Safety Assessment: Outcome of assessment of the extension of use of mono- and diglycerides (E 471)

Reference number RP1057

Food Standards Agency (FSA) and Food Standards Scotland (FSS)

Regulated Product Dossier Assessment

Assessment finalised: 19/09/2023

Summary

An application was submitted to the Food Standards Agency (FSA) and Food Standards Scotland (FSS) in April 2021 from Apeel Sciences, USA ("the Applicant") for the authorisation of an extension of use authorisation for mono- and di- glycerides of fatty acids to include a number of fruits. This Application is being considered within the food additives regime.

The Applicant intends to apply mono- and diglycerides of fatty acids to form a lipid-based layer to extend the quality and shelf life of produce by regulating moisture loss and oxidation using their product, Edipeel. The application is seeking to extend the usage of mono- and diglycerides within the food category: 4.1.1 'Entire fresh fruits and vegetables' to include passionfruit, kiwi, apples, pears, stone fruit (peaches, nectarines and plums), cherries, berries (strawberries and blueberries), cucumbers, asparagus, tomatoes, and peppers at the level of *quantum satis*.

To support the FSA and FSS in evaluating the dossier the Additives, Enzymes and other regulated products Joint Expert Group (AEJEG) were asked to provide a partial risk assessment on the information provided within the dossier and the supplementary information from the applicant. The AEJEG concluded that mono- and diglycerides of fatty acids were safe under the proposed conditions of use. The Committee on Toxicity (COT) also reviewed the AEJEG safety assessment agreeing with the conclusions of the AEJEG.

The views of the AEJEG and COT have been presented below within this safety assessment which represents the opinion of the FSA and FSS on this extension of use of mono- and diglycerides.

1. Introduction

The FSA and FSS have reviewed the partial safety assessment of the AEJEG for the extension of use of mono- and diglycerides of fatty acids (E 471) from Apeel Sciences, USA ("the Applicant") under food additives legislation. The Applicant intends to extend the quality and shelf life of produce by regulating moisture loss and oxidation using their product, Edipeel. To support the risk assessment by FSA and FSS, the AEJEG provided advice to the FSA and FSS, outlined in this opinion.

The dossier was evaluated on behalf of the FSA and the FSS by the AEJEG. In line with Article 3 of retained EU regulation 1331/2008 (REUL 1331/2008), the assessment has considered the aspects of the food additive and its extension of use. This, and the guidance put in place by EFSA for food additive applications, has formed the basis and structure for the assessment (EFSA, 2012).

With thanks to the members of the AEJEG during the course of the assessment who were; Dr Allain Bueno, Dr Claude Lambré, Dr Martin Rose, Dr Olwenn Martin and Professor Qasim Chaudry.

Following the review by the AEJEG at their meeting in February 2022, further information was requested from the Applicant concerning the justification for the proposed maximum level and the sources of the mono- and diglycerides of fatty acids, in order to address information gaps in the dossier and complete the risk assessment.

In addition, following further review by the COT at their meeting in March 2022, further information was requested from the Applicant regarding the applicability of the chosen method to determine particle size.

This document presents the conclusions of the AEJEG assessment on the safety of the extension of use of mono- and diglycerides of fatty acids in food category 4.1.1 to include passionfruit, kiwi, apples, pears, stone fruit (peaches, nectarines and plums), cherries, berries (strawberries and blueberries), cucumbers, asparagus, tomatoes, and peppers at the level of *quantum satis*.

Quantum satis is defined within REUL 1333/2008 as where no maximum numerical level is specified and substances shall be used in accordance with good manufacturing practice, at a level not higher than is necessary to achieve the intended purpose and provided the consumer is not misled.

2. Assessment

2.1 Identity and characterisation

E471 consists of a mixture of saturated 1- and 2- monoacylglycerides. Common components within E471 utilised for surface treatment include 1-monopalmitin and 2-monopalmitin. 1- and 2- monoacylglycerides can also be combined with other saturated diacylglycerides of various chain lengths to create E471. Regardless of these steps, the product meets the specifications for E471 set out in Regulation (EC) No. 231/2012 (as retained EU legislation) and supplied within this application. Three batch analyses were also provided which corroborate this claim.

The current EU and UK Specifications, as provided by the Applicant are listed in Table 1, the reference to JECFA vol.4 refers to JECFA (2006), the combined compendium of food additive specifications, volume 4.

Table 1: EU specifications for E 471 as provided by the Applicant, which are applicable within the United Kingdom as retained EU legislation

Parameter	Specifications for E 471
Description	
Physical Appearance	Pale yellow to pale brown oily liquid; white or slightly off-white hard waxy solid (flakes, powders or small beads)
Definition	
Mono- and diesters	Not less than 70 %
Identification	
Infrared absorption spectrum	Characteristic of a partial fatty acid ester of a polyol
Test for glycerol	Passes the test (JECFA vol. 4)

Table 2 continued: EU specifications for E 471 as provided by the Applicant, which are applicable within the United Kingdom as retained EU legislation

Parameter	Specifications for E 471
Test for fatty acids	Passes the test (JECFA vol. 4)
Solubility**	Insoluble in water, soluble in ethanol and toluene at 50 °C
Purity* **	
Water content	Not more than 2 % (Karl Fisher method)
Acid value	Not more than 6
Free glycerol	Not more than 7 weight %
Polyglycerols	Not more than 4 % diglycerol and not more than 1 % higher polyglycerols both based on total glycerol content
Total glycerol	Not less than 16 % and not more than 33 %
Arsenic*	Not more than 3 mg/kg
Lead*	Not more than 2 mg/kg
Cadmium*	Not more than 1 mg/kg
Mercury*	Not more than 1 mg/kg
Sulphated ash	Not more than 0.5 % determined at 800 ± 25 °C

* – Tested on a semi-annual basis

** – Approximate solubility is interpreted according to the descriptive terms presented in the JECFA (Joint FAO/WHO Expert Committee on Food Additives) general method for solubility (JECFA, 2006).

