arsenic, cadmium and lead while the analyses for mercury were performed according to EPA 7470A protocol using a PerkinElmer FIMS 100 cold vapour atomic absorption (CVAA) analyser. A further report on the analysis of arsenic, cadmium, lead and mercury in 63 samples of GEWR was provided indicating that arsenic and cadmium are below 0.1 mg/kg, while the average level of lead is 0.13 mg/kg with a maximum value of 1.38 mg/kg and the average level of mercury is 0.09 mg/kg with a maximum value of 0.26 mg/kg. Based on these data, the IBO proposed the following limits for these toxic elements: 1 mg/kg for arsenic, 1 mg/kg for cadmium, 2 mg/kg for lead and 1 mg/kg for mercury (Documentation provided to EFSA No 10).

A third IBO provided analytical levels for lead, mercury, cadmium and arsenic in two commercial samples of GEWR manufactured from the crude wood rosin sourced from the pine species *P. halepensis* and *P. brutia* using the AOAC2015.01 method by ICP-MS (Documentation provided to EFSA No. 11). The LOQs were 0.01, 0.01, 0.005 and 0.001 mg/kg for arsenic, lead, mercury and cadmium, respectively. Arsenic, lead and mercury were not detected in any sample. Cadmium was detected only in one of the samples (0.005 mg/kg). The IBO proposed the following limits for these toxic elements justified by the analytical data and the lowest technologically achievable levels (interpreted as the LOQ): 1 mg/kg for arsenic, 0.5 mg/kg for cadmium, 1 mg/kg for lead and 0.5 mg/kg for mercury. These limits are based on the highest measured value or LOQ, whichever is highest, applying a margin of 100 times that value.

3.2.2. Impurities potentially present in glycerol

The following was requested in the European Commission call for data[8]:

- analytical data on current levels in commercial samples of the food additive E 445 of impurities of toxicological concern (e.g. butanetriols, acrolein, chlorinated compounds and 3-monochloropropane-1,2-diol), as identified in the EU specifications of the food additive glycerol (E 422)[8], which can be used in the manufacturing process of E 445.
- the lowest technologically achievable level for impurities of toxicological concern (e.g. butanetriols, acrolein, chlorinated compounds and 3-monochloropropane-1,2-diol) in order to adequately define their maximum limits in the specifications of E 445.
- analytical data on current levels in commercial samples of the food additive E 445 of any impurity present in glycerol (as mentioned in the call for data on the food additive glycerol (E 422)[8]), which can be used in the manufacturing process of E 445;
- the lowest technologically achievable level for any impurity which could be formed during the manufacturing processes of glycerol and be present in E 445, in order to adequately define their maximum limits in the specifications of E 445.

The three IBOs confirmed that glycerol used for the manufacturing of E 445 meets the specifications for E 422 (Documentation provided to EFSA No 7, 8, 10).

At the time of the European Commission call for data, information on potentially impurities of glycerol was requested (EFSA ANS Panel, 2017). As a follow-up of the EFSA re-evaluation of glycerol E 422, the specifications for this food additive were evaluated and some recommendations for their amendment were made (EFSA FAF Panel, 2022).

3.2.2.1. Chlorinated compounds

Results of the analysis in five commercial samples of GEWR for 1,3-dichloro-2-propanol (dichlorohydrin) and epichlorohydrin were submitted by one IBO (Documentation provided to EFSA No 2). The method of analysis used was gas chromatography–electron capture detection (GC–ECD). In all analysed samples the tested chlorinated compounds were below the LODs of 2 and 2.3 mg/kg for epichlorohydrin and dichlorohydrin, respectively. Five commercial samples of GEWR were also analysed for 3-monochloropropane diol (3-MCPD) (Documentation provided to EFSA No 10). The method used was ISO 18363-2 for determination by gas chromatography–mass spectrometry (GC–MS). All analysed samples were below the LOQ of 0.1 mg/kg. The IBO proposed a specification limit in E 445 for the sum of free 3-MCPD and 3-MCPD fatty acid esters, expressed as 3-MCPD, of 0.1 mg/kg.

Another IBO did not provide analytical data and indicated that wood rosin does not contain acylglycerols or diacylglycerols, and since these precursors are not present, it is highly unlikely that 3-MCPD and its esters and/or glycidyl esters could be formed during the manufacture of GEWR. The only possible source of these contaminants is the glycerol (that makes up 10% of GEWR) itself and it meets the specifications for the food additive E 422. It was also indicated that even if 3-MCPD and its esters were present in the glycerol starting material, they would be removed during the purification step (steam stripping) in GEWR manufacturing (Documentation provided to EFSA No 11).

3.2.2.2. Acrolein and butanetriols

Analytical levels of acrolein and butanetriols in five commercial samples of GEWR were submitted by one IBO (Documentation provided to EFSA No 1). The method of analysis used was GC–MS with selective ion monitoring (SIM). None of the tested samples had a concentration above the LODs of 25 and 430 mg/kg for acrolein and butanetriols, respectively.

3.2.2.3. Glycidol

Analytical levels of glycidol in five commercial samples of GEWR were submitted (Documentation provided to EFSA No 10). The analytical method used was ISO 18363-2 for determination by GC–MS. All analysed concentrations were below the LOQ of 0.1 mg/kg. The IBO proposed a specification limit for glycidyl fatty acid esters, expressed as glycidol, in E 445 of 0.1 mg/kg.

[8] Call for technical data on the permitted food additive glycerol (E 422) https://ec.europa.eu/food/safety/food_improvement_agents/additives/re-evaluation_en

Another IBO indicated that if glycidol esters were present in the glycerol starting material, they would be removed during the purification step (steam stripping) in GEWR manufacturing (Documentation provided to EFSA No 11).

3.2.3. Fractions of toxicological relevance in GEWR (E 445)

The following was requested in the European Commission call for data[8]:

- analytical data on the concentrations of the toxicologically relevant fractions of 'glycerol monoesters', 'free resin acids' and 'neutrals' from a GEWR preparation equivalent to the GEWR which was subject to the toxicological testing.

3.2.3.1. Free resin acids

Concentration data for free resin acids in five commercial samples of GEWR manufactured from *P. palustris/P. elliottii* wood rosin was submitted by one IBO (Documentation provided to EFSA No 2). The methods of analysis used for the quantification of the free resin acids fraction was gel permeation chromatography (GPC) in accordance with OECD 118. Two different detectors were used for comparison purposes (i.e. ultraviolet detector (UV detector) and differential refractometer detector (RI detector)). Quantitation was by relative peak areas percent. Results were comparable between RI and UV detectors. The concentration of free resin acids in GEWR ranged between 3.3% and 4.0%.

The IBO also submitted results on measurements of free resin acids performed by acid number analysis. These results were consistent with the measurements by GPC which indicates that no GPC interferences due to low molecular weight neutrals are co-eluting with resin acids.

Concentration data on free resin acids in five samples of GEWR manufactured from *P. palustris/ P. elliottii* wood rosin have been submitted by another IBO (Documentation provided to EFSA No 4, 11). The acid number determined by ASTM D-465 was calculated and abietic acid was used to represent all free resin acids because according to the IBO it is the most abundant and has the same molecular weight as most of the other free resin acids (pimaric, isopimaric, levopimaric, palustric, sandaracopimaric and communic acids). The average of the free resin acid in the samples was 3.4% (range: 2.6–4.2%).

3.2.3.2. Glycerol monoesters of resin acids

Concentration data for glycerol monoesters[9] in 42 commercial samples of GEWR manufactured from *P. palustris*/*P. elliottii* were submitted by one IBO (Documentation provided to EFSA No 2). The method of analysis was GPC using an RI detector. The concentration of glycerol monoesters in GEWR was reported to range between 2.5% and 6%. Based on the results a specification of not more than 8.0% for glycerol monoester content in E 445 was proposed (Documentation provided to EFSA No 2).

The IBO also stated that 'GPC results represent upper limits for both glycerol monoesters and free resin acids, due to the presence of trace neutral components that may elute in the same regions and be integrated with these peaks. If specifications are established for one or both of these fractions, reference should be made to this fact'. (Documentation provided to EFSA No 2).

Concentration data for glycerol monoesters in commercial GEWR samples manufactured from P. *palustris/P. elliottii* using two different analytical methods were submitted by another IBO (Documentation provided to EFSA No 11). In the first method, glycerol monoabietate was synthesised and used as a standard in the analysis of GEWR samples using high-performance liquid chromatography–mass spectrometry (HPLC–MS). In the second method, comprehensive two-dimensional gas chromatography coupled with high-resolution time-of-flight mass spectrometry (GCxGC–TOF MS) was used to assess the composition of the GEWR samples.

In the HPLC–MS method, glycerol monoabietate was the only compound quantified at a concentration of 0.1% in GEWR manufactured from *P. palustris/P. elliottii* wood rosin (Documentation provided to EFSA No 11).

Semi-quantitative analysis by GCxGC-TOF MS did not detect glycerol monoabietate at the limit of detection of 0.1%. Glycerol monodehydroabietate was determined at a concentration of 0.46% (based on the total peak area in the total ion current) in GEWR manufactured from *P. palustris/P. elliottii* wood rosin (Documentation provided to EFSA No 11).

