

GRAS Notice (GRN ) No. 648

<http://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/default.htm>

**ORIGINAL SUBMISSION**

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#648

GRN 000648

April 7, 2016

Office of Food Additive Safety (HFS-200)  
Center for Food Safety and Applied  
Nutrition (CFSAN)  
Food and Drug Administration  
5100 Paint Branch Parkway  
College Park, MD  
U.S.A. 20740-3835

Re: GRAS Notification for Mixture of Monoacylglycerides

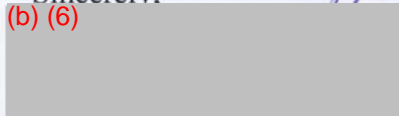
Dear Sirs:

On behalf of my client, Apeel Sciences, I am hereby submitting the attached GRAS Notification for a mixture of monoacylglycerides to be used as a food ingredient in the applications and under the conditions of use described therein. In compliance with 21 C.F.R. §170.36 (b) (proposed), we are enclosing an original, two paper copies and one computer disc containing an electronic copy of this notice. The disc has been scanned and found to be free of virus or other malware.

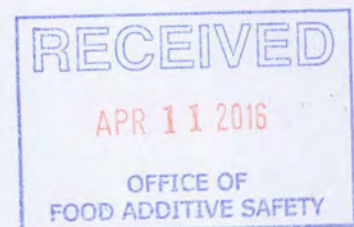
Should you have any questions, please do not hesitate to contact me.

Sincerely,

(b) (6)



Mark L. Itzkoff



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## GRAS NOTIFICATION

### I. Claim of GRAS Status

#### A. Claim of Exemption from the Requirement for Premarket Approval Requirements Pursuant to Proposed 21 CFR § 170.36(c)(1)

Apeel Sciences has determined that a mixture of monoacylglycerides (i.e., monoglycerides or fatty acid monoesters of glycerol) (Edipeel™) 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 is exempt from the requirement of premarket approval.

Signed,

(b) (6)

Mark L. Itzkoff

Counsel for Apeel Sciences

4/7/16  
Date

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**B. Name and Address of Notifier:**

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819 Reddick Street  
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USA

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Email: [james@apeelsciences.com](mailto:james@apeelsciences.com)

**C. Name of the notified substance:**

Mixture of monoacylglycerides.

**D. Conditions of use:**

A mixture of monoacylglycerides (i.e., monoglycerides or fatty acid monoesters of glycerol) (Edipeel™), primarily 2,3-dihydroxypropyl palmitate and 1,3-dihydroxypropan-2-yl palmitate, is intended for use as a surface-finishing agent and/or texturizer [21 CFR 170.3(o)(30) and (32)<sup>1</sup>], 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. It will be used at levels consistent with current Good Manufacturing Practice and is self limiting for technological reasons. The intended use of Edipeel™ on fruits and vegetables is estimated to result in a maximum daily (90<sup>th</sup> percentile) intake of 218 mg/person/day.

**E. Basis for GRAS Determination:**

In accordance with 21 CFR 170.30, the intended use of the subject mixture of monoacylglycerides (Edipeel™) has been determined to be Generally Recognized As Safe (GRAS) based on scientific procedures. A comprehensive search of the scientific literature on monoacylglycerides, such as 2,3-dihydroxypropyl palmitate and 1,3-dihydroxypropan-2-yl palmitate and other related compounds, was utilized for this assessment. There exists sufficient qualitative and quantitative scientific evidence, including human and animal data to determine safety-in-use for Edipeel™. Monoacylglycerol derivatives are components of dietary fats commonly found in food and are also endogenously formed in the human body. The two primary components of Edipeel™ (2,3-dihydroxypropyl palmitate and 1,3-dihydroxypropan-2-yl palmitate) each range from 0 to 100% in the final Edipeel™ product.

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<sup>1</sup>*Surface-finishing agents*: Substances used to increase palatability, preserve gloss, and inhibit discoloration of foods, including glazes, polishes, waxes, and protective coatings. *Texturizers*: Substances which affect the appearance or feel of the food.

It is well established and recognized that monoacylglycerides, 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 (i.e., monoglycerides). The free fatty acids released can be further used for triglyceride synthesis. Given the metabolic sequel described above, and by applying scientific procedures, it can be concluded that a mixture of monoacylglycerides would not pose any health hazards different from commonly consumed dietary oils derived from plants or animals.

On the basis of scientific procedures<sup>2</sup>, Apeel Sciences considers the consumption of a mixture of monoacylglycerides as an added food ingredient to be safe at levels up to 218 mg/day. The estimated daily intake of a mixture of monoacylglycerides from the intended use levels of Edipeel™, if ingested daily over a lifetime, is considered safe.

#### **F. Availability of Information:**

The data and information that form the basis for this GRAS determination will be sent to the U.S. Food and Drug Administration (FDA) upon request or will be available for FDA review and copying at reasonable times at the offices of:

The Law Office of Mark Itzkoff  
600 New Hampshire Ave., NW  
Suite 500  
Washington DC 20037

## **II. Detailed Information About the Identity of the Notified Substance:**

Edipeel™ will be used to coat the surface of fresh produce, forming an ultrathin natural barrier to protect produce from external stressors (e.g., moisture loss and oxidation).

### **Identity of the Notified Substance**

Edipeel™ is a mixture of monoacylglycerides that are found in nature.

