Apeel Sciences

October 9, 2019

#886

Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5001 Campus Drive
College Park, MD 20740-3835

Subject: Apeel Sciences GRAS Notice Submission for a Mixture of Monoacylglycerides Derived from Grape Seed

To Whom It May Concern:

Apeel Technology, Inc. ('Doing Business As' Apeel Sciences) is submitting the enclosed Generally Recognized as Safe (GRAS) Notification for a mixture of monoacylglycerides derived from grape seed. We trust that all information needed to review this GRAS Notification is enclosed.

Please direct all correspondence regarding this GRAS Notification to the following Apeel Sciences employees:

- Kathryn Davis, Senior Regulatory Manager, [redacted]; and
- Allison Gebbie, Regulatory Affairs Manager, [redacted].

Sincerely,

[redacted]

Jenny Du
VP of Operations
Apeel Sciences

Enclosure:
One (1) CD-ROM with all submission files

RECEIVED
OCT 15 2019
OFFICE OF
FOOD ADDITIVE SAFETY

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

GENERALLY RECOGNIZED AS SAFE *(GRAS)* NOTICE (Subpart E of Part 170)

Form Approved: OMB No. 0910-0342; Expiration Date: 09/30/2019
(See last page for OMB Statement)

FDA USE ONLY

GRN NUMBER	DATE OF RECEIPT
~~000827~~ 000886	RECEIVED OCT 15 2019 OFFICE OF FOOD ADDITIVE SAFETY
ESTIMATED DAILY INTAKE	INTENDED USE FOR INTERNET
NAME FOR INTERNET	
KEYWORDS	

Transmit completed form and attachments electronically via the Electronic Submission Gateway *(see Instructions)*; OR Transmit completed form and attachments in paper format or on physical media to: Office of Food Additive Safety *(HFS-200)*, Center for Food Safety and Applied Nutrition, Food and Drug Administration,5001 Campus Drive, College Park, MD 20740-3835.

SECTION A – INTRODUCTORY INFORMATION ABOUT THE SUBMISSION

1. Type of Submission *(Check one)*
☒ New ☐ Amendment to GRN No. _____ ☐ Supplement to GRN No. _____

2. ☒ All electronic files included in this submission have been checked and found to be virus free. *(Check box to verify)*

3 Most recent presubmission meeting *(if any)* with FDA on the subject substance *(yyyy/mm/dd)*: N/A

4 For Amendments or Supplements: Is your amendment or supplement submitted in response to a communication from FDA? *(Check one)* ☐ Yes ☐ No If yes, enter the date of communication *(yyyy/mm/dd)*: _____

SECTION B – INFORMATION ABOUT THE NOTIFIER

1a. Notifier

Name of Contact Person	Position or Title
Jenny Du	VP of Operations

Organization *(if applicable)*: Apeel Technology, Inc. ('Doing Business As' Apeel Sciences)

Mailing Address *(number and street)*: 71 S. Los Carneros Rd.

City	State or Province	Zip Code/Postal Code	Country
Goleta	California	93117	United States of America

Telephone Number	Fax Number	E-Mail Address
(805) 203-0146 ext. 7000		[redacted]

1b. Agent or Attorney *(if applicable)*

Name of Contact Person	Position or Title
Katie Davis	Senior Regulatory Manager

Organization *(if applicable)*: Apeel Technology, Inc. ('Doing Business As' Apeel Sciences)

Mailing Address *(number and street)*: 71 S. Los Carneros Rd.

City	State or Province	Zip Code/Postal Code	Country
Goleta	California	93117	United States of America

Telephone Number	Fax Number	E-Mail Address
(805) 203-0146 ext. 7090		[redacted]

SECTION C GENERAL ADMINISTRATIVE INFORMATION

1. Name of notified substance, using an appropriately descriptive term

Mixture of monoacylglycerides derived from grape seed

2. Submission Format: *(Check appropriate box(es))*

☐ Electronic Submission Gateway

☒ Electronic files on physical media

☐ Paper

If applicable give number and type of physical media

One (1) CD-ROM

3. For paper submissions only:

Number of volumes _____

Total number of pages _____

4. Does this submission incorporate any information in CFSAN's files? *(Check one)*

☐ Yes *(Proceed to Item 5)* ☒ No *(Proceed to Item 6)*

5. The submission incorporates information from a previous submission to FDA as indicated below *(Check all that apply)*

☐ a) GRAS Notice No. GRN _____

☐ b) GRAS Affirmation Petition No. GRP _____

☐ c) Food Additive Petition No. FAP _____

☐ d) Food Master File No. FMF _____

☐ e) Other or Additional *(describe or enter information as above)* _____

6. Statutory basis for conclusions of GRAS status *(Check one)*

☒ Scientific procedures *(21 CFR 170.30(a) and (b))* ☐ Experience based on common use in food *(21 CFR 170.30(a) and (c))*

7. Does the submission (including information that you are incorporating) contain information that you view as trade secret or as confidential commercial or financial information? (see 21 CFR 170.225(c)(8))

☐ Yes *(Proceed to Item 8*

☒ No *(Proceed to Section D)*

8. Have you designated information in your submission that you view as trade secret or as confidential commercial or financial information *(Check all that apply)*

☐ Yes, information is designated at the place where it occurs in the submission

☐ No

9. Have you attached a redacted copy of some or all of the submission? *(Check one)*

☐ Yes, a redacted copy of the complete submission

☐ Yes, a redacted copy of part(s) of the submission

☐ No

SECTION D INTENDED USE

1. Describe the intended conditions of use of the notified substance, including the foods in which the substance will be used, the levels of use in such foods, and the purposes for which the substance will be used, including, when appropriate, a description of a subpopulation expected to consume the notified substance.

A mixture of monoacylglycerides derived from grape seed is intended to be a component of a surface-finishing agent, such as that described in GRN 648, creating an edible physical barrier against moisture loss and oxidation to protect the freshness and extend the shelf life of fresh fruits and vegetables. This substance is intended to be used at levels of Good Manufacturing Practice (GMP).

2. Does the intended use of the notified substance include any use in product(s) subject to regulation by the Food Safety and Inspection Service (FSIS) of the U.S. Department of Agriculture?

(Check one)

☐ Yes ☒ No

3. If your submission contains trade secrets, do you authorize FDA to provide this information to the Food Safety and Inspection Service of the U.S. Department of Agriculture?

(Check one)

☐ Yes ☐ No , you ask us to exclude trade secrets from the information FDA will send to FSIS.

SECTION E – PARTS 2 -7 OF YOUR GRAS NOTICE

(check list to help ensure your submission is complete – PART 1 is addressed in other sections of this form)

☒ PART 2 of a GRAS notice: Identity, method of manufacture, specifications, and physical or technical effect (170.230).

☒ PART 3 of a GRAS notice: Dietary exposure (170.235).

☒ PART 4 of a GRAS notice: Self-limiting levels of use (170.240).

☐ PART 5 of a GRAS notice: Experience based on common use in foods before 1958 (170.245).

☒ PART 6 of a GRAS notice: Narrative (170.250).

☒ PART 7 of a GRAS notice: List of supporting data and information in your GRAS notice (170.255)

Other Information

Did you include any other information that you want FDA to consider in evaluating your GRAS notice?

☐ Yes ☒ No

SECTION F – SIGNATURE AND CERTIFICATION STATEMENTS

1. The undersigned is informing FDA that Jenny Du
(name of notifier)

has concluded that the intended use(s) of Mixture of monoacylglycerides derived from grape seed
(name of notified substance)

described on this form, as discussed in the attached notice, is (are) not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act based on your conclusion that the substance is generally recognized as safe recognized as safe under the conditions of its intended use in accordance with § 170.30.

2. Jenny Du agrees to make the data and information that are the basis for the conclusion of GRAS status available to FDA if FDA asks to see them; agrees to allow FDA to review and copy these data and information during customary business hours at the following location if FDA asks to do so; agrees to send these data and information to FDA if FDA asks to do so.
(name of notifier)

71 S. Los Carneros Rd., Goleta, CA, 93117, USA
(address of notifier or other location)

The notifying party certifies that this GRAS notice is a complete, representative, and balanced submission that includes unfavorable, as well as favorable information, pertinent to the evaluation of the safety and GRAS status of the use of the substance.The notifying party certifies that the information provided herein is accurate and complete to the best or his/her knowledge. Any knowing and willful misinterpretation is subject to criminal penalty pursuant to 18 U.S.C. 1001.

3. Signature of Responsible Official, Agent, or Attorney	Printed Name and Title	Date (mm/dd/yyyy)
[redacted]	Jenny Du, VP of Operations	10/10/2019

SECTION G LIST OF ATTACHMENTS

List your attached files or documents containing your submission, forms, amendments or supplements, and other pertinent information. Clearly identify the attachment with appropriate descriptive file names (or titles for paper documents), preferably as suggested in the guidance associated with this form. Number your attachments consecutively. When submitting paper documents, enter the inclusive page numbers of each portion of the document below.

Attachment Number	Attachment Name	Folder Location (select from menu) (Page Number(s) for paper Copy Only)
	Cover Letter_Apeel Sciences_GRASN Monoacylglycerides from Grape Seed_2019 10 10.p	inistrati
	Apeel Sciences_GRASN for a Mixture of Monoacylglycerides Derived from Grape Seed_2019 10 10.p	issio

OMB Statement: Public reporting burden for this collection of information is estimated to average 170 hours per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to: Department of Health and Human Services, Food and Drug Administration, Office of Chief Information Officer, **PRAStaff@fda.hhs.gov**. (Please do NOT return the form to this address). An agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a currently valid OMB control number.

Generally Recognized as Safe (GRAS) Notice for a Mixture of Monoacylglycerides Derived from Grape Seed

October 10, 2019

Prepared for:

Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5001 Campus Drive
College Park, MD 20740-3835

Prepared by:

Apeel Technology, Inc. ('Doing Business As' Apeel Sciences)

Table of Contents

Part 1 §170.225 Signed Statements and Certification 3
1.1 Name and Address of Notifier 3
1.2 Common Name of Notified Substance 3
1.3 Conditions of Use 4
1.4 Basis for GRAS 4
1.5 Availability of Information 5
1.6 Freedom of Information Act, 5 U.S.C. Section 552 6

Part 2 §170.230 Identity, Method of Manufacture, Specifications, and Physical or Technical Effect 6
2.1 Introduction 6
2.2 Background 6
2.3 Identity 7
2.3.1 Chemical Structure 7
2.3.2 Chemical and Physical Characteristics 8
2.4 Method of Manufacture 8
2.5 Product Specifications 9
2.6 Physical or Technical Effect 11

Part 3 §170.235 Dietary Exposure 11
3.1 Estimated Daily Intake 11
3.1.1 Fruit 11
3.1.2 Vegetables 13
3.1.3 Total Estimated Daily Intake 14
3.2 Maximum Limit of Residues 14
3.2.1 Ethyl Acetate 16
3.2.2 Heptane 17
3.2.3 Palladium 17
3.2.4 Arsenic 18
3.2.5 Lead 18
3.2.6 Cadmium and Mercury 18

Part 4 §170.240 Self-Limiting Levels of Use 18

Part 5 §170.245 Experience Based on Common Use in Food Before 1958 18

Part 6 §170.250 Narrative 18
6.1 Natural Occurrence and Approved Uses 18
6.2 Regulatory and Other Assessments 20
6.2.1 FDA GRAS Review 20
6.2.2 Cosmetic Ingredient Review 22
6.2.3 Digestion and Metabolism 22
6.3 Safety Studies 24
6.4 Expert Panel Evaluation 27
6.5 Summary 28
6.6 Conclusion 29

Part 7 §170.255 List of Supporting Data and Information 31

List of Tables

Table 1-1. Illustrative Maximum Quantities of a Mixture of Monoacylglycerides Derived from Grape Seed Applied to the Surfaces of Certain Fruits and Vegetables 4
Table 2-1. Chemical and Physical Characteristics of two Representative Monoacylglycerides Derived from Grape Seed 8
Table 2-2. Specifications for a Mixture of Monoacylglycerides Derived from Grape Seed 10
Table 3-1. Reported RFEP Fruit Sources as Percentage of Total Fruit Intake 12
Table 3-2. Average Daily Intake of Apples, Strawberries, and Grapes Among Consumers Reporting (Grams) 12
Table 3-3. Reported VEP Vegetable Sources as Percentage of Total Vegetable Intake 13
Table 3-4. Average Daily Intake of Baked/Boiled Potatoes, String Beans, Among Consumers Reporting (Grams) 14
Table 3-5. Maximum Daily Exposure Limits for Residues in a Mixture of Monoacylglycerides Derived from Grape Seed 16
Table 6-1. Summary of Long-Term Clinical Studies of Diacylglycerol Oil 25

List of Figures

Figure 2.1. Generalized chemical structures of monoacylglycerides. 7
Figure 2.2. Chemical structures of two representative monoacylglyceride derivatives derived from grape seed. 8
Figure 2.3. Schematic of the manufacturing process for a mixture of monoacylglycerides derived from grape seed. The solid boxes indicate unit operations and dashed boxes indicate ingredients and processing aids. 9

List of Appendices

Appendix I. Test Methods for Batch Analysis

Appendix II. Batch Analysis

Part 1 §170.225 Signed Statements and Certification

Apeel Sciences has determined that a mixture of monoacylglycerides (i.e., monoglycerides or fatty acid monoesters of glycerol) derived from grape seed is Generally Recognized As Safe (GRAS), consistent with Section 201(s) of the *Federal Food, Drug, and Cosmetic Act*. This determination is based on scientific procedures as described in the following sections, under the conditions of its intended use in selected food. Therefore, the use of a mixture of monoacylglycerides derived from grape seed is exempt from the requirement of premarket approval. In accordance with 21 CFR Part 170, Subpart E, consisting of §170.203 through §170.285, Apeel Sciences provides the information herein to support the view that a mixture of monoacylglycerides derived from grape seed is GRAS.