[9] For the sake of simplicity only the 'glycerol monoesters' is used in the present opinion when referring to glycerol monoesters of resin acids.

3.2.3.3. Neutrals

According to one IBO (Documentation provided to EFSA No 10), neutrals in GEWR manufactured from *P. palustris*/*P. elliottii* can be grouped into three main classes for purposes of characterisation: (1) monoterpene (C10) and sesquiterpene (C15) neutrals; (2) diterpene (C20) neutrals; and (3) high molecular weight neutrals.

Gel permeation chromatography (GPC) was used to isolate the volatile fraction from samples of GEWR that was analysed by GC–MS for component identification. The fraction consisted mostly of components with a lower molecular weight than the diterpene resin acids (< 320 Da), although there was some carry-over of compounds in the GPC with higher molecular weight. Ninety-eight peaks were resolved of which 54 were identified and 44 could not be identified by the MS software. Some peaks had multiple coelutions and the identification was based on highest probability. Components with a MW < 230 Da correspond to monoterpenes and essentially all of the sesquiterpenes, and components with a MW > 230 Da are diterpene neutrals, plus some other unrelated constituents in both cases. However, no quantification of the major components or the whole fraction was provided.

Five samples of GEWR produced from *P. palustris* and *P. elliottii* were analysed using standard ASTM-1065 method for the concentration of neutrals and unsaponifiables (Documentation provided to EFSA No 11). The average percentage of neutrals in GEWR was 14.6% (13.7–15.2). However, no identification of the major components of this fraction was provided.

3.2.4. Chemical composition of GEWR originating from *P. halepensis* and *P. brutia*

The following was requested in the European Commission call for data[8]:

- Detailed information on the chemical composition of GEWR originating from *P. halepensis* and *P. brutia* (and potentially other pine species), in particular on the concentrations of the toxicologically relevant fractions of 'glycerol monoesters', 'free resin acids' and 'neutrals. Those data are needed to determine whether GEWR originating from *P. halepensis* and *P. brutia* (and potentially other pine species, provided that appropriate data are provided) is chemically (compositionally) equivalent to GEWR originating from *P. palustris* and *P. elliottii*, and, consequently, whether read across of toxicological data obtained with GEWR originating from *P. palustris* and *P. elliottii* would be possible.

Information on the concentration of glycerol monoesters', 'free resin acids' and 'neutrals' in GEWR manufactured from *P. halepensis* and *P. brutia* wood rosin has been provided by one IBO (Documentation provided to EFSA No 3, 11). Information on concentration of 'glycerol monoesters', 'free resin acids' and 'neutrals' in GEWR manufactured from *P. halepensis* and *P. brutia and P. palustris/ P. elliottii* (five samples of each of the two group species) using the same methodology were provided and is reported in Table 2.

Table 2: Summary of concentration of 'glycerol monoesters', 'free resin acids' and 'neutrals' in GEWR

	GEWR manufactured from *P. palustris* and *P. elliottii*	**GEWR manufactured from *P. halepensis* and *P. brutia***
Glycerol monoesters	HPLC–MS (glycerol monoabietate): 0.1%	HPLC–MS (glycerol monoabietate): 0.01%
	GC&GC–TOF MS: 0.46%	GC&GC–TOF MS: < 0.1%
Free resin acids	Average: 3.5%	Average: 2.6%
Neutrals	Average: 14.6%	Average: 14.4%

GEWR: glycerol esters of wood rosin; HPLC–MS; high-performance liquid chromatography–mass spectrometry; GCxGC-TOF MS: gas chromatography coupled with high-resolution time-of-flight mass spectrometry.

GCxGC–TOF MS chromatograms for analysis of neutrals/unsaponifiables and IR spectra for five samples of GEWR manufactured from *P. halepensis* and *P. brutia* and five samples of GEWR manufactured from *P. palustris/P. elliottii* were submitted. The GCxGC–TOF MS results were presented as plan pictures ('heat contour' plots) in the TIC mode (total ion current) but with no substance identification and no quantitative evaluation of the different components in the mixtures. The IR spectra were compared for correlation using proprietary software, within each of the five sample sets but not between the five sets. There was no discussion on the similarities/differences between the two

sample sets other than the concluding statement that 'The GCxGC-TOF MS and the IRs show substantial similarities between the compound classes in both sets of samples and within the sets' (Documentation provided to EFSA No 3).

3.3. Proposed revision to existing EU specifications for glycerol esters of wood rosin (E 445)

3.3.1. Toxic elements

The potential exposure to impurities from the use of GEWR (E 445) can be calculated by assuming that the impurity is present in the food additive up to a limit value, and then by calculation pro-rata to the estimates of exposure to the food additive itself.

With regard to the dietary exposure to the food additive, the Panel considered the exposure calculations for E 445 as presented in the re-evaluation of the food additive (EFSA ANS Panel, 2018). Since the ANS Panel noted that GEWR (E 445) is authorised and used in a certain type of flavoured drinks, to which consumers may be brand loyal, the refined brand-loyal scenario was considered as the most relevant exposure scenario for the safety evaluation of this food additive (EFSA ANS Panel, 2018). For the current assessment, the highest exposure levels for the mean and 95th percentile among the different population groups were considered, i.e. 1.07 and 4.06 mg/kg bw per day, for toddlers, respectively (Table 3).

Table 3: Summary of dietary exposure to GEWR (E 445) from its use as a food additive in the brand-loyal refined exposure scenario, in six population groups (minimum–maximum across the dietary surveys in mg/kg bw per day) (EFSA ANS Panel, 2018)

	Infants (12 weeks–11 months)		Toddlers (12–35 months)		Children (3–9 years)		Adolescents (10–17 years)		Adults (18–64 years)		The elderly (≥ 65 years)	
	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max
Brand-loyal scenario												
Mean	0	0.2	0.02	1.07	0.09	1.01	0.14	0.77	0.06	0.36	0.03	0.10
95th percentile	0	1.32	0.10	4.06	0.46	3.24	0.58	2.04	0.3	1.39	0.13	0.42

bw: body weight; GEWR: glycerol esters of wood rosin.

The level of the impurity in the food additive combined with the estimated intakes of E 445, presented in Table 3, could result in an exposure which can be compared with the following reference points (RPs) or health-based guidance values (HBGVs) (Table 4) for the undesirable impurities potentially present in E 445.

Table 4: Reference points/health-based guidance values for impurities and constituents potentially present in E 445

Impurity/constituent/ HBGV/RP (ug/kg bw)	Basis/Reference
Lead (Pb)/0.5 ($BMDL_{01}$)	The reference point is based on a study demonstrating perturbation of intellectual development in children with the critical response size of 1 point reduction in intelligence quotient (IQ). The EFSA CONTAM Panel mentioned that a 1-point reduction in IQ is related to a 4.5% increase in the risk of failure to graduate from high school and that a 1-point reduction in IQ in children can be associated with a decrease of later productivity of about 2%. A risk cannot be excluded if the exposure exceeds the $BMDL_{01}$ (MOE lower than 1) EFSA CONTAM Panel (2010).
Mercury (Hg)/4 (TWI)	The HBGV was set using kidney weight changes in male rats as the pivotal effect. Based on the $BMDL_{10}$ of 0.06 mg/kg bw per day, expressed as mercury, and an uncertainty factor of 100 to account for inter and intra species differences, with conversion to a weekly basis and rounding to one significant figure, a TWI for inorganic mercury of 4 μg/kg bw per week, expressed as mercury was established. EFSA CONTAM Panel (2012)

Impurity/constituent/ HBGV/RP (ug/kg bw)	Basis/Reference
Cadmium (Cd)/2.5 (TWI)	The derivation of the reference point is based on a meta-analysis to evaluate the dose–response relationship between selected urinary cadmium and urinary beta-2-microglobulin as the biomarker of tubular damage recognised as the most useful biomarker in relation to tubular effects. A group based $BMDL_5$ of 4 μg Cd/g creatinine for humans was derived. A chemical specific adjustment factor of 3.9 was applied to account for human variability in urinary cadmium within each dose-subgroup in the analysis resulting in a reference point of 1.0 μg Cd per g creatinine. In order to remain below 1 μg Cd/g creatinine in urine in 95% of the population by age 50, the average daily dietary cadmium intake should not exceed 0.36 μg Cd/kg bw, corresponding to a weekly dietary intake of 2.5 μg Cd/kg bw. EFSA CONTAM Panel (2009a)
Arsenic (As)/0.3–8 ($BMDL_{01}$)	The reference point is based on a range of benchmark dose lower confidence limit ($BMDL_{01}$) values between 0.3 and 8 μg/kg bw per day identified for cancers of the lung, skin and bladder, as well as skin lesions. In general, the MOE should be at least 10,000 if the reference point is based on carcinogenicity in animal studies. However, as the BMDL for as is derived from human studies, an interspecies extrapolation factor (i.e. 10) is not needed, i.e. an MOE of 1,000 would be sufficient. EFSA CONTAM Panel (2009b); EFSA Scientific Committee (2012a,b)

HBGV: health-based guidance value; RP: reference point; $BMDL_{01}$: benchmark dose (lower confidence limit); TWI: tolerable weekly intake.