### **Chemical Structure**

The generalized chemical structures of the constituents of Edipeel™ are presented in Figure II.B.1, while chemical structures of 2,3-dihydroxypropyl palmitate (PA-1G) and 1,3-dihydroxypropan-2-yl palmitate (PA-2G) (the majority components of Edipeel™) are presented in Figure II.B.2. It is noteworthy to highlight that Edipeel™ is derived from edible fatty acid sources commonly found in nature.

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<sup>2</sup> 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.



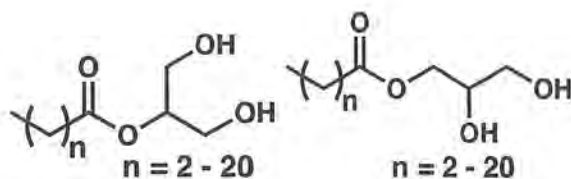


Figure II.B.1. Generalized chemical structures of Edipeel™.

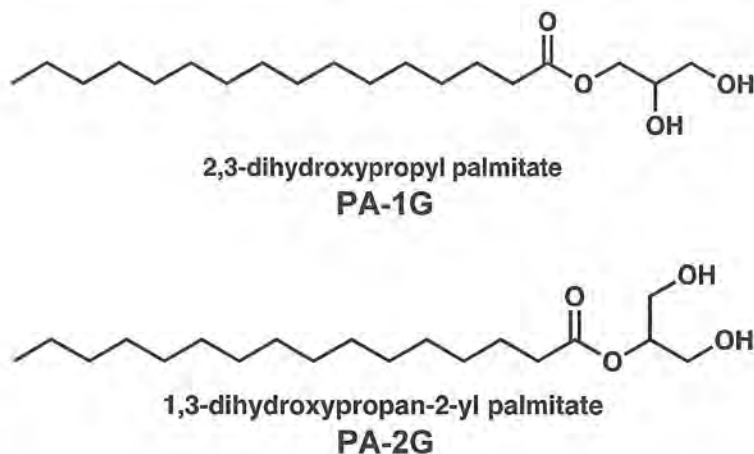


Figure II.B.2. Chemical structures of two Edipeel™ derivatives.

#### Trade Name

The subject of this notification will be marketed as Edipeel™.

#### Common or Usual Name of Substance

The common or usual name of the substance is glycerolipids.

#### Chemical and Physical Characteristics

The chemical and physical characteristics of two of the monoacylglycerides of which Edipeel™ is largely comprised are detailed in Table II.E.1. The IR spectra of the two derivatives are presented Section F.

**Table II.E.1. Chemical and Physical Characteristics of the Primary Components of Edipeel™.**

Property/Parameter	PA-1G	PA-2G
CAS Registry No.	542-44-9	23470-00-0
Chemical Name	2,3-dihydroxypropyl palmitate, also 1,2-dihydroxypropan-3-yl palmitate	1,3-dihydroxypropan-2-yl palmitate
Empirical Formula	C <sub>19</sub> H <sub>38</sub> O <sub>4</sub>	C <sub>19</sub> H <sub>38</sub> O <sub>4</sub>
Molecular Weight	330.51	330.51
Physical State	Solid	Solid
Density (g/mL)	1.09	1.09

### Infrared (IR) Spectra

**Figure II.F.1. Attenuated total reflectance (ATR)-IR spectrum for PA-1G.**

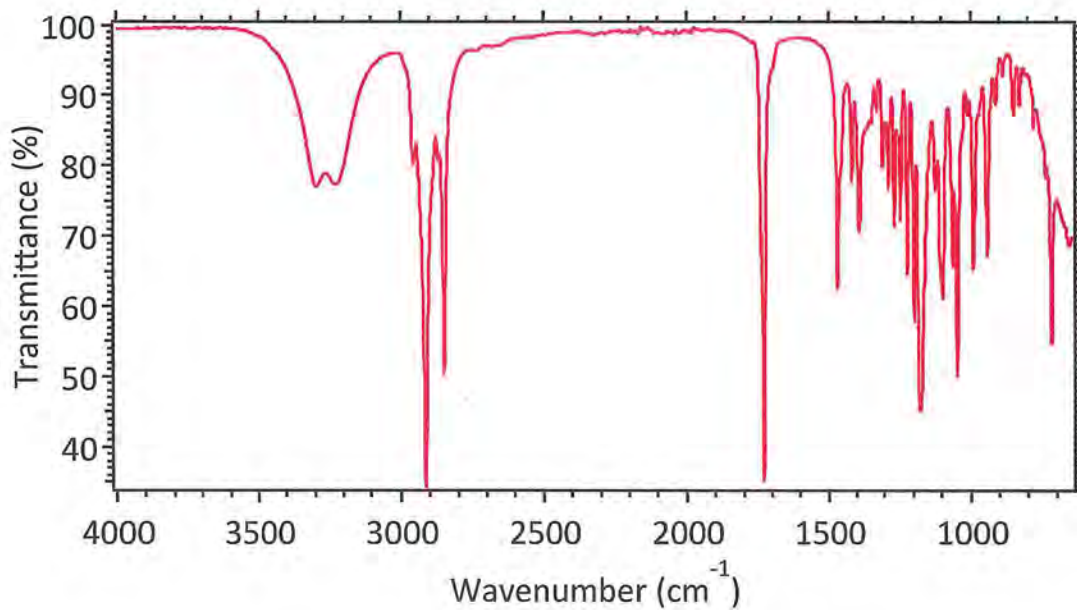
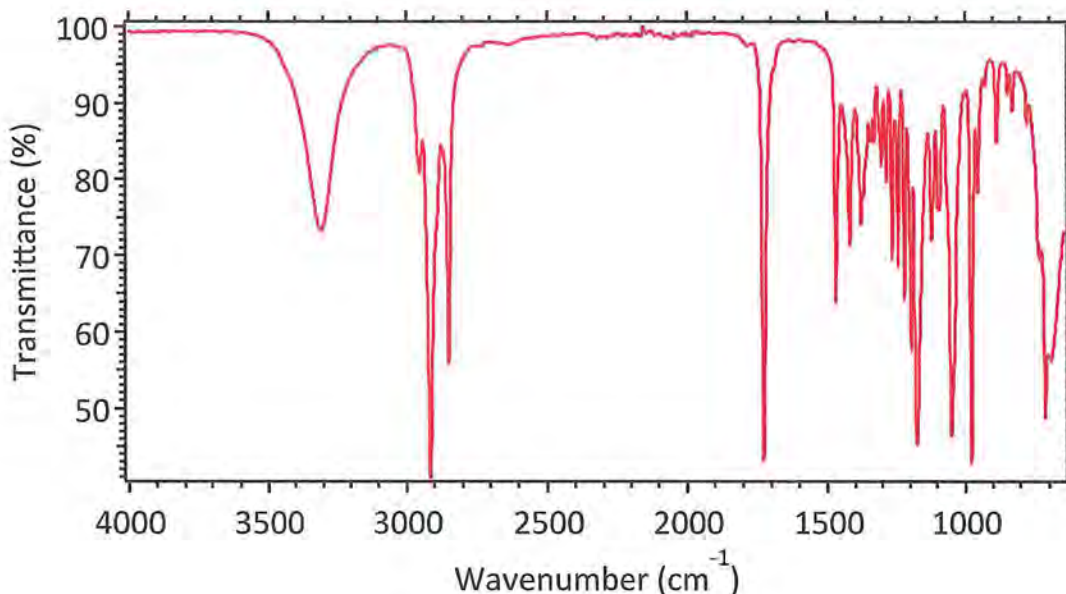