*** – In the EU specifications, purity criteria apply to the additive free of sodium, potassium, and calcium salts, however these substances may be present up to a maximum level of 6 % (expressed as sodium oleate).

2.2 Particle Size Information

The Applicant does not expect the particle size to exceed 50% nano-particles and hence it is not considered a nano-material. The Applicant stated that the ‘average particle diameter of E471 additive, when dispersed in water, is greater than 100

nanometers (nm) in any direction, as measured using small-angle X-ray scattering (SAXS) with instruments that can measure sufficiently small scattering angles (< 0.005 Å-1). This was corroborated by transmission electron microscopy (TEM) measurement.'

The Applicant provided further information on the use of transmission electron microscopy paired with smalls angle X-ray scattering as a method of assessing particle size. The Applicant discussed how a high shear mixing in water results in the dispersion of E471 particles within the liquid-state. Due to this, special techniques are required to accurately measure the particle size of the additive. This entails directly observing the additive E471 in the liquid-state under cryogenic conditions. This is known as cryo-TEM. Image analysis software such as ImageJ (NIH) can then be used to assess the physical properties of the additive. When the instrument can achieve a sufficiently small scattering angle (q < 0.005 Å-1), small angle X-ray scattering (SAXS) can be used to measure physical properties of a material in a liquid state including E471 in aqueous solution. SAXS when coupled with statistical modelling software (Igor) can be used to corroborate the results of cryo-TEM. The Applicant reported the diameter of E471 recorded by TEM as 135.6 ± 36.1 nm whilst via SAXS the diameter was reported as 129.6 nm ± 29.8 nm. The Applicant reported that the vesicle size reported by SAXS is consistent with the result produced by cryo-TEM.

The Applicant claimed that both TEM and SAXS are recognized by the European Commission's 2019 Joint Research Centre Science for Policy Report as acceptable methods for measurement of particle size (Rauscher et al., 2019).

The Applicant discussed that upon deposition of E471 on fresh produce a solid coating is formed and the thickness of the coating can be measured through more appropriate methods such as scanning electron microscopy (SEM). The Applicant stated that 'Direct imaging via SEM of a fractured coating can be further analyzed using image analysis software, such as ImageJ (NIH). Analysis of images gathered through SEM, show film thicknesses between 330 nm and 1.3 microns in thickness after deposition'.

The AEJEG further considered that mono- and di- glycerides of fatty acids are easily digestible therefore a nanoparticle assessment would not be required.

2.3 Manufacturing Process

The Applicant provided an overview of the manufacturing processes as indicated within EFSA's 2017 re-evaluation on the safety of E471 (EFSA, 2017). Due to the risk of presence of unregulated contaminants which can be produced within different processes, a more detailed description of the manufacturing process was requested by FSS/FSA.

The Applicant stated that 'Apeel Sciences commercially sources or manufactures E471 to create fruit and vegetable coatings. In either case, E471 is produced from the existing process to manufacture mono- and diglycerides: by heating vegetable oils or fats with excess glycerol, or by direct esterification of glycerol with fatty acids. The proportion of monoester is dependent on the proportion of glycerol and on the temperature of reaction. It is usually in the range of 30–60%. Mono-and diglycerides with more than 90% monoester content are produced by high vacuum distillation or other techniques (EFEMA, 2019).'

E471 is manufactured according to current Good Manufacturing Practices (GMP) for food. Where solvents are required in the process, only food grade solvents are used. Where a definition of "food grade" has been established by the Food Chemicals Codex (FCC) or JECFA, only reactants or solvents complying with the monograph are used. Where no monograph has been published, American Chemical Society (ACS) or United States Pharmacopeia (USP) reagent grade standards are used to establish food grade specifications.

The final E471 coating formulations may be comprised entirely of mono- and diglycerides of fatty acids manufactured by Apeel Sciences, a combination of Apeel Sciences-manufactured and commercially sourced mono- and diglycerides of fatty acids, or solely of commercially sourced mono- and diglycerides. Regardless of whether the mono- and diglycerides are manufactured by Apeel Sciences or commercially sourced, all final formulations will meet the EU specifications for E471.'

Analytical information was provided by the Applicant to corroborate this statement and the AEJEG was satisfied that E471 in the present Application meets the specifications as laid out in Table 1 and the relevant retained EU regulation.

Information on the source of the mono-and diglycerides (E471) relevant to this Application has been provided which includes typical food oils.

