2-04
17 March 2004

DRAFT ASSESSMENT REPORT

APPLICATION A505

DIACYLGLYCEROL OIL

DEADLINE FOR PUBLIC SUBMISSIONS to FSANZ in relation to this matter:
28 April 2004
(See 'Invitation for Public Submissions' for details)

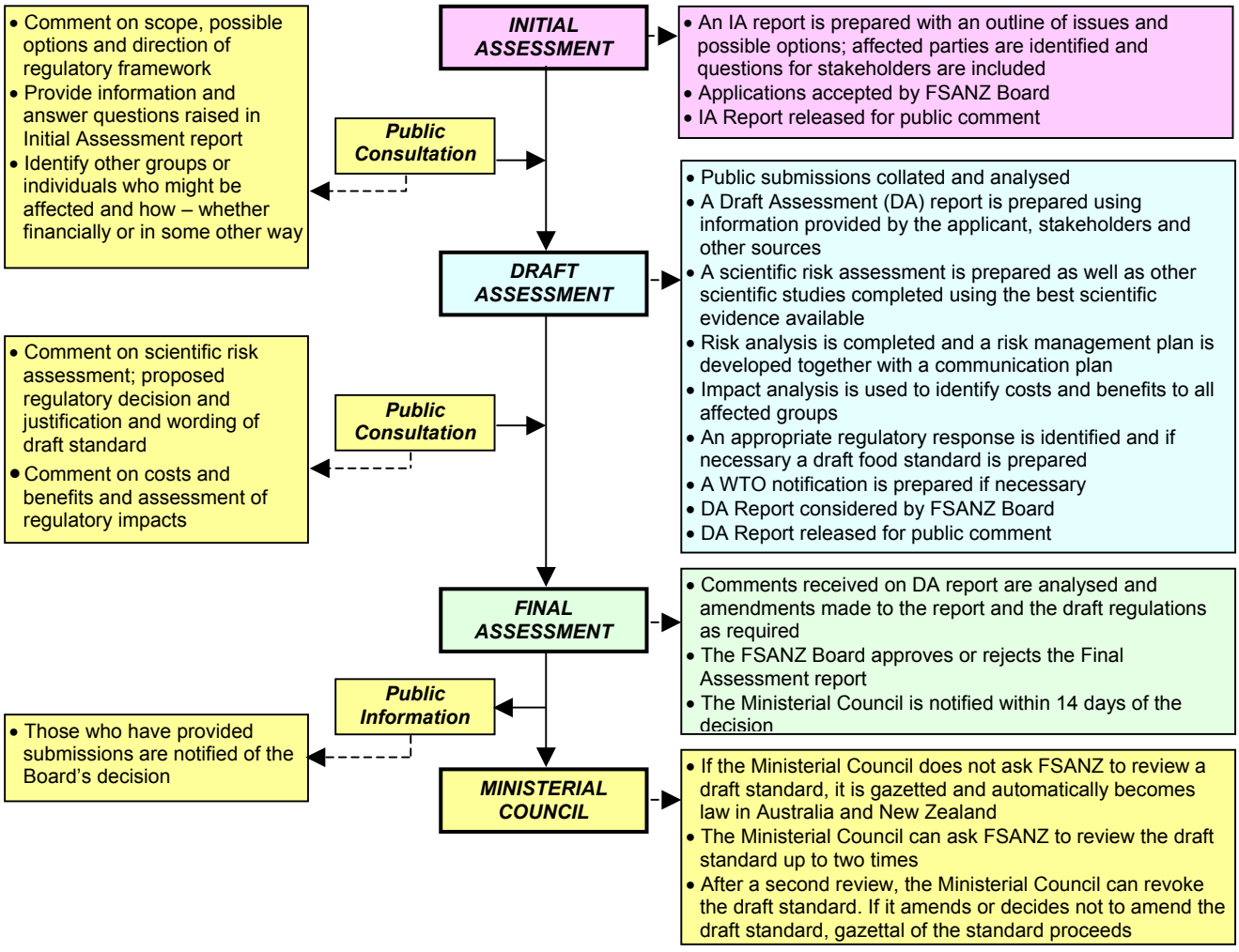
FOOD STANDARDS AUSTRALIA NEW ZEALAND (FSANZ)