The risk assessment of the undesirable impurities helps inform whether there could be a possible health concern if these impurities and constituents would be present at the limit values in the food additive. The assessment is performed by calculating the margin of exposure (MOE) by dividing the RP (e.g. BMDL Table 4) by the exposure estimate (Table 3), or by estimating the contribution of the use of E 445 to the HBGV (expressed as percentage of the HBGV).

The results of analyses for lead, mercury, cadmium and arsenic in E 445 are reported in Section 3.2.1.

The analytical levels in two samples of GEWR manufactured from *P. halepensis* and *P. brutia* provided by one IBO were below the LOQs of 0.01, 0.005, 0.001 and 0.01 mg/kg for lead, mercury, cadmium and arsenic, respectively. One sample had a detectable level of cadmium (0.005 mg/kg). Proposed limits for these toxic elements were also provided (Table 5) (Documentation provided to EFSA No 11). The other IBO reported analytical results on the toxic elements in 63 samples of GEWR indicating that arsenic and cadmium are below 0.1 mg/kg, while the average level of lead is 0.13 mg/kg with a maximum value of 1.38 mg/kg, and the average level of mercury is 0.09 mg/kg with a maximum value of 0.26 mg/kg. The IBO proposed to maintain the limits as in the current specifications for lead, mercury and cadmium and decrease arsenic to 1 mg/kg as the other IBO (Table 5) (Documentation provided to EFSA No 10).

Table 5: Proposed limits by IBOs

	Pb (mg/kg)	Hg (mg/kg)	Cd (mg/kg)	As (mg/kg)
Documentation provided to EFSA No 11	1	0.5	0.5	1
Documentation provided to EFSA No 10	2	1	1	1

The Panel performed the risk assessment that would result if these toxic elements were present in the food additive E 445; (i) at the current limit in the EU specifications and also; (ii) limit proposed by an IBO as 1 mg/kg for lead, 0.5 mg/kg for mercury, 0.5 mg/kg for cadmium and 1 mg/kg for arsenic. The outcome of the risk assessment for the different scenarios is presented in Table 6.

Table 6: Risk assessment for toxic elements

(i) Considering the presence of toxic elements at the current limits of the EU specifications for E 445 (Commission Regulation (EU) No 231/2012)				
Exposure to E 445 (mg/kg bw per day)	**MOE for Pb at 2 mg/kg**	**% of the TWI for Hg at 1 mg/kg**	**% of the TWI for Cd at 1 mg/kg**	**MOE for as at 3 mg/kg**
1.07[(a)]	234	0.2	0.3	94–2,492
4.06[(b)]	62	0.7	1.1	24–656
(ii) Considering the presence of toxic elements at the proposed limit by an IBO				
Exposure to E 445 (mg/kg bw per day)	**MOE for Pb at 1 mg/kg**	**% of the TWI for Hg at 0.5 mg/kg**	**% of the TWI for Cd at 0.5 mg/kg**	**MOE for as at 1 mg/kg**
1.07[(a)]	467	0.1	0.1	280–7,477
4.06[(b)]	123	0.4	0.6	73–1,970

bw: body weight; TWI: tolerable weekly intake; MOE: margin of exposure; IBO: interested business operator.
(a): Highest exposure level among the different population groups (refined brand-loyal scenario – toddlers – mean) (Table 3).
(b): Highest exposure level among the differ.ent population groups (refined brand-loyal scenario – toddlers – 95th percentile) (Table 3).

The potential exposure to these impurities from the use of E 445 was compared with the available HBGV and RP (Table 4). For As, in both scenarios, i.e. (i) the maximum current limit in the EU specification and (ii) the proposed limit by an IBO, the lower end of the range of calculated MOE values was below the target value of 1,000. For the other toxic elements (cadmium, mercury, lead), the limit values proposed by the IBO do not give rise to safety concerns (Table 6).

The Panel considered that the maximum limits in the EU specifications for toxic elements should be established based on actual levels in the commercial food additive. If the European Commission decides to revise the current limits in the EU specifications, the estimates of toxic elements intake as above could be considered.

3.3.2. Impurities potentially present in glycerol

Limited information on the presence of potentially impurities of glycerol in E 445 has been submitted (Section 3.2.2). The three IBOs confirmed that glycerol used for the manufacturing of E 445 meets the specifications for glycerol as food additive E 422.

In addition, it was also indicated that even if these potential impurities were formed during the manufacturing process of E 445, they would be removed during its purification steps (steam stripping) in GEWR manufacturing.

Hence, the Panel considered that if the glycerol used for the manufacturing process of E 445 complies with the specifications of E 422 in Commission Regulation (EU) No 231/2012, there is no need for setting a specification limit in Commission Regulation (EU) No 231/2012 for the content of potential impurities in E 445 from the use of glycerol. Revisions to the specifications of E 422 were recommended in the follow-up of the re-evaluation of E 442 (EFSA FAF Panel, 2022).

3.3.3. Fractions of toxicological relevance in E 445

According to the information available at the time of the re-evaluation of E 445 (EFSA ANS Panel, 2018), in GEWR derived from wood rosin from *P. palustris* (longleaf pine) and *P. elliottii* (slash pine) the total glycerol esters averaged 84.3% (ranging from 79.7% to 86.3%), being the sum of glycerol di- and triesters determined to be 82.0% (ranging from 78.3% to 83.9%). The ANS Panel noted that ester bonds in GEWR are largely resistant to hydrolysis in the gut, the majority of orally applied GEWR being excreted unchanged in the faeces. Only a small fraction, most likely the glycerol monoesters of wood rosin, seems to undergo hydrolysis and that the absorbed components were hydrolysis products from glycerol monoesters of wood rosin present in GEWR.

The ANS Panel also noted that the absorption rate of dehydroabietic acid (a representative of the resin acids fraction) is ~ 40% after oral exposure. GEWR contain a fraction of residual free resin acids and resin acids which might be released in the gastro-intestinal tract after hydrolysis of glycerol monoesters or of esters with other alcohols in GEWR.

Similarly, the ANS Panel noted that the fraction of 'neutrals' (including small amounts of esters of resin acids with alcohols other than glycerol) in GEWR is of particular interest, since these compounds may be absorbed or be hydrolysed to compounds which may be absorbed.

Therefore, the ANS Panel considered that GEWR may be more specifically characterised by indicating the maximum levels of the fraction of glycerol monoesters of resin acids and of the fraction of neutrals (non-acidic other saponifiables and unsaponifiables) in the definition. Setting limits for glycerol monoesters of resin acids might be relevant for the specifications. The ANS Panel noted that the concentration of the total of free resin acids in GEWR is limited by the acid value,[10] which according to the EU specifications for E 445 should not exceed 9 (EFSA ANS Panel, 2018).

The ANS panel recommended:

- an update of the definition of GEWR (E 445) in the EU specifications. It should be indicated that GEWR (E 445) (i) contains, besides the mentioned glycerol di- and triesters, a residual fraction of glycerol monoesters and (ii) contain residual free resin acids and neutrals (non-acidic other saponifiable and unsaponifiable substances).
- setting limits for glycerol monoesters of resin acids and neutrals in the EU specifications.
- setting a maximum level for free neoabietic acid of 0.05 weight percent (limit of quantitation) in the EU specifications, while the concentration of the total of free resin acids in GEWR being limited already by the acid value of the existing specifications.

The recommendations from the ANS Panel were the reason for requesting additional data on the fractions of toxicological relevance in E 445 in order to amend the EU specifications for E 445 accordingly.

A summary of the data submitted (Section 3.2.1) by only two of the IBOs for GEWR produced from *P. palustris* and *P. elliottii* is presented in Table 7.

Table 7: Summary of the data submitted by IBOs

	Documentation provided to EFSA No 2, 10	Documentation provided to EFSA No 3, 11	
Free resin acids (%)	3.3–4.0[(a)]	2.6–4.2[(b)]	
Glycerol monoesters (%)	2.5–6[(c)]	0.1[(d)]	0.01[(e)]
Neutrals (%)	Not quantified	13.7–15.2[(f)]	

IBO: interested business operator.
(a): By gel permeation chromatography in accordance with OECD 118.
(b): By acid number using ASTM D-465.
(c): By Gel permeation chromatography.
(d): By HPLC–MS.
(e): By GCxGC–TOF MS.
(f): ASTM-1065 method.

The Panel noted a substantial difference among the results of the concentration of glycerol monoesters determined in GEWR by applying three different methods for the detection and quantification. Since GPC is a methodology predominantly based on size or approximate molecular weight, this method could overestimate the amounts of glycerol monoesters due to overlapping with other components with similar molecular weight or size. The Panel considered that the reported results by GPC (of up to 6%) is an overestimation of the presence of glycerol monoesters in GEWR.

Based on the data submitted (Table 7), the Panel was not able to propose a specific maximum concentration for the fractions of glycerol monoesters and neutrals that can define better the GEWR used as E 445. However, the Panel considered that the constituents of these fractions should be as low as technologically achievable.