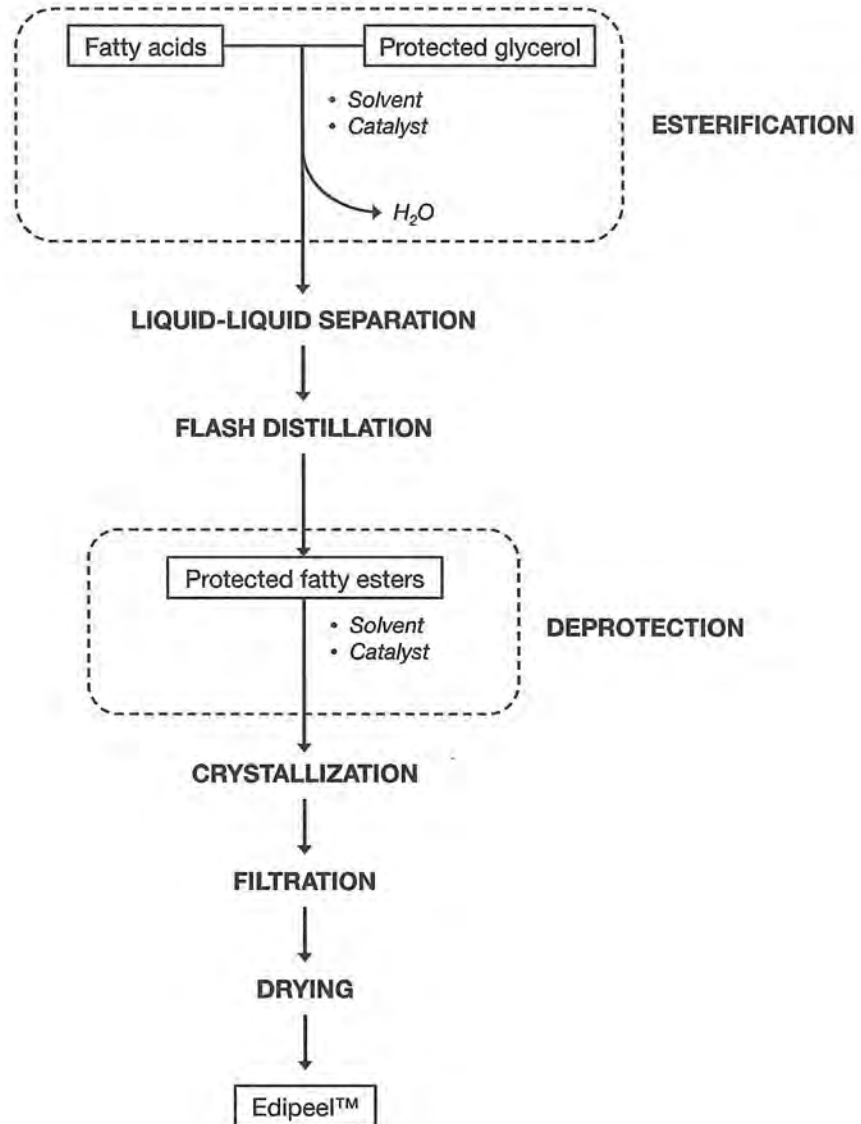
Figure II.F.2. ATR-IR spectrum for PA-2G.