FSANZ's role is to protect the health and safety of people in Australia and New Zealand through the maintenance of a safe food supply. FSANZ is a partnership between ten Governments: the Commonwealth; Australian States and Territories; and New Zealand. It is a statutory authority under Commonwealth law and is an independent, expert body.

FSANZ is responsible for developing, varying and reviewing standards and for developing codes of conduct with industry for food available in Australia and New Zealand covering labelling, composition and contaminants. In Australia, FSANZ also develops food standards for food safety, maximum residue limits, primary production and processing and a range of other functions including the coordination of national food surveillance and recall systems, conducting research and assessing policies about imported food.

The FSANZ Board approves new standards or variations to food standards in accordance with policy guidelines set by the Australia and New Zealand Food Regulation Ministerial Council (Ministerial Council) made up of Commonwealth, State and Territory and New Zealand Health Ministers as lead Ministers, with representation from other portfolios. Approved standards are then notified to the Ministerial Council. The Ministerial Council may then request that FSANZ review a proposed or existing standard. If the Ministerial Council does not request that FSANZ review the draft standard, or amends a draft standard, the standard is adopted by reference under the food laws of the Commonwealth, States, Territories and New Zealand. The Ministerial Council can, independently of a notification from FSANZ, request that FSANZ review a standard.

The process for amending the *Australia New Zealand Food Standards Code* is prescribed in the *Food Standards Australia New Zealand Act 1991* (FSANZ Act). The diagram below represents the different stages in the process including when periods of public consultation occur. This process varies for matters that are urgent or minor in significance or complexity.



INVITATION FOR PUBLIC SUBMISSIONS

FSANZ has prepared a Draft Assessment Report of Application A505; and prepared draft variations to the *Australia New Zealand Food Standards Code* (the Code).

FSANZ invites public comment on this Draft Assessment Report based on regulation impact principles and the draft variation to the Code for the purpose of preparing an amendment to the Code for approval by the FSANZ Board.

Written submissions are invited from interested individuals and organisations to assist FSANZ in preparing the Final Assessment for this Application. Submissions should, where possible, address the objectives of FSANZ as set out in section 10 of the FSANZ Act. Information providing details of potential costs and benefits of the proposed change to the Code from stakeholders is highly desirable. Claims made in submissions should be supported wherever possible by referencing or including relevant studies, research findings, trials, surveys etc. Technical information should be in sufficient detail to allow independent scientific assessment.

The processes of FSANZ are open to public scrutiny, and any submissions received will ordinarily be placed on the public register of FSANZ and made available for inspection. If you wish any information contained in a submission to remain confidential to FSANZ, you should clearly identify the sensitive information and provide justification for treating it as commercial-in-confidence. Section 39 of the FSANZ Act requires FSANZ to treat in-confidence, trade secrets relating to food and any other information relating to food, the commercial value of which would be, or could reasonably be expected to be, destroyed or diminished by disclosure.

Submissions must be made in writing and should clearly be marked with the word 'Submission' and quote the correct project number and name. Submissions may be sent to one of the following addresses:

Food Standards Australia New Zealand
PO Box 7186
Canberra BC ACT 2610
AUSTRALIA
Tel (02) 6271 2222
www.foodstandards.gov.au

Food Standards Australia New Zealand
PO Box 10559
The Terrace WELLINGTON 6036
NEW ZEALAND
Tel (04) 473 9942
www.foodstandards.govt.nz

Submissions should be received by FSANZ **by 28 April 2004**.

Submissions received after this date may not be considered, unless the Project Manager has given prior agreement for an extension.