3.3.4. Source material of wood rosin used for the manufacturing process of E 445

In the re-evaluation of E 445 (EFSA ANS Panel, 2018), the ANS Panel concluded that, based on the data available at that time on the chemical composition of GEWR originating from *P. halepensis* and

[10] mg of KOH per gram of sample.

P. brutia, read across of toxicological data from *P. palustris* and *P. elliottii* for the assessment of GEWR from *P. halepensis* and *P. brutia* was not possible and a safety assessment could not be performed.

Information of the concentration of the fractions of 'glycerol monoesters', 'free resin acids' and 'neutrals' in GEWR originating from *P. halepensis* and *P. brutia* has been submitted and compared to GEWR originating from *P. palustris* and *P. elliottii* (Section 3.2.2).

The Panel concluded that the concentration of the fractions of neutrals and free resin acids were in the same range for GEWR originating from *P. halepensis* and *P. brutia* as for GEWR originating from *P. palustris* and *P. elliottii*. The concentration reported for the fraction of glycerol monoesters was significantly lower for GEWR originating from *P. halepensis* and *P. brutia*.

Clear differences in the plan profile plots of the GCxGC–TOF MS data on the neutrals/ unsaponifiables of GEWR manufactured from *P. halepensis* and *P. brutia* and GEWR manufactured from *P. palustris* and *P. elliottii* were noted by the Panel. Since identification and quantification of the different components in the mixtures was not provided, it was not possible to conclude on the significance or otherwise of these differences. The additional submitted IR spectra do not allow to conclude that the major constituents of the fractions of 'glycerol monoesters', 'free resin acids' and 'neutrals' in GEWR originating *P. halepensis* and *P. brutia* are the same as for GEWR manufactured from *P. palustris* and *P. elliottii*, for which toxicity data are available.

There is not a substantial difference in the presence of toxic elements in GEWR manufactured from *P. halepensis* and *P. brutia and* GEWR manufactured from *P. palustris* and *P. elliottii* (see Section 3.2.1).

3.3.5. Summary of the proposed revisions to the EU specifications

Based on the information provided by the IBOs (Documentation provided to EFSA No 1, 2, 4, 7–12) and the considerations of the Panel presented in the above sections, the Panel recommends the following revisions of the existing EU specifications for GEWR (E 445) as listed in Table 8. The Panel noted that the choice of maximum limits for impurities in the EU specifications is in the remit of risk management.

Table 8: Proposal for a revised version of the existing EU Specifications for GEWR (E 445)

	Commission Regulation (EU) No 231/2012 E 445	**Comment/justification for revision**
Synonyms	Ester gum	
Definition	A complex mixture of tri- and diglycerol esters of resin acids from wood rosin. The rosin is obtained by the solvent extraction of aged pine stumps followed by a liquid–liquid solvent refining process. Excluded from these specifications are substances derived from gum rosin, and exudate of living pine trees, and substances derived from tall oil rosin, a by-product of kraft (paper) pulp processing. The final product is composed of ~ 90% resin acids and 10% neutrals (non-acidic compounds). The resin acid fraction is a complex mixture of isomeric diterpenoid monocarboxylic acids having the empirical molecular formula of $C_{20}H_{30}O_2$, chiefly abietic acid. The substance is purified by steam stripping or by counter current steam distillation	A complex mixture of glycerol di- and triesters of resin acids from wood rosin, with residual fractions of glycerol mono esters, neutrals (non-acidic saponifiable and unsaponifiable substances) and between 2% and 5% of residual free resin acids (complex mixture of isomeric diterpenoid monocarboxylic acids). It is manufactured in a two-stage process: At first, refined wood rosin is obtained by solvent extraction from aged pine stumps (*Pinus palustris* and *Pinus elliottii* species*) followed by a liquid–liquid solvent refining process. In a second step, the refined wood rosin is esterified with glycerol (E 422) and the final product is purified by steam stripping or by counter current steam distillation. Excluded from these specifications are substances derived from gum rosin, an exudate of living pine trees, and substances derived from tall oil rosin, a by-product of kraft (paper) pulp processing
Description	Hard, yellow to pale, amber-coloured solid	Unchanged
Identification		
Solubility	Insoluble in water, soluble in acetone	Unchanged
Infrared absorption spectrum	Characteristic of the compound	Unchanged

	Commission Regulation (EU) No 231/2012 E 445	Comment/justification for revision
Purity		
Specific gravity of solution	$[d]^{20}_{25}$ not less than 0.935 when determined in a 50% solution in d-limonene (97%, boiling point 175.5–176°C, d^{20}_4: 0.84)	Unchanged
Ring and ball softening range	Between 82°C and 90°C	To be deleted on the basis of the considerations of the Panel**
Acid value	Not less than 3 and not more than 9	To be deleted since a value for free resin acids is proposed in the definition
Hydroxyl value free neoabietic acid	Not less than 15 and not more than 45	Unchanged To include a maximum limit based on the re-evaluation of E 445 (EFSA ANS Panel, 2017)
Arsenic	Not more than 3 mg/kg	Maximum limit to be lowered on the basis of the information provided by IBOs and on the considerations of the Panel
Lead	Not more than 2 mg/kg	Maximum limit to be lowered on the basis of the information provided by IBOs and on the considerations of the Panel
Mercury	Not more than 1 mg/kg	Maximum limit to be lowered on the basis of the information provided by IBOs and on the considerations of the Panel
Cadmium	Not more than 1 mg/kg	Maximum limit to be lowered on the basis of the information provided by IBOs and on the considerations of the Panel
Test for absence of tall oil rosin (sulfur test)	When sulfur-containing organic compounds are heated in the presence of sodium formate, the sulfur is converted to hydrogen sulfide which can readily be detected by the use of lead acetate paper. A positive test indicates the use of tall oil rosin instead of wood rosin	Unchanged

*: The Panel proposed to limit E 445 to be manufactured only from the species *P. palustris* and *P. elliotti* based on the conclusion on the ADI established by the Panel (see Discussion section).

**: The Panel noted that the entry under the purity specifications, for the softening range to be between 82°C and 90°C as measured using the ring and ball method, seems to have little or no relevance with respect to purity of the additive E 445. No link has been made between the softening temperature and the purity of E 445, e.g. linking with the identified impurities in E 445 and/or the fractions of free resin acids, glycerol monoesters and neutrals. The softening temperature did not feature in the previous 2018 EFSA re-evaluation of E 445 and it does not feature as a parameter of importance in the current follow-up to that re-evaluation.

3.4. Biological and toxicological data

3.4.1. Summary of the biological and toxicological data of the EFSA opinion (EFSA ANS Panel, 2018)

A summary of the main conclusions for the biological and toxicological data from the assessment of the ANS Panel during the re-evaluation of E 445 (EFSA ANS Panel, 2018) is presented below.

"ADME studies have shown that ester bonds in GEWR are largely resistant to hydrolysis in the gut, the majority of orally applied GEWR being excreted unchanged in the faeces.

Only a small fraction, most likely the glycerol monoesters of wood rosin, seems to undergo hydrolysis. Studies with 14C-labelled GEWR in rats on excretion of radioactivity via faeces, bile, urine and exhaled air and disposition in the carcass gave evidence for a low absorption rate of ≤ 5% of the applied dose. Most of the absorbed radioactivity was eliminated in the bile and excreted via faeces. Results of HPLC analysis of faeces and bile suggested that absorbed components were hydrolysis products from glycerol monoesters of wood rosin present in GEWR.

A few ADME data were available for free resin acids which might be released in the gastro-intestinal tract after hydrolysis of glycerol monoesters or of esters with other alcohols in GEWR. Furthermore, GEWR contain a fraction of residual free resin acids. The experiments in rats with radiolabelled

dehydroabietic acid revealed an absorption rate of approximately 40% after oral exposure. Most of the radioactivity was excreted via the bile, minor amounts via the urine and only traces were exhaled. Tetrahydroabietic acid and isopimaric acid exhibited an excretion pattern similar to dehydroabietic acid.

No studies on acute oral toxicity of GEWR were available.

No treatment-related effects were detected in a 13-week feeding study conducted with GEWR originated from P. palustris *(longleaf pine) and* P. elliottii *(slash pine), representative of the food additive on the market, according to current standards after oral exposure of male and female F344 rats to 0, 625, 1,250 or 2,500 mg GEWR/kg bw per day. From this study, the Panel identified a no observed adverse effect level (NOAEL) of 2,500 mg GEWR/kg bw per day, the highest dose tested.*

GEWR, when tested as a mixture, did not show genotoxic potential in the core battery of adequately performed in vitro tests (i.e. bacterial reversion and in vitro micronucleus assays) recommended in the EFSA guideline on genotoxicity testing strategy (EFSA Scientific Committee, 2011*). Negative results were also obtained in limited in vitro chromosomal aberration and UDS assays. Positive results, of unclear biological significance, were only reported in a limited in vivo study. Based on the overall weight evidence, the Panel considered GEWR as non-genotoxic.*

When individual compounds from the residual free resin acids fraction, which accounts only for 2.3–2.8% of GEWR, were evaluated, an equivocal mutagenic outcome was only observed for neoabietic acid in limited bacterial and yeast mutation assays (Nestmann et al., 1979*; Nestmann and Lee,* 1983*). The Panel also noted that according to the information provided by one interested party levels of free neoabietic acid in GEWR are below the limit of quantitation of 0.05 wt. %. Overall, the Panel concluded that GEWR (E 445) containing free neoabietic acid below the limit of quantitation (0.05 wt. %) when used as a food additive are of no concern for genotoxicity.*

There were no studies available concerning chronic toxicity or carcinogenicity of GEWR.