Signed,

[redacted] 10/10/2019

Jenny Du, ~~Ph.D.~~ Date
VP of Operations
Apeel Sciences

1.1 Name and Address of Notifier

Apeel Technology, Inc. ('Doing Business As' Apeel Sciences)
71 S. Los Carneros Rd.
Goleta, CA 93117
USA

1.2 Common Name of Notified Substance

The substance proposed by Apeel Sciences for classification as Generally Recognized as Safe (GRAS), is a mixture of monoacylglycerides (i.e., monoglycerides or fatty acid monoesters of glycerol) derived from grape seed for use as an edible barrier (i.e., surface-finishing agent or glazing agent) on the surface of raw fruits and vegetables. The United States (US) Food and Drug Administration (FDA) has previously reviewed the GRAS status of monoacylglycerides for use as an edible barrier on the surface of raw fruits and vegetables (GRN 648). A letter dated December 20, 2016 from the FDA acknowledges that the FDA has no further questions concerning the determination that the substance is GRAS for these uses. The method of manufacture and product specifications for a mixture of monoacylglycerides have changed since the filing of GRN 648 following further evaluation of plant-based options for sourcing monoacylglycerides. As such, the basis for the determination of GRAS status of a mixture of monoacylglycerides derived from grape seed is described in the following sections.

It should be noted that although the substance is referred to as 'a mixture of monoacylglycerides' throughout this document (which is consistent with the nomenclature used in GRN 648), the substance may also be identified as 'mono- and diglycerides' in the US Code of Federal Regulations (CFR) and international regulatory bodies such as the Codex Alimentarius.

1.3 Conditions of Use

A mixture of monoacylglycerides derived from grape seed is intended to be a component of a surface-finishing agent [21 CFR 170.3 (o)(30)[1]], such as that described in GRN 648, creating an ultra-thin and edible physical barrier against moisture loss and oxidation to protect the freshness and extend the shelf life of fresh produce such as fruits (e.g., berries, grapes, stone fruit, citrus, bananas, mangoes, avocados) and vegetables (e.g., bulbs, stems, pods, flowers and flower buds, legumes, roots, tubers) when applied to their surfaces. It will be used at levels consistent with current Good Manufacturing Practice (cGMP) and is self-limiting for technological reasons. The intended use of a mixture of monoacylglycerides derived from grape seed on fruits and vegetables is estimated to result in an average daily intake of 109 mg/person/day and a maximum daily intake of 218 mg/person/day.[2] Illustrative quantities of a mixture of monoacylglycerides derived from grape seed applied to the surfaces of certain fruits and vegetables are presented in Table 1-1. The highest amount of a mixture of monoacylglycerides derived from grape seed applied to fruit and vegetables to achieve its intended use (i.e., in the case of potential unintentional over-application) is 1.52 grams per kilogram of produce.

Table 1-1. Illustrative Maximum Quantities of a Mixture of Monoacylglycerides Derived from Grape Seed Applied to the Surfaces of Certain Fruits and Vegetables

Fruit or Vegetable	Illustrative Maximum Amount Applied
Apples	108 g / 100 kg apples (100 g / 93 kg apples)
Grapes	118 g / 100 kg grapes (100 g / 85 kg grapes)
Strawberries	118 g / 100 kg strawberries (100 g / 85 kg strawberries)
Green Beans	152 g / 100 kg green beans (100 g / 66 kg green beans)

1.4 Basis for GRAS

Pursuant to 21 CFR 170.30, the intended use of a mixture of monoacylglycerides derived from grape seed has been determined to be Generally Recognized As Safe (GRAS) based on scientific procedures. Therefore, the intended use of a mixture of monoacylglycerides derived from grape seed is exempt from the requirement of premarket approval under the Federal Food, Drug, and Cosmetic Act (FD&C Act). A comprehensive search of the scientific literature on monoacylglycerides was utilized for this assessment. There exists sufficient qualitative and quantitative scientific evidence, including human and animal data, to determine safety-in-use for monoacylglycerides. Monoacylglyceride derivatives are components of dietary fats commonly found in food and are also endogenously formed in the human body. A mixture of monoacylglycerides derived from grape seed will consist primarily of saturated monoglycerides of varying chain lengths.

[1] *Surface-finishing agents*: Substances used to increase palatability, preserve gloss, and inhibit discoloration of foods, including glazes, polishes, waxes, and protective coatings.

[2] As outlined in the dietary exposure calculation in Section 3.1, the maximum, or high end, consumer daily intake is estimated as twice the average daily consumer intake.

It is well established and recognized that monoacylglycerides, including those derived from grape seed, the subject of the present GRAS assessment, are formed in the gastrointestinal tract from the generally accepted metabolic pathway for the breakdown of triglycerides (i.e., lipolysis). The hydrolysis of triglycerides by lipases proceeds through the formation of monoacylglycerides. The free fatty acids released can be further used for triglyceride synthesis. Given the metabolic sequence described above, and by applying scientific procedures, it can be concluded that monoacylglycerides would not pose any health hazards different from commonly consumed dietary oils derived from plants or animals.

On the basis of scientific procedures,[3] Apeel Sciences considers the consumption of a mixture of monoacylglycerides derived from grape seed as an added food ingredient to be safe at levels up to 218 mg/day. The estimated daily intake of monoacylglycerides derived from grape seed for the intended use as a component of an edible coating for fruits and vegetables, if ingested daily over a lifetime, is considered safe.

A comprehensive search of the scientific literature for safety and toxicity information on monoacylglycerides was previously conducted for GRN 648 and reviewed by FDA. A search of recent literature published since the submission of GRN 648 and through July 2018 did not yield information that would change the safety conclusions provided for GRN 648; therefore, the conclusions reached for GRN 648 are relevant and applicable to this GRAS assessment. Based on a critical evaluation of the pertinent data and information summarized here, Apeel Sciences has determined by scientific procedures that the addition of a mixture of monoacylglycerides derived from grape seed to fruits and vegetables, when not otherwise precluded by a Standard of Identity, meeting the specifications cited in Table 2-2, and manufactured according to current Good Manufacturing Practice, is Generally Recognized As Safe (GRAS) under the conditions of intended use in selected foods, as specified herein.

As per 21 CFR 184.1505, mono- and diglycerides that consist of a mixture of glyceryl mono- and di-esters, and minor amounts of triesters, are affirmed as GRAS for direct addition to food. In coming to its decision that the mixture of monoacylglycerides derived from grape seed is GRAS, Apeel Sciences relied upon the conclusions that neither the mixture of monoacylglycerides nor any of its constituents pose any toxicological hazards or safety concerns at the intended use levels, as well as on published toxicology studies and other articles relating to the safety of the end product. Other qualified and competent scientists, reviewing the same publicly available toxicological and safety information, would reach the same conclusion.

1.5 Availability of Information

The data and information that serve as the basis of the GRAS assessment will be made available to the United States (US) Food and Drug Administration (FDA) for review and copying, in an electronic format that is accessible for the US FDA's evaluation or on paper, upon request during customary business hours at the offices of:

Apeel Sciences
71 S. Los Carneros Road
Goleta, CA 93117
USA

[3] 21 CFR §170.3 Definitions. (h) Scientific procedures include those human, animal, analytical, and other scientific studies, whether published or unpublished, appropriate to establish the safety of a substance.

To the best of our knowledge, the GRAS assessment is a complete, representative, and balanced submission. The assessment includes unfavorable as well as favorable information known to Apeel Sciences and pertinent to the evaluation and safety of the use of the substance. We note that we are not aware of any unfavorable information that is relevant to the GRAS assessment.

1.6 Freedom of Information Act, 5 U.S.C. Section 552

It is Apeel Sciences' view that none of the information is exempt from disclosure under the Freedom of Information Act, 5 U.S.C. Section 552.

Part 2 §170.230 Identity, Method of Manufacture, Specifications, and Physical or Technical Effect

2.1 Introduction

A comprehensive search of the scientific literature was conducted through July 2018 for safety and toxicity information on monoacylglycerides and other related compounds was utilized to determine the Generally Recognized As Safe (GRAS) status of a mixture of monoacylglycerides (i.e., monoglycerides or fatty acid monoesters of glycerol) derived from grape seed for its intended use as a component of a surface-finishing agent [21 CFR 170.3 (o)(30)], creating a thin and edible physical barrier against moisture loss and oxidation to protect the freshness and extend the shelf-life of fresh fruits and vegetables. The exposure from added monoacylglycerides derived from grape seed in the proposed food uses in the total US population is estimated as 109 mg/person/day of a mixture of monoacylglycerides for average consumers and 218 mg/person/day for high-end consumers.

2.2 Background

Grape species have economic importance in both the fresh and processed fruit markets. A large amount of grape seeds are produced from the waste of processed fruit products, like juice, jam and wine. These waste products, such as peels, seeds and pulps, represent about 50% to 60% of the raw processed fruit (Al Juhaimi et al., 2016). In recent years, grape seed has attracted attention due to it being a rich source of oil that has both nutritional and bioactive constituents (Kamel et al., 1985; Göktürk Baydar & Akkurt, 2001; Hassanein & Abedel-Razek, 2009). For example, it has been established that grape seed oil is rich in unsaturated fatty acids, such as linoleic acid (72% to 76%) (Citil et al., 2010; Akın & Altındişli, 2011). Grape seed oil is regularly consumed as a component of salad dressings, marinades, deep frying, flavored oils, baking, and is also found widely in a variety of cosmetic products (Akın & Altındişli, 2011).

For centuries, edible films and coatings, such as wax on various fruits, have been used to prevent loss of moisture and to create a shiny fruit surface for aesthetic purposes (Embuscado and Huber, 2009). These practices were accepted long before their associated chemistries were understood and are still carried out in the present day. There are several different types of edible packaging, and a diverse array of edible barrier compositions. Among the different types of hydrophobic film-forming barrier materials are fatty acids and alcohols, and acetylated glycerides. Edible barriers based on hydrophobic substances such as lipids were developed specifically for limiting moisture migration from foods. The different lipid-based film forming or barrier compounds include lecithins, mono- and diglycerides, and mono- and diglyceride

esters, among other compounds. These substances are used as emulsifiers and surface-active agents. Given the beneficial properties of mono- and diglycerides, Apeel Sciences intends to use a mixture of monoacylglycerides derived from grape seed as an ultra-thin, invisible, tasteless, and completely edible coating (or a component thereof) formulated to serve as an inert, physical diffusion barrier that covers the surface of fresh produce to help reduce moisture loss and oxidation.

2.3 Identity

A mixture of monoacylglycerides derived from grape seed are intended to be used as a component of an edible coating formulated to be applied to the surface of raw produce, forming an ultra-thin edible barrier to protect produce from external, abiotic stressors (e.g., moisture loss and oxidation). A related substance is the subject of a previous GRAS Notification (GRN 648).

Common name:	Mono- and diglycerides of fatty acids
Commercial or Trade name:	Not applicable
Synonyms:	Mono- and diacylglycerols; fatty acid esters of glycerol
International Numbering System (INS) No:	471
CAS Registry Number:	67701-31-9, 31566-31-1, 123-94-4, 621-61-4, 67701-33-1, 68990-53-4, 85251-77-0, 67254-73-3

2.3.1 Chemical Structure

The generalized chemical structures of monoglycerides are presented in Figure 2.1. The chemical structures for 2,3-dihydroxypropyl stearate (SA-1G) and 1,3-dihydroxypropan-2-yl stearate (SA-2G) (i.e., n = 16), representative monoacylglyceride compounds derived from grape seed, are presented in Figure 2.2.

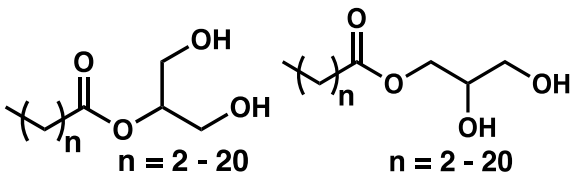
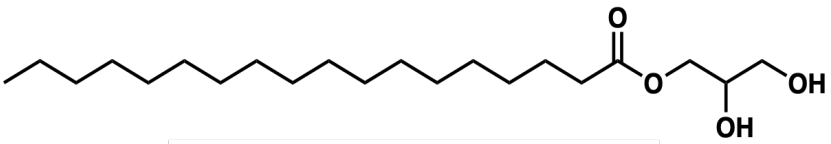
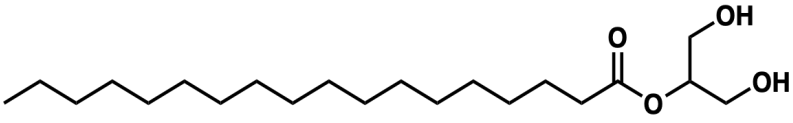


Figure 2.1. Generalized chemical structures of monoacylglycerides.



2,3-dihydroxypropan-1-yl stearate (SA-1G)



1,3-dihydroxypropan-2-yl stearate (SA-2G)

Figure 2.2. Chemical structures of two representative monoacylglyceride derivatives derived from grape seed.

2.3.2 Chemical and Physical Characteristics

The chemical and physical characteristics of two representative monoglycerides derived from grape seed are detailed in Table 2-1.