While FSANZ accepts submissions in hard copy to our offices, it is more convenient and quicker to receive submissions electronically through the FSANZ website using the Standards Development tab and then through Documents for Public Comment. Questions relating to making submissions or the application process can be directed to the Standards Management Officer at the above address or by emailing slo@foodstandards.gov.au.

Assessment reports are available for viewing and downloading from the FSANZ website. Alternatively, requests for paper copies of reports or other general inquiries can be directed to FSANZ's Information Officer at either of the above addresses or by emailing info@foodstandards.gov.au.

Further Information

Further information on this Application and the assessment process should be addressed to the FSANZ Standards Liaison Officer at one of the following addresses:

Food Standards Australia New Zealand
PO Box 7186
Canberra BC ACT 2610
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Executive Summary and Statement of Reasons

FSANZ received a cost-recovered application on 10 June 2003 from ADM Kao LLC, a joint venture business between the Archer Daniels Midland Company and the Koa Corporation to approve the use of diacylglycerol oil (DAG-oil) as a novel food under Standard 1.5.1 - Novel Foods of the *Australia New Zealand Food Standards Code* (the Code). DAG-oil mainly consists of diglycerides.

Regulatory Problem

Novel foods may not be sold as food or for use as food ingredients unless listed in the Table to clause 2 of Standard 1.5.1. DAG-oil is considered a non-traditional food because it has no history of significant human consumption in Australia or New Zealand. Diglycerides are currently approved as food additives and are a by-product in edible oils, but their safety (e.g. when DAG-oil is >80% of the edible oils) has not yet been determined within the context of the Australian and New Zealand diet. In these circumstances, DAG-oil is considered to be a novel food and should be considered under Standard 1.5.1.

The current definition of edible oils under Standard 2.4.1 refers only to triglycerides. Therefore, according to the current definition DAG-oil is not an edible oil. Thus, the Application relates also to a matter that warrants a variation to Standard 2.4.1.

Objective

The objective of this Application is to establish if the Code should be changed to allow the use of DAG-oil as a novel food ingredient in various foods. Before DAG-oil can enter the food supply in Australia and New Zealand, FSANZ must undertake an assessment of the potential risk to public health and safety of dietary exposure to DAG-oil.

Relevant issues

A range of issues was considered during the assessment of the Application.
Safety studies in animals and humans did not indicate any specific safety concerns with DAG-oil. Based on the safety assessment it is concluded that it is not necessary to set an upper limit for the use of DAG-oil.

The nutrition assessment concluded that although DAG-oil has no demonstrated nutritional benefits, there is also no evidence to indicate an adverse impact on population nutrition Thus DAG-oil can be considered nutritionally adequate for general consumption.

DAG-oil may be used as a food ingredient and has similar uses to triacylglycerol oils. It may be used in foods including spreads, salad dressings, mayonnaise, bakery products, fried foods, beverages, soups, sauces, and gravies.

As the risk to public health and safety is determined to be low it would be sufficient to rely on general labelling provisions to manage any minimal safety or nutritional risk associated with the consumption of DAG-oil.

Regulatory Options

The only regulatory options identified were to approve or not approve the use of DAG-oil as a novel food. The regulatory impact analysis shows that benefits accrue to industry, in terms of enhanced market opportunities and trade, and to consumers through, potentially, a greater choice of foods, if the use of DAG-oil was approved.

Consultation

Nine submissions were received in response to the Initial Assessment Report. Seven submissions supported the Application, one did not support it and one was non-committal until FSANZ had performed a risk analysis. Two submissions raised the issue that DAG-oil should not be considered as a novel food, since it is not considered non-traditional. Further issues raised in the submissions relate to safety, nutrition and efficacy.

FSANZ is now seeking public comment in order to assist in assessing this Application at Final Assessment. Comments that would be useful could cover:

- scientific aspects of this Application;
- other issues including labelling of foods and food products containing DAG-oil.

