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Supporting document 1

Risk and technical assessment – Application A1191

Mono- and diglycerides of fatty acids (INS 471) as glazing agent for fruits and vegetables

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Executive summary

FSANZ received an application from Apeel Sciences to extend the use of the currently permitted food additive used at Good Manufacturing Practice (GMP) mono- and diglycerides of fatty acids as a glazing agent for fresh fruits and vegetables. The relevant food class is 4.1.2 (surface treated fruits and vegetables) in the table to section S15—5 of the Australia New Zealand Food Standards Code (the Code). In the Code, mono- and diglycerides of fatty acids is a food additive able to be used at GMP since it is listed in the tables to section S16—2 but not for fresh fruit and vegetables.

An assessment of data provided by the applicant has concluded that the food additive mono- and diglycerides of fatty acids performs the technological purpose of a glazing agent to extend the shelf life of various treated fresh fruits and vegetables. A comparison of results indicate mono- and diglycerides of fatty acids performs better than untreated product, and conventional waxes and resins. As an already permitted GMP food additive, it complies with internationally accepted specifications of identity and purity, which are primary sources of specifications in Schedule 3.

FSANZ concurs with the conclusion of JECFA (1974 a, b) that mono-and diglycerides, including those in INS 471, do not differ significantly from dietary lipids and a numerical ADI is not required. Estimated mean dietary exposure for Australian and New Zealand populations to mono- and diglycerides of fatty acids if used as a glazing agent on surface treated fruits and vegetables represents 0.6-0.8% of mean total fat intake, which is within normal daily variation.

The assessment also considered the potential for allergenicity due to the possible use of oils and fats derived from allergenic sources to produce the food additive. Based on the available evidence and taking into account the purification processes used during production, there are unlikely to be allergenicity concerns related to use of the food additive, including extending its use as a glazing agent for fresh fruits and vegetables.
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1 Introduction

FSANZ received an application from Apeel Sciences to extend the use of the currently permitted food additive used at Good Manufacturing Practice (GMP) mono- and diglycerides of fatty acids as a glazing agent for fresh fruits and vegetables. The relevant food class is 4.1.2 (surface treated fruits and vegetables) in the table to section S15—5 of the Australia New Zealand Food Standards Code (the Code).

The technological purpose relevant to the application is for the extension of use of the food additive to perform the technological purpose of a 'glazing agent' as defined in Schedule 14 of the Code. That is, the food additive 'imparts a coating to the external surface of a food'. In the case of this application the treated food is fresh fruits and vegetables. The reason for applying a surface coating to fresh fruits and vegetables is to extend the commercial shelf life of the treated produce.

1.1 Objectives of the assessment

The objectives of this assessment are to:

- determine whether mono- and diglycerides of fatty acids perform the technological purpose in the quantity and form proposed
- evaluate any potential public health and safety concerns that may arise.

2. Food technology assessment

2.1 Characterisation of mono- and diglycerides of fatty acids

2.1.1 Identity of the food additive

Mono- and diglycerides of fatty acids is a currently permitted food additive in the Code, having the Codex Alimentarius food additive International Numbering System (INS) number of 471 (CXG 36-1989). In the Code, mono- and diglycerides of fatty acids is a food additive able to be used at GMP since it is listed in the tables to section S16—2.

The identity of the food additive is well recognised since it has monographs in internationally recognised sources of specifications. These include the Combined Compendium of Food Additives of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), the Food Chemicals Codex (FCC) and the European Commission Regulation (EU) No 231/2012 specifications for food additives. These are primary sources of specifications in section S3—2 of the Code (further details below).

As detailed in the JECFA specification definition, the food additive is 'a mixture of mono- and diglyceryl esters of long chain, saturated and unsaturated fatty acids that occur in food fats'. The food additive may also contain triglycerides, 'free glycerol, free fatty acids, soap and moisture'. The food additive includes a wide range of chain lengths and isomers of mono- and diglycerides. The application contains generalised chemical structures of such mono- and diglycerides of fatty acids. The chemical reaction below also provides a simple schematic of the structures of both monoglycerides and diglycerides. It is noted that the R group comes from the fatty acid, and can be saturated or unsaturated and in the case of the diglyceride, they do not need be the same, i.e. R can be R' and R'' in the structure.

Glycerol + Fatty Acid $\xrightarrow{H^+ \text{ or } OH^-}$ Monoglyceride + Diglyceride + H_2O
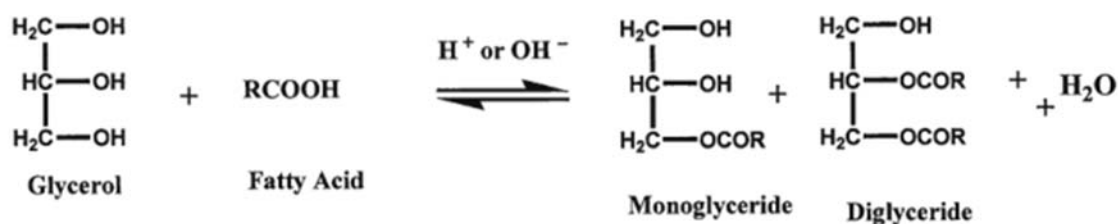
A summary of the identity of the food additive is provided in Table 1. It is noted that since the food additive contains a wide range of possible mono- and diglycerides of fatty acids some of the information provided, including from the application, is on some of the general and likely families of such glycerides within the food additive.

Table 1   *Identity of the food additive, mono- and diglycerides of fatty acids and possible general families of mono- and diglycerides in the preparation*

Common name	Mono- and diglycerides of fatty acids
Alternative names	glycerolipids; glycerol esters of fatty acids; names of examples of specific glycerides: glyceryl monostearate, glyceryl monopalmitate, glyceryl monooleate; examples of alternate names for specific glycerides: monostearin, monopalmitin, monoolein,
INS number	471
Examples of general families of mono- and diglycerides with their CAS[1] numbers	
C14–18, mono- and diglycerides, saturated	67701-33-1
C14–22, monoglycerides, saturated	68990-53-4
C16–18, mono- and diglycerides, saturated	85251-77-0

2.1.2   Chemical and physical properties of the food additive

As would be expected of glycerides of fatty acids, the food additive is not directly soluble in water. It is soluble in ethanol, chloroform and benzene. However, when it is used for the proposed purpose as a glazing agent on fresh fruits and vegetables it is dispersed in water before it is applied to the surface. The applicant notes and provides experimental evidence that the food additive forms a thin film but does not react directly with the surface of the treated food, i.e. it is inert. The appearance of the food additive is stated in the JECFA specification to be white or cream coloured hard fats of waxy appearance, plastic products or viscous liquids, though for its use for the proposed purpose of the application it would need to be a liquid so it can readily be dispersed in water.

2.1.3   Product specifications

As indicated in section 2.1.1, the food additive has been permitted for use as a GMP food additive in the Code for many years as well as around the world. It therefore has specifications of identity and purity in many international sources of food additive specifications. In the Code, primary sources of specifications, which cover the food additive are listed in section S3—2, being the Combined Compendium of JECFA food additive specifications (JECFA 2017) (para S3—2(1)(b)), Food Chemicals Codex (United States Pharmacopeia 2018) (para S3—2(1)(c) and the EU Commission Regulation No 231/2012 (EU Commission Regulation 2012) (para S3—1(b)(d)). The relevant JECFA and Food Chemicals Codex specifications are contained as appendices in the application. The applicant's food additive meets the requirements of these two monographs, with data of three

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[1] Chemical Abstract Services number

non-consecutive samples provided. An assessment confirmed that these data also complied with the EU Commission food additive specification regulation. These results are summarised in Table 2.

Table 2 *Results of three non-consecutive samples of the food additive compared to specification requirements for JECFA, Food Chemicals Codex and EU Commission regulations*

Parameter	JECFA	FCC[a]	EU Regs[b]	Results
Alpha monoglycerides % w/w	≥ 30	-	-	57.6, 56.1, 58.8
Free glycerol % w/w	≯[c] 7	≯ 7	≯ 7	<5, <5, <5
Water content %	≯ 2	-	≯ 2	0.30, 0.20, 0.36
Acid value[2]	≯ 6	≯ 6	≯ 6	4.6, 2.8, 2.2
Sulphated ash % w/w		≯ 0.5[d]	≯ 0.5	<0.1, <0.1, <0.1
Soap, (as sodium oleate) % w/w	≯ 6	-	-	<0.156, <0.156, <0.156
Lead mg/kg	≯ 2	≯ 2	≯ 2	<0.0965, <0.0923, <0.0931
Arsenic mg/kg	-	≯ 3	≯ 3	<0.0965, <0.0923, <0.187
Cadmium mg/kg	-	-	≯1	<0.0965, <0.0923, <0.187
Mercury mg/kg	-	-	≯1	<0.0914, <0.265, <0.0944

Notes
a   Food Chemicals Codex
b   EU Commission Regulation No 231/2012
c   ≯ = not more than, in the FCC, written as NMT
d   Residue on ignition

2.1.4   Analytical method of detection

The food additive has been permitted as a GMP food additive for many years. Therefore, there are widely available methods of analysis for detecting and quantifying mono- and diglycerides added to food as well for confirming its consistency with the purity specifications.