There were no studies available on the endpoint reproductive and developmental toxicity of GEWR.

The Panel noted that, wood rosin has been classified as skin sensitiser (Karlberg et al., 1999*). However, there is evidence from experimental data that the esterification of rosins with glycerol reduces their skin allergenic activity (Shao et al.,* 1993*; Gaefvert et al.,* 1994*). Furthermore, data on allergic reactions on food containing GEWR have not been identified.*

In 1994, the SCF established an ADI of 12.5 mg/kg bw per day for GEWR from the NOAEL of 2,500 mg/kg bw per day from a 13-week study in rats by applying an UF of 200 to take into account the 90-day duration (SCF, 1994*). JECFA concluded in 1996 that the documented data on sub chronic oral toxicity studies and the studies confirming the non-bioavailability of GEWR were adequate to establish an ADI of 0–25 mg/kg bw per day for GEWR by applying an UF of 100 to the NOAEL of 2,500 mg/kg bw per day, although there were no studies available on chronic or reproductive toxicity (JECFA,* 1996b*). This ADI was confirmed in 2013 (JECFA,* 2013a*).*

Based on the overall toxicity database and given the absence of reproductive and developmental toxicity data, the Panel considered that the current ADI of 12.5 mg/kg bw per day for GEWR (E 445) as established by the SCF in 1994 should be temporary pending the provision of such data."

3.4.2. Toxicological data submitted

The following was requested in the European Commission call for data[8]:

The limitations in the toxicological database of GEWR (E 445) need to be decreased to allow EFSA to establish an ADI for this food additive.

- A reproductive and developmental toxicity study, in accordance with the applicable OECD test guidelines, should be conducted using a test material which is representative of the food additive present on the market and taking into account the recommendations made by EFSA for the update of the specifications.

As a result of the European Commission call for data, the IBOs submitted three studies described below and summarised in the tables in Appendix A.

Reproductive toxicity studies

An oral (dietary) combined repeat dose toxicity study with a reproduction/developmental toxicity screening test on test item 'Rosin, glycerol ester' was performed in rats (Documentation provided to EFSA No. 5). Information on the *Pinus* species from which the test item was derived was not provided.

The study was in accordance with OECD TG 422 (22 March 1996), and in compliance with GLP. Concentrations of 0, 3,000, 7,500 and 18,000 mg/kg diet (equal to 0, 181, 449 and 1,087 mg/kg bw

per day for males and 0, 229, 538 and 1,281 mg/kg bw per day for females, respectively, during the 14 days pre-pairing phase) were administered. The dietary concentration given to the high-dose females during gestation and lactation was decreased to 15,000 mg/kg diet to account for the expected increase in food consumption during these phases. Doses in high dose dams during gestation ranged from 1,087 to 1,167 mg/kg bw per day and during lactation (to PND 5) was equal to 1, 711 mg/kg bw per day.

No treatment-related systemic toxicity in male and female parental rats was observed up to 1,087 and 1,281 mg/kg bw per day, respectively. No reproductive or developmental toxicity occurred in parental animals or offspring treated until termination of the study on postnatal day 5, including no treatment-related effects on mating, fertility and parturition indices, parental organ weights and pathology or offspring macroscopic abnormalities. The Panel considered that the test item was not systemically toxic and did not adversely affect reproduction and development up to an oral dose of at least 1,087 mg/kg bw per day.

GEWR from *P. palustris* and *P. elliottii* was tested in a dietary reproduction/developmental toxicity screening study in rats, in accordance with OECD TG 421 (29 July 2016), and in compliance with GLP (Documentation provided to EFSA No 3). From the start of the study, 14 days before mating, the test substance was administered at concentration of 0, 0.8, 1.8 and 3.4%. Beginning on gestation day (GD) 20, the females in groups 2–4 were administered dietary concentrations at half the original concentrations (0, 0.4, 0.9 and 1.7%, respectively) to account for increased food consumption during the lactation period. Doses equal to 0, 414, 976 and 1,842 mg/kg bw per day for males and 0, 532, 1,184 and 2,347 mg/kg bw per day for females were achieved.

No reproductive or developmental toxicity was observed in male and female parental animals and offspring up to a dietary concentration of 1.8% of the test item (equal to 976 mg/kg bw per day for males and 1,184 mg/kg bw per day for females). All females in the control, low-and mid-dose groups became pregnant at the first or second *oestrus* after mating was initiated and produced viable litters at birth. The study authors concluded that there were no adverse effects at high dose. However, only 9 of the 12 females in the high-dose group (3.4%; equivalent to 1,842 mg/kg bw per day for males and 2,347 mg/kg bw per day for females) were pregnant. In one of these cases, the failure was likely due to the male, which was found to have small testes and epididymides at macroscopic evaluation and severe testicular atrophy at microscopic evaluation. In the other two cases, the reason(s) for the infertility remained unexplained. It is noted that mean absolute weight of testes and seminal vesicles were reduced at the high dose (not statistically significant). There were no treatment-related histopathological findings in testis by qualitative cell and spermatogenesis stage-dependent evaluation; sperm analyses were not conducted. Given the low number of animals used in screening studies (OECD TG 421), the possibility that there was an adverse effect on male fertility at the high dose of 1,842 mg/kg bw per day (above the standard limit dose of 1000 mg/kg bw per day, as indicated in OECD TG 421) cannot be excluded. Therefore, the Panel considered that the NOAEL in this study was 976 mg/kg bw per day.

It is noted that a previous study conducted in accordance with OECD TG 422, for which the test item source was not identified, reported no treatment-related effects on fertility or on absolute weight of testes and seminal vesicles up to the highest dose of 1,087 mg/kg bw per day; this is consistent with the lack of effects in the present study at the mid dose of 976 mg/kg bw per day in males.

Developmental studies

An oral dietary prenatal developmental toxicity study in rats was performed with 'Rosin, glycerol ester' (Documentation provided to EFSA No 6). Information on the *Pinus* species from which the test item was derived was not provided.

The study was in accordance with OECD TG 414 (22 January 2001) and in compliance with GLP. The test substance was administered at concentrations of 0, 3,000, 7,500 or 15,000 mg/kg diet (equal to 0, 241, 608 or 1,228 mg/kg bw per day, respectively) from GD 3–19.

Dietary exposure from before implantation (GD 3; time-mated rats) did not elicit maternal toxicity, and the number of implantations and of implants surviving to termination on GD 20 were unaffected up to a dose of 1,228 mg/kg bw per day. Litter size, fetal weights, placental weights, percentages of males and females, and pre- and post-implantations losses were comparable to control values in all treated groups. No test substance-related external, soft tissue or skeletal abnormalities were detected. The Panel considered that the test substance did not adversely affect pregnancy or *in utero* development up to an oral dose of 1,228 mg/kg bw per day.

Overall, the Panel considered that GEWR tested up to the limit dose of 1,000 mg/kg bw per day does not have any adverse effects on development and reproduction and is not systemically toxic.

The Panel noted that the test item used in the dietary reproduction/developmental toxicity screening study in rats (Documentation provided to EFSA No 3) was generated from *P. palustris* and *P. elliottii*.

According to the IBO, the test item in the prenatal developmental toxicity study and in the combined repeat dose toxicity study with reproduction/developmental toxicity screening test in rats (Documentation provided to EFSA No 11 and No 12) met the same substance identification profile as samples sourced from *P. halepensis* and *P. brutia* species. The Panel noted that the actual pine species used to derive the test item are not known and therefore cannot relate the outcome of the studies to a *Pinus* species.

4. Discussion

GEWR (E 445) was re-evaluated by the EFSA ANS Panel in 2018. Based on the toxicity database and given the absence of reproductive and developmental toxicity data at the time of the re-evaluation, the ANS Panel concluded that the ADI of 12.5 mg/kg bw per day for GEWR (E 445) as established by the SCF in 1994 should be temporary pending the provision of such data (EFSA ANS Panel, 2018). The conclusions of the assessment were restricted to GEWR derived from *P. palustris* (longleaf pine) and *P. elliottii* (slash pine) and with a chemical composition in compliance with GEWR used in the toxicological testing.

The data gaps and uncertainties identified by the ANS Panel during the re-evaluation of GEWR (E 445) required a follow-up by the European Commission by means of a subsequent call for additional data. The present opinion deals with the assessment of the data provided by IBOs.

With regard to the dietary exposure to the food additive, the Panel considered the exposure calculations for E 445 as presented in the re-evaluation of the food additive (EFSA ANS Panel, 2018). Since the ANS Panel noted that GEWR (E 445) is authorised and used in a certain type of flavoured drinks, to which consumers may be brand loyal, the refined brand-loyal scenario was considered as the most relevant exposure scenario for the safety evaluation of this food additive. For the current assessment, the highest exposure levels for the mean and 95th percentile among the different population groups were considered, i.e. 1.07 and 4.06 mg/kg bw per day, for toddler, respectively.