Table 2-1. Chemical and Physical Characteristics of two Representative Monoacylglycerides Derived from Grape Seed

Property/Parameter	1-Monostearin	2-Monostearin
CAS Registry No.	123-94-4	621-61-4
Chemical Name	2,3-dihydroxypropyl stearate, also 1-stearoyl-*rac*-glycerol	1,3-dihydroxypropan-2-yl stearate, also 2-stearoylglycerol
Empirical Formula	$C_{21}H_{42}O_4$	$C_{21}H_{42}O_4$
Molecular Weight (g/mol)	358.68	358.68
Physical State	Solid	Solid
Density (g/mL)	1.03	1.03

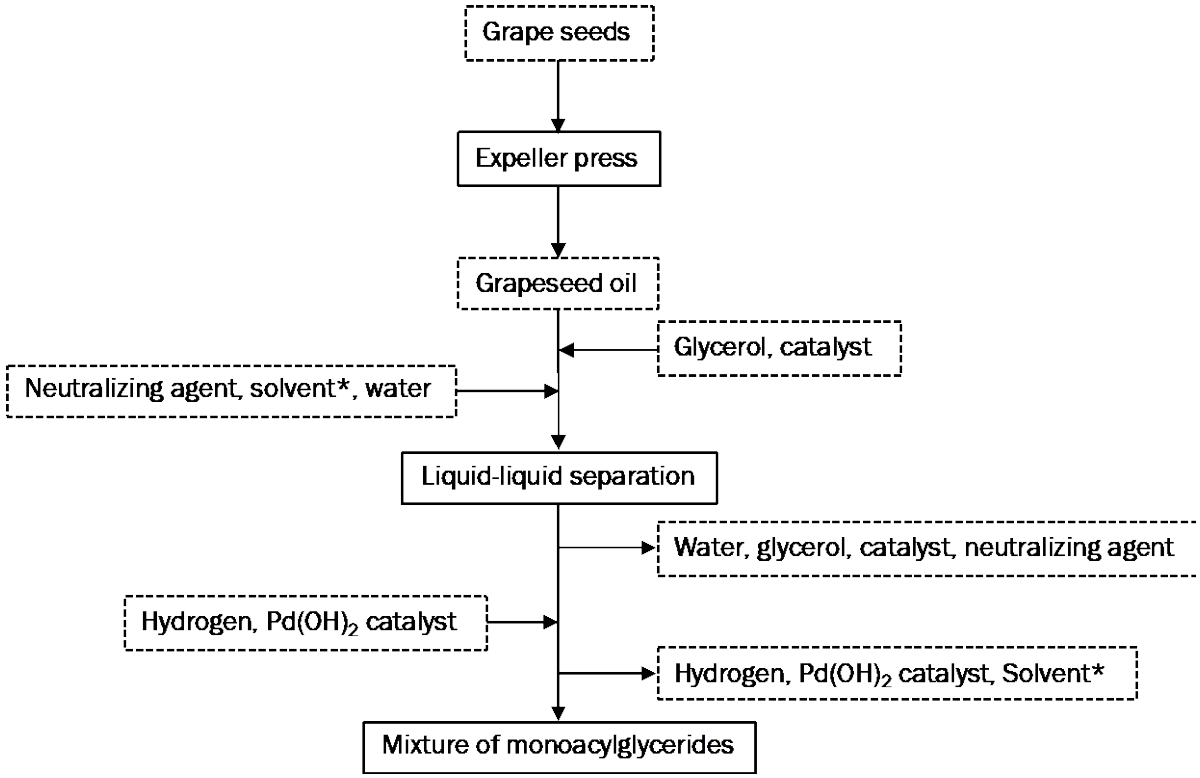
2.4 Method of Manufacture

Grapeseed oil, a mixture of triglycerides containing common dietary fatty acids, is extruded from grape seeds by mechanical pressing. The oil is processed to generate monoacylglycerides containing exclusively the same naturally occurring fatty acids in the grape seed oil in their saturated forms. Common dietary fatty acids that naturally occur in grapeseed oil (Kamel et al., 1985)—linoleic acid, oleic acid, palmitic acid, stearic acid, alpha-linolenic acid, and palmitoleic acids—are processed to generate the saturated forms as indicated by the low iodine number in the specifications in Table 2-2.

The catalyst used to generate the monoacylglycerides and the neutralizing agent subsequently used to quench the catalyst are both safe and suitable for use in food. A liquid–liquid extraction with water and heptane or ethyl acetate[4] is performed to remove unreacted glycerol, residual catalyst, and the residual neutralizing agent. The saturated forms of the naturally occurring fatty acids are then formed in the presence of hydrogen gas and a catalyst (20% w/w palladium hydroxide supported on carbon).

All processing aids used are allowed for food applications in the US, as described in Section 3.2. Only food grade solvents are used. Where a definition of "food grade" has been established by the Food Chemicals Codex (FCC), 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 manufacturing process is depicted in Figure 2.3.



* – Heptane or ethyl acetate may be used interchangeably as the solvent.

Figure 2.3. Schematic of the manufacturing process for a mixture of monoacylglycerides derived from grape seed. The solid boxes indicate unit operations and dashed boxes indicate ingredients and processing aids.

2.5 Product Specifications

Food grade specifications have been established by Apeel Sciences for the mixture of monoacylglycerides derived from grape seed, as presented in Table 2-2. The methods of analysis conform to standardized test methods or are third-party or in-house methods that have been validated by accredited third party

[4] Heptane or ethyl acetate may be used interchangeably in the manufacturing process.

laboratories or Apeel Sciences, respectively. The test methods used for batch analysis are listed in Appendix I.

Table 2-2. Specifications for a Mixture of Monoacylglycerides Derived from Grape Seed

Parameter	Specifications
Physical appearance	Varies in consistency from yellow liquids through white- to pale yellow-colored plastics to hard, ivory-colored or white to pale yellow solids (flakes, powders or small beads)
Total glycerides	Not less than 90 weight %
Mono- and diesters	Not less than 70 weight %
α-Monoglyceride content	Not less than 30 weight %
Total glycerol	16–33 weight %
Free glycerol	Not more than 7 weight %
Soap (as sodium oleate)	Not more than 6 weight %
Residue on ignition	Not more than 0.5 % determined at 800 ± 25 °C
Acid value (1)	Not more than 6
Iodine value	Not more than 4
Water content	Not more than 2 % (Karl Fisher method)
Processing Aid Residuals	
Ethyl acetate (2)	Not more than 21,000 mg/kg
Heptane (2)	Not more than 23,000 mg/kg
Heavy Metals	
Palladium (from Pd/C catalyst)	Not more than 10 mg/kg
Arsenic (3)	Not more than 3 mg/kg
Lead (3)	Not more than 2 mg/kg
Cadmium (3)	Not more than 1 mg/kg
Mercury (3)	Not more than 1 mg/kg

1 mg/kg = 1 ppm
(1) – Acid value can be converted to free fatty acid ("FFA") content, where FFA (wt. %) = Acid value ÷ 1.99.
(2) – Heptane and ethyl acetate may be used interchangeably in the manufacturing process.
(3) – Tested on a semi-annual basis.

The safety of the residuals and exposure limits from the literature are discussed in Section 3.2. The analytical results for three non-consecutive batches of a mixture of monoacylglycerides derived from grape seed are provided in Appendix II to illustrate conformance with the specifications presented in Table 2-2.

2.6 Physical or Technical Effect

A mixture of monoacylglycerides derived from grape seed is intended to be used as a coating (or component thereof) on the exterior of fruits (e.g., berries, grapes, stone fruit, citrus, bananas, mangoes, avocados) and vegetables (e.g., bulbs, stems, pods, flowers and flower buds, legumes, roots, tubers), such as the coating that is the subject of GRN 648. A mixture of monoacylglycerides derived from grape seed will be used as a surface-finishing agent [21 CFR 170.3 (o)(30)], creating a thin and edible physical barrier against moisture loss and oxidation to protect the freshness and extend the shelf-life of fresh (i.e., raw) produce. It will be used at levels consistent with current Good Manufacturing Practice and is self-limiting for technological reasons. The quantity of monoacylglycerides derived from grape seed required to achieve the technical function is inherently self-limiting given the unique characteristics of each fruit and vegetable and varies with the specific application. For example, overapplication of the substance may damage the fruit or vegetable, while underapplication will prohibit the maximum shelf life benefits from being achieved. It is recognized that there are Standard of Identity requirements, located in Title 21 of the Code of Federal Regulations, and as such, Apeel Sciences does not intend to refer to those foods by the commonly recognized names.

The quantity of the mixture of monoacylglycerides derived from grape seed recommended for use varies with the specific application (e.g., crop type, regional climate). Quantities illustrative of maximum amounts of a mixture of monoacylglycerides derived from grape seed applied to the surfaces of certain fruits and vegetables are listed in Table 1-1. These loadings are atypically elevated but are used in the subsequent dietary intake calculations as an overestimation of potential exposure to monoacylglycerides derived from grape seed.

As described below, the weight of evidence clearly supports the safety and GRAS status of the mixture of monoacylglycerides derived from grape seed, when produced in accordance with cGMP to a food-grade specification, for its intended use in fruits and vegetables at levels up to 152 g of per 100 kg of produce (i.e., 100 g per 66 kg produce). No studies were identified showing any adverse effects when this amount of a mixture of monoacylglycerides is added to food. The application quantity of 152 g per 100 kg of crop is a conservatively high application quantity and is used as the illustrative maximum use level for any crop throughout this GRAS assessment.

Part 3 §170.235 Dietary Exposure

3.1 Estimated Daily Intake

3.1.1 Fruit

A mixture of monoacylglycerides derived from grapeseed will be applied to the external surfaces (i.e., peels) of fruits that may be consumed with peels or without peels. Fruits where the peel or rind is removed or discarded before the food is consumed include bananas, some citrus fruit, certain varieties of squash, and avocados. A mixture of monoacylglycerides derived from grape seed are not expected to migrate through the fruit skin into the edible portions of these foods. While these monoacylglycerides may be used on produce with edible peels (e.g., apples, grapes), it is expected that the monoacylglycerides will remain on the peel and will not be present in juice extracted from these or other fruits. The primary source of consumer exposure to a mixture of monoacylglycerides in Apeel products will be raw fruit with edible peels (RFEP).

In a recent report, Kimmons et al. (2009) reviewed data from the 2003–2004 National Health and Nutrition Examination Survey (NHANES) and determined the dietary contribution of fruits and vegetables from multiple sources. As shown in Table 3-1, below, the study reported on the ten most reported fruit and vegetable sources for adolescents, men over 19 years of age, and women over 19 years of age.

Table 3-1. Reported RFEP Fruit Sources as Percentage of Total Fruit Intake

Fruit	**Adolescents Age 12–18 Years**	**Men Age ≥ 19 Years**	**Women Age ≥ 19 Years**
Apples, raw	9.9	9.7	8.5
Grapes, raw	3.8	3.5	3.4
Strawberries, raw	Not Reported	1.6	2.2
Total	13.7	14.8	14.1
% Total fruit intake represented by top 10	72.4	66.9	61.6
% RFEP in top 10	18.9	22.1	22.9

It is reasonable to assume that the percentage of RFEP in the total fruit intake is close to the percentage in the top 10 fruit sources, *i.e.*, less than 25%. Thus, for the purpose of this estimate, we used 25% as the percentage of RFEP in the diet.

The US Department of Agriculture (USDA) has reported the results on the intake of various fruits and vegetables (Smiciklas-Wright et al., 2002). The reported intake for apples, strawberries, and grapes among consumers who reported eating these fruits are presented in Table 3-2, below.

Table 3-2. Average Daily Intake of Apples, Strawberries, and Grapes Among Consumers Reporting (Grams)

	All Consumers	**2-5**	**6-11**	**12 – 19**		**20 – 39**		**40 – 59**		**60 and Over**	
				M	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Apples	14	19	18	11	10	13	11	14	14	19	14
Grapes*	12	27	17	9	13	10	9	11	9	12	10
Strawberries*	3	2	3	2	3	2	4	3	4	4	4

* - Study reported only combined intake for raw fruit and fruit juice.

The coating will be applied to apples at a loading of 108 g / 100 kg fruit (i.e., 100 g / 93 kg fruit); for grapes and strawberries the loading would be 118 g / 100 kg fruit (100 g / 85 kg fruit) (see Table 1-1). According to the USDA data presented above, the average daily consumption for apples and strawberries was 14 and 3 grams, respectively, with 60-year-old males reporting the highest average consumption level for both fruit at 19 and 4 grams, respectively.

Using 14 g per day of apples, 12 g per day of grapes, and 3 g per day of strawberries as the average daily consumption, the intake of the coating is calculated as follows:

i. Apples

$$(14 \text{ g/day})(100 \text{ g} / 93 \times 10^3 \text{ g apples}) = 15.1 \text{ mg/day}$$

ii. Grapes

$$(12 \text{ g/day})(100 \text{ g} / 85 \times 10^3 \text{ g apples}) = 14.1 \text{ mg/day}$$

iii. Strawberries

$$(3 \text{ g/day})(100 \text{ g} / 85 \times 10^3 \text{ g apples}) = 3.5 \text{ mg/day}$$

The total intake from these sources would be 33 mg per day.

As shown in Table 3-1, these 3 fruits represent RFEP in the top 10 fruit sources and that the top 10 represent between 61.6% and 72.4% of daily fruit consumption. It is reasonable to assume that the percentage of RFEP in all fruit consumed is similar to the percentage in the top 10 sources. Using 61.6% as the minimum concentration of the top 10 sources, the daily intake of a mixture of monoacylglycerides derived from grape seed from all fruit would be:

$$(33 \text{ mg/day})/(61.6\%) = 54 \text{ mg/day}$$

Assuming that a high-end consumer eats twice as much fruit as the average consumer, the daily intake for the high-end consumer would be:

$$2 \times (54 \text{ mg/day}) = \underline{108 \text{ mg/day}}$$

3.1.2 Vegetables

A similar calculation can be used to determine the intake of a mixture of monoacylglycerides derived from grape seed from its use on vegetables with edible peels (VEP). The Kimmons et al. (2009) report cited in Table 3-1 also includes data on the 10 most common vegetable sources. This information is set forth in Table 3-3, below.

Table 3-3. Reported VEP Vegetable Sources as Percentage of Total Vegetable Intake

Vegetables	**Adolescents Age 12–18 Years**	**Men Age ≥ 19 Years**	**Women Age ≥ 19 Years**
White potato, baked/boiled	13.1	8.8	8.6
Beans, various	4.6	5.3	3.7
Beans, string	Not Reported	2.0	2.3
Total	17.7	16.1	14.6
% Total vegetable intake represented by top 10	70.1	57.4	54.9
Percent VEP in top 10	25.2	28.0	26.6

Thus, the top 10 vegetables represent 55% to 70% of the total amount of vegetables in the diet. Using an analogous assumption as was used to calculate the intake of a mixture of monoacylglycerides derived from grape seeds resulting from use on fruit, it is reasonable to estimate that VEP will represent 30% of vegetable intake.

The USDA has also published data on the consumption of vegetables (Smiciklas-Wright et al., 2002). For potatoes and string (i.e., green) beans, the consumption levels are listed in Table 3-4, below.

Table 3-4. Average Daily Intake of Baked/Boiled Potatoes, String Beans, Among Consumers Reporting (Grams)

	All Consumers	2-5	6-11	12 – 19		20 – 39		40 – 59		60 and Over	
				M	F	M	F	M	F	M	F
Baked Potatoes	8	3	4	7	5	10	8	9	10	12	10
Boiled Potatoes	5	2	1	3	2	4	2	7	5	11	8
String Beans	7	5	5	4	3	7	6	9	7	10	9
Total	20	10	10	14	10	21	16	25	22	33	27

Coating quantities for green beans calls for the use of 152 g per 100 kg green beans (100 g per 66 kg of green beans) (see Table 1-1). Using this application load, the average consumer's intake would be as follows:

(20 g vegetables/day)(100 g Edipeel™/66 x 10^3 g green beans) = 30 mg/day

Assuming that the top 10 reported vegetables are 55% of the vegetables consumed and that the percentage of VEP in the diet is the same as the percentage of VEP in the top 10, then the amount of a mixture of monoacylglycerides derived from grape seed that may be consumed from its use on vegetables would be:

(30 mg/day)/(55%) = 55 mg/day

It is important to note that this estimate is very conservative since the use of monoacylglycerides derived from grape seed on potatoes accounts for more than half of the amount consumed through vegetable consumption, and this estimate assumes that consumers eat the entire potato, including the peel. In practice, the peel is often removed before or after frying or boiling potatoes and many consumers do not eat the peel when eating baked potatoes.