The applicant uses its own in-house analytical method for the determination of total glycerides, alpha-monoglycerides and free glycerol using ultra high-performance liquid chromatography (UHPLC) with an evaporative light scattering detector (ELSD).

2.2   Manufacturing process

The details on the manufacturing process to produce the food additive is provided in the application. Mono- and diglycerides of fatty acids are produced from heating oils or fats with excess glycerol, or by direct esterification of glycerol with fatty acids (EFEMA 2015).

The proportion of monoester thus produced is dependent on the proportion of glycerol and the temperature of reaction and is usually in the range of 30-60%. Products with more than 90% monoester content are produced by high vacuum distillation or other techniques.

The applicant's mono- and diglycerides of fatty acids are derived only from plants, and not animal sources of fats and oils. That is not the case for specifications of mono- and diglycerides of fatty acids where no distinction is made relating to the source of the fats and

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[2] The acid value is the defined as the amount of potassium hydroxide in mg required to neutralise 1 gm of the substance (usually a fat or oil). It is a measure of the carboxylic acid groups in a fatty acid or blend of fatty acids.

oils.

2.3 Technological purpose of the food additive

The technological purposes of the food additive is listed by the Codex Alimentarius guidance document CXG 36-1989 as: antifoaming agent, emulsifier, glazing agent and stabiliser. Section 1.1.2—11 of the Code requires that food additives (substances 'used as a food additive') perform one or more of the technological purposes listed in Schedule 14, which includes emulsifier, glazing agent and stabiliser.

The technological purpose relevant to the application is for the extension of use of the food additive to perform the technological purpose of a 'glazing agent' as defined in Schedule 14. That is, the food additive 'imparts a coating to the external surface of a food'. In the case of this application the treated food is fresh fruits and vegetables. The reason for applying a surface coating to fresh fruits and vegetables is to extend the commercial shelf life of the treated produce.

Once fresh untreated fruits and vegetables are harvested they are subject to a range of different processes that ultimately lead to an end of their shelf life. These processes include a loss of moisture, tissue softening (due to ripening), which leads to visual deterioration, a breakdown of cell materials due to respiration, a reduction in inherent volatile flavour compounds that are characteristic of the product with sometimes the formation of other unacceptable flavour compounds and a decrease in organic acid levels.

Relevant parameters linked to extending shelf life of such products are to preserve the quality and maintain acceptable appearance of the product so it is still desired by consumers. Extending the shelf life of food is always an ongoing challenge for all parts of the food supply chain as it has major benefits for all sectors. These main benefits are:

- to maintain the quality (includes appearance) and nutritional value
- to reduce wastage of product that is no longer commercially acceptable
- to provide a gas barrier, to limit gas exchange, both in and out of the product
- to reduce water loss which causes shrinkage.

2.4 Technological justification of the food additive

Currently waxes and resins are permitted to be used as glazing agents for fresh fruits and vegetables around the world, including in Australia and New Zealand due to permissions in the Code. However, there are some more fragile and delicate fruits and vegetables, such as berries, that are not appropriate to coat with such agents. That is, they cannot withstand the coating process using a rough brush-bed surface or the considerable heat needed to be applied to dry or set the wax or resin.

Mono- and diglycerides of fatty acids however can be readily applied to a wider range of fresh fruits and vegetables, as different types of coating techniques can be tailored for the specific product. The surface of fragile fruits or vegetables can be sprayed or dipped with an aqueous solution of mono- and diglycerides of fatty acids that can be more readily used compared to waxes and resins. The mono- and diglycerides of fatty acids are colourless, odourless and tasteless when applied to the surface of treated fruits and vegetables, similar to currently permitted waxes and resins. The applicant has performed efficacy studies on more than 30 fresh fruits and vegetables demonstrating shelf life extensions and superior performance (visually assessed) compared to alternative commercial glazing agents. Visual time lapse studies of these treated products are available on the applicant's website. The details of studies for some of the products, being asparagus, lemons and avocados are also

provided in the application. The results of these efficacy studies have been assessed and are summarised in Table 3. As can be readily seem from these studies, treatment with mono- and diglycerides of fatty acids as a glazing agent on various fresh fruits and vegetables provides superior shelf life compared to untreated and conventional wax treatment.

The levels of use of the food additive as glazing agents on the different fresh fruits and vegetables is GMP, as the levels will vary on the different produce treated as well as the seasonality, variety, climatic conditions and the desired shelf life extension. Therefore the use levels will be determined by the individual processor to meet their requirements. GMP is also the limit of use for the food additive to treat fresh fruits and vegetables in the Codex General Standard for Food Additives.

Table 3   *Summary of efficacy data comparison for some treated fruits and vegetables provided in the application*

Fruit or vegetable	Storage time (days)	Untreated	Treated with INS 471	Alternate treatment, with conventional wax
Asparagus	1	Acceptable	Acceptable	-
	7	Unacceptable, dehydrated stems	Acceptable	-
	12	Unacceptable, further deterioration	Acceptable	-
Lemons	1	Acceptable	Acceptable	Acceptable
	31	Some signs of spoilage	Acceptable	Some signs of spoilage
	60	Unacceptable, significant signs of dehydration and discolouration	Acceptable	Unacceptable, significant signs of dehydration and discolouration
Avocados	1	Acceptable	Acceptable	-
	10	Ripe, acceptable for sale	Acceptable, still unripe	-
	30	Unacceptable, significant signs of spoilage	Acceptable	-

2.5   Food technology conclusion

An assessment of data provided by the applicant has concluded that the food additive mono- and diglycerides of fatty acids performs the technological purpose of a glazing agent to extend the shelf life (visual assessment) of various treated fresh fruits and vegetables compared to untreated product and conventional waxes and resins applied as glazing agents. Analysis of some of the treated fruits and vegetables concludes that mono- and diglycerides performs better than other permitted glazing agents. This is an extension of use of the currently already permitted GMP food additive. As an already permitted GMP food additive it complies with internationally accepted specifications of identity and purity which are primary sources of specifications in Schedule 3.

3   Hazard assessment

3.1   Background

Schedules 15 and 16 of the Code currently permit the use of the food additive mono- and diglycerides (INS 471) for a range of foods. The purpose of this application is to request permission to use the food additive as a glazing agent in the food class 4.1.2 (surface treated fruits and vegetables).

Mono- and diglycerides as a food additive were approved, within the category 'Emulsifiers and stabilizers' by JECFA in 1963 and 1973. An ADI of "not limited" was established at the 17th JECFA meeting in 1973, on the basis that mono- and diglycerides differ little from food. JECFA did not identify any further information as being required or desirable for mono- and diglycerides (JECFA, 1974a). This assessment is therefore limited to information that postdates that assessment.

3.1.1 Evaluation of the submitted data

FSANZ has assessed the submitted evidence on the safety of the food additive mono- and diglycerides of fatty acids (INS 471), and information from other sources.

The data are considered suitable to assess the hazard of INS 471 and include information on kinetics and metabolism, genotoxicity, toxicity in laboratory animals, and a study in human volunteers. Most of the studies were conducted using dietary diacylglycerol (diglyceride) oil that is considered sufficiently similar to the food additive that is the subject of this application.

3.1.2 Characteristics of the food additive

The chemical characteristics of the food additive are discussed in detail in Section 2.

3.2   Toxicokinetics and metabolism

Mono- and diglycerides are normally present in food, and are also formed from triglycerides by the action of endogenous lipases in the gastrointestinal tract. They are absorbed by enterocytes and transported to the liver, where they may be reformed into triglycerides or metabolized into carbon dioxide, acetate and ketones (JECFA 1974a; Yang and Kuksis 1991; Lien et al 1997; Høy and Xu 2001).

On that basis, JECFA (1974 a,b) concluded that mono- and diglycerides used as food additives do not significantly differ from those naturally present in the diet, although saturated long-chain fatty acids, if present, may have lower digestibility than unsaturated fatty acids if fed alone. No new information was submitted or located by literature search that contradicts the conclusions of JECFA (1974a,b).