2.4 Presence of Impurities

The Applicant stated that E471 complies with the specifications set out in the retained EU Regulation No. 231/2012. Three batch analyses were also provided to corroborate this claim. Based on this information, contaminants were present at levels compliant with the relevant regulation. The Applicant provided information on the methods of analysis used to quantify the presence of the various contaminants of interest, presented in Table 2.

Table 3: Analytical Methods of Detection for E471 Analysis

Parameter	**Test Method***
Physical appearance	Apeel TM-0004 (Visual inspection)
Mono- and diesters	Apeel TM-0029 (GC-FID)
Infrared absorption spectrum	Apeel TM-0028 (FTIR)
Test for glycerol	Satisfied by Apeel TM-0029
Test for fatty acids	Satisfied by Apeel TM-0019
Solubility	Solubility, JECFA vol. 4 (General Methods: Appearance and Physical Properties) (JECFA, 2006)
Total glycerol	Calculated from Apeel TM-0029
Free Glycerol	Apeel TM-0029 (GC-FID)
Polyglycerols	Apeel TM-0030 (GC-FID)
Sulphated ash	<USP 281> Eurofins Method: ROI_S United States Pharmacopeia, Twenty-ninth Revision, , United States Pharmacopeial Convention, Inc.: Rockville, Maryland (2005). United States Pharmacopeia, 41st Revision - National Formulary 36th Edition. USP Convention. Rockville, MD (2017) (modified).
Acid value	Apeel TM-0019 (Titration based on DIN EN ISO 660)

Parameter	Test Method*
Water content	Apeel TM-0012 (Karl Fischer titration based on ASTM D6304-16e1)
Arsenic	Apeel TM-0021 (ICP-OES)
Mercury	Apeel TM-0021 (ICP-OES)
Lead	Apeel TM-0021 (ICP-OES)
Cadmium	Apeel TM-0021 (ICP-OES)

* – Apeel TMs are test methods that are developed in-house and validated or verified according to USP <1225> and <1226>.

The AEJEG was satisfied with the information provided by the Applicant and considered that based on the information provided, the presence of contaminants would not pose a risk to health.

2.5 Methods of analysis in food

Regarding methods of analysis in food the Applicant stated that: 'To extract the E 471 from the surface, treated fruits or vegetables are repeatedly rinsed 10 times each with a total of 10 mL of dichloromethane (DCM). The 10 mL of DCM is re-collected after each rinse and reused for the subsequent rinse on the same produce. A rinse of the next produce within a given group is then conducted using 10 mL of fresh DCM. The resulting DCM solution collected from each individual produce is then analyzed by UPLC-ELSD by dilution of the DCM solution into methanol.'

2.6 Stability of the additive

E471 is considered physically and chemically stable when dry and under ambient conditions. The Applicant discussed potential degradation of the product due to extremely elevated temperatures or moisture commenting that with the production of free glycerol there would be no safety concerns. The applicant further discussed how dissolving the additive into water or aqueous, ethanolic solutions for prolonged periods can promote partial conversion of E471 to free fatty acids and glycerol which does not impact safety.

2.7 Stability of the additive on food

The Applicant provided their own stability study demonstrating no chemical change of the product on food at room temperature. The Applicant commented that hydrolysis of E471 to glycerol and fatty acids may occur during cooking which would

not pose any safety hazard to the consumer. The AEJEG considered the information provided satisfactory.

2.8 Existing authorisations and risk assessments

The Applicant discussed previous authorisations and risk assessments performed by the European Food Safety Authority (EFSA) and the Joint FAO/WHO Expert Committee on Food Additives (JECFA).

EFSA concluded in 2017 that there was no need to set a numerical Acceptable Daily Intake (ADI) concentration and that there was no risk to health for the European population at current levels. E471 is widely used for purposes such as an emulsifier, starch complexing agent and aerating agent. As a Group 1 food additive it is authorised for use in all categories where Group 1 additives are authorised. In 2019 it was authorised for the surface treatment of some fruits and vegetables within the category 4.1.1 ‘Entire fruits and vegetables’.

JECFA concluded in 1973 that there is no risk to human health from exposure stating that ‘mono- and di-glycerides differed little from food so that their use need not to be limited’ (JECFA, 1974a). JECFA set a ‘not limited’ ADI (JECFA, 1974b).

The applicant discussed a report published by The Federation of the American Societies for Experimental Biology (FASEB, 1975) in which the safety data for partial mono- and di-acyl glycerides had been reviewed, concluding they are safe.

Within the United States E471 is generally recognised as safe (GRAS) to eat as an additive under Title 21 of the US Code of Federal Regulations. Mono- and di-acyl glycerides are not limited in their usage apart from at levels consistent with good manufacturing practice and can be used within food for multiple purposes. In addition, in the United States E471 is used as a surface finishing agent, again at levels consistent with good manufacturing practice.

2.9 Proposed use levels

E471 is currently authorised as a surface coating on the fruit and vegetables citrus fruit, melons, pineapples, bananas, papayas, mangoes, avocados and pomegranates. The Applicant requested an extension within the food category 4.1.1. (Entire fresh fruit and vegetables) to include the further fruits and vegetables: passionfruit, kiwi, apples, pears, stone fruit (peaches, nectarines, and plums), cherries, berries (strawberries and blueberries), cucumbers, asparagus, tomatoes, and peppers at levels of *quantum satis*. The Applicant noted that the maximum use level proposed at 1,520 mg/ kg fruit or vegetables is 5-30 times greater than normal use levels. The Applicant's normal and illustrative estimated degree of overestimation of usage are provided in Table 3.