Assuming that a high-end consumer would eat twice as much as the average consumer, the quantity per day would be:

2 x (55 mg/day) = <u>110 mg/day</u>

3.1.3 Total Estimated Daily Intake

The total amount of a mixture of monoacylglycerides derived from grape seed consumed by a high-end consumer from both fruit and vegetable applications would be:

(108 mg/day) + (110 mg/day) = **218 mg/day**

3.2 Maximum Limit of Residues

As discussed in the manufacturing process section of this assessment, the starting materials, grape seeds, are pressed into grape seed oil. To ensure levels of pesticide residues and regulated allergens are within acceptable limits, the grape seed oil or the final mixture of monoacylglycerides derived from grape seed are tested. Impurities or byproducts present in the oil (or formed over the course of the manufacturing process) are removed by heating and/or liquid–liquid separation of the intermediates, and purification of the finished product.

The raw materials used to make the mixture of monoacylglycerides derived from grape seed are used only at the levels necessary to achieve the desired technical effect and are used in accordance with cGMP. Each batch of monoacylglycerides is tested for impurities. The impurity limits listed in Table 2-2 are based on Apeel Sciences' historical manufacturing data and the exposure limits established by government agencies and other standard-setting bodies.

A search of the literature for established regulatory and recommended exposure limits was conducted for each of the processing aids listed in the manufacturing specifications (ethyl acetate, heptane, and palladium from the palladium hydroxide on carbon catalyst) and for heavy metals (Table 2-2). The corresponding ethyl acetate, heptane, and palladium residue levels for the mixture of monoacylglycerides derived from grape seed are set at thresholds that are well below safe exposure limits. Specifically, the solvent and palladium residue levels have been set to no more than 10 % of the most conservative recommended exposure limit from the literature for each solvent (as described in the sections below). The limits for heavy metals in Table 2-2 are adopted from the mono- and diglyceride specifications established by the EU (and in some cases, also by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the FCC).

The findings in the toxicology reports are based on the estimated maximum daily intake of the surface-finishing agent of 218 mg/person/day (i.e., 3.63 mg/kg bw/day for an adult of 60 kg). This intake value is based on data published by the USDA and a 2003–2004 US NHANES. The estimated daily intake calculation for the US population is described in Section 3.1, wherein the supporting data used to calculate the maximum daily intake of 218 mg/person/day is also provided.

To illustrate how the residual limits from Table 2-2 translate to a maximum exposure level for a given residue as a result of consumption of the surface-finishing agent on fruit or vegetables, the maximum daily intake (218 mg/person/day) is used in the following example calculation:

If

$$1\ ppm = \frac{1\ mg}{1\ kg}$$

then,

$$\frac{218\ mg\ MAG\ consumed}{person/day} \times \frac{21{,}000\ mg\ Solvent\ X}{kg\ MAG} \times \frac{1\ kg}{1 \times 10^6\ mg} = \frac{4.58\ mg\ Solvent\ X}{person/day}$$

where the abbreviation "MAG" refers to monoacylglycerides derived from grape seed.

The maximum daily exposure limits for each of the solvent and heavy metal residues are listed in Table 3-5, below.

Table 3-5. Maximum Daily Exposure Limits for Residues in a Mixture of Monoacylglycerides Derived from Grape Seed

Residual	Manufacturing Limit (ppm)	Daily Exposure Limit (mg/person/day)*
Ethyl acetate	21,000	4.58
Heptane	23,000	5.01
Palladium	10	0.0022
Arsenic	3	0.00065
Lead	2	0.00044
Cadmium	1	0.00022
Mercury	1	0.00022

* – The daily exposure limit is calculated based on the total amount of monoacylglycerides derived from grape seed that are consumed by a high-end consumer (218 mg/person/day).

For each of the solvent residues and palladium, the toxicological evaluation is summarized below. The heavy metal (arsenic, lead, cadmium, mercury) exposure limits for a mixture of monoacylglycerides derived from grape seed are set at the most conservative exposure limits identified in the literature, as summarized in the sub-sections below.

3.2.1 Ethyl Acetate

Ethyl acetate is the most common naturally occurring ester in wine, occurring at concentrations of around 100 mg/L. In the US, ethyl acetate is permitted as a secondary direct food additive as a solvent in the decaffeination of coffee and tea in accordance with 21 CFR 173.228, as a solvent for modified hop extraction (21 CFR 172.560(b)(4)), and in the preparation of sucrose fatty acid esters (21 CFR 172.859(a)). It is also GRAS as a synthetic flavoring substance and adjuvant in the US (21 CFR 182.60). At the International Conference on Harmonisation (ICH), the US FDA recommended that ethyl acetate be considered a Class 3 solvent (US FDA, 2012), which equates to a permissible daily exposure (PDE) limit of 50 mg or more per day.

Ethyl acetate was evaluated by JECFA at its 46th meeting, in 1996 (JECFA, 1997). The experts concluded that there was no safety concern at current levels of intake when it is used as a flavoring agent. A previously set acceptable daily intake (ADI) of 0 to 25 mg/kg bw (bw = body weight) (JECFA, 1967) was maintained. JECFA (1997) noted that ethyl acetate is completely hydrolyzed in the human body to ethanol and acetic acid, which are endogenous intermediates in human metabolism.

Additionally, ethyl acetate is acceptable as an extraction solvent for food (29th report of the Scientific Committee on Food) (SCF, 1997) and authorized for use as a flavoring in all categories of flavored foods in the EU (Regulation (EU) No. 872/2012 adopting the list of flavoring substances).

In a 2002 evaluation, the Organization for Economic Co-operation and Development (OECD) concluded that ethyl acetate was of low priority for further work (OECD, 2002).

The maximum consumption of ethyl acetate residues in the mixture of monoacylglycerides from use on fruit and vegetables derived from grape seed (21,000 ppm) corresponds to only 9.2% of the PDE of 50 mg/day established by the US FDA and the ICH.

3.2.2 Heptane

n-Heptane has been identified as a volatile constituent in foods, including duck meat, fried bacon, nectarines, heated peanut oil, and crab and shrimp meat (NIH, 2014). The US FDA, ICH, and the USP established a PDE of 50 mg/day (FDA, 2012; ICH, 2011; USP, 2016*a*). JECFA evaluated heptanes in 1970 and noted that the solvent should be used only in accordance with good manufacturing practice, with the expectation that this will result in minimal residues in the final food (JECFA, 1970). The manufacturing specification limit for heptane in the mixture of monoacylglycerides derived from grape seed (23,000 ppm) corresponds to a maximum daily consumption of 5.01 mg/person/day, which is only 10% of the FDA, USP, and ICH's established ADI.

3.2.3 Palladium

In the US, palladium is allowed for use a as catalyst for modified hop extraction (21 CFR 172.560(b)(6)). Further, the USP established an oral daily dose permissible daily exposure (PDE) of 100 micrograms/day for drug products (USP, 2016*b*). The US FDA also permits the use of palladium in the fabrication of prosthetic dental devices (21 CFR 872.3060).

In 2012, EFSA assessed palladium for use as a component of an oxygen absorber incorporated in the walls of PET bottles and/or in their closure liner (EFSA, 2012*a*). The experts reported that consumers are mainly exposed to palladium from jewelry and dental restorations and from drug products as metal residue from the production process. Palladium levels detected in food ranged from 0.3 μg/kg fresh weight (milk and poultry) to 15 μg/kg fresh weight (honey sample collected from a polluted area) (EMEA, 2008; EFSA, 2012*b*).

The EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (EFSA CEF Panel) concluded that palladium was non-genotoxic and that a low exposure to palladium, resulting from concentrations up to 50 μg/kg in food, was not of toxicological concern (EFSA, 2012*b*; EFSA, 2014).

As an example, one 150 g apple treated with the mixture of monoacylglycerides derived from grape seed at a concentration of 152 g per 100 kg of apples (where 152 g per 100 kg of produce is the most conservative quantity of monoacylglycerides applied to any type of produce), where the manufacturing specification limit for is 10 ppm (10 mg/kg) palladium. The single apple would thus carry 2.28 μg palladium. This corresponds to 15.2 μg palladium per kg of apples (or any other fruit or vegetable), which is more than 3 times lower than the 50 μg/kg limit in food established by the EFSA CEF Panel.

Additionally, the oral PDE for palladium has been established by the ICH, the European Medicines Agency (EMEA), and the USP at 0.002 mg/kg bw/day (ICH, 2014; EMEA, 2008; USP, 2016*b*), which corresponds to 0.12 mg/person/day for a 60 kg person. The manufacturing specification limit for palladium in the mixture of monoacylglycerides derived from grape seed (10 ppm) is 1.8% of the PDE established by the ICH, EMEA, and the USP.

3.2.4 Arsenic

The mono- and diglycerides (INS 471) specifications from the FCC and EU (Regulation (EU) No 231/2012) allow not more than 3 mg/kg (3 ppm) of arsenic residue. Additionally, arsenic is allowed at not more than 3 ppm in the US FDA specification for the color additive FD&C Blue No. 2 (21 CFR 74.102). The manufacturing specification limit for arsenic in the mixture of monoacylglycerides derived from grape seed is not more than 3 ppm, which is equivalent to the amount allowed by the FCC specifications.

3.2.5 Lead

The US FDA specifies a lead limit of not more than 10 ppm in two GRAS substances, glyceryl palmitostearate (21 CFR 184.1329) and gum ghatti (21 CFR 184.1333). The mono- and diglycerides (INS 471) specifications from the FCC, JECFA (JECFA, 2006), and EU (Regulation (EU) No 231/2012) allow not more than 2 mg/kg (2 ppm) of lead residue. The manufacturing specification limit for lead in the mixture of monoacylglycerides derived from grape seed is equivalent to the amount allowed by the FCC, JECFA, and EU specifications.

3.2.6 Cadmium and Mercury

As specified in GRAS Notice 648, a limit of 1 ppm was established for both cadmium and mercury for a mixture of monoacylglycerides. Additionally, the mono- and diglycerides (INS 471) specifications from the European Food Emulsifiers Manufacturers Association (EFEMA) Index of Food Emulsifiers (EFEMA, 2015) and EU (Regulation (EU) No 231/2012) allow not more than 1 mg/kg (1 ppm) of cadmium residue, and not more than 1 mg/kg (1 ppm) of mercury residue. The manufacturing specification limit for each of cadmium and mercury in the mixture of monoacylglycerides derived from grape seed is not more than 1 ppm, which is equivalent to the amount specified in the EFEMA Index of Food Emulsifiers and the amount allowed by EU specifications.

Part 4 §170.240 Self-Limiting Levels of Use

The use of grape seed monoglycerides on fresh (i.e., unprocessed) agricultural produce is self-limiting for technological reasons, such as appearance on produce and/or the effect on the produce's flavor profile, either of which could affect consumer acceptability. The quantity of monoacylglycerides derived from grape seed required to achieve the technical function is also inherently self-limiting given the unique characteristics of each fruit and vegetable and varies with the specific application. For example, overapplication of the substance may damage the fruit or vegetable, while underapplication will prohibit the maximum shelf life benefits from being achieved.

Part 5 §170.245 Experience Based on Common Use in Food Before 1958

Not applicable.

Part 6 §170.250 Narrative

6.1 Natural Occurrence and Approved Uses

The subject of this GRAS assessment is monoacylglycerides: glycerides in which each glycerol molecule has formed an ester bond with exactly one fatty acid molecule. These molecules are also known as

monoacylglycerols, monoglycerides, and fatty chain monoesters of glycerol. Thus, it is a type of glyceride molecule, also known as a lipid or fat. Monoglycerides usually occur in foods in small amounts in the region of 1% (including diglycerides). These molecules are found in plant oils or animal fats. Monoacylglycerides can be created by breaking down a triglyceride by removing two of its fatty acids or they can be manufactured synthetically. The available information suggests that approximately 20 to 25% of the total human milk fatty acids are palmitic acid, of which 70% is esterified with glycerol at the sn-2 position (Innis et al., 1993, 1994; Lien et al., 1997).

Commonly consumed vegetable fats and oils are known to contain triacylglycerols and small amounts of diacylglycerols and monoacylglycerols (D'alonzo et al., 1982). There is some evidence that further amounts of these partial glycerides, including monoglycerides, may be formed during the preparation of certain foods. Therefore, apart from any addition of these substances to food for technological purposes, these glycerides will always be present in the food as consumed (NAS, 1960). Monoglycerides are added to processed food to act as emulsifiers, binders, thickeners, texturizers. On the ingredient list of many processed sweets, such as baked goods, gum, and ice cream, these fats are simply labeled as monoglycerides or as monoacylglycerols. In bakery products, monoglycerides are useful in improving loaf volume and texture, and as anti-staling agents. Monoglycerides are also used in beverages, chewing gum, shortening, whipped toppings, margarine, and confections (Sikorski and Kolakowska, 2002; Hui, 2008).

As per 21 CFR 184.1505, mono- and diglycerides that consist of a mixture of glyceryl mono- and diesters, and minor amounts of triesters are affirmed as GRAS for direct addition to food. These substances are prepared from fats or oils or fat-forming acids and are derived from edible sources. Mono- and diglycerides are, in many cases (although not exclusively), manufactured by the reaction of glycerin (i.e., glycerol) with fatty acids, or the reaction of glycerin with triglycerides in the presence of an alkaline catalyst. The products are further purified to obtain a mixture of glycerides, free fatty acids, and free glycerol that contains at least 90 percent-by-weight glycerides. The ingredients are used in food with no limitation other than current Good Manufacturing Practice and can be used in food for multiple purposes, for example as: a dough strengthener; an emulsifier and emulsifier salt; a flavoring agent and adjuvant; a formulation aid; a lubricant and release agent; a solvent and vehicle; a stabilizer and thickener; a surface-active agent; a surface-finishing agent; and a texturizer.