Differing data on toxic elements (lead, mercury, cadmium and arsenic) in samples of GEWR were submitted by IBOs (Section 3.2.1). One IBO proposed the following limits for these toxic elements: 1 mg/kg for arsenic, 1 mg/kg for cadmium, 2 mg/kg for lead and 1 mg/kg for mercury. A second IBO proposed 1 mg/kg for arsenic, 0.5 mg/kg for cadmium, 1 mg/kg for lead and 0.5 mg/kg for mercury. The Panel considered the potential presence of lead, cadmium, mercury and arsenic in E 445 at (i) at the current limit in the EU specifications and also; (ii) at the lowest limits proposed by the IBOs as 1 mg/kg for lead, 0.5 mg/kg for mercury, 0.5 mg/kg for cadmium and 1 mg/kg for arsenic (Table 5).

The potential exposure to these impurities from the use of E 445 was compared against the available HBGVs and RPs. The resulting figures (see Table 6) show that for arsenic, in both scenarios (i.e. (i) and (ii) mentioned above) the lower end of the range of calculated MOE values was below the target value of 1,000. For the other toxic elements (cadmium, mercury, lead), the limit values proposed by the IBO do not give rise to safety concerns.

The Panel noted that the maximum limits in the EU specifications for toxic elements should be established based on actual levels in the commercial food additive. Considering the results of the estimation calculated by the Panel (Table 6) and the fact that the food additive is not the only potential dietary source of toxic elements, the Panel recommended that the maximum limits be lowered on the basis of the information provided by the IBOs and on the considerations of the Panel (see Table 8).

The Panel considered that if the glycerol used for the manufacturing process of E 445 complies with the specifications of E 422 in Commission Regulation (EU) No 231/2012, as stated by the IBOs, there is no need for setting a specification limit in Commission Regulation (EU) No 231/2012 for the content of potential impurities in E 445 from the use of glycerol.

Data on the concentrations of the fractions of 'glycerol monoesters', 'free resin acids' and 'neutrals' in GEWR manufactured from *P. palustris* and *P. elliottii* were submitted by two IBOs (Section 3.2.1) and a summary is presented in Table 7 (Section 3.3.3). The Panel noted a substantial difference among the results of the concentration of glycerol monoesters determined in GEWR by applying three different

methods for the detection. GPC used by one of the IBO is a methodology predominantly based on size or approximate molecular weight, and it could overestimate the amounts of glycerol monoesters due to overlapping with other components with similar molecular weight or size. No quantitative information on the fraction of neutrals was submitted by one of the IBOs and it could not be compared with the data submitted by the other IBO. The concentration of free resin acids reported by both IBOs was in the same range. Therefore, based on the data submitted, the Panel was not able to propose a specific maximum concentration for the fractions of glycerol monoesters and neutrals that can define better the GEWR used as E 445. However, the Panel considered that the constituents of these fractions should be as low as technologically achievable.

Based on the information provided by the IBOs and the considerations presented in this assessment, the Panel recommended some modifications of the existing EU specifications, mainly a revision of the definition of the food additive E 445 as presented in Table 8.

In the call for data from EU Commission 'detailed information on the chemical composition of GEWR originating from *P. halepensis* and *P. brutia* (and potentially other pine species)', in particular on the concentrations of the toxicologically relevant fractions of glycerol monoesters, free resin acids and neutrals have been requested. The response, received from only one IBO, was limited to the total concentrations of the known fractions of toxicological relevance (see Section 3.2.4) and no further information to support read across was provided after request from EFSA (Documentation provided to EFSA No 11). The data submitted showed that the concentration of the fractions of neutrals and free resin acids were in the same range for GEWR originating from *P. halepensis* and *P. brutia* as for GEWR originating from *P. palustris* and *P. elliottii*. The concentration reported for the fraction of glycerol monoesters was significantly lower for GEWR originating from *P. halepensis* and *P. brutia*. Because the major constituents of the fractions were not identified, the Panel could not exclude the presence of constituents which might affect the toxicity of GEWR from *P. halepensis* and *P. brutia*. Therefore, the Panel considered that read across of toxicological data from GEWR from *P. palustris* and *P. elliottii* for the assessment of GEWR from *P. halepensis* and *P. brutia* was not possible.

GEWR was tested for effects on fertility and developmental toxicity according to OECD TG 421, 422 and 414. The Panel considered that GEWR tested up to the limit dose of 1,000 mg/kg bw per day does not have any adverse effects on development and reproduction and is not systemically toxic.

The Panel noted that only the test item used in the dietary reproduction/developmental toxicity screening study in rats (OECD TG 421) (Documentation provided to EFSA No 3) was identified as being generated from *P. palustris* and *P. elliottii*. According to the IBO, the test item in the prenatal developmental toxicity study and in the reproduction/developmental toxicity screening test in rats (OECD TG 414 and TG 422) (Documentation provided to EFSA No 11 and No 12) met the same substance identification profile as samples sourced from *P. halepensis* and *P. brutia* species. The Panel noted that the actual *Pinus* species used to derive the test item were not known, and therefore could not relate the outcome of the studies to a *Pinus* species.

In 1994, the SCF established an ADI of 12.5 mg/kg bw per day for GEWR from the NOAEL of 2,500 mg/kg bw per day from a 13-week study in rats by applying an uncertainty factor (UF) of 200 to take into account the 90-day duration (SCF, 1994). In the absence of reproductive and developmental toxicity data, the ANS Panel (2018) changed the ADI established by SCF to be a temporary ADI.

Considering the available toxicological database evaluated during the re-evaluation of GEWR (E 445) by the ANS Panel in 2018 and the studies submitted by the IBOs following the call for data to address the data gap for GEWR from *P. palustris* and *P. elliottii*, the Panel established an ADI of 10 mg/kg bw per day based on the NOAEL of 976 mg/kg bw per day from the newly available dietary reproduction/developmental toxicity screening study in rats and applying an uncertainty factor of 100. The NOAEL of 976 mg/kg bw per day is the lowest NOAEL in the available studies even of longer duration. Since GEWR from *P. palustris* and *P. elliottii* were tested in the toxicity studies considered to establish the ADI and in the absence of detailed information on the chemical composition (major constituents) in GEWR generated from other *Pinus* species, the ADI was restricted to the GEWR (E 445) manufactured from *P. palustris* and *P. elliottii*.

The Panel concluded that the mean and the high exposure levels (P95) of the brand-loyal refined exposure scenario and the maximum permitted level (MPL) scenario did not exceed the new ADI in any of the population groups from the use of GEWR (E 445) as a food additive.

5. Conclusions

The Panel concluded that the technical data provided by the interested business operator support an amendment of the specifications for GEWR (E 445) laid down in Commission Regulation (EU) No 231/2012, as presented by the recommendations made in Table 8.

The Panel established an ADI for GEWR (E 445) manufactured from *P. palustris* and *P. elliottii*. of 10 mg/kg bw per day based on the NOAEL of 976 mg/kg bw per day from a dietary reproduction/ developmental toxicity screening study in rats and applying an uncertainty factor of 100.

The Panel concluded that there is no safety concern for the use of GEWR (E 445), at either the maximum permitted levels or at the reported uses and use levels.

6. Documentation as provided to EFSA

1) Submission of data response to the European Commission call for technical data on the permitted food glycerol esters of wood rosin (GEWR) (E 445). Submitted by CARAGUM to the European Commission.
2) Submission of data response to the European Commission call for technical data on the permitted food glycerol esters of wood rosin (GEWR) (E 445). Submitted by Intertek to the European Commission.
3) A Dietary Reproduction/Developmental Toxicity Screening Study of Pinova Ester Gum 8BG in Rats, Study No. 1936–012, 2020. Submitted by Intertek to the European Commission.
4) Submission of data response to the European Commission call for technical data on the permitted food glycerol esters of wood rosin (GEWR) (E 445). Submitted by T&R Chemicals to the European Commission.
5) Oral (Dietary) Combined Repeat Dose Toxicity Study with Reproduction/Developmental Toxicity Screening Test in the Rat (OECD 422), study number 41302570; 2014. Submitted by Megara Resins to the European Commission.
6) Oral (Dietary) Pre-Natal Developmental Toxicity Study in the Rat, Study number 41403694, 2016. Submitted by Megara Resins to the European Commission.
7) Additional information submitted in response to a request from EFSA. Submitted by CARAGUM May 2022.
8) Additional information submitted in response to a request from EFSA. Submitted by T&R Chemicals May 2022.
9) Additional information submitted in response to a request from EFSA. Submitted by CARAGUM August 2022.
10) Additional information submitted in response to a request from EFSA. Submitted by Intertek November 2022.
11) Additional information submitted in response to a request from EFSA. Submitted by T&R Chemicals January 2023.
12) Additional information submitted in response to a request from EFSA. Submitted by T&R Chemicals March 2023.