Table 4: Illustrative normal and potential degree of overestimation of E471 applied to the surfaces of certain fruits and vegetables

Fruit or Vegetable	Normal Use Level for Applied E471 (g E471 per 100 kg crop)	Degree of Overestimation (Maximum Use Level * ÷ Normal Use Level)
Cucumbers	12	13
Asparagus	27	6
Tomatoes	10	15
Peppers	10	15
Apples	11	14
Pears	11	14
Strawberries	21	7
Passion fruit	8	19
Peaches	5	30
Nectarines	5	30
Kiwi	15	10
Blueberries	30	5
Plums	5	30
Cherries	25	6

*152 grams of E471 per 100 kg is the highest application load of any crop. To best illustrate the safety of E471 as a food additive, this value is assumed to be the maximum use level for all fruits and vegetables in the dietary exposure assessment described in the subsequent sections.

The AEJEG agrees that the large degree of overestimation between the maximum levels and the normal use levels and the Applicant taking forward the maximum use level for their exposure assessment increased the conservative nature of the safety assessment.

Concerns were raised on the introduction of increased fat to the diet from this additive however this was deemed beyond the remit of the AEJEG.

The AEJEG requested clarification on the technical reasons and origin of the proposed maximum use level. The Applicant provided an excerpt from the proposed usage levels section of an extension of use request to the European commission from 2017. This included a statement that: 'These loadings of Edipeel® are atypically elevated (i.e. 3 to 30 times higher than what is typically used for a given crop) but are used in the subsequent dietary intake calculations as an overestimation of potential exposure to Edipeel®'. As mentioned above, the AEJEG considered that the use of the maximum proposed level for the exposure assessment increased the conservative nature of the exposure assessment.

2.10 Dietary exposure assessment

EFSA revaluated the safety of E471 in 2017. In this re-evaluation they performed an exposure assessment. This assessment was used as a basis for the Applicant's own assessment for exposure to existing and proposed uses.

The food categories considered for the exposure assessment were those for which E471 is authorised. The Applicant listed these as:

- Using the most detailed level possible in the nomenclature of the EFSA Comprehensive European Food Consumption Database (FoodEx classification system).
- Excluding food categories for which restrictions/exceptions were not referenced in FoodEx, i.e., categories 1.7.6 Cheese products (reclassified under 01.7.5); 2.3 Vegetable oil pan spray; 6.6 Batters; 6.7 Precooked or processed cereals; 8.3.3 Casings and coatings and decorations for meat;

12.1.2 Salt substitutes; 12.2.4 Fruit wine and made wine; 14.2.5 Mead; 14.2.7.2 Aromatised wine-based drinks; 14.2.7.3 Aromatised wine/product cocktails.

- Considering the whole food categories, even if restrictions/exceptions apply, for 7.1. Bread and rolls, except products in 7.1.1 and 7.1.2; 8.3.2 Heat-treated processed meat, except foie gras, foie gras entier, blocs de foie gras, libamaj , libamaj egeszben; 13.1.3 Processed cereal-based foods and baby foods for infants and young children as defined by Directive 2006/125/EC only biscuits and rusks, cereal-based foods, baby foods; 13.1.4 Other foods for young children, only when sold as powder; 13.1.5.1 Dietary foods for infants for special medical purposes and special formulae for infants from birth onwards in specialised diets, particularly those devoid of proteins; 13.1.5.2 Dietary foods for babies and young children for special medical purposes as defined in Directive 1999/21/EC from birth onwards in specialised diets, particularly those devoid of proteins; 17.0 Food supplements.
- Foods belonging to the general food category 18, Processed foods not covered by categories 1–17, excluding foods for infants and young children, e.g., processed foods, prepared or composite dishes as well as food items under FCs 13.2, 13.3 and 13.4, were reclassified under food categories in accordance to their main component and included as such in the exposure assessment. The use levels available for FCs 13.2, 13.3, 13.4 and 18 were not considered in the exposure assessment.

Category 4.1.1 (Entire fruits and vegetables) is not included in the exposure assessment conducted by EFSA in 2017 as the authorisation for these products was granted in 2019.

In the refined scenario, 24 additional food categories were not taken into account as no use levels were provided. 10 further categories were not taken into account as they were provided by a food additive producer and not deemed suitable for this exposure assessment. The forementioned categories were however included in the maximum level exposure scenario. For remaining food categories the refinements

considering the restrictions/exceptions as set in Annex II to Regulation (EC) No 1333/2008 (as retained EU law) were applied.

In the regulatory maximum level exposure scenario, 41 food categories were included and for the refined (brand loyal and non-brand loyal) scenarios 31 categories were included. Compared to this scenario, three additional food categories (17.1, 17.2 and 17.3) were included in the food supplement scenario whilst two additional categories (13.1.5.1 and 13.1.5.2) were included.

Two sets of concentration data were used in EFSA's assessment. Maximum permitted levels (MPLs) based upon levels set in retained EU legislation or maximum reported concentrations provided to EFSA by industry where E471 is authorised for use at levels *quantum satis*. EFSA considered that these exposure estimates were the most conservative possible assuming that populations will be exposed to MPLs for over a long period of time.