### Manufacturing Process

Edipeel™ is manufactured according to current good manufacturing practices (cGMP). The processing agents and starting materials used in the production of the monoacylglycerides are food grade chemicals (where available) and/or high purity chemicals. In general, the manufacturing process involves the functionalization of commonly found, edible fatty acids of various chain lengths to arrive at the Edipeel™ product. The manufacturing process consists of two major reaction steps: (1) catalyzed esterification of fatty acids with protected glycerol; and (2) catalyzed deprotection of the protected fatty esters from step #1. The residual solvents and reactants are monitored to ensure the product complies with the appropriate food regulations. The reaction input materials and associated separation steps are detailed in Figure II.G.1.

Figure II.G.1. Edipeel™ manufacturing process.



## Product Specifications

Food grade specifications of the mixture of monoacylglycerides (Edipeel™) are presented in Table II.H.1. Edipeel™ is a white to pale yellow powder, primarily containing a mixture of 2,3-dihydroxypropyl palmitate and 1,3-dihydroxypropan-2-yl palmitate. Analytical data from three non-consecutive manufacturing lots for Edipeel™ are presented in Table II.H.2.

**Table II.H.1. Food Grade Specifications for Edipeel™**

<b>Parameters</b>	<b>Specifications/Limits</b>
<b>Description</b>	White to pale yellow powder
<b>Free Glycerol</b>	Not more than 0.2 weight %
<b>Free Fatty Acid</b>	Not more than 0.5 weight %
<b>Mixture of Monoacylglycerides</b>	Not less than 91 weight %
<b>1,3-Bis(benzyloxy)propan-2-yl esters of fatty acids*</b>	Not more than 2 weight %
<b>Diacylglycerides*</b>	Not more than 7 weight %
<b>Total Fatty Acid Esters</b>	Not less than 99 weight %
<b>Ethyl acetate</b>	Not more than 100 ppm
<b>Acetonitrile</b>	Not more than 5 ppm
<b>Toluene</b>	Not more than 5 ppm
<b>Hexane</b>	Not more than 25 ppm
<b>Palladium</b>	Not more than 0.1 ppm
<b>Arsenic**</b>	Not more than 0.2 ppm
<b>Lead**</b>	Not more than 1 ppm
<b>Cadmium**</b>	Not more than 1 ppm
<b>Mercury**</b>	Not more than 1 ppm

\* - Diacylglyceride content in the final product is typically controlled in the manufacturing process to be not more than 2 weight %. However, given the negligible toxicity of this component (see Section IV for more details) we have set the limit for this residual to 7 weight % to allow for variations in the final product both in process and physical properties.

\*\* - Tested on a semi-annual basis.



**Table II.H.2. Analytical Data for Residuals from Three Non-Consecutive Manufacturing Lots for Edipeel™**

Parameters	Specification	Lot No.: (b) [REDACTED]	Lot No.: (b) [REDACTED]	Lot No.: (b) [REDACTED]
Total Free Glycerol	≤ 0.20 wt. %	n.d.	n.d.	n.d.
Total Free Fatty Acid	≤ 0.50 wt. %	n.d.	n.d.	n.d.
1,3-Bis(benzyloxy)propan-2-yl esters of fatty acids	≤ 3 wt %	n.d.	n.d.	n.d.
Diacylglycerides	≤ 22 wt %	n.d.	n.d.	n.d.
Ethyl Acetate	≤ 100 ppm	91 ppm	58 ppm	28 ppm
Acetonitrile	≤ 5 ppm	< 3 ppm	< 3 ppm	< 3 ppm
Toluene	≤ 5 ppm	< 4 ppm	< 4 ppm	< 4 ppm
Hexane	≤ 25 ppm	< 3 ppm	< 3 ppm	< 3 ppm
Palladium	≤ 0.1 ppm	< 0.046 ppm	< 0.050 ppm	< 0.046 ppm
Arsenic	≤ 0.2 ppm	< 0.046 ppm	< 0.050 ppm	< 0.046 ppm
Lead	≤ 1 ppm	< 0.046 ppm	< 0.050 ppm	< 0.046 ppm
Cadmium	≤ 1 ppm	< 0.098 ppm	< 0.11 ppm	< 0.099 ppm
Mercury	≤ 1 ppm	< 0.58 ppm	< 0.62 ppm	< 0.58 ppm

n.d. – Not detected.

### Intended Technical Effects

Edipeel™ is intended to be used as a coating on fruits (e.g., berries, grapes, stone fruit, citrus, bananas, mangoes, avocados) and vegetables (e.g., legumes, roots, tubers). Edipeel™ will be used as a surface-finishing agent and/or texturizer [21 CFR 170.3 (o)(30) and (32)], creating a thin and edible physical barrier against moisture loss and oxidation to protect the freshness and extend the shelf-life of fresh produce. Edipeel™ is intended to cover the surface of fresh produce as an extra-thin layer of "invisible, tasteless, and completely edible" protection. It will be used at levels consistent with current Good Manufacturing Practice and is self limiting for technological reasons. 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.

### **III. Summary of the Basis for the Notifier's Determination that Mixture of Monoacylglycerides is GRAS**

A comprehensive search of the scientific literature for safety and toxicity information on the mixture of monoacylglycerides and other related compounds was conducted through February 2016 and was also utilized for this review. Based on a critical evaluation of the pertinent data and information summarized here, Apeel Sciences has determined by scientific procedures that the addition of the mixture of monoacylglycerides to fruits and vegetables, when not otherwise precluded by a Standard of Identity, meeting the specification cited above, 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 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 product. Other qualified and competent scientists, reviewing the same publicly available toxicological and safety information, would reach the same conclusion.