Draft Statement of Reasons

At Draft Assessment, FSANZ recommends the approval of the use of DAG-oil as a novel food for the following reasons.

- There are no public health and safety concerns associated with the use of DAG-oil as proposed;
- from a nutritional perspective, DAG-oil is considered nutritionally adequate for general consumption;
- DAG-oil has similar uses to triacylglycerol-based oils;
- appropriate labelling requirements have been proposed;
- the proposed changes to the Code are consistent with the section 10 objectives of the FSANZ Act;
- the submissions in relation to this Application have been addressed;
- there are no more cost-effective alternatives available other than changing Standard 1.5.1; and
- the Regulatory Impact Statement indicates that, for the preferred option, namely, to approve the use of DAG-oil as a novel food, the benefits of the proposed amendment outweigh the costs.

1. Introduction

An Application was received from ADM Kao LLC, a joint venture business between the Archer Daniels Midland Company and the Kao Corporation, on 10 June 2003 seeking approval for the use of diacylglycerol oil (DAG-oil), marketed as ENOVA^{tm} Oil, derived from vegetable oils as a novel food ingredient in various food applications under Standard 1.5.1 – Novel Food of the Code. Work commenced on this Application on 10 June 2003.

2. Regulatory Problem

Under the current food standards, novel foods are required to undergo pre-market assessment, as per Standard 1.5.1 – Novel Foods. The purpose of Standard 1.5.1 is to ensure that non-traditional foods that have features or characteristics that may raise safety concerns will undergo a risk-based safety assessment before they are offered for retail sale in Australia or New Zealand.

A Novel Food is defined in Standard 1.5.1 – Novel Foods as:

> *A non-traditional food for which there is insufficient knowledge in the broad community to enable safe use in the form or context in which it is presented, taking into account –*
>
> *a) the composition or structure of the product; or*
> *b) levels of undesirable substances in the product; or*
> *c) known potential for adverse effects on humans; or*
> *d) traditional preparation and cooking methods; or*
> *e) patterns and levels of consumption of the product.*
>
> *Non-traditional food means a food which does not have a history of significant human consumption by the broad community in Australia or New Zealand.*

It is proposed to use DAG-oil as a novel food ingredient in various foods. It is considered a non-traditional food because it has no history of significant human consumption in Australia or New Zealand. Diglycerides are currently approved as food additives and are a by-product in the manufacture of edible oils, but their safety when used in foods at levels which would result in high dietary exposure (i.e., when DAG-oil is >80% of the edible oil) has not yet been determined within the context of the Australian and New Zealand diet. In these circumstances, DAG-oil is considered to be a novel food and should be considered under Standard 1.5.1.

Therefore, the Application relates to a matter that warrants a variation to Standard 1.5.1.

In Standard 2.4.1 – Edible Oils, the definition of edible oil is:

> *Edible oil means the triglycerides of fatty acids of plant or animal origin.*
>
> *Edible oils may contain incidental amounts of free fatty acids, unsaponifiable constituents and other lipids.*

DAG-oil mainly consists of diglycerides and therefore is, according to the current definition not an edible oil. Therefore, the Application relates also to a matter that warrants a variation to Standard 2.4.1.

3. Objective

The objective of this Application is to establish if it is appropriate to change the Code to allow the use of DAG-oil as a novel food ingredient in various foods. Before DAG-oil can enter the food supply in Australia and New Zealand, FSANZ must undertake a safety assessment that considers the potential health impact of dietary exposure to DAG-oil on consumers. For approval, an amendment to the Code must be agreed by the FSANZ Board, and subsequently be notified to the Australia and New Zealand Food Regulation Ministerial Council (Ministerial Council). An amendment to the Code may only be gazetted once the Ministerial Council process has been finalised.

In assessing the Application to vary Standard 1.5.1 to approve the use of DAG-oil as novel food, FSANZ is required by its legislation to meet three primary objectives which are set out in section 10 of the FSANZ Act. These are:

- the protection of public health and safety;
- the provision of adequate information relating to food to enable consumers to make informed choices; and
- the prevention of misleading or deceptive conduct.