Reported use levels (defined as in the refined exposure assessment scenario) include:

a) Brand loyal scenario: Conducted under the assumption that consumers will be exposed to E471 over a long time period at maximum permissible levels for one food category. Calculated by:

    - Combining food consumption with the maximum of the reported use levels for the main contributing food category at the individual level.
    - The mean of typical reported use levels for remaining food categories.

b) Non-loyal brand scenario: Conducted under the assumptions that consumers will be exposed long term to E471 at mean levels reported in food. This exposure assessment used the mean of the typical reported use levels for all food categories.

In the conservative MPL scenario mean exposure ranged from 36 mg/kg bw/day in adolescents to 432 mg/kg bw/day in infants. For 95th percentile consumers exposure ranged from 86 mg/kg bw/day to 845 mg/kg bw/day in infants.

For the refined, brand loyal scenario mean exposure ranged from 23 mg/kg bw/day in adolescents to 252 mg/kg bw/day in children. For the 95th percentile consumers exposure ranged from 61 mg/kg bw/day in adolescents to 620 mg/kg bw/day in toddlers.

In the non-brand loyal scenario mean exposure to E471 ranged from 9 mg/kg bw/day in adults and the elderly to 69 mg/kg bw/day in toddlers. For 95th percentile consumers exposure ranged from 18 mg/kg bw/day in the elderly to 128 mg/kg bw/day in toddlers.

Regarding specific scenarios performed by EFSA, in a refined scenario accounting for foods for special medical purposes, mean exposures ranged for infants from 42-67 mg/kg bw/day for infants and 22-66 mg/kg bw/day for toddlers. For 95th percentile consumers the exposures ranged from 84-123 mg/kg bw/day for infants and 80-123 mg/kg bw/day for toddlers. For food supplement consumers only, in the brand loyal scenario mean exposure to E471 was 26-100 mg/kg bw/day for children and 21-33 mg/kg bw/day in adults. For 95th percentile consumers it ranged from 64-124 mg/kg bw/day for children and 52-66 mg/kg bw/day for adults.

EFSA did not include exposure to mono- and di-acyl glycerides from the natural diet as quantifying these levels was not possible. EFSA concluded however, that exposure to E471 can be compared to fat consumed due to similarities in the metabolic breakdown products between mono- di- and tri- glycerides. The lower bound reference of the reference intake for fat is 20% energy and the upper bound 35% (EFSA, 2010). Using this information, the intake of fat would range from 741 to 1,296 mg/kg bw, assuming 2,000 kcal/day as the reference energy intake for adults (Retained Regulation (EU) 1169/2011 on food information to consumers) and a body weight of 60 kg.

The Applicant provided an example of how for adults in the non-brand loyal scenario at mean exposure levels of 10-26 mg/kg bw/day and 21-58 mg/kg bw/day at the 95th percentile of exposure this would translate to 0.8%-3.5% and for 95th percentile a maximum 7.8% contribution to an adult's daily recommended fat intake.
With regards to the main contributing food groups, the Applicant stated that 'EFSA received reported use levels of E471 in foods from industry in response to a public call for data. This data is presented in Appendix A to the 2017 EFSA Re-evaluation of mono- and di-glycerides of fatty acids (E471) as a food additive. The highest mean of the typical use levels of E471 occur in the following food categories: vegetable oil pan spray (100,000 mg/kg), cocoa and chocolate products as covered by Directive 2000/36/EC (43,725 mg/kg), other creams (27,342 mg/kg), processed nuts (25,000 mg/kg), and chewing gum (20,000 mg/kg). According to the Mintel's Global New Products Database, and as reported by EFSA (2017), E471 was labelled on 33,090 food and beverage products as an ingredient in the EU. The main foods were chilled desserts, edible ices, bread and bread products, sandwiches/wraps, baking ingredients and mixes, pasta (including fresh and dry pasta, gnocchi and stuffed pasta), and margarines. The percentage of food and beverage products per food subcategory that are labelled with E471 ranged from less than 0.1% to about 75% (in the case of "dairy based ice cream & frozen yoghurt" subcategory).'

The Applicant further commented that: 'Some methods of making fruit and vegetable juices involve removing the peels, in which case, it is expected that the product will remain on the peel and will not be present in the juice extracted from these fruits and vegetables. However, we recognize that it is possible for juice made by masticating fruits and vegetables to contain elements of the peel. In such situations, we expect that the E471 would be carried over into the fruit or vegetable juice with the peel. As such, the dietary exposure assessment provided in Section 2.2.8.2 of the dossier dated April 20, 2021 uses a conservative approach that assumes that in all cases, the peel is consumed—even for fruits and vegetables where the peel is not typically consumed or where the peel is non-edible. Additionally, considering that the JECFA considered in its 17th report in 1973 that "mono-and di-glycerides differed little from food so that their use need not to be limited" (JECFA, 1974a), and an ADI "not limited" had been thus established by JECFA (JECFA, 1974b), the potential for carryover from the peel is not of concern.'

After reviewing the information supplied by the Applicant regarding EFSA's 2017 application the AEJEG considered that the assessment was satisfactory to act as a basis for a further exposure assessment of the proposed extension of use.