JECFA has reviewed mixtures of mono- and diglyceryl esters of long chain saturated and unsaturated fatty acids (JECFA No. 471) that occur in food fats and determined the acceptable daily intake (ADI) for man to be "not limited"[5] when used as an emulsifier and stabilizer (JECFA, 1974). Similarly, the Federation of American Societies for Experimental Biology (FASEB, 1975) reviewed the safety data for partial mono- and diacylglycerol and concluded that these ingredients present no safety concerns at the intended use levels. Similarly, the European Food Safety Authority recently re-evaluated the safety of use of mono- and diglycerides of fatty acids (E 471) in foods and maintained the conclusion that there was no need for a numerical ADI (EFSA, 2017).

[5] An ADI without an explicit indication of the upper limit of intake (i.e., "not limited") may be assigned to substances of very low toxicity, especially those that are food constituents or that may be considered as foods or normal metabolites in man. An additive having a "not limited" ADI must meet the criteria of good manufacturing practice - for example, it should have proven technological efficacy and be used at the minimum level of efficacy, it should not conceal inferior food quality or adulteration, and it should not create a nutritional imbalance.

6.2 Regulatory and Other Assessments

6.2.1 FDA GRAS Review

The FDA reviewed GRAS Notice 124, the subject of which is grape seed extract (GSE), for use in beverages and beverage bases, breakfast cereals, fats and oils, frozen dairy desserts and mixes, grain products, milk (whole and skim), milk products, processed fruits, and fruit juices at levels ranging from 0.01 to 0.08%; and in oil/water systems as an antioxidant and as an emulsifier. The notifier cited a variety of published studies conducted with grape seed extract products, or components, in humans and various animal species and draws the following conclusions: acute, subchronic and chronic oral toxicity studies conducted in animals fed GSE, or substances that are components of GSE, showed no relevant compound-related toxicological effects; genotoxicity studies conducted *in vivo* or *in vitro* with commercial GSE or substances that are components of GSE demonstrated no mutagenic effects; clinical, epidemiological, and nutritional studies conducted with substances that are components of GSE demonstrated no significant adverse effects. The notifier concluded that GSE, meeting food grade specifications and produced in compliance with good manufacturing practice, is GRAS, through scientific procedures, as an antioxidant and/or emulsifier in conventional foods under the conditions of its intended use. In a letter dated August 1, 2003, FDA indicated they had 'no further questions' regarding this finding.[6]

The FDA has reviewed GRAS Notice 648, a mixture of monoacylglycerides that are applied as a surface-finishing agent and/or texturizer [21 CFR 170.3(o)(30) and (32)] to the surface of fruits and vegetables. In December of 2016, the FDA responded to GRAS Notice 648 that the agency had "no questions regarding Apeel Sciences' conclusion that monoglycerides are GRAS under its intended conditions of use." Monoacylglycerides derived from grape seed are intended for the same use as described in GRAS Notice 648, at the same proposed levels of use.

Diacylglycerol oil, the subject of two GRAS notices (GRN 56[7] and GRN 115[8]), contains a significant amount of monoacylglycerides (5%). The toxicity-related studies and findings described in these GRAS notices also support the safety of monoacylglycerides. In these GRAS notices (GRN 56 and GRN 115), diacylglycerol oil intake at 15.8 g/person/day was considered to be safe. This also indicates that the resulting intake of monoacylglycerides (i.e., 790 mg/person/day) from the uses of diacylglycerol oil is safe. The notifier for GRN 56 and GRN 115 informed the FDA that diacylglycerol oil is GRAS for use as a substitute for vegetable oils in bakery products, salad dressings, mayonnaise, pizza, breakfast/snack/power bars, soups and gravies, meal replacements, and frozen dinner entrees. The subject diacylglycerol oil is manufactured by esterification of fatty acids derived from natural edible plant oils and either monoacylglycerol or glycerol. The diacylglycerol oil has been reported to contain > 80% diacylglycerols, < 20% triacylglycerols and < 5% monoacylglycerols (Morita and Soni, 2009). In GRN 56 and GRN 115, several studies conducted with diacylglycerol oil—including published absorption and metabolism studies; unpublished acute, subchronic, and chronic toxicity studies; an unpublished mutagenicity study; and published and unpublished clinical studies designed to study the effects of diacylglycerol oil on circulating lipid levels—are described. The notifier reported that a published study shows that this composition of fat is readily hydrolyzed to monoglycerides and fatty acids in the gastrointestinal tract. The main metabolic product is 1-monoglyceride, which is further hydrolyzed into free fatty acids and glycerol, while the minor product, 2-monoglyceride, is re-esterified into triglycerides. The notifier estimated that the expected consumption of diacylglycerol oil from its uses would range from approximately 5.11 g/person/day (0.09 g/kg bw/day)

[6] Available at: http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=124.
[7] Available at: http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=56.
[8] Available at: http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=115.

for mean user consumption and 11.50 g/person/day (0.21 g/kg bw/day) for the 90th percentile user consumption. The diacylglycerol oil contains not more than 5% monoacylglycerol. Thus, the intake of monoacylglycerol from the uses of diacylglycerol oil will be, at most, approximately 256 to 575 mg/person/day. All of these GRAS notices received no question letters from the FDA. Apeel Sciences has reviewed the studies cited in GRNs 56 and 115 and has determined that the findings or conclusions from these studies support the safety of monoacylglycerides at the intended use levels proposed by Apeel Sciences in this GRAS assessment.

The subject of GRN 269,[9] polyglycerol fatty acid esters (PGFA) with a degree of polymerization (DP) of 11 to 40 for the polyglycerol backbone [PGFA (DP 11-40)] is expected to contain esters of mono- and diglycerides. From the proposed use of PGFA in conventional food and zinc- or iron-providing dietary supplements, an upper bound total maximum estimated intake of PGFA was estimated as 195 mg/person/day or 2.79 mg/kg bw/day. In GRN 269, it is described that the polyglycerides are reacted with one of the following fatty acids: stearic acid, palmitic acid, oleic acid, or coconut fatty acid. With the exception of palmitic acid, all of the above fatty acids are listed in 21 CFR 172.854, the food additive regulation for PGFAs. The notice also discusses and provides reasoning for considering palmitic acid also to be safe for the intended use. As regards the safety of such non-polyglycerol components, including palmitic acid, the notice states that FDA recognizes the GRAS status of many fatty acids and substances that produce fatty acids when metabolized. For example, ascorbyl palmitate is GRAS as a chemical preservative in food, as per 21 CFR 182.3149. Additionally, as per 21 CFR 184.1505, mono- and diglycerides made from lauric, linoleic, myristic, oleic, palmitic and stearic acids are GRAS for a variety of uses in food. Thus, FDA regulations support the safety and GRAS status of the intended use of stearic acid, palmitic acid, oleic acid, or coconut fatty acid in the production of PGFAs.

In GRN 269, the notifier discusses absorption, distribution, metabolism, and excretion pertaining to PGFA (DP 11–40). Published reports show that lower polymerized PGFAs (3–10) are hydrolyzed to their polyglycerols and fatty acids, with the fatty acids being metabolized through known biochemical pathways and the polyglycerols being largely excreted unchanged. The notifier anticipated that the highly polymerized PGFAs (11–40), will also be hydrolyzed with their polyglycerols either not being absorbed or being partially absorbed and excreted since the increased molecular size of the polyglycerols, coupled with the apparent resistance of polyglycerols to enzymatic attack, will probably further decrease, if not prevent, absorption. Following its review, the FDA responded to the notifier that the agency has no questions. There are several remarks in this notice with regards to the metabolic by-products of PGFA esters (i.e., glycerol and fatty acid) being safe and non-toxic. These would be the same metabolic by-products of consumption of monoacylglycerides derived from grape seed.

In a GRAS notice on high 2-palmitic vegetable oil to FDA (GRN 131[10]) for use of a triglyceride mixture made specifically for use in infant formulas to closely mimic both the proportion of fatty acids in human breast milk and their arrangement on the glycerol backbone, several safety related studies are summarized. The triglyceride mixture is prepared by interesterification technology. The principal fatty acids of the mixture, oleic and palmitic acids, are consumed as components of fat in infant formulas. In this GRAS notice, the metabolic fate of ^{14}C-labeled palmitic acid esterified to glycerol in the sn-1 and -3 or sn-2 positions were compared in suckling and weanling rats. No apparent differences were noted in the rate of metabolism or distribution of radioactivity in the body between rats dosed with palmitic acid in either the sn-1 and -3, or sn-2 positions. This GRAS notice also summarized several other safety studies that supported the use

[9] Available at: http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=269.
[10] Available at: http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=131.

of this triglyceride mixture in infants. The FDA filed this and another similar GRAS (GRN 192[11]) notice on high 2-palmitic acid vegetable oil for use in infant formulas without any questions.

The intake of monoacylglycerides of 218 mg/person/day from the intended uses proposed by Apeel Sciences in GRN 648 is less than 1/6th of the intake of monoacylglycerides from uses of diacylglycerol oil. Apeel reviewed the studies cited in these notices and determined that they confirm that consumption of a mixture of monoacylglycerides as an added food ingredient is safe at the proposed maximum exposure level of 218 mg/day.

6.2.2 Cosmetic Ingredient Review

In an extensive review, the Cosmetic Ingredient Review (CIR) Expert Panel summarized the safety information of 43 glyceryl monoesters as cosmetic ingredients (CIR, 2004). The report states that glyceryl monoesters have been approved by FDA for use as direct or indirect food additives. Following its oral ingestion, glyceryl monoesters (monoglycerides) are metabolized to free fatty acids and glycerol, both of which are available for the re-synthesis of triglycerides. This review also cited that the safety of mono- and diglycerides in food has been reviewed by the Food Protection Committee of the National Academy of Sciences National Research Council Food and Nutrition Board (National Academy of Sciences, 1960). The Food Protection Committee concluded that there appears to be no reason to question the safety of mono-, di-, or triglycerides of lauric acid (i.e., glyceryl laurate, glyceryl dilaurate, or glyceryl trilaurate [trilaurin]) as food additives.

The CIR Panel concluded that, although mammalian genotoxicity data on the glyceryl monoesters were not available, these esters are not likely genotoxic agents based on the chemical structures of these compounds and negative Ames test data. Limited carcinogenicity data were negative, and data on the glyceryl monoester glyceryl stearate indicated that 5% glyceryl stearate in acetone was not a tumor promoter in Swiss mice. Based on the available animal and clinical data included, the CIR Expert Panel concluded that the glyceryl monoesters (described in the report) are safe as cosmetic ingredients in the present practices of use and concentration. The Panel also concluded that the available data are insufficient to support the safety of glyceryl arachidonate in cosmetic formulations.

6.2.3 Digestion and Metabolism

The majority of dietary fat is supplied in the form of triacylglycerols that must be hydrolyzed to fatty acids and monoacylglycerols before being absorbed. It is well established that the pancreas produces and secretes digestive enzymes in the upper gastrointestinal (GI) tract. The stomach plays an important role in fat digestion because of its churning action, which helps to create an emulsion. Once inside the intestine, fat is mixed with bile and is further emulsified. The emulsion is then acted upon by lipases secreted by the pancreas. Pancreatic lipase catalyzes the hydrolysis of fatty acids from positions 1 and 3 to yield 2-monoacylglycerols (Tso, 1985). The free fatty acids and monoglycerides are absorbed by the enterocytes of the intestinal wall. In general, fatty acids with chain length of < 14 carbons enter directly into the portal vein system and are transported to the liver. Fatty acids with 14 or more carbons are re-esterified within the enterocyte and enter the circulation via the lymphatic route as chylomicrons.

The available information indicates that approximately 28% of the β-monoglyceride (2-monoglyceride) is isomerized to α-monoglyceride (1-monoglyceride), and approximately 75% of the α-monoglyceride is

[11] Available at: http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=192.

further hydrolyzed to free glycerol. Free glycerol enters the intestinal wall independent of the lipids, and it has no further use in terms of lipid absorption. The free fatty acids and glycerol are available for the re-synthesis of triglycerides. β-Monoglycerides are not hydrolyzed because of their transfer to a water-soluble phase and, also, because of enzyme specificity. However, they can be acylated directly to triglyceride (Mattson and Volpenhein, 1964). In another study, Mattson and Beck (1965) reported that the results with triglycerides of known composition demonstrate that the hydrolysis of triglyceride by pancreatic lipase is a series of directed stepwise reactions from triglyceride to 1,2-diglyceride to 2-monoglyceride. This route is the same regardless of whether the fatty acid is palmitic, stearic, or oleic acid.

The digestion and absorption of *long-chain triglycerides*, the major form of dietary lipids, is a highly efficient process involving several distinct steps such as emulsification, hydrolysis by lipases into fatty acids and monoacylglycerols, dispersion of these products in to an aqueous environment, and uptake by enterocytes. Following ingestion, long-chain triglycerides are broken down by buccal, gastric, pancreatic, and intestinal lipases to form two free long-chain fatty acids and sn2-monoacylglycerol. The middle fatty acid remains attached to the glycerol backbone (Hoy and Xu, 2001). The long chain fatty acid and sn2-monoacylglycerols are packaged into micelles for transport through the blood. The micelles contain bile salts, phospholipids, and other emulsifiers that help in binding to enterocytes. After incorporation into micelles, absorption into the intestinal mucosa can occur throughout the small intestine.

There is preferential absorption of sn2-monoacylglycerol over the free fatty acid. In the mucosa, the sn2-monoacylglycerol serves as a template for triacylglyceride formation, and the long chain fatty acids are converted into acyl-Coenzyme A (CoA) derivatives in the presence of acyl-CoA synthetase (an enzyme specific for fatty acids with more than 12 carbon atoms). Once formed, the long chain fatty acid acyl-CoA's are packaged into micelles, containing emulsifiers, bile salts, and phospholipids, followed by re-esterification back onto the sn2-monoacylglycerol to reform triacylglycerols. Following this, the triacylglycerols are packaged by the intestinal cells into lipoprotein complexes or chylomicrons, which are secreted into the lymphatic system and eventually enter the systemic circulation. In an attempt to elucidate nutritional characterization of diacylglycerol oil, it was demonstrated that 1,3-diacylglycerol is hydrolyzed to 1- (or 3-) monoacylglycerol and fatty acids via the intermediate 1- (or 3-) monoacylglycerol, whereas triacylglycerol is hydrolyzed to 2-monoacylglycerol and fatty acids. Based on these observations, Watanabe and Tokimitsu (2004) hypothesized that the limited availability of 2-monoacylglycerol for re-esterification retards chylomicron-triacylglycerol transport during diacylglycerol oil ingestion.