**IV. Basis for a Conclusion that Mixture of Monoacylglycerides is GRAS for its Intended Use.**

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## 1. INTRODUCTION

A comprehensive search of the scientific literature for safety and toxicity information on monoacylglycerides and other related compounds was utilized to determine the Generally Recognized As Safe (GRAS) status of the mixture of monoacylglycerides (i.e., monoglycerides or fatty chain monoesters of glycerol) (Edipeel™) for its intended use as a surface-finishing agent and/or texturizer [21 CFR 170.3 (o)(30) and (32)], creating a thin and edible physical barrier against moisture loss and oxidation to protect the freshness and extend the shelf-life of fresh produce. The exposure from added Edipeel™ in the proposed food uses in the total U.S. population is estimated as 218 mg/person/day of a mixture of monoacylglycerides for high end consumers. As described below, the weight of evidence clearly supports the safety and GRAS status of the mixture of monoacylglycerides, 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 Edipeel™ per 100 kg of produce (i.e., 100 g Edipeel™ per 66 kg produce)<sup>3</sup>. No studies were identified showing any adverse effects when this amount of a mixture of monoacylglycerides is added to food.

### 1.1. Background

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. A packaging material such as a film, a thin layer, or a coating is an integral part of a food and is consumed with the food, and qualifies as "edible" packaging. As there are several types of edible packaging, compositions of edible barriers are very diverse. 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 within foods. The different lipid based film former or barrier compounds used include lecithins, mono- and diglycerides, mono- and diglyceride esters, etc. The substances are used as emulsifiers and surface-active agents. Given the beneficial properties of mono- and diglycerides, Apeel Sciences intends to use monoacylglycerides (Edipeel™) on selected food products, such as fruits and vegetables, to cover the surface of fresh produce as an extra-thin layer of "invisible, tasteless, and completely edible" protection.

### 1.2. Description, Manufacturing and Specifications

The subject of this GRAS assessment, Edipeel™, is a mixture of monoacylglycerides, primarily containing 2,3-dihydroxypropyl palmitate and 1,3-dihydroxypropan-2-yl palmitate. The chemical structures of these two monoacylglycerides are presented in Figure II.B.2, while the chemical and physical characteristics of the two constituents are detailed in Table II.E.1. Edipeel™ is manufactured as per current good manufacturing processes (cGMP) that

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<sup>3</sup> Edipeel™ will initially be supplied to customers in 100 g packages. For illustrative purposes, the corresponding minimum mass of produce that can be treated using 100 g of Edipeel™ is provided in parentheses, where relevant, throughout this document.



involves the functionalization of commonly found, edible fatty acids of various chain lengths to arrive at the Edipeel™ product. The specifications of the final product are established. The standard food grade specifications and batch analysis data are presented in Table II.H.1 and Table II.H.2. For additional details, please see Section II.

### 1.3. 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 acylglycerols, monoacylglycerols, monoglycerides, and fatty chain monoesters of glycerol. Depending on the position of the ester bond on the glycerol bond, any monoacylglycerol is either a 1-monoacylglycerol or a 2-monoacylglycerol. 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, and can be created by breaking down a triglyceride by removing two of its fatty acids, and can also 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 at the sn-2 position (Innis et al., 1993, 1994; Lien et al., 1997). In contrast, the palmitic acid present in vegetable oil and other non-milk fats are primarily esterified at the sn-1 and sn-3 positions (Innis et al., 1993).

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 labeled as simply 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, including palmitic acid, and are derived from edible sources. Mono- and diglycerides are manufactured by the reaction of glycerin 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 glycerin 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.



Under 21 CFR 172.832, monoglyceride citrate, a mixture of glyceryl monooleate and its citric acid monoester, is cleared for use as a food additive and may be safely used as a synergist and solubilizer for antioxidants in oils and fats. Similarly, as per 21 CFR 172.755, stearyl monoglyceridyl citrate may be safely used in food as an emulsion stabilizer in or with shortenings containing emulsifiers. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) has reviewed mixtures of mono- and di-glyceryl esters of long chain saturated and unsaturated fatty acids (JECFA No. 471) that occur in food fats and determined the acceptable daily intake for man to be "not limited"<sup>4</sup> when used as a 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. Additionally, two GRAS notices (GRN 56 and 115) on diacylglycerol oil that contains small amounts (<5%) of monoglyceride received no question letters from the FDA.

#### **1.4. Technical effects**

Edipeel™, a mixture of monoacylglycerides, is intended for use as a surface-finishing agent and/or texturizer [21 CFR 170.3 (o) (30) and (32)], creating a thin and edible physical barrier against moisture loss and oxidation to protect the freshness and extend the shelf-life of fresh produce. Edipeel™ will be used to cover the surface of fresh produce as an extra-thin layer of "invisible, tasteless, and completely edible" protection.

#### **1.5. Estimated Daily Intake from the Intended Uses**

Edipeel™ is intended to be used as a coating on fruits and vegetables. When used as directed, Edipeel™ will be applied to the outside of the peel of fruits and vegetables. The quantity of Edipeel™ recommended for use varies with the specific application. Quantities illustrative of maximum amounts of Edipeel™ applied to the surfaces of certain fruits and vegetables are listed in Table IV.1.5.1. These loadings of Edipeel™ are atypically elevated, but are used in the subsequent dietary intake calculations as an overestimation of potential exposure to Edipeel™.

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<sup>4</sup> 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.