2.11 Product usage as a surface treatment for fruits and vegetables

The applicant proposed a maximum use level of 1,520 mg E471 /kg fruit or vegetables which is used as a basis of dietary exposure from this new proposed use for any fruit and vegetable. The maximum use level was described as 5-30 times higher than the amount of E471 required to achieve the desired technological effect as shown in Table 2. The AEJEG considered that this would produce a conservative result when in comparison to normal use levels.

The exposure assessment performed by the Applicant utilising the Food Additives Intake Model (FAIM version 2) is summarised below in Table 4.

Table 5: Total estimated dietary exposure to E471 when used at maximum levels on unprocessed fruits and vegetables (Category 4.1 – Unprocessed Fruit and Vegetables) Using FAIM Tool Version 2

Age Group	Exposure Range for Mean Consumption Across Dietary Surveys (mg/kg bw/day)		Exposure Range for 95th Percentile Consumption Across Dietary Surveys (mg/kg bw/day)	
	Min	Max	Min	Max
Infants	11.26	17.60	29.81	53.35
Toddlers	14.94	26.87	30.37	55.08
Children	6.89	25.22	18.57	48.85
Adolescents	3.40	11.69	9.27	24.45
Adults	3.61	12.36	9.74	24.41
The Elderly	4.59	12.55	10.70	22.51

Whilst the Applicant stated that migration from the peel to the flesh of the fruit is highly unlikely, to produce a conservative assessment the Applicant has assumed in this assessment that the peel is consumed for all fruits even those with inedible peel.

For adults at mean exposure levels of E471 from the total diet in addition to category 4.1 at levels of 1,520 mg E471/kg fruit or vegetables, total dietary exposure ranged from 3.61-12.36 mg/kg bw/day and at the 95th percentile of consumption 9.74-24.41

mg/kg bw/day. Following the indication by EFSA in 2017 that the reference intake for total fat is 20-35% of an individual's total energy intake based on a 2,000 kcal diet the Applicant presents that E471 from category 4.1 would contribute 0.95-1.6% of fat intake at mean levels of consumption and 1.8-3.3% at the 95th percentile of exposure.

The AEJEG noted that the use of European average weights and average calorie intake values would produce different results than from using UK values however this would be unlikely to affect the outcome of the exposure assessment.

The AEJEG noted that in assessing this dossier they had not considered the nutritional impact of the added fat content to the fruits and vegetables that would occur when consumers ingest E471 coated produce.

The AEJEG was satisfied with this exposure assessment and considered it to be conservative. The AEJEG also acknowledged that EFSA considered that no numerical ADI was required.

2.12 Biological and toxicological data

The Applicant provided the following information regarding the biological and toxicological data relevant to this application:

'The 2017 EFSA Re-evaluation of mono- and di-glycerides of fatty acids (E471) as a food additive reviewed studies of short-term and subchronic toxicity, genotoxicity, chronic and carcinogenic toxicity, and reproductive and developmental toxicity of E471. Diacylglycerol is used in several of the toxicity studies described in the Re-evaluation, and the EFSA Panel considered that the results of these toxicological studies can be used for the assessment of E471.'

A summary of EFSA's review of these studies is provided below.

Short-term and subchronic toxicity studies were conducted with rats and hamsters. In one 90-day study, rats were fed diets containing different ratios of heated to unheated di- and tri-acylglycerol oils. Clinical pathology, ophthalmic examination,

and macroscopic and microscopic examinations of the rats showed some changes that were not considered to be dose or treatment related, and ultimately, the authors identified a no observed effect level of heated diacylglycerol or triacylglycerol oil of 5.5% in the diet, the highest dose tested. In a 28-week study with male Golden Syrian hamsters that were dosed with different concentrations of glyceryl monostearate (ranging from 0% to 15%), there were no adverse effects reported, where the highest dose tested was 7,500 mg glycerol monostearate/kg bodyweight per day. However, the EFSA Panel noted that this study had several limitations: a limited number of organs were examined and there was no information on clinical chemistry and haematological parameters with diacylglycerol or glyceryl monostearate.

In vitro and *in vivo* studies were conducted to assess the mutagenicity of diacylglycerol having a composition rich in unsaturated fatty acids (> 95%). EFSA states that no genotoxicity of diacylglycerol (rich in unsaturated fatty acids (> 95%)) was observed in adequately conducted studies, which included a bacterial reverse mutation assay (Ames test), an *in vitro* test for the induction of chromosomal aberrations, and an *in vivo* bone marrow micronucleus test, thus covering the basic requirements required for the assessment of genotoxicity. As such, the EFSA Panel considered that the diacylglycerol tested in these studies was not of genotoxic concern.

In a chronic toxicity study with mice that were fed a diet containing diacylglycerol oil for 24 months, the authors concluded that diacylglycerol at dietary concentrations up to 6.0% (equivalent to 7,800 and 10,020 mg/kg bodyweight per day in males and females, respectively) for 24 months produced no signs of systemic toxicity and had no effect on the incidence of neoplastic findings.