Metabolism studies indicate that—based on chain length—medium and long chain triglycerides are readily broken down into medium- and long-chain fatty acids and absorbed via the portal or lymphatic route. Medium-chain fatty acids are directly absorbed into the portal vein, preferentially oxidized in the liver, and ultimately metabolized to carbon dioxide, acetate, and ketones. Long chain fatty acids and sn2 long-chain monoacylglycerols are packaged into micelles, which are then absorbed across the intestinal mucosa. The sn2-monoacylglycerols and long-chain fatty acids are reformed into triacylglycerols, and secreted into the lymphatic system as chylomicrons, for eventual uptake into the adipose tissues for storage and later release as an energy source. It is well known that the intestine is capable of assimilating dietary fat via phosphatidic acid and monoacylglycerol pathways of acylglycerol synthesis, which under normal conditions contribute about 20% and 80%, respectively, to the total chylomicron triacylglycerol formation (Yang and Kuksis, 1991).

The available studies indicate that, regardless of their constituent fatty acid, monoacylglycerols are typically well absorbed (Lien et al., 1997). However, the free fatty acid absorption depends on their

structure, with mono- and polyunsaturated fatty acids and saturated fatty acids with chain lengths of 12 carbons or less being better absorbed than long-chain saturated fatty acids (Lien et al., 1997). In the small intestine, triacylglycerols esterified with palmitic acid at the sn2 position are converted to free fatty acids and 2-monopalmitin (the 2-monoacylglycerol), which is readily absorbed (Lien et al., 1997).

As the subject of the present GRAS assessment covers monoacylglycerides primarily in the 8- to 18-carbon chain length, the above discussion is applicable to the metabolism of these constituents. The above discussion also suggests that, similar to triacylglycerol oil, both of these constituents are readily digested, absorbed and metabolized. The available information also indicate that different fatty acid chains or positioning are unlikely to affect the overall oral toxicity, as the fatty acid portions of molecules are largely cleaved prior to absorption by mucosal cells.

6.3 Safety Studies

The safety of the intended use of monoacylglycerides by Apeel Sciences is also based on available animal and human studies. As described in the review article by Morita and Soni (2009), in a dose-response 90-day rat study, the effects of feeding rats a diet containing unheated and heated diacylglycerol oil (containing 5% monoacylglycerides) was assessed. The results of the subchronic toxicity study of unheated and heated diacylglycerol oil in rats did not reveal any adverse effects, as evaluated by a series of clinical parameters and histological evaluation of a number of tissues (Morita et al., 2008). The “no observed adverse effect level” (NOAEL) for male and female rats was determined to be 3,226 and 4,488 mg/kg bw/day respectively for unheated diacylglycerol oil. The lowest NOAEL value for diacylglycerol oil (male) is 3,226 mg/kg bw/day. The corresponding intake of monoacylglycerides due to diacylglycerol oil intake (where diacylglycerol oil contains 5% monoacylglycerides) was calculated to be 161 mg/kg bw/day, or 9,660 mg/person/day for an individual weighing 60 kg. The maximum monoacylglycerides exposure determined by Apeel Sciences for use on fruit and vegetables of 218 mg/person/day is approximately 1/44th of the monoacylglyceride intake for the lowest NOAEL determined in these studies for unheated diacylglycerol oil. This finding suggests that the proposed use of monoacylglycerides as a component of an edible coating for fruits and vegetables is unlikely to cause adverse effects following human consumption.

Similarly, the results of the chronic animal toxicity studies show that dietary administration of diacylglycerol oil (containing 5% monoacylglycerides) at levels up to 5.3% to rats for 2 years (Soni et al., 2001), or administration at 9.5% to Beagle dogs for 1 year (Chengelis et al., 2006), had no adverse effects. In the life-time exposure rat study, dietary exposure to diacylglycerol oil did not affect cumulative survival rate, clinical parameters, or tissue histopathology. The highest dose tested was determined to be the NOAEL: 1.77 and 2.35 g/kg bw/day for male and female rats, respectively. For safety assessment purposes, the low NOAEL value of 1,770 mg/kg bw/day in male rats was assessed. Furthermore, dietary exposure of diacylglycerol oil to dogs for one year did not reveal any evidence of toxicity. The NOAEL for the dog study was also the highest dose tested in male and female dogs. The corresponding lower NOAEL of 2,300 mg/kg bw/day in female dogs was assessed. The corresponding amount of monoacylglycerides in rats would amount to 88.5 mg/kg bw/day (5,310 mg/person/day for a 60 kg person), while in dogs it would be 115 mg/kg bw/day (6,900 mg/person/day for a 60 kg person). These intake levels from chronic studies in rats and dogs are approximately 24 and 32 times (respectively) greater than the monoacylglycerides intake of 218 mg/person/day from its intended uses proposed by Apeel Sciences. Therefore, these rat and dog studies support the safety of the monoacylglycerides for the intended use as a component of an edible coating for fruits and vegetables.

The findings of the longer-term duration clinical studies with diacylglycerol oil are summarized in Table 6-1. From the available clinical studies, a total of 12 longer-term clinical studies were identified in which over 800 subjects participated. The studies lasted from 3 to 12 months. In these studies, the physiological effects of diacylglycerol oil were examined in normal subjects as well as in some patient groups. In the majority of these studies, diacylglycerol oil was used as a source of dietary fat. Of the 12 longer duration clinical studies of diacylglycerol oil, 6 were double-blind trials. These studies provided an opportunity to assess the safety and tolerability of diacylglycerol oil in a diverse population. Collectively, these studies are of sufficient quality and consistency to draw certain conclusions regarding the safety of diacylglycerol oil. In five studies, the intake of diacylglycerol oil was *ad libitum*, as a complete substitute for oil for up to 12 months. The parameters investigated in these studies included lipid profile, serum chemistry, and hematology parameters. No treatment-related adverse effects of diacylglycerol oil were noted. In most of these studies, diacylglycerol oil consumption was in the range of 10 to 35 g/day for several months (also described in GRN 115). As diacylglycerol oil contains 5% monoacylglyceride, the corresponding intake of monoacylglycerides in these studies ranged from 500 to 1,750 mg/person/day. These intake estimates are more than 2 to 8 times higher than the estimated intake for a mixture of monoacylglycerides derived from grape seed (218 mg/person/day).

Table 6-1. Summary of Long-Term Clinical Studies of Diacylglycerol Oil

Reference	**Study Design; Subjects; Sex**	**Dose; Duration**	**Estimated Intake (mg/kg-bw/day)**	**Safety Results; Comments**
Kawashima et al. (2008)	Double-blind parallel; 155–157 /group; M,F	Normal cooking oil substituted with DAG oil for 12 months	*Ad libitum*	No adverse effects; well tolerated
Yasunaga et al. (2002; unpublished)	Randomized dietary trial; overweight; 198F	Normal edible oil substituted with DAG oil for 1 year	*Ad libitum*	No adverse effects; well tolerated
Katsuragi et al. (1999)	Open label; overweight; 89M, 20F	Normal edible oil substituted with DAG oil for 9 months	*Ad libitum*	No adverse effects; well tolerated
Maki et al. (2002)	Double-blind parallel; overweight/obese; 62–65/group; M, F	Normal cooking oil substituted with DAG oil (substituting ~15% of dietary energy) for 24 weeks		No adverse effects; well tolerated
Matsuyama et al. (2006)	Open label; obese children (7–17 year); 4M, 7F	Normal cooking oil substituted with DAG oil (24 g/day) for 5 months		No adverse effects; well tolerated
Nagao et al. (2000)	Double-blind parallel; 19/group; M	10 g DAG oil/day for 16 weeks		No adverse effects; well tolerated
Watanabe et al. (2001)	Double-blind parallel; 12–15/group; M	20 g DAG oil/day for 3 months	300	No adverse effects; well tolerated
Teramoto et al. (2004)	Open label; uremic adults on dialysis; 7M, 3F	Normal cooking oil substituted with DAG oil (9.8 g/day) for 3 months	*Ad libitum*	No adverse effects; well tolerated

Reference	Study Design; Subjects; Sex	Dose; Duration	Estimated Intake (mg/kg-bw/day)	Safety Results; Comments
Shoji et al. (2008)	Double-blind parallel; obese; 77–78 /group; M, F	Normal cooking oil substituted with DAG oil for 3 months	*Ad libitum*	No adverse effects; well tolerated
Yamamoto et al. (2006)	Single-blind controlled; Type II diabetic with hypertriglyceridemia; 24	Normal cooking oil substituted with DAG oil for 3 months		No adverse effects; well tolerated
Yamamoto et al. (2001)	Single-blind parallel; Type II diabetic with hypertriglyceridemia; 3–5/gender/group; M, F	Normal cooking oil substituted with DAG oil (~10.6 g/day) for 12 weeks		No adverse effects; well tolerated
Yasunaga et al. (2004)	Double-blind parallel; 18–21 /group; M, F	DAG oil incorporated in diet for 12 weeks	Up to 500	No adverse effects; well tolerated

DAG = diacylglycerol; adapted from Morita and Soni (2009). The full list of references for these studies is provided in the list of references (Part 7).

Human clinical studies with diacylglycerol oil showed no adverse effects at levels ranging from 9.8 to 44 g/person/day (Teramoto et al., 2004). The resulting intake of monoacylglycerides of 490 to 2,200 mg/person/day in these studies is 2-to 10-fold higher than the monoacylglycerides intake of 218 mg/person/day from its intended uses proposed by Apeel Sciences. Thus, findings from these human studies also support the safety of chronic exposure to monoacylglycerides from the proposed uses of monoacylglycerides described in this GRAS assessment. The evidence from the human clinical studies adds to the totality of available evidence from other studies to support the conclusion, of the safety of monoacylglycerides as described in this GRAS assessment.

6.4 Expert Panel Evaluation

An ad hoc GRAS Panel was assembled by Apeel Sciences. Only scientists who are qualified by scientific training and experience to evaluate the safety of food ingredients were included. Specifically, the ad hoc GRAS Panel consisted of the following qualified scientific experts:

- Ashley Roberts, Ph.D. President, ARToxicology Inc
- Nadia Moore, Ph.D, DABT, ERT, J.S. Held Principal Toxicologist
- Robert Mathews, Ph.D., Keller & Heckman Senior Toxicologist

Based on a critical evaluation of the publicly available data summarized herein, the ad hoc GRAS Panel members, whose signatures appear below, have individually and collectively concluded that a mixture of monoacylglycerides (i.e., monoglycerides or fatty acid monoesters of glycerol) derived from grape seed, meeting the specifications cited above, and when used as a component of a surface-finishing agent [21 CFR 170.3(o)(30)] to create a thin and edible physical barrier against moisture loss and oxidation to protect the freshness and extend the shelf-life of fresh fruits and vegetables, when not otherwise precluded by Standards of Identity as described in this dossier, and, resulting in an intake of up to 218 mg of the monoacylglycerides per person per day for a high-end consumer, is Generally Recognized as Safe (GRAS).

It is also our opinion that other qualified and competent scientists reviewing the same publicly available toxicological and safety information would reach the same conclusion. Therefore, we have concluded that a mixture of monoacylglycerides derived from grape seed, when used as described, is GRAS, based on scientific procedures.

Signatures

DocuSigned by:
[redacted]
8813FF6C30204CE...

Ashley Roberts, Ph.D. — Date: 10/2/2019

DocuSigned by:
[redacted]
AB90E9C1BC3D468...

Nadia Moore, Ph.D, DABT, ERT — Date: 10/2/2019

DocuSigned by:
[redacted]
A440EC40D02D413...

Robert Mathews, Ph.D. — Date: 10/2/2019

6.5 Summary

Apeel Sciences intends to use a mixture of monoacylglycerides (i.e., monoglycerides or fatty chain monoesters of glycerol) derived from grape seed as a surface-finishing agent [21 CFR 170.3(o)(30)], creating a thin and edible physical barrier against moisture loss and oxidation to protect the freshness and extend the shelf-life of agricultural products such as fruits (e.g., berries, grapes, stone fruit, citrus, bananas, mangoes, avocados) and vegetables (e.g., legumes, roots, tubers) when applied to their surfaces. A mixture of monoacylglycerides derived from grape seed will be applied at a maximum loading of 152 g /100 kg produce (100 g / 66 kg produce) to fruits and vegetables. The mixture of monoacylglycerides derived from grape seed is prepared by extruding the grape seeds by mechanical pressing followed by processing of the grapeseed oil to generate monoacylglycerides containing exclusively the same naturally occurring fatty acids in the grapeseed oil in their saturated forms. The mixture of monoacylglycerides is manufactured as per current Good Manufacturing Practices (cGMP) using food grade ingredients and processing aids. Food grade specifications for the mixture of monoacylglycerides derived from grape seed have been established (Table 2-2). The intended use of a mixture of monoacylglycerides derived from grape seed will result in a maximum intake of 218 mg monoacylglycerides/person/day for a high-end consumer.

The constituents of a mixture of monoacylglycerides derived from grape seed are found in nature. As per 21 CFR 184.1505, mono- and diglycerides that consist of a mixture of glyceryl mono- and diesters and minor amounts of triesters are affirmed as GRAS for direct addition to food. The mono- and diglycerides are permitted for use in food with no limitation other than current Good Manufacturing Practice. These esters can be used in food for multiple purposes, for example, as: a dough strengthener; an emulsifier and emulsifier salt; a flavoring agent and adjuvant; a formulation aid; a lubricant and release agent; a solvent and vehicle; a stabilizer and thickener; a surface-active agent; a surface-finishing agent; and a texturizer.

The constituents of the mixture of monoacylglycerides derived from grape seed are present in the diet as components of conventional dietary oils and as approved food additives (i.e., mono-and diglycerides), and are formed from normal lipid metabolism following the consumption of dietary fat. There is no evidence that the presence of monoglycerides or diglycerides of food fats have any deleterious effect on cells or tissues. The JECFA has evaluated mixtures of mono- and diglyceryl esters of long chain saturated and unsaturated fatty acids (JECFA No. 471) that occur in food fats and established an acceptable daily intake as “not limited” when used as an emulsifier and stabilizer. Similarly, the FASEB reviewed the safety data of partial mono- and diacylglycerol and concluded that these ingredients present no safety concerns at the intended use levels. Further, a GRAS notice (GRN 648) for the use of a mixture of monoacylglycerides as a surface-finishing agent and/or texturizer on the surface of fruits and vegetables received a ‘no questions letter’ from the FDA. Monoacylglycerides derived from grape seed are intended for the same use as described in GRAS Notice 648, at the same proposed levels of use. Two further GRAS notices (GRN 56 and 115) for diacylglycerol oil that contains monoglycerides received ‘no question letters’ from the US FDA. Additionally, GRN 124 which describes the safety of grape seed extract as an antioxidant and/or emulsifier in conventional foods under the conditions of its intended use also received a ‘no questions letter’ from the US FDA.