In developing and varying standards, FSANZ must also have regard to:

- the need for standards to be based on risk analysis using the best available scientific evidence;
- the promotion of consistency between domestic and international food standards;
- the desirability of an efficient and internationally competitive food industry;
- the promotion of fair trading in food; and
- any written policy guidelines formulated by the Ministerial Council.

4. Background

4.1 Properties of DAG-oil

DAG-oil is manufactured from natural edible plant oils such as soybean, canola (rapeseed) or corn oil and is composed largely of randomised diacylglycerols (DG). DAG-oil contains approximately 80% DG, 20% triacylglycerol, 5% monoacylglycerol and <0.2% emulsifiers (polyglycerol esters of fatty acids) and antioxidants (ascorbyl palmitate and tocopherol). The main constituent fatty acids of DAG-oil are oleic (C18:1), linoleic (C18:2), and linolenic (C18:3) acids, present as 1,3- and 1,2-diacylglycerols at a ratio of 7:3, respectively. The constituents of DAG-oil are already present in the Australian and New Zealand diets as components of conventional dietary oils, as approved food additives i.e. mono-and diglycerides, and occur as metabolites of normal lipid metabolism following the consumption of dietary fat.

4.2 Other materials used in the manufacture of DAG-oil

The enzyme lipase sourced from *Rhizomucor miehei*, which is produced in an *Aspergillus oryzae* host, is an approved processing aid in Standard 1.3.3 – Processing Aids. Furthermore, citric acid is an approved food additive in Standard 1.3.1. The ion exchange resin used as an absorbent for the enzyme is polymerised phenol-formaldehyde ion exchange resin functionalised with triethylenetetramine. This resin is listed in the Table to clause 8 in Standard 1.3.3. Residues of the resin are removed during the refining process.

4.3 Proposed uses

The substance is proposed to be used in:

- edible oil (100%),
- salad dressings (23-28%),
- mayonnaise (26-30%),
- viscous dressings 23-28%),
- fat spreads / margarine (25-50%),
- baked products (bread, cakes, crackers and cookies, croissants, pastries; 0.5-1%),
- pizza (3-5%),
- health bars (1-3%), and
- health drinks (1-3%).

4.4 Approvals in other countries

In several countries DAG-oil is permitted in various foods. It is also under consideration in other countries. The relevant regulations are:

- In Japan, DAG-oil was approved for food use by the Ministry of Health and Welfare on May 20, 1998. It is used as cooking oil, and as an ingredient in margarine, dressings for salads, canned tuna, curry roux and some baked goods.

- In the United States, an independent Expert Panel determined DAG-oil to be GRAS in spreads and cooking oils (2000). The U.S. FDA subsequently granted a GRAS amendment in 2003 to expand the original uses of DAG-oil to include baked goods, pizza, mayonnaise, salad dressings, health bars (breakfast, snack and power bars), meal replacements, frozen entrées, and soups, soup mixes and gravies.

- The EU considers DAG-oil as a novel food / food ingredient, because it meets the definition of a novel food pursuant to the EU Novel Food regulation. The Application has been reviewed for an initial assessment by the Committee on Safety Assessment of Novel Foods of the Ministry of Health, Welfare and Sports of the Netherlands in December 2002. The Committee has set the safe level of exposure at 140 gram per 70 kilograms of body weight per day. As the next step in the EU regulatory process this initial assessment is now under review by the EC and the EU member states, including the UK Advisory Committee on Novel Foods and Processes for final regulatory approval.

- An application for the approval of DAG-oil as a novel food / food ingredient has been submitted to the Health Canada regulatory agency for pre-market notification to permit DAG-oil for use as cooking oil and as an ingredient in baked goods, pizza, fats and oils, health bars, meal replacements, frozen entrees, and soups, soup mixes and gravies. Health Canada is currently assessing the application.