In vitro *intestinal digestion of 1,3-diglyceride and 1-monoglyceride oils (Martin et al 2014). Regulatory status: not GLP*

The test substances for this study were concentrated diolein oil (DO), concentrated monoolein oil (MO), a 1:1 mixture of DO and MO and, as a representative triolein-rich oil (TO), olive oil. Test substances were emulsified in Trizma-maleate buffer. A simulated biliary secretion was prepared using lecithin, bile salts, cholesterol, $CaCl_2$, NaCl and Trizma-maleate buffer. Test substances and the simulated biliary secretion were emulsified together, prior to the addition of pancreatin in Trizma-maleate buffer to start the simulation of intestinal

digestion. Digestion was conducted in triplicate at 37°C with continuous stirring, and aliquots were taken at 0, 5, 10, 30 and 60 minutes. Lipids were extracted from the aliquots and quantified. The rate of lipolysis was, in increasing order, TO<DO<DO:MO<MO. At the end of the digestion, the DO:MO mixture was hydrolysed to digestable products to the same extent as MO. The bioaccessibility of DO, MO and DO:MO were all considered to be comparable.

Bioavailability study on diacylglycerol microemulsion in Sprague Dawley rats (Chen et al 2013). Regulatory status: Not GLP

Diacylglycerol (diglyceride) oil (DAG), with 18:1 and 18:2 fatty acids predominating, was prepared as a microemulsion (DAGM) using food-grade surfactants. Male Sprague Dawley rats, 5 weeks old at receipt, were acclimatised to laboratory environmental conditions and to a fat-free diet for one week prior to study start. Food was withheld for 12 h before the first treatment. On the first day of study, rats were randomly assigned to two groups, with nine rats in each group. One group was gavaged with 10 mL/kg bw DAGM, equivalent to 2.5 mL/kg bw DAG. The other group was gavaged with a combination of 2.5 mL/kg bw DAG and 7.5 mL/kg bw distilled water. Blood was collected from the tail vein of 3 rats/group at 0, 6, and 24 h after dosing for lipid analysis. Rats were maintained in metabolic cages with free access to water and fat-free diet, and gavaged daily with their assigned test substance. Food intake was measured daily and bodyweight was recorded every three days. Faeces were collected on the last day of study for lipid analysis. The digestibility coefficient of the DAG was calculated to be $58.8 \pm 14.33\%$, and to equal the absorption. The group mean serum fatty acid concentration remained stable in the DAG group, but increased at 6 h after dosing in the DAGM group, Compared with the DAG group, the DAGM group had significantly higher serum fatty acid concentration at 6 h after dosing ($P = 0.043$). However, there was no significant difference in group mean serum fatty acid concentration at 0 or 24 h. There was no significant difference in group mean bodyweights at Day 0 or Day 14, although total food consumption was significantly higher in the DAG group. Although there was no significant difference between the two groups for fat intake or dried faeces mass, the levels of fat excretion and fat content of dried faeces of the DAGM group were both significantly less than those of the DAG group. There was a significantly higher level of fat absorption in the DAGM group than in the DAG oil group ($P = 0.001$).

3.3   Toxicity studies

3.3.1   Studies in experimental animals

3.3.1.1 *Acute toxicity studies*

No information was located concerning the acute toxicity of monoglycerides.

Morita and Soni (2009) reviewed three acute oral gavage studies of diacylglycerol oil in rats. No acute toxicity was observed in any of the studies at the highest dose administered, which was 5000 mg/kg bw in one study and 15,000 mg/kg bw in the other two studies.

Acute oral toxicity study of diacylglycerol microemulsion in ICR mice. Huang et al (2011). Regulatory status: Not GLP

Diacylglycerol oil (DAG) containing 87% diglycerides, 2% monoglycerides and 11% triglycerides was used to formulate a DAG microemulsion (DAGM) containing 25% w/w DAG, with the balance being distilled water and food-grade surfactants.

ICR mice, 20/sex, ranging from 18 to 22 g at time of receipt, were used for this study. Mice

were maintained under standard laboratory environmental conditions and acclimatized for one week. Mice were randomly assigned to two groups of 10/sex/group and fasted for 12 h prior to being gavaged with either DAGM at 20 mL/kg bw or water at 20 mL/kg bw. Mice were monitored for 14 d after dosing. All mice survived the 14 d observation period and no abnormal clinical observations were recorded.

3.3.1.2 Subchronic toxicity studies

No subchronic studies of monoglycerides were located.

30-day repeat-dose study of diacylglycerol microemulsion in Sprague Dawley rats (Huang et al 2011). Regulatory status: Not GLP

The test article for this study was the same DAG microemulsion (DAGM) as that used in the acute mouse study summarised in the previous subsection.

Male and female Sprague Dawley rats ranging from 60 to 80 g bw were acclimatised to standard laboratory environmental conditions for one week before being assigned to groups of 10/sex/group. The treatment groups were gavaged daily for 30 d with DAG at 6.7, 10 or 20 mL/kg bw. DAGM was diluted in water but it is not clear whether the control group was administered water, or gavaged. Rats were subject to daily observations. Body weights were recorded on study days 0, 7, 14, 21, and 30. Food and water consumption were recorded weekly. On Day 30, rats were weighed and then decapitated. Organ weights were recorded for liver, kidneys, brain, heart, lungs, spleen, adrenals and gonads. Histopathological examination was carried out on liver, kidney, brain and gonads. Haematology and serological assessment were also conducted, although details of the blood collection are not described.

Treatment with DAGM had no significant effect on body weights in either sex, and there were no treatment-related histopathological findings. Statistically significant but biologically minimal changes in group mean values for haematology and serum chemistry parameters did not show dose-response relationships and are considered to be unrelated to treatment. There were dose-related increases in group mean values for relative liver weight in females treated with 20 mL/kg bw/day and males treated with ≥10 mL/kg bw/day. The group mean value for relative kidney weight was also significantly increased in females treated with 20 mL/kg bw/day. However there were no corresponding histopathological changes or changes in clinical pathology parameters. No adverse effects were observed at any dose.

90-day dietary study of diacylglycerol oil in rats (Morita et al 2008a). Regulatory status: GLP; designed to be in general accordance with OECD, ICH and US-FDA Redbook guidelines.

This study was conducted to investigate the effects of heating DAG; unheated DAG was used as the control substance. The basal diet was fat-free, and oils were added up to a total of 5.5% w/w. Two additional groups were fed diets containing either 5.5% unheated triacylglycerol oil or 5.5% heated triacylglycerol. Male and female Sprague Dawley rats, 40 days old at receipt, were housed individually under standard environmental and husbandry conditions, and acclimatised for 16 days prior to the start of the study. Food and water were provided *ad libitum*. Rats were randomised to the six groups, 10/sex/group. All animals were observed twice daily for mortality/moribundity, and subject to cageside observations once daily. Detailed clinical observations were made weekly, from one week prior to study start, and including the day of necropsy. Bodyweights were recorded weekly from two weeks prior to study start, and including the day of scheduled necropsy. A comprehensive functional observational battery (FOB) was conducted on all rats prior to study start and during Week 12 of study. Urine and blood was collected from all animals prior to termination for

comprehensive analyses. Ophthalmic examinations were conducted on all rats prior to study start and during Week 11 of study. All animals were killed with isoflurane and subject to complete necropsy, with a comprehensive list of organs and tissues preserved for histopathological examination. Organ weights were recorded for adrenal glands, brain, epididymides, heart, kidneys, liver, lungs, ovaries, pituitary, prostate, salivary gland, seminal vesicles, spleen, testes, thymus, thyroid with parathyroids, and uterus.

All rats survived to scheduled necropsy, and there were no treatment-related clinical observations. There were no toxicologically significant effects on body weights or body weight gains, food consumption, ophthalmic findings, FOB and motor activity, clinical pathology values, organ weights, gross necropsy findings or findings on histopathological examinations. The dose, 5.5% w/w corresponded to 3178 to 4120 mg/kg bw/day.

12-month dietary study of DAG in Beagle dogs (Chengelis et al 2006c). Regulatory status: GLP; study design based on US-FDA Redbook guidelines.

This study was conducted using DAG oil with a diglyceride content of >82%, although it was assumed to be 100% purity for dose calculation purposes. The triacylglycerol (TAG) oil was >95% pure. The first control group were fed a standard laboratory diet containing 9.5% lipid. The second control group were fed a diet containing 9.5% TAG. The treatment groups were fed diets containing 1.5, 5.5 or 9.5% DAG, with the difference made up with TAG so that all dogs received 9.5% dietary lipid, although it is not clear whether the energy content was the same across groups. Dose concentration, homogeneity and stability were confirmed by analysis.