References

EFSA ANS Panel (EFSA Panel on Food Additives and Nutrient Sources Added to Food), 2012. Guidance for submission for food additive evaluations. EFSA Journal 2012;10(7):2760, 60 pp. https://doi.org/10.2903/j.efsa.2012.2760

EFSA ANS Panel (EFSA Panel on Food Additives and Nutrient Sources added to Food), 2017. Scientific opinion on the re-evaluation of glycerol (E 422) as a food additive. EFSA Journal 2017;15(3):4720, 64 pp. https://doi.org/10.2903/j.efsa.2017.4720

EFSA ANS Panel (EFSA Panel on Food Additives and Nutrient Sources added to Food), 2018. Scientific Opinion on the re-evaluation of glycerol esters of wood rosin (E 445) as a food additive. EFSA Journal 2018;16(7):5370, 47 pp. https://doi.org/10.2903/j.efsa.2018.5370

EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), 2009a. Scientific Opinion on arsenic in food. EFSA Journal 2009;7(10):1351, 199 pp. https://doi.org/10.2903/j.efsa.2009.1351

EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), 2009b. Scientific opinion on cadmium in food. EFSA Journal 2009;7(10):980, 139 pp. https://doi.org/10.2903/j.efsa.2009.98

EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), 2010. Scientific Opinion on lead in food. EFSA Journal 2010;8(4):1570, 151 pp. https://doi.org/10.2903/j.efsa.2010.1570

EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), 2012. Scientific Opinion on the risk for public health related to the presence of mercury and methylmercury in food. EFSA Journal 2012;10(12):2985, 241 pp. https://doi.org/10.2903/j.efsa.2012.2985

EFSA FAF Panel (EFSA Panel on Food Additives and Flavourings), Younes M, Aquilina G, Castle L, Engel K, Fowler P, Frutos Fernandez MJ, Gundert-Remy U, Gürtler R, Husøy T, Manco M, Mennes W, Moldeus P, Passamonti S, Shah R, Waalkens-Berendsen I, Wölfle D, Wright M, Cheyns K, Mirat M, Rincon AM and Fürst P, 2022. Scientific Opinion on the follow-up of the re-evaluation of glycerol (E 422) as a food additive. EFSA Journal 2022;20 (6):7353, 18 pp. https://doi.org/10.2903/j.efsa.2022.7353

EFSA Scientific Committee, 2009. Guidance of the Scientific Committee on Transparency in the Scientific Aspects of Risk Assessments carried out by EFSA. Part 2: general Principles. EFSA Journal 2009;7(7):1051, 22 pp. https://doi.org/10.2903/j.efsa.2009.1051

EFSA Scientific Committee, 2011. Scientific Opinion on genotoxicity testing strategies applicable to food and feed safety assessment. EFSA Journal 2011;9(9):2379, 69 pp. https://doi.org/10.2903/j.efsa.2011.2379

EFSA Scientific Committee, 2012a. Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured data. EFSA Journal 2012;10(3):2579, 32 pp. https://doi.org/10.2903/j.efsa.2012.2579

EFSA Scientific Committee, 2012b. Scientific Opinion on the applicability of the Margin of Exposure approach for the safety assessment of impurities which are both genotoxic and carcinogenic in substances added to food/feed. EFSA Journal 2012;10(3):2578, 5 pp. https://doi.org/10.2903/j.efsa.2012.2578

Gäfvert E, Shao LP, Karlberg AT, Nilsson U and Nilsson JL, 1994. Allergenicity of rosin (colophony) esters (II). Glyceryl monoabietate identified as contact allergen. Contact Dermatitis, 31, 11–17. Available online: https://pubmed.ncbi.nlm.nih.gov/7924286/

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1996a. Toxicological Evaluation of Certain Food Additives. Glycerol ester of wood rosin. WHO Food Additive Series No. 37. Available online: https://inchem.org/documents/jecfa/jecmono/v37je01.htm

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1996b. Toxicological Evaluation of Certain Food Additives. Glycerol ester of wood rosin. WHO Food Additive Series No. 35. Available online: https://inchem.org/documents/jecfa/jecmono/v35je01.htm

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2000. Safety evaluation of certain food additives and contaminants. 976. Lead. WHO Food Additives Series No. 44, 39 pp. Available online: https://apps.who.int/iris/bitstream/handle/10665/44521/9789241660648_eng.pdf?sequence=1&isAllowed=y

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2013a. Evaluation of certain food additives and contaminants. Seventy-seventh report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series No. 983, 23 pp. Available online: https://apps.who.int/iris/bitstream/handle/10665/98388/9789241209830_eng.pdf?sequence=1&isAllowed=y

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2018. Residue Monograph prepared by the meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), 86th Meeting 2018. Glycerol ester of wood rosin. Available online: https://www.fao.org/3/ca3737en/ca3737en.pdf

Karlberg AT, Basketter D, Goossens A and Lepoittevin JP, 1999. Regulatory classification of substances oxidized to skin sensitizers on exposure to air. Contact Dermatitis, 40, 183–188. https://doi.org/10.1111/j.1600-0536.1999.tb06030.x

Nestmann ER, Lee EGH, Mueller JC and Douglas GR, 1979. Mutagenicity of resin acids identified in pulp and papermill effluents using the Salmonella/mammalian-microsome assay. Environmental Mutagenesis, 1, 361–369. https://doi.org/10.1002/em.2860010408

Nestmann ER and Lee EG, 1983. Mutagenicity of constituents of pulp and paper mill effluent in growing cells of Saccharomyces cerevisiae. Mutation Research, 119, 273–280. https://doi.org/10.1016/0165-7992(83)90172-0

SCF (Scientific Committee for Food), 1994. Opinion expressed 1992. Report of the Scientific Committee for Food.32nd Series. Food Science and Techniques, 37 pp. Available online: https://aei.pitt.edu/40841/1/32nd_food.pdf

Shao LP, Gäfvert E, Karlberg AT, Nilsson U and Nilsson JL, 1993. The allergenicity of glycerol esters and other esters of rosin (colophony). Contact Dermatitis, 28, 229–234. https://doi.org/10.1111/j.1600-0536.1993.tb03408.x

Abbreviations

ADI	acceptable daily intake
ADME	absorption, distribution, metabolism and excretion
ANS	EFSA Scientific Panel on Food Additives and Nutrient Sources added to Food
ASTM	American Society for Testing Materials
BMDL	benchmark dose (lower confidence limit)
bw	body weight
CAS	Chemical Abstracts Service
CONTAM	EFSA Panel on Contaminants in Food Chain

CVAA	cold vapour atomic absorption analyser
FAF	EFSA Scientific Panel on Food Additives and Flavourings
FAO	Food and Agriculture Organization of the United Nations
FCs	Food categories
GC	gas chromatography
GC–ECD	gas chromatography–electron capture detection
GD	gestation day
GCxGC–TOF MS	gas chromatography coupled with high-resolution time-of-flight mass spectrometry (GCxGC-TOF MS)
GEWR	glycerol esters of wood rosin
GLP	Good Laboratory Practice
GPC	gel permeation chromatography
HBGV	health-based guidance value
HPLC	high-performance liquid chromatography
IQ	intelligence quotient
IR	infrared
IBO	interested business operator
ICP-AES	inductively coupled plasma atomic emission spectroscopy
ICP-OES	inductively coupled plasma optical emission spectroscopy
ICP-MS	inductively coupled plasma mass spectrometry
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LOD	limit of detection
LOQ	limit of quantification
3-MCPD	3-monochloropropane diol
MOE	margin of exposure
MPL	maximum permitted level
MS	mass spectrometry
NOAEL	no observed adverse effect level
OECD	Organization for Economic Co-operation and Development
PND	postnatal day
RI	refractometer
RP	reference points
SC	Scientific Committee of EFSA
SCF	Scientific Committee on Food
SIM	selective ion monitoring
TIC	total ion current
TWI	tolerable weekly intake
UDS	unscheduled DNA synthesis
UF	uncertainty factor
UV	ultraviolet
WHO	World Health Organization

Appendix A – Summary tables of the toxicity data submitted in the call for data

Study ID	
Reference (title, number, year)	Oral (Dietary) Combined Repeat Dose Toxicity Study with Reproduction/Developmental Toxicity Screening Test in the Rat (OECD 422), study number 41302570; 2014 (Documentation provided to EFSA No 5)
Source (published/unpublished)	Unpublished
Overview of the study and guideline	
Good Laboratory Practice (yes/no)	Yes
Guideline studies (if yes, specify)	OECD 422, adopted 22 March 1996
Animal model	
Species and strain	Wistar Han™: RccHan™: WIST strain rats
Disease models (e.g. diabetes, allergy, obesity)	No
Housing conditions	
Housing condition	Groups of four at the beginning, in pairs during mating, individuals for female after successful mating
Diet name and source (if reported)	Certified Rodent Diet PMI 5002
Treatment	
Test material	Rosin, glycerol ester, CAS NO. 8050-31-5
Provider	
Compound purity	100%
Vehicle used	
Dose regimen (dose level or concentration per group, and frequency) and achieved doses if available	Dietary concentrations of 0, 3,000, 7,500 and 18,000 mg/kg diet (equal to a 0, 181, 449 and 1,087 mg/kg bw per day, respectively, for males and 229, 538 and 1,281 mg/kg bw per day, respectively, for females during the pre-pairing phase). The dietary concentration given to the high dosage females during gestation and lactation was decreased to 15,000 mg/kg diet to account for the expected increase in food consumption during these phases.
Route of administration (diet, drinking water, gavage)	Diet
Period of exposure (pre-mating, mating, gestation, lactation, adult)	Females: from pre-mating (14 days) to 5 days post-partum Male: from pre-mating (14 days) until day 43 or 44
Duration of the exposure	8–9 weeks
Study design	
Sex and age at the start of the treatment	12 weeks
Number animals/sex/group	12 males and 12 females in 4 groups
Measured endpoints	According to guideline
Time of measurement/observation period	According to guideline
Methods to measure the endpoints	According to guideline
Statistical analysis	
Statistical methods	Williams Test for parametric data or the Shirley Test for non-parametric data. If no dose response was found but the data shows non-homogeneity of means, the data were analysed by a stepwise Dunnett's (parametric) or Steel (non-parametric) test to determine significant difference from the control group. Where the data were unsuitable for these analyses, pair-wise tests were performed using the student t-test (parametric) or the Mann–Whitney U test (non-parametric).