5. Relevant Issues

5.1 Safety Considerations

A detailed report on the safety issues associated with DAG-oil is provided at **Attachment 2.**

Diglycerides are currently approved as food additives and are a by-product in manufacture of edible oils, however the safety when used in foods at levels which would result in high dietary exposure (i.e., when DAG-oil is >80% of the edible oil) needed to be determined within the context of the Australian and New Zealand diet prior to approval of its use as a food ingredient in Australia and New Zealand.

An acute study in rats, a 4-week short-term study in rats, a long-term study in rats, a long-term study in dogs, and *in vitro* mutagenicity studies on diglycerides were available for the assessment of safety of DAG-oil. Furthermore, in a series of human studies the physiological effects of DAG-oil were examined in normal subjects and some patient groups. These studies were assessed in detail. Most studies were not considered relevant for the safety assessment of DAG-oil because of improper study design and / or measurement of relevant parameters was not performed. However a few studies were considered relevant for the safety assessment, these included one acute study in healthy males, a 8-week study in healthy female subjects, a 12-week study in healthy human subjects and a 24-week study in obese human subjects.

The metabolism of DAG-oil is comparable to that of partial glycerides (monoglycerides) and triglycerides. Depending upon their composition, and the overall diet composition, they are partially or completely hydrolysed in the intestinal lumen by lipases and the resulting products are absorbed for re-esterification to triglycerides and / or oxidised as a source of energy, to varying degrees.

The safety studies in animals and humans did not indicate any specific safety concerns with DAG-oil. There is no specific data available on allergenic potential or on reproductive toxicity, however the available studies do not suggest that DAG-oil has allergenic potential or has any effects on reproduction and development. DAG-oil has no mutagenic or carcinogenic potential. No specific adverse effects were observed in human studies. Other studies, conducted in sub-population groups largely to examine efficacy, did also not indicate any adverse health effects. These studies were conducted in individuals on haemodialysis, in people with type II diabetes and in obese and hyperlipidemic children.

One animals study has indicated an increase in serum fatty acids in the portal vein following DAG-oil consumption. This increased level of portal vein free fatty acids (FFA) after DAG-oil consumption, could result in increased insulin resistance. Animal and human studies did not reveal an increase in serum FFA and the chronic study in rats did not indicate any long-term effects on the lipid profile, therefore there is no indication that DAG-oil would result in an increased risk for insulin resistance.

In conclusion, it is not necessary to set an upper limit for the use of DAG-oil.

5.2 Nutritional Implications

A nutrition assessment has been undertaken to determine whether substituting DAG-oil for triacylglycerol (TAG)-based oil will significantly alter the health and nutritional status of Australian and New Zealand populations. A detailed version of the nutrition assessment is provided at **Attachment 3,** with the outcomes summarised below.

The findings of the nutrition assessment demonstrate that DAG-oil does not have any nutritional attributes that are substantially different from TAG-based oils. There is evidence of an increased level of β-oxidation associated with DAG-oil intake; however, the body's homeostatic processes appear to accommodate this change, resulting in the same level of energy expenditure, fat storage and fat excretion from the intake of DAG-oil as occurs with the intake of TAG-based oils.

The nutrition assessment also identifies a decreased serum TAG levels over time with DAG-oil consumption. However, the influence of DAG-oil on other risk factors linked to the development of chronic diseases appears to be limited, and at best, only marginally greater than the influence provided by TAG-based oils of similar fatty acid composition.

It is concluded that although DAG-oil has no demonstrated nutritional benefits, there is also no evidence to indicate an adverse impact on population nutrition, thus DAG-oil can be considered nutritionally adequate for general consumption.