Dogs were 7 to 8 weeks old at receipt and were acclimatised to standard laboratory housing conditions for 14 days prior to assignment to groups, 4/sex/group. Dogs were pair-housed for the first four weeks of the study, and individually housed, with daily periods of co-housing for social interaction, for the rest of the study. Water was provided *ad libitum.* Food was offered *ad libitum* for the first three weeks of the study, and then for progressively shorter times until Week 7, when access to food was reduced to 1-2 h, which was maintained to the end of the study. All dogs were checked for mortality/moribundity twice daily, and observed prior to feeding and 1 to 2 h after feeding from Week 7. Detailed physical examinations were conducted weekly from prior to study start. Body weights were recorded twice weekly from Week -2 to Week 2, and weekly thereafter. Food consumption was recorded from the separation of dogs into individual cages. Blood for clinical pathology evaluation was collected prestudy, during Weeks 13, 26 and 39, and prior to scheduled necropsies. Ocular examinations were conducted prestudy, in Weeks 12, 25 and 38, and in Week 51. Electrocardiographs(ECGs) were recorded prestudy, in Week 25 and in Week 51. Following study start, ECGs were recorded 1-3 h after dosing. All dogs were subject to complete necropsies with collection of a comprehensive list of tissues and organs for histopathology. Fresh weights were recorded for adrenals, brain, epididymides, kidneys, liver, ovaries, testes and thyroids (with parathyroids), as sex-appropriate.

All dogs survived to scheduled necropsies, and no test article-related clinical signs were observed. Body weights and body weight gains were similar between DAG-treated dogs and both control groups throughout the study. Food consumption values for the TAG control group and the DAG-treated dogs were frequently lower than those for the control group fed the standard diet, possibly reflecting a difference in palatability. Treatment with DAG had no adverse effects on group mean values for haematology or clinical chemistry parameters when compared to the TAG control group. However both TAG and DAG groups exhibited higher group mean values for serum levels of alkaline phosphatase activity, cholesterol and triglycerides when compared to dogs in the standard diet control group, but not when compared to each other. In the absence of any corresponding microscopic changes or a

dose-response relationship, these differences in group mean values for alkaline phosphatase, cholesterol and triglycerides were not considered to be toxicologically significant, and were attributed to the different diets. No treatment-related effects on ophthalmological findings, ECGs, gross necropsy findings, organ weights, organ weight ratios or microscopic findings were found. It was concluded that the chronic dietary NOEL was 9.5% w/w DAG in the diet.

3.3.1.3 Chronic and carcinogenicity studies

No carcinogenicity studies of monoglycerides were submitted or located by literature search.

24-month dietary carcinogenicity study of diacylglycerol oil in mice (Chengelis et al 2006a). Regulatory status: GLP; study design based on US-FDA Redbook guidelines.

Diacylglycerol oil for this study was >82% pure, with most of the balance made up of triglycerides, but for dose calculation purposes, was assumed to be 100% DAG. The study included two control groups. One control group was fed a standard rodent diet containing 4.5% dietary fat. The second control group was given a triglyceride oil with the same fatty acid content as the test article. The triglyceride oil was administered in the diet as 6% w/w, while the DAG was administered as 1.5, 3.0 or 6.0% w/w, with the balance made up to 6% using the triglyceride oil. It is not clear whether the doses were of equivalent energy content. Dose concentration, homogeneity and stability of the lipids in the diets were confirmed by analysis.

Mice of Crl:CD®-1(ICR)BR strain were 34 days old on receipt. They were acclimatised to standard laboratory environmental conditions for 15 days prior to study start. Prestudy assessments included body weights, physical examinations, ophthalmic examinations and physical examinations. Mice were housed individually during the study, and water was provided *ad libitum*. Mice were randomised to groups of 50/sex/group. All mice were subject to twice-daily mortality/moribundity checks and daily cageside observations. Detailed clinical observations were conducted weekly from one week prior to study start. Body weights and food consumption were recorded weekly from prior to study start through to Week 13, and recorded every two weeks thereafter. Haematological assessment was conducted on blood from 10 mice/sex/group in Week 51 and prior to necropsy. All mice were subject to complete necropsy and a comprehensive list of organs and tissues were preserved for histopathology. Organ weights were recorded for adrenals, brain, epididymides, heart, kidneys, liver, ovaries, spleen, testes, thyroids and uterus, as sex-appropriate.

When compared to the second control group, which received 6% triglyceride in the diet, mice fed DAG showed no treatment-related effects on survival, clinical observations, ophthalmic findings, body weights, organ weights, group mean haematology values, gross and microscopic lesions, latency of neoplasms or incidence of neoplasms. When compared to the first control group, which was fed a standard diet containing 4.5% lipid, both the second control group and the DAG-treated groups exhibited lower survival, higher body weights, lower food consumption and greater frequency of gross and microscopic lesions. These differences were attributed to the greater fat consumption. It was concluded that the effects of DAG consumption at up to 6% were no different to those of triglycerides alone, and the NOEL for DAG in the diet was 6%, approximately equivalent to 7.4 g/kg bw/day in males and 9.8 g/kg bw/day in females.

24-month dietary carcinogenicity study of diacylglycerol oil in rats (Chengelis et al 2006b). Regulatory status: GLP; study design based on US-FDA Redbook guidelines.

The diacylglycerol oil for this study was the same as that for the acute study in mice described previously, and for dose calculation purposes, was likewise assumed to be 100% DAG. In the main study, food intake was restricted for two control groups and three treatment groups. The first control group in the main study was fed a standard rodent chow containing 4.5% dietary fat, whereas the second control group was given a triglyceride oil in the diet at 5.5% w/w. DAG was administered to the treatment groups as 1, 2.75 or 5.5% w/w, with the balance made up to 5.5% using the triglyceride oil. A further two groups were in the satellite study in which food intake was *ad libitum*. These groups were another control group fed diet containing 5.5% triglyceride oil, and a group fed DAG at 5.5%. Groups and treatments are shown in Table 4. Dose concentration, homogeneity and stability of the lipids in the diets were confirmed by analysis.

Table 4   Study groups (50/sex/group) and treatments

Group	Treatment	Dietary fat/oil treatment		
		Standard	TG (%)	DAG (%)
Main (restricted intake) groups				
1	Control-1	4.5	-	-
2	Control-2	-	5.5	0
3	DAG-low	-	4.5	1
4	DAG-mid	-	2.75	2.75
5	DAG-high	-	0	5.5
Ad libitum satellite groups				
6	Control 3	-	5.5	0
7	DAG-high	-	0	5.5

Rats of Crl:CD®(SD)-IGS BR strain (Charles River Sprague Dawley) were 28 days old on receipt. They were acclimatised to standard laboratory environmental conditions for 16 days prior to study start. Prestudy assessments included body weights, physical examinations, ophthalmic examinations and physical examinations, and rats from which the main study subjects were selected were placed on the restricted diet regimen for the seven days prior to study start. Rats were housed individually during the study, with *ad libitum* water, and randomised to groups of 50/sex/group for the main study, and 65/sex/group for the satellite study. All rats were subject to twice-daily mortality/moribundity checks and daily cageside observations. Detailed clinical observations were conducted weekly from one week prior to study start. Body weights were recorded weekly from approximately one week prior to study start through to Week 68, and every two weeks thereafter. Food consumption was recorded daily for the main study rats, whereas for the satellite study rats, food consumption was recorded weekly until Week 68 and every two weeks thereafter. Ocular examinations were conducted on all rats in Week 51 and all male rats in Week 104. Ocular examinations were also conducted on 10 rats/group in Week 67 for satellite study females, Week 91 for satellite study males, and Week 95 for main study females. Five rats/sex/group were scanned with a densitometer to determine lean and non-lean body mass after 8 and 16 months (Weeks 34 and 65) on study. In addition, scanning was conducted on 5 female rats/group in satellite study in week 86; 5 male/rats/group in the satellite study in Week 91; 5 female rats/sex/group in the main study in Week 94, and 5 male rats/sex/group in the main study in Week 103. Haematological assessment was conducted on blood from 10 rats/sex/group from all groups in Week 51; from satellite study group females in Week 91; from satellite study group males in Week 95; and from all main study rats prior to scheduled necropsy. All rats were subject to complete necropsy and a comprehensive list of organs and tissues were preserved for histopathology. Organ weights were recorded for adrenals, brain, epididymides, heart, kidneys, liver, ovaries, spleen, testes, thyroids and uterus, as sex-appropriate.