In another chronic toxicity study, rats were fed diets containing different concentrations of diacylglycerol oil. At the end of 105 weeks, the cumulative survival rate, occurrence of clinical signs, body weights and food consumption measurements were similar in all groups and did not reveal any adverse effects of diacylglycerol oil, and urinalysis did not reveal any treatment-related effects. While a significantly higher incidence of mammary gland tumours was noted in female rats

exposed to the higher concentration of diacylglycerol oil (5.3% in the diet), because it was within the range of tumours in historical controls, it was not considered related to diacylglycerol oil treatment. The authors determined that the NOAEL was the highest dose tested (1,770 and 2,350 mg/kg bodyweight per day for males and females, respectively), and the EFSA Panel agreed with this conclusion.

To assess carcinogenic effects, rats were fed diacylglycerol oil in the diet for 24 months. After reviewing haematology and serum chemistry tests, ophthalmic findings, incidence of preneoplastic or neoplastic lesions, and testing on several organs, the authors concluded that there was not a higher risk of carcinogenic effects in the rats fed a diet containing diacylglycerol oil as compared to the rats fed a diet in which all dietary fat came from triacylglycerol.

Additionally, in a two-generation reproduction toxicity study and a prenatal developmental toxicity study in rats that were fed a diet containing diacylglycerol oil, no parental, reproductive, or developmental effects were observed, where the NOAEL of both studies was the highest dose tested (4,630 mg diacylglycerol/kg bodyweight per day).'

The AEJEG was satisfied with this toxicological information.

3. Discussion

3.1 Overall conclusions of the applicant on safety

Regarding the overall conclusions on safety the Applicant has stated that:

'In accordance with the current authorizations, proposed use level, and publicly available scientific literature about mono- and diglycerides of fatty acids (E471), it can be established that the extension of use as a surface treatment on additional entire fresh fruit and vegetables (specifically, passionfruit, kiwi, apples, pears, stone fruit (peaches, nectarines, and plums), cherries, berries (strawberries and blueberries), cucumbers, asparagus, tomatoes, and peppers) is safe, as summarized below.

E471 is currently authorized in 84 food categories in the EU and the UK, including certain fresh fruits: citrus fruit, melons, pineapples, bananas, papayas, mangoes, avocados, and pomegranates. Fresh fruits and vegetables are particularly susceptible to perishability, as they contain 80-90% water. Water progressively evaporates from the fruit or vegetable, resulting in a loss of quality and limited shelf-life. Additionally, after harvest, there is metabolic loss that leads to a progressive maturation and eventual senescence and decay of fruits and vegetables. As part of the effort to reduce food waste and loss throughout the food system, edible coatings, such as E471, can contribute to extending the shelf-life of fruits and vegetables by providing a layer of protection that stays with the produce through its entire journey along the supply chain, slowing moisture loss and solute migration, gas exchange or respiration (and ethylene production), and oxidation. As shown in the shelf-life extension studies, E471 coatings allow fruits and vegetables to maintain firmness and quality for longer, and slow mass loss (and thus retain moisture) and oxidation, compared to untreated produce. Importantly, this extension of shelf-life means that consumers gain improved access to a more diverse array of healthy, fresh fruits and vegetables, and consumers will have more time to consume the produce they purchase while reducing food waste at the consumer level.

E471 is proposed for use as a surface treatment to extend the postharvest shelf-life of additional fresh fruits and vegetables at levels of *quantum satis*. A conservative overestimation of the highest amount of E471 that would be used to treat any fruit or vegetable is 1,520 mg E471 per kg of produce. This use level was applied to assess dietary exposure from the surface treatment of all fresh fruits and vegetables in Food Category 4.1 – Unprocessed fruit and vegetables using the Food Additives Intake Model (FAIM version 2), and that all peels are consumed, even for fruits and vegetables for which the peel is not customarily consumed. The resulting estimated maximum mean and highest level (95th percentile) exposures for a European adult are 12.36 and 24.41 mg/kg bodyweight per day, respectively. In its 2017 Re-evaluation of E471, EFSA indicated that the reference intake for total fat is 20-35% of the total energy intake, corresponding to 741 to 1,296 mg/kg bodyweight per day for a 2,000 kcal/day diet. The contribution of E471 applied on fruits and vegetables to the fat intake, in the present very conservative scenario, would be 0.95-1.6% at the mean intake level and 1.8-3.3% in the highest (95th percentile) intake level.

Additionally, EFSA concluded that there was no need for setting an ADI for E471 (EFSA, 2017).

Mono- and diglycerides of fatty acids are subjected to hydrolysis by lipases in the gastrointestinal tract, liberating glycerol and free fatty acids (EFSA, 2017). Further, studies of short-term and subchronic toxicity, genotoxicity, chronic and carcinogenic toxicity, and reproductive and developmental toxicity of E471 indicate the following:

• No evidence for adverse effects in short-term and subchronic studies in rats and hamsters, even at 2,500 diacylglycerol/kg bodyweight per day in rats and 7,500 mg glyceryl stearate/kg bodyweight per day in hamsters, the highest doses tested;

• No indication of genotoxic concern based on *in vitro* and *in vivo* studies conducted to assess the mutagenicity of diacylglycerol;

• No indication of chronic toxicity or carcinogenicity in mice and rats at levels of 7,800 and 10,020 mg/kg bodyweight per day in male and female mice (respectively), 1,770 and 2,350 mg/kg bodyweight per day for male and female rats (respectively), and in a second rat study, 1,982 and 2,645 mg/kg bodyweight per day for administered diet male and female rats (respectively) and 1,946 and 2,507 mg/kg bodyweight per day for *ad libitum* diet male and female rats (respectively), all of which were the highest doses tested in the respective studies; and

• No parental, reproductive, or developmental effects at the highest dose tested in rats (EFSA, 2017).