It is well known that orally ingested fats or oils (triglycerides) undergo initial metabolism in the gastrointestinal tract and are broken down mainly by pancreatic lipase resulting in the formation of mono- and diacylglycerols and individual fatty acids, prior to its absorption. These parts are reassembled into triglycerides and carried into the body through the lymph system in chylomicrons. The absorbed lipids are stored, used for energy, or converted into other endogenous constituents. Since metabolism of dietary

oils results in the formation of monoglycerides, it is therefore considered unlikely that monoacylglycerides generated from grape seed would be metabolized any differently.

The totality of available evidence from the dietary consumption of oils that contain mono- and diglycerides, current approved uses of mono- and diglycerides, and animal and human studies of diacylglycerol oil that contains (< 5%) monoglycerides suggest that consumption of a mixture of monoacylglycerides derived from grape seed for the intended uses at proposed use levels is safe. On the basis of both scientific procedures[12] corroborated by history of exposure from natural dietary sources and approved uses, Apeel Sciences considers the consumption of a mixture of monoacylglycerides derived from grape seed as an added food ingredient to be safe at a daily consumption of up to 218 mg/day. Thus, information available within the scientific literature demonstrate that a mixture of monoacylglycerides derived from grape seed, offering a thin and edible physical barrier against moisture loss and oxidation to protect the freshness and extend the shelf-life of agricultural products when manufactured under cGMP, is safe for its intended uses.

6.6 Conclusion

In summary, based on the information provided above and the fact that the monoacylglyceride constituents of a mixture of monoacylglycerides derived from grape seed are found naturally and likewise produced during the normal metabolism of oils, it is concluded that scientific experts, generally, would recognize them to be as safe and as acceptable as other mono- and diglycerides. Further, Apeel Sciences believes that there are no significant questions regarding the safety of a mixture of monoacylglycerides derived from grapeseed that would appear to require additional safety studies. In light of the data and discussion presented above, Apeel Sciences respectfully concludes that a mixture of monoacylglycerides derived from grape seed, meeting the specifications cited above (Table 2-2), and when used as a component of a surface-finishing agent [21 CFR 170.3(o)(30)] to create a thin and edible physical barrier against moisture loss and oxidation to protect the freshness and extend the shelf-life of fresh fruits and vegetables, when not otherwise precluded by Standards of Identity, is GRAS, as demonstrated through scientific procedures.

The GRAS notices (Section 6.2) describing the safety and metabolic fate of polyglycerol fatty acid esters (GRN 269) and a mixture of monoacylglycerides (GRN 648)—along with the Code of Federal Regulations (21 CFR 182.3149 and 21 CFR 184.1505, respectively)—corroborate the safety of monoacylglycerides at use levels proposed by Apeel Sciences in GRN 648 and this GRAS assessment. In addition to the aforementioned GRAS notices, the Cosmetic Ingredient Review (CIR) Expert Panel described the safety information of 43 glyceryl monoesters, including as cosmetic ingredients. The report also states that glyceryl monoesters have been approved by FDA for use as direct or indirect food additives. The safety conclusions drawn by the CIR for glyceryl monoesters corroborate the safety of the mixture of monoacylglycerides derived from grape seed proposed by Apeel Sciences herein. Apeel is in agreement with the interpretation of the findings or conclusions drawn by the authors of the cited sources or references.

The cited studies in Section 6.3 were conducted with diacylglycerol and the findings from these studies showed a lack of adverse effects. Thus, these studies support the safety of diacylglycerol, where the diacylglycerol used in these studies contained 5% monoacylglycerides. Taking into account the

[12] 21 CFR §170.3 Definitions. (h) Scientific procedures include those human, animal, analytical, and other scientific studies, whether published or unpublished, appropriate to establish the safety of a substance.

monoacylglyceride content in diacylglycerol, Apeel Sciences concludes that the studies also support that there are no adverse effects resulting from the monoacylglyceride component thereof. Based on the findings from diacylglycerol studies containing 5% monoacylglycerides, Apeel Sciences concludes that the associated monoacylglyceride exposure can be considered safe.

Part 7 §170.255 List of Supporting Data and Information

ADM-KAO, 2002. GRAS notification for expanded use of diacylglycerol oil. GRAS Notice 115. Available at: http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=115.

Akın, A., Altındişli, A., 2011. Determination of fatty acid composition and lipid content of some grape cultivar seeds in Turkey. Biyol. Sci. Res. J. 4:13-15.

Al Juhaimi, F., Geçgel, Ü., Gülcü, M., Hamurcu, M., Özcan, M. M., 2017. Bioactive properties, fatty acid composition and mineral contents of grape seed and oils. S. Afr. J. Enol. Vitic., 38(1): 103-107.

Apeel Sciences, 2016. GRAS notification for monoglycerides. GRAS Notice 648. Available at: https://www.accessdata.fda.gov/scripts/fdcc/?set=GRASNotices&id=648.

Chengelis, C.P., Kirkpatrick, J.B., Marit, G.B., Morita, O., Tamaki, Y., Suzuki, H., 2006. A chronic dietary toxicity study of DAG (diacylglycerol) in Beagle dogs. Food Chem.Toxicol.44:81-97.

CIR, 2004. Cosmetic Ingredient Review. Final Report of the Amended Safety Assessment of Glyceryl Laurate, Glyceryl Laurate SE, Glyceryl Laurate/Oleate, Glyceryl Adipate, Glyceryl Alginate, Glyceryl Arachidate, Glyceryl Arachidonate, Glyceryl Behenate, Glyceryl Caprate, Glyceryl Caprylate, Glyceryl Caprylate/Caprate, Glyceryl Citrate/Lactate/Linoleate/Oleate, Glyceryl Cocoate, Glyceryl Collagenate, Glyceryl Erucate, Glyceryl Hydrogenated Rosinate, Glyceryl Hydrogenated Soyate, Glyceryl Hydroxystearate, Glyceryl Isopalmitate, Glyceryl Isostearate, Glyceryl Isostearate/Myristate, Glyceryl Isostearates, Glyceryl Lanolate, Glyceryl Linoleate, Glyceryl Linolenate, Glyceryl Montanate, Glyceryl Myristate, Glyceryl Isotridecanoate/Stearate/Adipate, Glyceryl Oleate SE, Glyceryl Oleate/Elaidate, Glyceryl Palmitate, Glyceryl Palmitate/Stearate, Glyceryl Palmitoleate, Glyceryl Pentadecanoate, Glyceryl Polyacrylate, Glyceryl Rosinate, Glyceryl Sesquioleate, Glyceryl/Sorbitol Oleate/Hydroxystearate, Glyceryl Stearate/Acetate, Glyceryl Stearate/Maleate, Glyceryl Tallowate, Glyceryl Thiopropionate, and Glyceryl Undecylenate. International Journal of Toxicology, 23(Suppl. 2):55–94.

Citil, O.B., Sezgin, M., Guler, G.O., Aktümsek, A., 2010. Fatty acid compositions of some feed raw materials in poultry diets. Asian J. Chem. 22:3785-3792.

COMMISSION IMPLEMENTING REGULATION (EU) No. 872/2012 of 1 October 2012 adopting the list of flavouring substances provided by Regulation (EC) No. 2232/96 of the European Parliament and of the Council, introducing it in Annex I to Regulation (EC) No. 1334/2008 of the European Parliament and of the Council and repealing Commission Regulation (EC) No. 1565/2000 and Commission Decision 1999/217/EC.

D'alonzo, R.P., Kozarek, W.J., Wade, R.L., 1982. Glyceride composition of processed fats and oils as determined by glass capillary gas chromatography. Journal of the American Oil Chemists' Society 59:292-295.

Danish Environmental Protection Agency, 2014. Survey of toluene - Part of the LOUS review Environmental project No. 1613. Copenhagen, Denmark.

EFEMA (European Food Emulsifier Manufacturers Association) index of food emulsifiers. June 2015. Available at: http://www.emulsifiers.org/ViewDocument.asp?ItemId=31.

EFSA (European Food Safety Authority), 2012*a*. Panel on Contaminants in the Food Chain (CONTAM); Scientific Opinion on the evaluation of the substances currently on the list in the annex to Commission Directive 96/3/EC as acceptable previous cargoes for edible fats and oils – Part II of III. EFSA Journal 2012;10(5):2703. [151 pp.] doi: 10.2903/j.efsa.2012.2703.

EFSA, 2012*b*. CEF Panel (EFSA Panel on Food Contact Materials, E., Flavourings and Processing Aids). Scientific Opinion on the safety evaluation of the active substances, sodium borohydride and palladium acetate for use in active food contact materials. EFSA Journal. 2012; 10(3).

EFSA, 2014. CEF Panel (EFSA Panel on Food Contact Materials, E., Flavourings and Processing Aids),. Scientific Opinion on the safety assessment of the active substances, palladium metal and hydrogen gas, for use in active food contact materials. EFSA Journal. 2014; 12(2).

EFSA Panel on Food Additives and Nutrient Sources Added to Food (ANS), 2017. Scientific Opinion: Re-evaluation of mono- and di-glycerides of fatty acids (E 471) as food additives. EFSA Journal 2017; 15(11):5045, 43 pp. doi: 10.2903/j.efsa.2017.5045.

EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2012. Scientific Opinion on the safety evaluation of the active substances, sodium borohydride and palladium acetate for use in active food contact materials. EFSA Journal 2012;10(3):2642, 15 pp. doi: 10.2903/j.efsa.2012.2642.

Embuscado, M.E., Huber, K.C., 2009. Editors Edible Films and Coatings for Food Applications. Springer. ISBN 978-0-387-92823-4. pp. 1-410.

EMEA (European Medicines Agency), 2008. Guideline on the Specification Limits for Residues of Metal Catalysts or Metal Reagents. London, UK: 2008. Committee for Medicinal Products for Human Use.

European Chemicals Bureau, 2002. European Union Risk Assessment Report – ACETONITRILE. EUROPEAN COMMISSION JOINT RESEARCH CENTRE. EUR 19839 EN. Luxembourg: Office for Official Publications of the European Communities, 2002.

European Chemicals Bureau, 2003. European Union Risk Assessment Report – TOLUENE. EUROPEAN COMMISSION JOINT RESEARCH CENTRE. EUR 20539 EN. Luxembourg: Office for Official Publications of the European Communities, 2003.

FASEB, 1975. Evaluation of the health aspect of glycerin and glycerides as food ingredients. Federation of American Societies for Experimental Biology, US Department of Commerce, National Technical Information Service, PB-254536.

FDA. Guidance for Industry. Q3C – Tables and List 2012. US Department of Health and Human Services. Food and Drug Administration. Center for Drug Evaluation and Research (CDER). Center for Biologics Evaluation and Research (CBER). February 2012. ICH.

Göktürk Baydar, N., Akkurt, M., 2001. Oil content and oil quality properties of some grape seeds. Turk J. Agric. Forestr. 25:161-168.

Hassanein, M.M.M., Abedel-Razek, A.G., 2009. Chromatographic quantitation of some bioactive minor components in oils of wheat germ and grape seeds produced as by-products. J. Oleo Sci. 58: 227-233.

Hoy, C. E., Xu, X., 2001. Structured Triacyglycerols. In Structured and Modified Lipids. (F. D. Gunstone, Ed.). Marcel Dekker, New York, NY. p. 209-239.

Hui, Y.H., 2008. Bakery Products: Science and Technology. John Wiley & Sons. pp. 350. ISBN 978-0-470-27632-7.

ICH (International Conference on Harmonisation of Technical Requirements for Pharmaceuticals for Human Use), 2011. Impurities: Guideline for residual solvents Q3C (R5). Step 4 version. International Conference on Harmonisation (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use.

ICH, 2014. Guideline for elemental impurities Q3D. Step 4 version. International Conference on Harmonisation (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use.

Innis, S.M., Dyer, R., Nelson, M., 1994. Evidence that palmitic acid is absorbed as sn-2 monoacylglycerol from human milk by breast-fed infants. Lipids 29(8):541-545.

Innis, S.M., Quinlan, P., Diersen-Schade, D., 1993. Saturated fatty acid chain length and positional distribution in infant formula: effects on growth and plasma lipids and ketones in piglets. American Journal of Clinical Nutrition 57(3):382-390.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1967. FAO Nutrition Meetings. Report Series No. 44A. WHO/Food Add./68.33 Toxicological evaluation of some flavouring substances and non-nutritive sweetening agents. Ethyl acetate. WHO, Geneva, Switzerland.

JECFA, 1970. Evaluation of food additives. Specifications for the identity and purity of food additives and their toxicological evaluation; some extraction solvents and certain other substances; and a review of the technological efficacy of some antimicrobial agents. 14th report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, Switzerland: 1970. 1970 JECFA meeting. In: Report No.: WHO Technical Report Series No. 462; FAO Nutrition Meetings Report Series No. 48, 1971.

JECFA, 1974. Mono- and diglycerides. In: JECFA. Toxicological Evaluation of Some Food Additives Including Anticaking Agents, Antimicrobials, Antioxicants, Emulsifiers and Thickening Agents. 17th JECFA Session, June 25-July 4, 1973, Geneva, Switzerland.

JECFA, 1997. WHO Technical Report Series 868. Evaluation of certain food additives and contaminants. Forty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives. WHO, Geneva, Switzerland.

JECFA, 2006. Mono- and Diglcyerides. In: JECFA, 2006. Combined Compendium of Food Additive Specifications: Analytical methods, test procedures and laboratory solutions used by and referenced in food additive specifications, Vol. 4.

JECFA, 2006. Mono- and Diglcyerides. In: JECFA, 2006. Combined Compendium of Food Additive Specifications: Food additives, E-O, Vol. 2.

Kamel, B.S., Dawson, H. & Kakuda, Y., 1985. Characteristics and composition of melon and grape seed oils and cakes. J. Am. Oil Chem. Soc. 62:881-883.

Kao Corporation, 2000. GRAS Notification for DAG. GRAS Notice 56. Available at: http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=56.