When compared to rats fed an equivalent amount of triglyceride in the diet and under the

same dietary regimen (restricted or *ad libitum*), rats fed DAG showed no treatment-related effects on survival, clinical observations, ophthalmic findings, body weights, organ weights, clinical pathology parameters, gross and microscopic lesions, latency of neoplasms or incidence of neoplasms. In the main study, when compared to the first control group fed a standard diet containing 4.5% lipid, both the second control group and the DAG-treated groups exhibited lower survival, higher body weights, higher percentage body fat, higher fat-related clinical chemistry parameters, higher incidence of lesions in a number of organs, and higher incidences of pituitary and mammary neoplasms. Rats in the satellite study, when compared to those on the main study, showed poorer survival, higher body weights, higher body fat, alterations in fat-related serum chemistry parameters, and higher incidences of lesions of heart, kidney and liver. However, there were no significant differences between rats fed DAG in an *ad libitum* regimen and those fed an equivalent amount of triglyceride in an *ad libitum* regimen. It was concluded that rats fed DAG instead of an equivalent amount of triglyceride did not exhibit higher risk of carcinogenic effects, or shorter latency to background neoplasms, than those fed triglyceride.

3.3.1.4 *Genotoxicity assays*

Genotoxicity studies of dietary diacylglycerol (DAG) oil (Kasamatsu et al 2005).Regulatory status: GLP; conducted in accordance with OECD and MHLW guidelines.

A suite of genotoxicity assays, comprising a bacterial reverse mutation assay, chromosomal aberration assay in Chinese hamster lung cells, and bone marrow micronucleus assay in ICR CD mice, was conducted using edible oil containing >80% diacylglycerol (DAG).

The bacterial reverse mutation assay was conducted using the plate incorporation method, with the standard bacterial strains and positive control substances. The solvent/negative control was DMSO. The experiments were conducted in the absence and presence of S9 mix for metabolic activation. Based on the dose-rangefinding experiment, the definitive experiment was conducted at doses up to 5000 µg/plate. No bacterial toxicity was observed at any dose level, and there was no increase in revertant colonies when compared to the negative control plates. The positive control substances induced the expected increases in revertant colonies, confirming the validity of the assay.

The chromosomal aberration assay was conducted at dose levels up to 5000 µg/mL. Standard positive control substances were used, and experiments were conducted with and without S9 mix. Both short-term (6 h) and continuous (24 or 48 h) exposures were conducted. Cells were processed to slides for examination of chromosomal aberrations. No cytotoxicity was observed, and there was no increase in frequency of cells with structural or numerical aberrations, in the presence or absence of S9 mix, when compared to negative controls. The positive control substances induced the expected increases in chromosomal aberrations, confirming the validity of the assay.

The bone marrow micronucleus assay was conducted in 8 week old male ICR mice. The paper does not specify how many mice were assigned to each group. The OECD guideline for this assay states that each group should contain at least 5 animals. DAG was suspended in olive oil to doses of 500, 1000 and 2000 mg/kg bw and administered by gavage twice, with 24 h between gavages, while control mice were treated with olive oil. Mice in a positive control group were treated once by intraperitoneal injection of cyclophosphamide at 40 mg/kg bw. Bone marrow smears were prepared 24 h after the last administration. Administration of DAG was not associated with any significant increase in micronucleated polychromatic erythrocytes (MNPCEs) when compared to negative controls. The PCE/NCE (polychromatic erythrocyte/normochromatic erythrocyte) ratio was comparable to that of negative controls for mice treated with ≤1000 mg/kg bw but was slightly decreased in mice treated with 2000

mg/kg bw. This decrease in the group mean value was attributed to intragroup variability, in that one animal was a significant outlier. The positive control substance, cyclophosphamide, induced a marked increase in incidence of MNPCEs, confirming the validity of the assay.

It was concluded on the basis of this battery of genotoxicity assays that DAG does not exhibit genotoxicity.

No genotoxicity assays of monoglycerides were located.

3.3.1.5 Developmental and reproductive studies

Two-generation reproductive toxicity study of diacylglycerol oil in Sprague Dawley rats (Morita et al 2008b). Regulatory status: GLP, conducted in general accordance with US-FDA Proposed Redbook 2000 guidelines.

The test article for this study was prepared from rapeseed oil and soybean oil, and comprised >86% DAG, and was administered daily by gavage at dose levels of 0, 1.25, 2.5 or 5 mL/kg bw/day. An additional group was administered a triacylglycerol (TAG) oil containing >91% TAG, at 5 mL/kg bw/day. The control group were administered corn oil which was >95% triglycerides, at 5 mL/kg bw/day. Corn oil was also used to make up the volume of oil administered to rats dosed with 1.25 or 2.5 mL/kg bw/day DAG.

Rats, 30/sex/group, were individually housed, except during mating, under standard laboratory environmental and husbandry conditions, and food and water were provided *ad libitum*. Both male and female rats were dosed for at least 70 days prior to mating. Rats were subject to cageside observations daily, except during the period of expected parturition, when females were observed twice daily. Body weights and food consumption were measured at least weekly, although during pregnancy and lactation, body weights and food consumption were recorded on gestation days (GDs) 0, 4, 7, 11, 14 and 20, and lactation days (LDs) 1, 4, 7, 14 and 21. At parturition, litters were sexed, pups were examined and the numbers of live and dead pups were recorded. Litters were culled to 8 pups (4/sex, if possible) on postnatal day (PND) 4. Pup survival was monitored daily and each pup was weighed and given a detailed physical examination on PNDs 1, 4, 7, 14 and 21. Pups that died before weaning were necropsied. The F1 generation also comprised 30 rats/sex/group, using at least one pup/sex/litter. Gavaging of the F1 generation commenced on PND 22. Selected male pups were examined for balanopreputial separation from PND 35 and selected female pups were examined for vaginal patency from PND 25. F_0 and F_1 males were subject to spermatogenic evaluations, including numbers and morphology, at termination, which was done at the time that their pups were weaned. F_0 and F_1 females were also killed at weaning of their pups. All F_1 not selected for breeding, and all F_2 pups, were killed at PND 21. All killed animals were subject to gross necropsy. Adrenal glands, brain, cervix, coagulating glands, epididymis, kidneys, liver, ovaries, oviducts, pituitary gland, prostate, seminal vesicles, spleen, testis, thymus, uterus, vagina, vas deferens, as appropriate for sex, and all gross lesions, were preserved for histopathological examination.

A small number of animals did not survive to scheduled termination, but these deaths did not show a dose-response relationship. Unscheduled deaths included one male and one female F_0 rat in the 50% DAG group; one F_1 male in each of the 50% DAG and 100% TAG groups; two F_1 females in the 25% DAG group; and two F_1 males in and one F_1 female in the 0% DAG group. No treatment-related clinical signs were observed in any rats that survived to scheduled termination. There were no treatment-related effects of DAG oil on body weights, body weight gains, food consumption, or feed efficiency. Treatment with TAG oil was associated with significantly lower group mean bodyweights in F_0 and F_1 males, but there was no corresponding effect in females. There were no gross or microscopic findings related

to treatment with DAG oil or TAG oil, and no treatment-related effects on organ weights, primordial follicles in females, spermatogenic assessment parameters, reproductive performance parameters, litter parameters, pup viability, pup growth, balanopreputial separation or vaginal patency. The authors concluded that the highest dose level of DAG oil, 5.0 mL/kg/day, was the NOAEL for systemic, reproductive and neonatal toxicity. This dose level is equivalent to 4630 mg/kg bw/day.

Developmental toxicity study of diacylglycerol oil in Sprague Dawley rats (Morita et al 2008c). Regulatory status: GLP, conducted in accordance with MHLW guidelines and stated to be in general accordance with ICH guidelines though these were not specified.

The test article for this study was the same as that used in the two-generation reproductive toxicity study summarized above. The control article was corn oil. Rats, 25 females/group, were dosed on gestation days (GDs) 6 to 17 inclusive at a fixed volume of 5 mL/kg bw/day, with dose levels of 0, 1.25, 2.5 or 5 mL/kg bw/day DAG, with the balance of the volume being corn oil. Rats were 70 days old at time of receipt and were acclimatized to standard laboratory environmental and husbandry conditions for 13 days prior to mating with resident male rats. During pregnancy, females were housed individually. Food and water were provided *ad libitum.* They were observed for mortality and moribundity twice daily, and cageside clinical observations were made prior to dosing and one hour after dosing. Individual body weights were recorded daily from GDs 6 to 18 inclusive, and also on GDs 0 and 20. Rats were killed on GD 20. Following termination, gravid uterus weight and net body weight were recorded, as well as number of corpora lutea, number of implantation sites, number and location of fetuses, and late and early resorptions. Uteri with no gross evidence of implantation were placed in 10% ammonium sulphide for detection of early embryonic loss. Live fetuses were examined, weighed, sexed and then killed and necropsied with particular attention to the heart and major blood vessels. Heads of approximately half the fetuses were preserved for dissection while the heads of the remaining fetuses were examined by mid-coronal slice. All fetal carcasses were eviscerated and preserved for examination of skeletons using Alizarin Red.