Based on the information presented in Apeel Sciences' dossier and in the 2017 EFSA Re-evaluation of E471, we ('The Applicant') conclude that the use of E471 as a surface treatment of additional fresh fruits and vegetables is not only safe, but also an integral strategy to reduce food loss and waste through the fresh produce supply chain, while delivering healthy, longer lasting produce to consumers.'

3.2 AEJEG conclusions

The AEJEG has considered the application using the key information presented above.

The AEJEG recognised the safe history of use of E471 within the EU and US. The AEJEG recognised that EFSA did not consider it appropriate to set a numerical ADI for the additive E471. The AEJEG concurred with this and considered the use of mono- and di-glycerides of fatty acids as coating agents for fruits and vegetables within the context of this application to be of low toxicological concern.

The AEJEG acknowledged the conservative nature of the Applicant's exposure assessment from authorised and proposed uses. The application of the maximum level to the whole food category to account for its omission in EFSA's 2017 re-evaluation has also been noted. The AEJEG considered that the exposures presented in this assessment did not constitute a risk to health.

From the information presented within this application RP1057, summarised above, the AEJEG overall concluded that no risk to health will be presented from the extension of use of E471 on the basis that the additive was considered of low toxicological concern, as indicated by EFSA's conclusion that a numerical ADI was not required, the conservative nature of the exposure assessment, and the resulting exposures from the proposed uses. It should be noted that the AEJEG did not consider the nutritional impact of added fat to the diet from the extension of use of this additive in the context of this application.

4. Conclusions

The FSA and FSS agreed with the assessment undertaken by the AEJEG on the safety of the extension of use of mono- and diglycerides of fatty acids to the fruits to include passionfruit, kiwi, apples, pears, stone fruit (peaches, nectarines and plums), cherries, berries (strawberries and blueberries), cucumbers, asparagus, tomatoes, and peppers at the level of *quantum satis* conducted by the AEJEG. The FSA and FSS agreed with the conclusions of the AEJEG that the extension of use is safe under the proposed conditions of use and at the anticipated levels of intake.

The AEJEG concluded that sufficient information had been provided to allow for an evaluation of the proposal for the extension of use of mono- and diglycerides of fatty acids (E 471) to include passionfruit, kiwi, apples, pears, stone fruit (peaches, nectarines and plums), cherries, berries (strawberries and blueberries), cucumbers, asparagus, tomatoes, and peppers at the level of *quantum satis*. The AEJEG noted that they had not considered the nutritional impact of added fat to the diet from the extension of use of this additive in the context of this application but raised no further concerns.

The FSA and FSS therefore conclude that the extension of use is safe under the proposed conditions of use and at the anticipated levels of intake as described within this safety assessment, noting the AEJEG had not considered the nutritional impact of added fat to the diet.

These conclusions were based on the information in the food additive dossier plus the supplementary information and could not have been reached without the data claimed as proprietary by the applicant.

5. References

EFEMA (European Food Emulsifier Manufacturers Association) index of food emulsifiers.4th March 2019. The European Food Emulsifier Manufacturers Association (EFEMA) is a non-profit making organisation founded in 1973 (emulsifiers.org)

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6. Abbreviations

Abbreviation	Definition
ACS	American Chemical Society
ADI	Acceptable daily intake
AEJEG	Additives Enzymes and Other Regulated Products Joint Expert Group
ASTM	American Society for Testing and Materials
COT	Committee on Toxicity in Food, Consumer Products and the Environment
DCM	Dichloromethane
DIN	Deutsches Institut für Normung (German Institute for standardization)
E471	Mono- and diacylglycerides of fatty acids
EC	European Commission
EFEMA	European Food Emulsifiers Manufacturers Association
EFSA	European Food Safety Authority
EN	European Standard
EU	European Union
FAIM	Food Additives Intake Model
FASEB	Federation of American Societies for Experimental Biology
FAO	Food and Agriculture Organisation
FCC	Food Chemicals Codex
FSA	Food Standards Agency
FSS	Food Standards Scotland
FTIR	Fourier transformed Infared spectroscopy
GMP	Good Manufacturing Practice
GC-FID	Gas Chromatography-Flame Ionisation Detection
GRAS	Generally recognised as safe
ICP-OES	Inductively coupled plasma optical emission spectroscopy
ISO	International Organization for Standardization
JECFA	Joint FAO/WHO Expert Committee on Food Additives

Abbreviation	Definition
Kg	Kilogram
Max	Maximum
mg/kg	Milligram per kilogram
mg/kg bw/day	Miligram per kilogram bodyweight per day
Min	Minimum
mL	Mililitre
MPL	Maximum permitted level
NOAEL	No observed adverse effect level
ppm	Parts per million
ROI	Residue on ignition
SAXS	Small-angle X-ray scattering
SEM	Scanning electron microscopy
TEM	Transmission electron microscopy
TM	Test method
UPLC-ELSD	Ultra-performance liquid chromatography coupled with evaporative light scattering detection
US	United States
USP	US pharmacopeia testing
WHO	World Health Organisation

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