**Table IV.1.5.1. Illustrative Quantities of Edipeel™ Applied to the Surfaces of Certain Fruits and Vegetables.**

Fruit or Vegetable	Maximum Amount of Edipeel™ Applied
Apples	108 g Edipeel™ / 100 kg apples (100 g Edipeel™ / 93 kg apples)
Grapes	117 g Edipeel™ / 100 kg grapes (100 g Edipeel™ / 85 kg grapes)
Strawberries	117 g Edipeel™ / 100 kg strawberries (100 g Edipeel™ / 85 kg strawberries)
Green Beans	152 g Edipeel™ / 100 kg green beans (Edipeel™ / 66 kg green beans)

**A. Fruit**

Edipeel™ will be applied to the 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, citrus fruit, squash, and watermelon. Edipeel™ is not expected to migrate through the fruit skin into the edible portions of these foods. While Edipeel™ may be used on apples, oranges or grapes, it is expected that the product will remain on the peel and not present in juice extracted from these or other fruits. The primary source of consumer exposure to Edipeel™ 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 IV.1.5.A.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 IV.1.5.A.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
Percent RFEP in top 10	18.9	22.1	22.8

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 will use 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 IV.1.5.A.2, below.

**Table IV.1.5.A.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
Strawberry*	3	2	3	2	3	2	4	4	4	8	7

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

Under the Edipeel™ instructions for use (see Appendices A and B), the coating will be applied to apples at a loading of 108 g Edipeel™/100 kg fruit (*i.e.*, 100 g Edipeel™/93 kg fruit); for grapes and strawberries the loading would be 118 g Edipeel™/100 kg fruit (100 g Edipeel™/85 kg fruit). 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 8 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:

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

ii. Grapes

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

iii. Strawberries

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

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

As shown in Table IV.1.5.A.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 Edipeel™ 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:

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

### A. Vegetables

A similar calculation can be used to determine the intake of Edipeel™ from its use on vegetables with edible peels (VEP). The Kimmons et al. (2009) report cited in Table IV.1.5.A.1 also includes data on the 10 most common vegetable sources. This information is set forth in Table IV.1.5.B.1, below.

**Table IV.1.5.B.1. 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	<u>Not Reported</u>	<u>2.0</u>	<u>2.3</u>
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 Edipeel™ 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 IV.1.5.B.2 below.

**Table IV.1.5.B.2. 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



Edipeel™'s instruction for use on green beans calls for the use of 152 g Edipeel™ per 100 kg green beans (100 g Edipeel™ per 66 kg of green beans). Using this application load, the average consumer's intake would be as follows:

$$(20 \text{ g vegetables/day})(100 \text{ g Edipeel}^{\text{TM}}/66 \times 10^3 \text{ g green beans}) = 30 \text{ mg/day}$$

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 Edipeel™ that may be consumed from its use on vegetables would be:

$$(30 \text{ mg/day})/(55\%) = \underline{55 \text{ mg/day}}$$

It is important to note that this estimate is very conservative since the use of Edipeel™ 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 Edipeel™ as the average consumer, the quantity per day would be:

$$2 \times (55 \text{ mg/day}) = \underline{110 \text{ mg/day}}$$

#### **B. Total**

The total amount of Edipeel™ consumed by a high end consumer from both fruit and vegetable applications would be:

$$(108 \text{ mg/day}) + (110 \text{ mg/day}) = \underline{218 \text{ mg/day}}$$

## **2. SAFETY RELATED INFORMATION**

### **2.1. Regulatory and Other Assessments**

#### **2.1.1. FDA GRAS Review**

The FDA has reviewed GRAS notices, particularly for oils (diacylglycerol oil- GRN 56<sup>5</sup> and GRN 115<sup>6</sup>; polyglycerol fatty acid esters- GRN 269<sup>7</sup>) containing small amounts of monoacylglycerol fatty acids. A copy of the complete GRAS notices submitted to the agency and FDA responses to these notices are available on the FDA GRAS Inventory website. These GRAS notices also included safety related information on monoacylglycerol. In addition to the three GRAS notices above, high 2-palmitic vegetable oil (subject of GRN 192) is also reported to contain <1% monoacylglycerides. All these GRAS notices received no question letters from the FDA.

<sup>5</sup> Available at: <http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=56>

<sup>6</sup> Available at: <http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=115>

<sup>7</sup> Available at: <http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=269>



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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 this GRAS notification, 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 0.3 to 0.5 g/kg bw/day at the 90<sup>th</sup> percentile (for an adult individual weighing 60 kg this will be 18 to 30 g/person/day). The intake of monoacylglycerol from the uses of diacylglycerol oil will be approximately 900 to 1500 mg/person/day. The FDA reviewed the notice and responded to the notifier that the agency has no questions.

The subject of GRN 269, 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 this notice, 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 (please see Section 1.3.). The available information suggests that metabolism releases these fatty acids, including palmitic acid. 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 as for Edipeel™ constituents.

In a GRAS notice on high 2-palmitic vegetable oil to FDA (GRN 131<sup>8</sup>) 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 <sup>14</sup>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 of triglyceride mixture in infants. The FDA filed this and another similar GRAS (GRN 192<sup>9</sup>) notice on high 2-palmitic acid vegetable oil for use in infant formulas without any question.

### 2.1.2. Cosmetic Ingredient Review

In an extensive review, the Cosmetic Ingredient Review (CIR) Expert Panel summarized the safety information of 43 glyceryl monoesters, including glyceryl palmitate, as cosmetic ingredients (CIR, 2004). Glyceryl palmitate is the monoester of glycerin and palmitic acid. 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 resynthesis 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

<sup>8</sup> Available at: <http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=131>

<sup>9</sup> Available at: <http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=192>



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.