All females survived until scheduled death. There were no treatment-related effects on maternal clinical signs, body weights, body weight gains, net body weights, or food consumption. Treatment with DAG had no effects on intrauterine growth, numbers of corpora lutea, numbers of implantation sites, numbers of viable fetuses, mean fetal weights, rates of preimplantation loss, numbers of resorptions, or fetal sex ratios. The numbers of fetuses available for examination were very similar between the dose groups. Treatment had no effect on rates of fetal malformation, which were low in all groups, within historical control ranges, and considered to be spontaneous in nature. It was concluded that the highest dose administered, 5 mL/kg bw/day DAG, equivalent to 4630 mg/kg bw/day, was the NOAEL for both maternal and fetal toxicity. A maternal maximum tolerated dose could not be identified.

No reproductive or developmental studies of monoglycerides were submitted or located by literature search.

3.3.1.6   Other studies in animals

One-month study comparing effects of dietary triacylglycerol oil and diacylglycerol oil on protein kinase C activation in male Wistar rats (Meguro et al 2007). Regulatory status: Not GLP

The percentage of DAG in the DAG oil used in the studies described in this publication is not stated in the text. It was compared to a triglyceride oil (TAG) with a similar profile of fatty

acids.

The test system for the in-life studies comprised male Wistar rats. They were housed under standard laboratory environmental conditions with *ad libitum* access to food and water. It is no clear whether they were group-housed or housed individually.

In the first experiment, the rats were 5 weeks old at study start and were randomised to four groups. The number of rats per group is not specified. The treatment groups were 5% DAG oil, 23% DAG oil, 5% TAG oil or 23% TAG oil in the diet. Rats were maintained on the diets for four weeks. Food intake was recorded three times each week and body weights were recorded weekly. At the end of the in-life phase rats were killed and tongue, oesophagus, stomach, small intestine caecum and colon were collected. The mucosa was removed and protein kinase C (PKC) activity was measured using a commercial assay kit.

In the second experiment, rats were 7 weeks old at study start and were randomised to two groups. Again, the number of rats per group was not specified. Rats were fed a diet containing either 10% DAG or 10% TAG. The design of the in-life phase was the same as that for Experiment 1, with the exception that faeces from each rat was collected for the last five days of the study, and only caecal and colonic contents were collected after the rats were killed. The faeces and collected tissues were processed for measurement of PKC activity.

In the third experiment, which also used rats of 7 weeks old, there were two groups, although once again the number of rats per group was not stated. Rats were fed either DAG oil or TAG oil for 9 days, but the level of the oil in the diet is not stated. Food intake was recorded every 3 or 4 days, and rats were weighed on Days 1 and 9. After 9 days, the rats were fasted for 18 h and then supplied with the diet for 1 h. At 0, 1 or 4 h after restoration of the diet to the rats, rats were killed and serum was collected for analysis. The number of rats killed at each time-point is not specified.

Finally, the publication includes an *in vitro* experiment to determine whether the addition of DAG oil or TAG oil to CaCo-2 cells affected PKC activity in the cells. Exposure of cells to the oils was to both the apical and basolateral surfaces of the cells.

No differences in PKC activity between DAG groups or TAG groups was detected in lingual, oesophageal, gastric, small intestinal, caecal, proximal colonic or distal colonic mucosa, or in caecal contents, colonic contents, faeces or serum. Neither oil had any effect on PKC expression in CaCo-2 cells.

This series of studies tends to support the other studies that show that substituting triglycerides with diglycerides has no effect, although the lack of information concerning the composition of the DAG oil and the sizes of the groups of rats limit the usefulness of this paper.

3.3.2   Human tolerance studies

No human tolerance studies of monoglycerides were submitted or located by literature search.

Morita and Soni (2009) reviewed a total of thirteen clinical studies of diacylglycerol oil in human beings. The studies included one double-blind crossover study, seven double-blind parallel studies, one randomized dietary trial, one single-blind parallel study, one single-blind controlled study, and three open-label studies. Study subjects included both sexes and ranged from healthy adults to overweight/obese adults and children, uraemic adults on

dialysis, and Type II diabetics with hypertriglyceridaemia. Study durations were up to 12 months, and dose levels were measured at up to 730 mg/kg bw/day, or *ad libitum*. In all studies, DAG was well tolerated with no associated adverse effects.

12-week intervention study replacing dietary triacylglycerol oil with diacylglycerol oil in healthy overweight volunteers (Telle-Hansen et al 2012). Regulatory status: Not GLP.

This study was not designed as a tolerance study, but conducted to determine whether replacing TAG oil in food items with DAG oil would influence metabolic markers in healthy, overweight volunteers. Twenty-three men and women, aged between 37 and 67 years, with BMI between 27 and 35, completed the study with ≥ 90% compliance. Subjects were assigned to either the TAG or DAG group. The oils were provided in margarine, mayonnaise and/or oil, and compliance was determined by return of empty containers of the lipid sources. Subjects were asked to maintain a stable weight and to refrain from the use of *n*-3 fatty acid supplements or other dietary supplements. The dietary intervention was continued for 12 weeks. Parameters measured prior to the start of the intervention, and at the end, included blood pressure, body weight, total body fat percentage, total body fat mass, trunk fat mass, and fat-free mass. Additionally, urine was collected and analysed for was analysed for 8-isoPGF$_{2\alpha}$ and creatinine, and blood was collected and analysed for serum lipids, glucose, insulin, C-reactive protein, liver enzymes, TSH, C-peptide α-tocopherol and TAG.

Substitution of dietary TAG with DAG for 12 weeks led to an improvement in 10-year cardiovascular risk score, through non-significant improvements in group mean values for a number of parameters including total body fat percentage, trunk fat mass, systolic blood pressure, total fat-free mass and serum activities of alanine aminotransferase, alkaline phosphatase and γ-glutamyl transferase. No adverse effects of consuming DAG rather than TAG were reported. The daily intake of DAG was 11.2 g per subject. The range in body weights in the DAG group was 80.2 to 102.9 kg, so the approximate daily intake of DAG ranged from 109 to 140 mg/kg bw/day.

3.4   Assessments by other agencies

JECFA

As described in section 3.1 , mono- and diglycerides as food additives were approved, within the category 'Emulsifiers and stabilizers' by JECFA in 1963 and 1973. An ADI of "not limited" was established at the 17th JECFA meeting in 1973 (JECFA, 1974a).

Codex

The Codex Alimentarius General Standard for Food Additives (GSFA) gives approval for mono- and diglycerides (INS 471) in 27 food categories, including use in foods for vulnerable subpopulations, in that approval exists for use in infant formulae, follow-on formulae and foods for special medical purposes. As an additive in GSFA Table 3, INS 471 is approved for use at GMP in 61 food categories. At its 42nd session, in July 2019, the Codex Alimentarius Commission adopted a recommendation from the Codex Committee on Food Additives to expand to use of INS 471 to include use as a glazing agent on surface-treated fruits and vegetables (Codex Alimentarius Commission, 2019).

European Union

In 2019, the European Union (EU) approved the use of mono- and diglycerides of fatty acids

at GMP for the surface treatment of citrus fruit, melons, pineapples, bananas, papayas, mangoes, avocados and pomegranates (EU 2019). This approval followed the assessment of mono- and diglycerides of fatty acids by EFSA in 2017. The EFSA Panel found no evidence of adverse effects and concluded that a numerical ADI was not required. However the EFSA Panel recommended that the European Commission consider reviewing a number of the specifications for mono- and diglycerides (which they call E 471) including arsenic, lead, mercury, cadmium, glycerol, residual solvents (specifically, tert-butanol and tert-pentanol), trans fatty acids, glycidyl esters, and erucic acid. The Panel also recommended that more data should be generated concerning 3-monochloropropane-1,2-diol (3-MCPD) and glycidyl esters because these can be produced under certain conditions form mono- and diglycerides (EFSA ANS Panel 2017). FSANZ notes that the European Commission has not revised the specifications for mono- and diglycerides and that the food additive that is the subject of the current application complies with the relevant JECFA, FCC and EU specifications.