5.3 Dietary Exposure

A detailed report on the dietary exposure assessment of DAG-oil is provided at **Attachment 4.**

DAG-oil is proposed for use in cooking oil, salad dressings, mayonnaise, viscous dressings, fat spreads/margarines, baked products (including bread, biscuits, cakes, crackers and cookies, croissants, pastries, pizza), health bars and health drinks (meal replacements). A dietary exposure assessment was undertaken to determine the potential dietary impact of allowing DAG-oil to be added to the above foods.

The Applicant proposes to use DAG-oil as a 1:1 (w/w) replacement for conventional triglyceride (TG) in edible vegetable cooking oil. A dietary exposure assessment was conducted for the general Australian and New Zealand populations (2+ and 15+ years respectively), and for the population considered at potential risk from higher exposures; children (2-12 years, Australia only). Food consumption data were derived from the 1995 Australian National Nutrition Survey (NNS) and the 1997 New Zealand NNS. DAG-oil concentration data were derived from levels proposed in the application.

Estimated mean and 95th percentile dietary exposures for consumers of DAG-oil in the Australian population (2+ years) from all proposed foods were 0.4 and 1.3 grams per kilogram of body weight per day (g/kg BW/day) respectively. Estimated mean and 95th percentile dietary exposures for consumers of DAG-oil in the New Zealand population (15+ years) from all proposed foods were 0.3 and 1.0 g/kg BW/day respectively.

Australian children (2-12 years) had estimated dietary exposures of 0.7 g/kg BW/day (mean) and 2.4 g/kg BW/day (95th percentile). The highest percentage contribution to dietary exposure was from oil and oil emulsions for all age groups.

Estimated mean and 95th percentile dietary exposures for consumers of DAG-oil in the Australian population (2+ years) from oil and oil emulsions only were 0.4 and 1.3 g/kg BW/day respectively. Estimated mean and 95th percentile dietary exposures for consumers of DAG-oil in the New Zealand population (15+ years) from oil and oil emulsions only were 0.2 and 0.7 g/kg BW/day respectively. Australian children (2-12 years) had estimated dietary exposures of 0.7 g/kg BW/day (mean) and 2.3 g/kg BW/day (95th percentile) from oil and oil emulsions only.

It is recognised that the estimated exposures to DAG-oil as an ingredient in all foods compared to DAG-oil being an ingredient in just oil and oil emulsions are similar. This is due to both methodological reasons and DAG-oil concentrations in the foods.

Estimated dietary exposures are usually compared to a reference health standard in order to determine the potential risk to health of the population or its subgroups. However, the metabolism of DAG-oil is comparable to that of partial glycerides (monoglycerides) and triglycerides. Furthermore, no adverse effects were observed in both animal and human studies that would indicate adverse health effects if DAG-oil were to be allowed in Australia and New Zealand. Therefore, the estimated exposures reported above are simply reported in grams per kilogram body weight per day.

5.4 Food Technology Assessment

A detailed report on the technical issues associated with DAG-oil is provided at **Attachment 5.**

DAG-oil may be used as a food ingredient and has similar uses to triacylglycerol oils. It may be used in foods including spreads, salad dressings, mayonnaise, bakery products, fried foods, beverages, soups, sauces, and gravies.

5.5 Labelling

Labelling is one of a number of risk management tools used by FSANZ. FSANZ uses labelling in the development of food standards if there is –

- a demonstrated risk to public health and safety; and/or
- a need to ensure the adequacy of information for informed choice; and/or
- the potential for misleading and deceptive conduct.

The safety (Attachment 2) and nutritional (Attachment 3) assessments for DAG-oil indicate that there are no specific safety concerns or nutritional implications associated with the consumption of DAG-oil. Therefore, as the risk to public health and safety is determined to be low it would be sufficient to rely on general labelling provisions to manage any minimal safety or nutritional risk associated with the consumption of DAG-oil.

5.5.1 The Adequacy of Information for Informed Choice and Misleading and Deceptive Conduct

The primary role of a food label is to provide information regarding the identity, nature and composition of a food. Consumers use labelling information to:

- identify individual food products; and
- make comparisons between products.