Katsuragi, Y., Toi, T., Yasukawa, T., 1999. Effects of dietary diacylglycerol on obesity and hyperlipedemia. Journal of Japanese Society of Human Dry Dock 14:258-262.

Kawashima, H., Takase, H., Yasunaga, K., Wakaki, Y., Katsuragi, Y., Mori, K., Yamaguchi, T., Hase, T., Matsuo, N., Yasukawa, T., Tokimitsu, I., Koyama, W., 2008. One-year ad libitum consumption of diacylglycerol oil as part of a regular diet results in modest weight loss in comparison with consumption of a triacylglycerol control oil in overweight Japanese subjects. J. Am. Diet. Assoc. 108, 57-66.

Kimmons, J., Gillespie, C., Seymour, J., Serdula, M., Blanck, H.M., 2009. Fruit and vegetable intake among adolescents and adults in the United States: Percentage meeting individualized recommendations. The Medscape Journal of Medicine 11(1): 26.

Lien, E.L., Boyle, F.G., Yuhas, R., Tomarelli, R.M., Quinlan, P., 1997. The effect of triglyceride positional distribution on fatty acid absorption in rats. Journal of Pediatric Gastroenterology Nutrition 25(2):167-174.

Loders Croklaan B.V., 2003. GRAS Notification for high 2-palmitic vegetable oil. GRAS Notice 131. Available at: https://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=131.

Maki, K.C., Davidson, M.H., Tsushima, R., Matsuo, N., Tokimitsu, I., Umporowicz, D.M., Dicklin, M.R., Foster, G.S., Ingram, K.A., Anderson, B.D., Frost, S.D., Bell, M., 2002. Consumption of diacylglycerol oil as part of a reduced-energy diet enhances loss of body weight and fat in comparison with consumption of a triacylglycerol control oil. American Journal of Clinical Nutrition 76, 1230-1236.

Matsuyama, T., Shoji, K., Watanabe, H., Shimizu, M., Saotome, Y., Nagao, T., Matsuo, N., Hase, T., Tokimitsu, I., Nakaya, N., 2006. Effects of diacylglycerol oil on adiposity in obese children: initial communication. Journal of Pediatrics Endocrinology and Metabolism 19, 795-804.

Mattson, F. H., Beck, L.W., 1965. The specificity of pancreatic lipase for the primary hydroxyl groups of glycerides. Journal of Biological Chemistry 219(2):735-740.

Mattson, F. H., Volpenhein, R.A., 1964. The digestion and absorption of triglycerides. Journal of Biological Chemistry 239:2772-2777.

Morita, O., Soni, M.G., 2009. Safety assessment of diacylglycerol oil as an edible oil: a review of the published literature. Food Chemical Toxicology 47(1):9-21.

Morita, O., Tamaki, Y., Kirkpatrick, J.B., Chengelis, C.P., 2008. Safety assessment of heated diacylglycerol oil: subchronic toxicity study in rats. Food and Chemical Toxicology 46, 2748-2757.

Nagao, T., Watanabe, H., Goto, N., Onizawa, K., Taguchi, H., Matsuo, N., Yasukawa, T., Tsushima, R., Shimasaki, H., Itakura, H., 2000. Dietary diacylglycerol suppresses accumulation of body fat compared to triacylglycerol in men in a double-blind controlled trial. Journal of Nutrition 130, 792-797.

National Academy of Sciences (NAS), 1960. The safety of mono- and diglycerides for use as intentional additives in food (NAS publication no. 251). Washington, DC: NAS.

NIH (National Institutes of Health), 2014. Hazardous Substances Data Bank: n-Heptane. Available at: https://toxnet.nlm.nih.gov/.

OECD (Organisation for Economic Co-operation and Development), 2002. Screening Information Dataset (SIDS) Initial Assessment Report. Ethyl acetate. CAS No 141-78-6. SIAM 14 (Paris, 26–28 March, 2002).

SCF (Scientific Committee for Food), 1996. Reports of the Scientific Committee for Food. 35th Series. Hexane used as an extraction solvent. Opinion expressed on 17 June 1994. Available at: https://ec.europa.eu/food/sites/food/files/safety/docs/sci-com_scf_reports_35.pdf.

SCF, 1997. Opinion on certain additives for use in foods for infants and young children in good health and in foods for special medical purposes for infants and young children (expressed on 21 March, 1997 and amended on 13 June, 1997). Available at: https://ec.europa.eu/food/sites/food/files/safety/docs/sci-com_scf_7_out06_en.pdf.

SCF, 1997. Opinion on the potential risk to human health arising from the transport in ships' tanks of oils and fats from substances proposed as acceptable previous cargoes (expressed on 20 September 1996). Annex VII to Document III/5693/96. DG III, European Commission, Brussels.

Shoji, K., Takase, H., Tomonobu, K., Hibi, M., Kudo, N., Katsuragi, Y., Meguro, S., Tokumitsu, I., 2008. Effect of diacylglycerol oil on body weight and fat in obese people. Progress Med. 28, 203-210.

Sikorski, Z.Z.E., Kolakowska, A., 2002. Chemical and Functional Properties of Food Lipids. CRC Press. pp. 218. ISBN 978-1-4200-3199-7.

Smiciklas-Wright, H., Mitchell, D.C., Mickle, S.J., Cook, A.J., Goldman, J.D., 2002. Foods Commonly Eaten in the United States: Quantities Consumed Per Eating Occasion and in a Day, 1994-1996. U.S. Department of Agriculture NFS Report No. 96-5, 252 pp. Available at: https://www.ars.usda.gov/ARSUserFiles/80400530/pdf/Portion.pdf.

Soni, M.G., Kimura, H., Burdock, G.A., 2001. Chronic study of diacylglycerol oil in rats. Food Chem.Toxicol.39:317-329.

Taiyo-Kagaku Co., Ltd., 2008. GRAS notification for Polyglycerol fatty acid esters with a degree of polymerization of 11 to 40 for the polyglycerol backbone. GRAS Notice 269. Available at: https://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=269.

Teramoto, T., Watanabe, H., Ito, K., Omata, Y., Furukawa, T., Shimoda, K., Hoshino, M., Nagao, T., Naito, S., 2004. Significant effects of diacylglycerol on body fat and lipid metabolism in patients on hemodialysis. Clinical Nutrition 23, 1122-1126.

Tso, P., 1985. Gastrointestinal digestion and absorption of lipid. Adv. Lipid Res. 21: 143-186.

US EPA, 2003. Toxicological Review of Acetone (CAS No. 67-64-1). In support of summary information on the integrated risk information system (IRIS). Washington, DC. US Environmental Protection Agency. May 2003. Report No.: EPA/635/R-03/004.

US EPA, 2015. Methane sulfonic acid: Decision document for requested exemption from the requirements of a tolerance for a food use inert ingredient. Memorandum from Mark I. Dow, through Kerry Leifer, to P.V. Shah, dated July 22, 2015.

USP (United States Pharmacopeia Convention), 2016*a*. Chapter 467: Residual Solvents. US Pharmacopeia 39 The National Formulary 34 Rockville, MD: U.S. Pharmacopoeia Convention; 2016.

USP, 2016*b*. Chapter 232: Elemental Impurities-Limits. US Pharmacopoeia 39 The National Formulary 34. Rockville, MD: U.S. Pharmacopoeia Convention; 2016.

Watanabe, H., Onizawa, K., Naito, S., Taguchi, H., Goto, N., Nagao, T., Matsuo, N., Tokimitsu, I., Yasukawa, T., Tsushima, R., Shimasaki, H., Itakura, H., 2001. Fat-soluble vitamin status is not affected by diacylglycerol consumption. Annals Nutrition and Metabolism 45, 259-264.

Watanabe, H., Tokimitsu, I., 2004. Digestion and absorption of diacylglycerol. In: Katsuragi, Y., Yasukawa, T., Matsuo, N., Flickinger, B.D., Toimitsu, I., Matlok, M.G. (Eds.), Diacylglycerol Oil. American Oil Chemists' Society, Champaign, pp. 30-45.

Yang, L.Y., Kuksis, A., 1991. Apparent convergence (at 2-monoacylglycerol level) of phosphatidic acid and 2-monoacylglycerol pathways of synthesis of chylomicron triacylglycerols. Journal of Lipid Research 32(7):1173-1186.

Yamamoto, K., Asakawa, H., Tokunaga, K., Watanabe, H., Matsuo, N., Tokimitsu, I., Yagi, N., 2001. Long-term ingestion of dietary diacylglycerol lowers serum triacylglycerol in type II diabetic patients with Hypertriglyceridemia. Journal of Nutrition 131, 3204-3207.

Yamamoto, K., Takeshita, M., Tokimitsu, I., Watanabe, H., Mizuno, T., Asakawa, H., Tokunaga, K., Tatsumi, T., Okazaki, M., Yagi, N., 2006. Diacylglycerol oil ingestion in type 2 diabetic patients with hypertriglyceridemia. Nutrition 22, 23-29.

Yasunaga, K., Glinsmann, W.H., Seo, Y., Katsuragi, Y., Kobayashi, S., Flickinger, B., Kennepohl, E., Yasukawa, T., Borzelleca, J.F., 2004. Safety aspects regarding the consumption of high-dose dietary diacylglycerol oil in men and women in a double-blind controlled trial in comparison with consumption of a triacylglycerol control oil. Food and Chemical Toxicology 42, 1419-1429.

Yasunaga, K., Tomonobu, K., Katsuragi, Y., 2002. Assessment of safety and physical complaints based on the long-term practical using evaluation by outside housewives. [Unpublished – cited in Morita and Soni, 2009].

Appendix I. Test Methods for Batch Analysis

The test methods used to analyze batches of a mixture of monoacylglycerides derived from grape seed are provided in the table below.

Table I-1. Test Methods for Batch Analysis

Parameter	**Test Method***
Physical appearance	Apeel TM-0004 (Visual inspection)
Total glycerides	Apeel TM-0014 (UHPLC-ELSD)
Mono- and diesters	
α-Monoglyceride content	
Total glycerol	Calculated from Apeel TM-0014 and Apeel TM-0008
Free glycerol	TM-0008 (UHPLC-ELSD)
Soap	Food Chemicals Codex, 11th Ed. *Soap,* Appendix VII
Residue on ignition	<USP 281> Eurofins Method: ROI_S United States Pharmacopeia, Twenty-ninth Revision, <281>, 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	Eurofins Method: FFA2_S Official Methods and Recommended Practices of the AOCS, Fifth Ed., Method Ca 5a-40, American Oil Chemists' Society, Champaign, Illinois (1997) (modified). United States Pharmacopeia, Thirty-Fifth Revision, <401 Fats & Fixed Oils>, USP Convention, Inc., Rockville, MD (2012). For extraction: Official Methods of Analysis of the AOAC International, 18th Ed, (2005), AOAC International, Gaithersburg, MD. Official Method 983.23 (modified).
Iodine value	Calculated in accordance with FCC monograph for Fully Hydrogenated Oils and Fats (FCC 10)
Water content	<USP 921> Eurofins Method: KFMO_S The United States Pharmacopeia, Thirty Seventh Revision, <921>, Method 1a, The United States Pharmacopeial Convention, Rockville, MD (2014) (modified).
Processing Aid Residuals	
Ethyl acetate	Apeel TM-0001 (Equivalent to <USP 467>)
Heptane	Apeel TM-0001 (Equivalent to <USP 467>)

Parameter	Test Method*
Heavy Metals	
Palladium	Inductively Coupled Plasma – Mass Spectrometry (ICP-MS) Eurofins Method: ICP_MS_S Official Methods of Analysis, Method 2011.19 and 993.14, AOAC INTERNATIONAL (modified). Pequette, L.H., Szabo, A., Thompson, J.J., "Simultaneous Determination of Chromium, Selenium, and Molybdenum in Nutritional Products by Inductively Coupled Plasma/Mass Spectrometry: Single-Laboratory Validation," Journal of AOAC International, 94(4): 1240 - 1252 (2011).
Arsenic	Inductively Coupled Plasma – Mass Spectrometry (ICP-MS) Galbraith Labs
Mercury	
Lead	
Cadmium	

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

Appendix II. Batch Analysis

Table II-1. Batch Analysis for Three Non-Consecutive Lots of a Mixture of Monoglycerides Derived from Grape Seed

Parameter	Specification	Lot #3067	Lot #3079	Lot #3106
Physical appearance	Varies in consistency from yellow liquids through white- to pale yellow-colored plastics to hard, ivory-colored solids.	White powder	White powder	White powder
Total glycerides	NLT 90 wt. %	100 wt. %	100 wt. %	100 wt. %
Mono- and diesters	NLT 70 wt. %	89.9 wt. %	90.4 wt. %	89.6 wt. %
α-Monoglyceride content	NLT 30 wt. %	57.6 wt. %	56.1 wt. %	58.8 wt. %
Total glycerol	16 – 33 wt. %	20.8 wt. %	20.6 wt. %	20.9 wt. %
Free glycerol	NMT 7 wt. %	< 5 wt. %	< 5 wt. %	< 5 wt. %
Soap (as sodium oleate)	NMT 6 wt. %	< 0.156 wt. %*	< 0.156 wt. %*	< 0.156 wt. %*
Residue on ignition	NMT 0.5 % at 800 ± 25°C	< 0.1%	< 0.1%	< 0.1%
Acid value	NMT 6	4.6	2.8	2.2
Iodine value	NMT 4	1.7	0.27	0.23
Water content	NMT 2%	0.30%	< 0.20%	< 0.36%
Processing Aid Residuals				
Ethyl acetate	NMT 21,000 ppm	BDL	BDL	BDL
Heptane	NMT 23,000 ppm	BDL	BDL	BDL
Heavy Metals				
Palladium (from Pd/C catalyst)	NMT 10 ppm	0.0561 ppm	0.0419 ppm	0.340 ppm
Arsenic	NMT 3 ppm	< 0.0965 ppm	< 0.0923 ppm	< 0.187 ppm
Lead	NMT 2 ppm	< 0.0965 ppm	< 0.0923 ppm	< 0.0931 ppm
Cadmium	NMT 1 ppm	< 0.0965 ppm	< 0.0923 ppm	< 0.187 ppm
Mercury	NMT 1 ppm	< 0.0914 ppm	< 0.265 ppm	< 0.0944 ppm

* – Soap reported as sodium stearate. Multiplying reported value by 0.99 provides the soap value as sodium oleate.
NLT – “Not less than”
NMT – “Not more than”
BDL – “Below detection limit”