United States of America

The US-FDA responded with a No Questions letter to a GRAS notification for the use of mono- and diglycerides as a surface-finishing agent on fresh fruits and vegetables (GRN 000648). However this does not constitute an assessment.

Other countries

Documents were provided as part of the application to show that Chile, China, Japan, Mexico and Peru have approved the use of mono- and diglycerides, either for all functional classes, or for functions including that of a surface treatment for fruits and vegetables (Chile 1997a,b; China National Health and Family Planning Commission 2018; Japan Ministry of Health, Labour and Welfare 2018; COFEPRIS (Mexico) 2018; DIGESA (Peru), 1998). However it is not clear whether the regulatory authorities in those countries conducted independent assessments, or based their approvals on the JECFA approval.

3.5   Potential allergen issues

The only potential for allergens to be present in the food additive comes from the possible use of oils and fats derived from allergen sources to produce the food additive. The applicant has confirmed that its version of the food additive is not produced from any oils or fats sourced from a major food allergen requiring mandatory declaration in section 1.2.3—4 of the Code. That is, it is not produced from a major food allergen such as soybean, peanut or sesame.

However, the specification for the food additive does not specify which oils or fats the food additive can be sourced from in its manufacture, nor which ones should not be used.

A permission for use of the food additive as an outcome of the application would permit the applicant's version of the food additive for which there are no allergenicity concerns, but it would also provide a generic permission where other sources of oils and fats could be used in the manufacture of the food additive. The source material used to produce the food additive can be, among others, coconut, palm, palm kernel, soya, rapeseed (Canola), sunflower, cottonseed, corn, olive, tallow and lard (EFSA 2017).

Of these potential sources, only soybean is a major food allergen. FSANZ concluded within Proposal P1031[3] (Allergen Labelling Exemptions) that soybean oil that has been fully refined

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[3] Proposal P1031 – Allergen Labelling Exemptions,
https://www.foodstandards.gov.au/code/proposals/Pages/P1031Allergenlabellingexemptions.aspx

i.e. degummed, neutralised, bleached and deodorised (N/RBD) so that potential allergen proteins have been removed presents negligible risk to soybean allergic consumers. Therefore, if fully refined soybean oil is used as the source to produce the food additive then no allergen safety concerns would apply.

Mono- and diglycerides of fatty acids have been used as a GMP food additive around the world for many years, and a literature internet search found no reports of food allergy arising from their consumption. The recent EFSA re-evaluation of the food additive published in 2017 noted that soya could be a source used in the production of mono- and diglycerides of fatty acids but did not raise any allergenicity concerns..

Based on the available evidence and taking into account the purification processes used during production, there are unlikely to be allergenicity concerns related to use of the food additive, including extending its use as a glazing agent for fresh fruits and vegetables.

4 Dietary Exposure Assessment

The objective of this dietary exposure assessment is to estimate the dietary exposure to mono- and diglycerides of fatty acids (INS 471) if applied as a glazing agent at the most conservative estimate of maximum use proposed by the applicant (152 g/100 kg produce) in the Food Class 4.1.2 (Surface treated fruits and vegetables).

Dietary exposure assessments (DEA) require data on the concentration of the chemical of interest in the food requested and consumption data for the foods that have been collected through a national nutrition survey. Consumption data used for this assessment were extracted using FSANZ's dietary computer modelling program Harvest[4].

A summary of the general FSANZ approach to conducting the dietary exposure assessment for this application is in Appendix 1. A detailed discussion of the FSANZ methodology and approach to conducting dietary exposure assessments is set out in *Principles and Practices of Dietary Exposure Assessment for Food Regulatory Purposes* (FSANZ 2009).

4.1 Dietary exposure assessment methodology

Dietary exposure assessments at FSANZ are conducted using a tiered approach. The hazard assessment concluded that mono-and diglycerides of fatty acids, including those in INS 471, do not differ significantly from dietary lipids and a numerical ADI is not required. Therefore, a deterministic DEA was conducted by FSANZ, with estimated exposure compared to total fat intake in order to estimate the potential proportional increase in fat intake from the application of mono- and diglycerides of fatty acids as a glazing agent on fruits and vegetables at the most conservative estimate of maximum use as proposed by the applicant.

Food Class 4 Fruits and Vegetables includes fungi, nuts, seeds, herbs and spices, therefore these foods were included in the dietary exposure assessment. Food classes for which mono- and diglycerides of fatty acids are currently permitted at GMP and naturally occurring sources were not included in the estimates of dietary exposures to mono- and glycerides of fatty acids. As food additive permissions in the Code apply to both Australia and New Zealand, dietary exposure assessments were undertaken for both countries.

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[4] Harvest is FSANZ's custom-built dietary modelling program that replaced the previous program, DIAMOND, which does the same calculations just using a different software program.

4.2 Food consumption data used

The food consumption data used for the dietary exposure assessments were:

- 2002 New Zealand National Children's Nutrition Survey (2002 NZ CNS) (Ministry of Health 2005)
- 2008-09 New Zealand Adult Nutrition Survey (2008 NZ ANS) (Ministry of Health 2011 a,b)
- 2011-12 Australian National Nutrition and Physical Activity Survey (2011-12 NNPAS) (ABS 2015).

The design of these nutrition surveys vary and the key attributes of each, including survey limitations, are set out in Appendix 1. Where significant uncertainties in the data exist, FSANZ uses conservative assumptions to ensure that the estimated dietary exposure is not an underestimate. Assumptions made in the dietary exposure assessment are also set out in Appendix 1.

The hazard assessment did not identify any target or at-risk groups for which there were specific safety considerations in relation to exposure to mono- and diglycerides of fatty acids. In addition, the food class requested in the application for the addition of mono- and diglycerides of fatty acids is consumed by all age groups of the Australian and New Zealand populations. Therefore the dietary exposure assessments were conducted for the general Australian and New Zealand populations based on day 1 of the dietary survey data available. For Australia, the population group used for the dietary exposure assessment was the population aged 2 years and above. For New Zealand the population groups were children (aged 5-14 years) and adults (aged 15 years and above).

4.3 Dietary Exposure Assessment Results

The mean consumption of food groups according to nutrition survey categorisation for Australia ('fruit products and dishes', 'vegetable products and dishes', 'seed and nut products and dishes') and New Zealand ('fruit', 'nuts and seeds', 'vegetables' (excluding potatoes, kumara and taro), 'potatoes, kumara and taro'), along with mean total fat intake by respondents to the surveys are detailed in Table 5 for Australia and Table 6 for New Zealand. Total estimated mean dietary exposures to mono- and diglycerides of fatty acids if applied to these foods range from 468 mg/respondent/day to 633 mg/respondent/day for the population groups assessed. This represents 0.6-0.8% of mean total fat intake for respondents in the surveys which is within normal daily variation.

Table 5 *Estimated dietary exposures to mono- and diglycerides of fatty acids from use as a glazing agent on fruits and vegetables for Australian respondents and percentage of mean total fat intake*

Country	Age Group	No. of resp.	Survey Food Group¥ Mean consumption (g/respondent/day)			Estimated mean dietary exposure to mono- and diglycerides of fatty acids (mg/respondent/day)**	Mean total fat intake (g/respondent/day)▽▽	Mean dietary exposure to mono- and diglycerides of fatty acids as a percentage of mean total fat intake (%)
			Fruit products and dishes	Seed and nut products and dishes	Vegetable products and dishes			
Australia*	2 years and above	12,153	146	6	156	468	72.8	0.6

* 2011-12 Australian National Nutrition and Physical Activity Survey. Based on day 1 consumption data only from all respondents.
¥ Consumption of herbs (dried) and spices not included as mean consumption <0.1 g/respondent/day.
** Based on mono- and diglycerides of fatty acids concentration of 152 g/100 kg produce.
▽▽ Based on day 1 consumption data only from all respondents. Data extracted from Harvest[4].

Table 6 *Estimated dietary exposures to mono- and diglycerides of fatty acids from use as a glazing agent on fruits and vegetables for New Zealand respondents and percentage of mean total fat intake*

Country	Age Group	No. of resp.	Survey Food Group¥¥ Mean consumption (g/respondent/day)				Estimated mean dietary exposure to mono- and diglycerides of fatty acids (mg/respondent/day)**	Mean total fat intake (g/respondent/day)▽▽	Mean dietary exposure to mono- and diglycerides of fatty acids as a percentage of mean total fat intake (%)
			Fruit	Nuts and seeds	Vegetables (excluding potatoes, kumara and taro)	Potatoes, kumara and taro			
New Zealand▽	5-14 years	3275	162	6	74	100	520	76.9	0.7
	15 years and above	4721	151	5	155	105	633	82.9	0.8

▽ 2002 New Zealand Children's Nutrition Survey and the 2008–09 New Zealand Adult Nutrition Survey. Based on day 1 consumption data only from all respondents.
¥¥ Herbs and spices were included in the Vegetables survey food group in the 2008-09 New Zealand Adult Nutrition Survey. Consumption of dried herbs and spices not expressly reported in the 2002 New Zealand National Children's Nutrition Survey.
*** Based on mono- and diglycerides of fatty acids concentration of 152 g/100 kg produce.
▽▽ Based on day 1 consumption data only from all respondents. Data extracted from Harvest[4].