Results	
Findings reported by the study author/s	No substance-related findings. One female in the high-dose group was not pregnant and one female in the control group appeared to be sub fertile, with total resorption of a litter that consisted of only one embryo. Females from all treatment groups showed a statistically significant increase in thyroid weight both absolute and relative to terminal body weight compared to the control, but here was no robust or statistically significant difference between the treatment groups. Furthermore, no histopathological correlates were evident in the thyroid, therefore the intergroup differences were considered not to be of toxicological significance. Males of the high-dose group showed a statistically significant increase in thymus weight both absolute and relative to terminal body weight. Females of the mid- and high-dose group and females of the mid-dose group also showed a statistically significant reduction in absolute and relative adrenal weight. The majority of individual values for these organs were within normal ranges for rats of the strain and age used and in the absence of any associated histopathological correlates the intergroup differences were considered not to be of toxicological significance. Females of the low-dose group showed a statistically significant increase in absolute and relative heart weight. In the absence of a similar effect in the mid- or high-dose group or any histopathological correlates, this finding was considered unrelated to the test item.
No observed adverse effect level, lowest observed adverse effect level, benchmark dose/benchmark dose lower bound	The no observed adverse effect level (NOAEL) for systemic toxicity and reproductive toxicity, was considered to be 1,087 and 1,281 mg/kg bw per day for males and females, respectively.
Further information	
Panel assessment: The Panel considered that the test item was not systemically toxic and did not adversely affect reproduction and development up to an oral dose of at least 1,087 mg/kg bw per day.	

bw: body weight; CAS: Chemical Abstracts Service.

Study ID	
Reference (title, number, year)	A Dietary Reproduction/Developmental Toxicity Screening Study in Rats, Study No. 1936-012, 2020 (Documentation provided to EFSA No 3)
Source (published/unpublished)	Unpublished
Overview of the study and guideline	
Good Laboratory Practice (yes/no)	Yes
Guideline study (if yes, specify)	OECD Guideline 421, adopted 29 July 2016
Animal model	
Species and strain	CD® [Crl:CD®(SD)] rats
Disease models (e.g. diabetes, allergy, obesity)	None
Housing conditions	
Housing condition	Individually housed, except during pairing, near parturition and during lactation
Diet name and source (if reported)	Meal Lab Diet ad libitum, tap water ad libitum
Treatment	
Test material	Glycerol ester of wood rosin (GEWR) (from *P. palustris* and *P. elliottii*)
Provider	Pinova, Inc.
Compound purity	100%
Vehicle used	Diet
Dose regimen (dose level or concentration per group, and frequency) and achieved doses if available	0, 0.8, 1.8 and 3.4 w/w%. Beginning on GD 20, the females in Groups 2 to 4 were administered dietary concentrations at half the original concentrations (0, 0.4, 0.9 and 1.7%, respectively) to account for increased food consumption during the lactation period. Calculated doses for males: 0, 414, 976 and 1,842 mg/kg bw per day Calculated doses for females: 0, 504, 1,208 and 2,211 mg/kg bw per day (premating); 0, 520, 1,167 and 2,366 mg/kg bw per day (pregnancy); 0, 544, 1,237 and 2,446 mg/kg bw per day (lactation).
Route of administration (diet, drinking water, gavage)	Diet
Period of exposure (pre-mating, mating, gestation, lactation, adult)	Female: from pre-mating (14 days) to lactation (LD 13). Male: from pre-mating (14 days) to euthanasia.
Duration of the exposure	50 days
Study design	
Sex and age at the start of the treatment	12 males and 12 females per group (total 48 male and 48 female) age 9 weeks at least
Number animals/sex/group	12 males and 12 females for 4 groups
Measured endpoints	According to guideline
Time of measurement/observation period	According to guideline
Methods to measure the endpoints	According to guideline
Statistical analysis	
Statistical methods	Group pair-wise comparisons (ANOVA); Fisher's Exact Test with Stepdown Sidak Adjustment for few fertility indexes

Results	
Findings reported by the study author/s	Nine of the 12 females in the high-dose group (3.4%; equivalent to 1,842 mg/kg bw per day for males and 2,347 mg/kg bw per day for females) were pregnant. In one of these cases, the failure was likely due to the male, which was found to have small testes and epididymides at macroscopic evaluation and severe testicular atrophy at microscopic evaluation. In the other two cases, the reason(s) for the infertility remained unexplained. These findings were considered not to be treatment-related by the authors.
No observed adverse effect level, lowest observed adverse effect level, benchmark dose/benchmark dose lower bound	Based on the absence of any test item-related effects, the no-observed-effect-level (NOEL) for reproductive and developmental toxicity, as well as general toxicity, was considered by the authors to be 3.4% (the highest concentration tested). This dietary concentration was equivalent to mean doses of 1,842 mg/kg bw per day for males and from 2,211 to 2,446 mg/kg bw per day for females.
Further information	
Mis-sexing of single pups was noted in two litters each of the control and the low-dose group. This does not affect the validity of the study.	
The Panel considered that due to lack of pregnancy in two females at the high dose, a more conservative NOAEL of 976 mg/kg bw per day (mid dose).	

bw: body weight; CAS: Chemical Abstracts Service.

Study ID	
Reference (title, number, year)	Rosin, glycerol ester CAS 8050-31-5: Oral (Dietary) Pre-Natal Developmental Toxicity Study in the Rat, Study number 41403694, 2016 (Documentation provided to EFSA No 6)
Source (published/unpublished)	Unpublished
Overview of the study and guideline	
Good Laboratory Practice (yes/no)	Yes
Guideline studies (if yes, specify)	OECD 414, adopted 22 January 2001
Animal model	
Species and strain	Sprague–Dawley Crl:CD® (SD) IGS BR strain rats
Disease models (e.g. diabetes, allergy, obesity)	No
Housing conditions	
Housing condition	Individually
Diet name and source (if reported)	Rodent PMI 5002 Ground diet and tap water *ad libitum*
Treatment	
Test material	Rosin, glycerol ester CAS 8050-31-5
Provider	
Compound purity	100%
Vehicle used	–
Dose regimen (dose level or concentration per group, and frequency) and achieved doses if available	0, 3,000, 7,500 or 15,000 mg/kg diet, equal to 0, 241, 608 or 1,228 mg/kg bw per day, respectively
Route of administration (diet, drinking water, gavage)	Diet
Period of exposure (pre-mating, mating, gestation, lactation, adult)	Between gestation days 3 and 19 (inclusive)
Duration of the exposure	17 days
Study design	
Sex and age at the start of the treatment	Female, adult, time-mated
Number animals/sex/group	24/female/group, 4 groups
Measured endpoints	According to the guideline
Time of measurement/observation period	According to the guideline
Methods to measure the endpoints	According to the guideline
Statistical analysis	
Statistical methods	Shapiro–Wilk normality test and Bartlett's test for homogeneity of variance and one way analysis of variance, followed by Dunnett's multiple comparison test or if unequal variances were observed. Kruskal–Wallis non-parametric analysis of variance; and a subsequent pairwise analysis of control values against treated values using the Mann–Whitney 'U' test, where significance was seen.
Results	
Findings reported by the study author/s	No treatment-related findings. Absent renal papilla was noted in one fetus in the intermediate dose and in two foetuses (from different litters) in the high-dose group. These findings are reported to be within the range of historical controls.
No observed adverse effect level, lowest observed adverse effect level, benchmark dose/benchmark dose lower bound	The no observed adverse effect level' (NOAEL) for the pregnant female and for prenatal developmental toxicity was considered to be 15,000 mg/kg diet equal to 1,228 mg/kg bw per day.

Further information
Panel assessment: The Panel considered that the test substance did not adversely affect pregnancy or in utero development up to an oral dose of 1,228 mg/kg bw per day.

bw: body weight; CAS: Chemical Abstracts Service.