## 2.2. 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  $\beta$ -monoglyceride (2-monoglyceride) is isomerized to  $\alpha$ -monoglyceride (1-monoglyceride), and approximately 75% of the  $\alpha$ -monoglyceride is 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 resynthesis of triglycerides.  $\beta$ -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 reesterification 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 sn-2 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 present GRAS assessment contains primarily 2,3-dihydroxypropyl palmitate (1-monopalmitin) and 1,3-dihydroxypropan-2-yl palmitate (2-monopalmitin), the above discussion is applicable to the metabolism of these constituents. The above discussion also suggests that, similar to triacylglycerol oil, both 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.



### 2.3. Safety Studies

In an extensive review, Morita and Soni (2009) reviewed the available information related to safety of diacylglycerol oil, an edible oil. As indicated earlier, diacylglycerol oil contains >80% diacylglycerols, <20% triacylglycerols and <5% monoacylglycerols. Although the amount of monoacylglycerols in diacylglycerol oil is small, the findings with this oil are applicable to the safety of monoacylglycerols. The reviewers described that feeding rats with unheated or heated diacylglycerol oil at levels up to 5.5% in diet for 90 days did not cause any toxic effects. In chronic studies, dietary administration of diacylglycerol oil (up to 5.3%) to rats for 2 years or at 9.5% to Beagle dogs for 1 year had no adverse effects. Genotoxicity studies of unheated and heated diacylglycerol oil did not reveal any genotoxic effects. Carcinogenicity studies in rodents demonstrate that diacylglycerol oil is non-carcinogenic. In a two-generation reproductive and developmental toxicity study, gavage administration of diacylglycerol oil at dose levels of 5 mL/kg bw/day did not reveal any adverse effects.

The physiological effects of diacylglycerol oil were examined in a total of 14 clinical studies involving over 800 volunteers, including normal subjects and some patient groups (Morita and Soni, 2009). In the majority of these studies, diacylglycerol oil was used as a source of dietary fat and the intake ranged from 9.8 to 44 g/person/day. These studies investigated possible effects of repeated long-term ingestion (1-12 months) of diacylglycerol oil. Of the 14 clinical studies of diacylglycerol oil, 8 were double-blind trials. Double-blind, placebo-controlled clinical trials are considered as the least likely to result in bias. These studies provide an opportunity to assess the safety and tolerability of diacylglycerol oil in a fairly diverse population. The reviewers concluded that there is sufficient qualitative and quantitative scientific evidence available from animal and human studies suggesting that intake of diacylglycerol oil is safe for human consumption when used in a manner similar to other edible oils. As described earlier, the uses of diacylglycerol oil will result in an intake of approximately 900 to 1500 mg monoacylglycerol/person/day. Thus, these studies support the consumption of monoacylglycerols (i.e., monoacylglycerides) at levels up to 1500 mg/person/day. As compared to this level, the resulting intake of 218 mg of a mixture of monoacylglycerides/person/day from the proposed uses of Edipeel™ is about 7-fold lower.

### 3. SUMMARY

Apeel Sciences intends to use a mixture of monoacylglycerides (i.e., monoglycerides or fatty chain monoesters of glycerol) (Edipeel™) as a surface-finishing agent and/or texturizer [21 CFR 170.3 (o) (30) and (32)], 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. Edipeel™ will be applied at a loading of 108 g Edipeel™/100 kg fruit (100 g Edipeel™/93 kg fruit) to apples, and at loading of 118 g Edipeel™/100 kg fruit (100 g Edipeel™/85 kg fruit) for grapes and strawberries. Edipeel™ is used to coat the surface of fresh produce, forming an ultrathin, natural barrier to protect produce from external stressors.



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The mixture of monoacylglycerides is prepared by acid-catalyzed esterification of commonly found, edible fatty acids with protected glycerol followed by catalyzed deprotection of the protected fatty esters. The mixture primarily contains 2,3-dihydroxypropyl palmitate and 1,3-dihydroxypropan-2-yl palmitate. It is manufactured as per current good manufacturing practices (cGMP) using food grade chemicals (where available) and/or high purity chemicals. Food grade specifications of the monoacylglycerides have been established. Analytical data from three non-consecutive manufacturing lots for Edipeel™ suggest that it is consistently produced. The intended use of Edipeel™ will result in intake of 218 mg of the monoacylglycerides/person/day for a high end consumer.

The constituents of Edipeel™, a mixture of monoacylglycerides, 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 are already present in diets as components of conventional dietary oils and as approved food additives (i.e., mono- and diglycerides), and occur as metabolites of normal lipid metabolism following the consumption of dietary fat. There is no evidence that the presence of monoglycerides or diglycerides of food fats has any deleterious effect on cells or tissues. The JECFA has evaluated mixtures of mono- and di-glyceryl esters of long chain saturated and unsaturated fatty acids (JECFA No. 471) that occur in food fats and determined an estimate of acceptable daily intake for man 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. Additionally, two GRAS notices (GRN 56 and 115) on diacylglycerol oil that contains small amounts (<5%) of monoglyceride received no question letters from 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. Given the metabolism of oils that results in the formation of mono-glycerides, there is no reason to indicate that mono-glycerides from Edipeel™ are metabolized any differently or pose any different health hazards.