5   Discussion

This application is for an extension of use of INS 471, already approved for a number of purposes as an emulsifier/stabilizer in the Code, as a surface treatment for fresh fruits and vegetables. JECFA (1974 a,b) concluded that mono- and diglycerides used as emulsifiers and stabilizers are not significantly different to dietary lipids and undergo the same metabolic processes. The Committee concluded that a numerical ADI was not required.

No new information was submitted or located by literature search that contradicts the conclusions of JECFA (1974 a, b) with regard to kinetics, metabolism, or toxicity. In the main, the reviewed studies were for dietary diacylglycerol (diglyceride) oil but results are applicable to INS 471. Studies specific to monoglycerides were not considered necessary because the mammalian body uses the same metabolic pathways to break down monoglycerides, diglycerides and triglycerides.

In EFSA's dietary exposure assessment, multiple scenarios were used to estimate chronic dietary exposure to mono- and diglycerides of fatty acids from foods in which the additive is permitted (EFSA ANS Panel 2017). The scenarios were: regulatory maximum level exposure assessment scenario; refined estimated exposure, i.e. brand-loyal consumer and non-brand-loyal consumer scenarios (using only industry reported use level data); and specific exposure scenarios (consumers of food supplements or foods for special medical purposes). When compared to recommended daily fat intake (20-35% total energy), estimated mean dietary exposure to the additive for adult populations using the non-brand-loyal exposure assessment scenario represented 0.8-3.5% of this recommended intake (EFSA ANS Panel 2017). Using a conservative deterministic approach, estimated mean dietary exposure for Australian and New Zealand populations to mono- and diglycerides of fatty acids from use as a glazing agent on surface treated fruits and vegetables represents 0.6-0.8% of mean total fat intake, which is within normal daily variation.

The assessment also considered the potential for allergenicity due to the possible use of oils and fats derived from allergenic sources to produce the food additive. Based on the available evidence and taking into account the purification processes used during production, there are unlikely to be allergenicity concerns related to use of the food additive, including extending its use as a glazing agent for fresh fruits and vegetables.

6   Conclusions

FSANZ concurs with the conclusion of JECFA (1974 a,b) that mono-and diglycerides, including those in INS 471, do not differ significantly from dietary lipids and a numerical ADI is not required. Estimated mean dietary exposure for Australian and New Zealand populations to mono- and diglycerides of fatty acids if used as a glazing agent on surface treated fruits and vegetables represents 0.6-0.8% of mean total fat intake, which is within normal daily variation.

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Appendix 1

A1.1   Dietary exposure assessments at FSANZ

A dietary exposure assessment is the process of estimating how much of a food chemical a population, or population sub group, consumes. Dietary exposure to food chemicals is estimated by combining food consumption data with food chemical concentration data. The process of doing this is called 'dietary modelling'.

Dietary exposure = food chemical concentration x food consumption

FSANZ's approach to dietary modelling is based on internationally accepted procedures for estimating dietary exposure to food chemicals. Different dietary modelling approaches may be used depending on the assessment, the type of food chemical, the data available and the risk assessment questions to be answered. In the majority of assessments, FSANZ uses the food consumption data from each person in the national nutrition surveys to estimate their individual dietary exposure. Population summary statistics such as the mean exposure or a high percentile exposure are derived from the ranked individual person's exposures from the nutrition survey. Summary consumption data are also derived from each respondents individual consumption amounts.

An overview of how dietary exposure assessments are conducted and their place in the FSANZ Risk Analysis Process is provided on the FSANZ website.

FSANZ has developed a custom-built computer program 'Harvest' to calculate dietary exposures. Harvest replaces the program 'DIAMOND' that had been used by FSANZ for many years. Harvest has been designed to replicate the calculations that occurred within DIAMOND using a different software package.

Further detailed information on conducting dietary exposure assessments at FSANZ is provided in *Principles and Practices of Dietary Exposure Assessment for Food Regulatory Purposes* (FSANZ 2009).

A1.2   Food consumption data used

The most recent food consumption data available were used to estimate dietary exposures to mono- and diglycerides of fatty acids for the Australian and New Zealand populations. The national nutrition survey (NNS) data used for these assessments were:
- The 2011-12 Australian National Nutrition and Physical Activity Survey (2011-12 NNPAS)
- The 2002 New Zealand National Children's Nutrition Survey (2002 NZ CNS)
- The 2008-09 New Zealand Adult Nutrition Survey (2008 NZ ANS)

The design of each of these surveys varies somewhat and key attributes of each are set out below. Further information on the National Nutrition Surveys used to conduct dietary exposure assessments is available on the FSANZ website.

A1.2.1   2011–12 Australian National Nutrition and Physical Activity Survey (2011-12 NNPAS)

The 2011–12 Australian National Nutrition and Physical Activity Survey (NNPAS) undertaken by the Australian Bureau of Statistics is the most recent food consumption data for Australia. This survey includes dietary patterns of a sample of 12,153 Australians aged 2 years and above. The survey used a 24-hour recall method for all respondents, with 64% of respondents also completing a second 24-hour recall on a second, non-consecutive day. The

collection dates of the data were May 2011 to June 2012 (with no enumeration between August and September 2011 due to the Census). Only the Day 1 24-hour recall data for all respondents (excluding supplements) were used for this assessment. Consumption and respondent data from the *Confidentialised Unit Record File*s (CURF) data set (ABS 2015) form part of the Harvest core data set. These data were used weighted in Harvest.

A1.2.2   2002 New Zealand National Children's Nutrition Survey (2002 NZ CNS)

The 2002 NZ CNS was a cross-sectional and nationally representative survey of 3,275 New Zealand children aged 5-14 years. The collection period for the data was during the school year from February to December 2002. The survey used a 24-hour food recall and provided information on food and nutrient intakes, eating patterns, frequently eaten foods, physical activity patterns, dental health, anthropometric measures and nutrition-related clinical measures. It was also the first children's nutrition survey in New Zealand to include a second day diet recall data for about 15% of the respondents, and dietary intake from both foods (including beverages) and dietary supplements (Ministry of Health 2005). Only the Day 1 24-hour recall data for all respondents (excluding supplements) were used for this assessment. These data are used weighted in Harvest.

A1.2.3   2008-09 New Zealand Adult Nutrition Survey (2008 NZ ANS)

The 2008 NZ ANS provides comprehensive information on the dietary patterns of a sample of 4,721 respondents aged 15 years and above. Collection of data for the survey occurred on a stratified sample over a 12-month period between October 2008-October 2009. The survey used a 24-hour recall methodology with 25% of respondents also completing a second 24-hour recall. The information collected in the 2008 NZ ANS included food and nutrient intakes, dietary supplement use, socio-demographics, nutrition related health, and anthropometric measures (Ministry of Health 2011 a,b). Only the Day 1 24-hour recall data for all respondents (excluding supplements) were used for this assessment. These data are used weighted in Harvest.

A1.3   Assumptions and limitations of the dietary exposure assessment

Assumptions made in the dietary exposure assessment include:
- All foods included in the Australian survey food groups (fruit products and dishes, vegetable products and dishes, seed and nut products and dishes) and New Zealand survey food groups (fruit, nuts and seeds, vegetables, potatoes, kumara and taro), are glazed with mono- and diglycerides of fatty acids, including foods that are peeled before consumption.
- All foods are glazed with mono- and diglycerides of fatty acids at the most conservative proposed maximum use level as indicated by the applicant of 152 g/100 kg produce.

Dietary exposure assessments based on 2011-12 NNPAS, 2002 NZ CNS and 2008 NZ ANS food consumption data provide the best estimation of actual consumption of a food and the resulting estimated dietary exposure assessment for the Australian population aged 2 years and above, as well as the New Zealand populations aged 5–14 years and 15 years and above, respectively. However, it should be noted that NNS data do have limitations. Further details of the limitations relating to dietary exposure assessments undertaken by FSANZ are set out in the FSANZ document, *Principles and Practices of Dietary Exposure Assessment for Food Regulatory Purposes* (FSANZ 2009).