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 from the intended uses of Edipeel™ at

proposed use levels is safe. On the basis of both scientific procedures<sup>10</sup> corroborated by history of exposure from natural dietary sources and approved uses, Apeel Sciences considers the consumption of a mixture of monoacylglycerides as an added food ingredient to be safe at a daily consumption of up to 218 mg/day. Thus, the available information and literature studies demonstrate that a mixture of monoacylglycerides from Apeel Sciences offers a thin and edible physical barrier against moisture loss and oxidation to protect the freshness and extend the shelf-life of agricultural products and, when manufactured under the highest standards of food purity, is safe for its intended uses.

#### 4. CONCLUSION

In summary, based on the information provided above and the fact that the monoacylglyceride constituents of Edipeel™ are essentially the same as found naturally as well produced naturally during the metabolism of oils and will be handled metabolically similarly, it is concluded that scientific experts, generally, would recognize them to be as safe and as acceptable as other mono- and diglycerides. Further, we believe that there are no significant questions regarding the safety of monoacylglycerides 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 (Edipeel™), meeting the specifications cited above, and when used as a surface-finishing agent and/or texturizer [21 CFR 170.3 (o) (30) and (32)] 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.

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<sup>10</sup> 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.



## 5. REFERENCES

- Branen, J., Davidson, P.M., 2004, Enhancement of nisin, lysozyme, and monolaurin antimicrobial activities by ethylenediaminetetraacetic acid and lactoferrin. *International Journal of Food Microbiology* 90:63-74.
- Bunkova, L., Bunka, F., Janis, R., Krejci, J., Dolezalkova, I., Pospisil, Z., Ruzicka, J., Tremlova B., 2011, Comparison of antibacterial effect of seven 1-monoglycerides on food-borne pathogens or spoilage bacteria. *Acta Veterinaria* 80:29-39.
- 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
- 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.
- 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.
- 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.
- Hoy, C. E., Xu, X., 2001. Structured Triacylglycerols. 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.
- 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.
- 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.



- JECFA, 1974. Mono- and diglycerides. In: JECFA. Toxicological Evaluation of Some Food Additives Including Anticaking Agents, Antimicrobials, Antioxidants, Emulsifiers and Thickening Agents. 17th JECFA Session, June 25-July 4, 1973, Geneva, Switzerland.
- 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.
- 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.
- 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.
- Oh, D.H., Marshall, D.L. 1993. Antimicrobial activity of glycerol monolaurate or lactic acid against *Listeria monocytogenes*. *International Journal of Food Microbiology* 20: 239-246.
- Kabara, J.J., Swieczkowski, D.M., Conley, A.J., Truant J.P., 1972. Fatty acids and derivatives as antimicrobial agents. *Antimicrobial Agents and Chemotherapy* 2: 23-28.
- Schlievert, P.M., Deringer, J.R., Kim, M.H., Projan, S.J., Novick, R.P., 1992. Effect of glycerol monolaurate on bacterial growth and toxin production. *Antimicrobial Agents and Chemotherapy* 36:626-631.
- 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: [www.barc.usda.gov/bhnrc/foodsurvey/Products9496.html](http://www.barc.usda.gov/bhnrc/foodsurvey/Products9496.html).
- Tso, P., 1985. Gastrointestinal digestion and absorption of lipid. *Adv. Lipid Res.* 21: 143-186.
- 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.

**Appendix A**  
**Sample Edipeel™ Package Label**

☒



Natural plant-based postharvest protection

blueberry  
formula

apeelsciences.com  
Santa Barbara, California

100 grams  
TEST SAMPLE

☒

THIS PRODUCT REQUIRES SPECIAL MIXING  
AND APPLICATION PROCEDURES.

Call 877-926-5184  
for instructions.

STORE IN A COOL, DRY ENVIRONMENT  
PRODUCED FEBRUARY 23, 2016

INGREDIENT: Glycerolipids derived from recycled  
plant materials

*This product has negligible nutritional value.*

☒



100 grams  
TEST SAMPLE

☒

☒

**Figure A.1. Sample Edipeel™ Blueberry Formula package label (top: front label; bottom: rear label).**



**Appendix B**  
**Sample Edipeel™ Application Instructions**

**A. *Edipeel™ Application Instructions – Strawberry Treatment***

One 100 g package of *Edipeel Strawberry Formula* is to be used to treat 85 kg of strawberries.

1. Dispense the entirety of the 100 g package of *Edipeel Strawberry Formula* into the liquid reservoir of the spraying system.
2. Add at least 1.25 L of 190 proof, food-grade ethanol to the liquid reservoir of the spray system containing the *Edipeel Strawberry Formula*. Ensure that the *Edipeel* powder is completely dissolved into the solution before application.
3. Feed *Edipeel* solution into spray system and operate per manufacturer's instructions.
4. Allow the surface of Edipeel-treated strawberries to dry prior to consumption of the strawberries.

**B. *Edipeel™ Application Instructions – Green Bean Treatment***

One 100 g package of *Edipeel Green Bean Formula* is to be used to treat 66 kg of green beans.

1. Dispense the entirety of the 100 g package of *Edipeel Green Bean Formula* into the liquid reservoir of the spraying system.
2. Add at least 1.25 L of 190 proof, food grade ethanol to the liquid reservoir of the spray system containing the *Edipeel Green Bean Formula*. Ensure that the *Edipeel* powder is completely dissolved into the solution before application.
3. Feed *Edipeel* solution into spray system and operate per manufacturer's instructions.
4. Allow the surface of Edipeel-treated green beans to dry prior to consumption of the green beans.

**SUBMISSION END**