Consumer information needs in relation to the identity and composition of a food, are likely to increase when, for example:

- a food, treatment or process to which the food has been exposed, has little or no (established long) history of safe use; or
- a food has been the subject of a public concern.

DAG-oil is considered to be a non-traditional food because it has no history of significant human consumption in Australia and New Zealand. Diglycerides are currently approved as food additives and are a by-product of edible oils but this is the first time it has been presented as an edible oil in Australia and New Zealand. Therefore, it can be assumed that consumers in Australia or New Zealand will have little understanding and knowledge about the product.

To ensure consumers are able to make an informed choice about DAG-oil it is appropriate that the specific format in which the information is presented should be prescribed to achieve consistent and uniform disclosure by manufacturers to prevent misleading and deceptive conduct. Uniform disclosure is necessary to enhance consumer confidence in locating and using the information when making a purchasing decision.

5.5.2 Recommendation

It is recommended that in order to assist consumers in making an informed choice about DAG-oil and to prevent misleading and deceptive conduct that:

- the prescribed name of 'Diacylglycerol oil' be included on the label so that consumers can easily identify DAG-oil.
- the prescribed name 'Diacylglycerol oil' be prescribed in the ingredient list so that consumers are clear that the oil present is different to other generic oils permitted in the Code; and
- the use of the generic name for fats and oils in the ingredient list cannot be used for DAG-oil.

6. Regulatory Options

FSANZ is required to consider the impact of various regulatory (and non-regulatory) options on all sectors of the community, which includes consumers, food industries and governments in Australia and New Zealand. The benefits and costs associated with the proposed amendment to the Code will be analysed using regulatory impact principles.

There are no options other than a variation to the Code available to permit a novel food to be sold as food.

Therefore, the following two regulatory options are available for this Application:

Option 1. Maintain the status quo and not approve the use of DAG-oil as a novel food.

Option 2. Amend the Code and approve the use of DAG-oil as a novel food

7. Impact Analysis

Parties possibly affected by the options outlined include:

- the edible oil industry;
- those sectors of the food industry wishing to produce and market food products produced using DAG-oil;
- consumers; and
- government agencies enforcing the food regulations.

The draft regulatory options are as follows:

7.1 Option 1. Not permit the use of DAG-oil as a novel food

On the basis of this Draft Assessment, there are no perceived benefits to government, consumers or industry by maintaining the *status quo* and not giving specific permission in the Code for the use of this ingredient.

On the basis of this Draft Assessment, there is no perceived cost for the government, however lack of approval in Australia or New Zealand may be construed as a non-tariff barrier to trade unless it is based on public health and safety considerations. Industry may also suffer from the non-availability of this ingredient.

Parties potentially disadvantaged by not permitting this substance, are the manufacturers of DAG-oil and producers who wish to use it in the manufacture of their final food products and potential consumers who cannot buy the product.

7.2 Option 2. Permit the use of DAG-oil as a novel food

On the basis of this Draft Assessment, industry and consumers would benefit from this option. This option would result in no cost to government, industry or consumers, if its safety can be ensured.

Approval of DAG-oil would promote international trade in food products.

8. Consultation

8.1 Public Submissions

FSANZ conducted an Initial Assessment on A505 – Diacylglycerol oil – and public comments on the Application were called for on 27 August 2003. A total of 9 submissions were received and are summarised in **Attachment 6**. **Seven** submissions **supported** the application, **one did not support** it and one was non-committal until FSANZ had performed a risk analysis.

Two submissions raised the issue that DAG-oil should not be considered as a novel food, arguing that it should not be considered non-traditional. Further issues raised in the submissions relate to safety, nutrition and efficacy. These issues are considered below.

8.1.1 DAG-oil as a novel food

Australian Food and Grocery Council argues that FSANZ needs to determine before accepting the Application as a novel food, that it is a non-traditional food and that there is not sufficient knowledge in the broad community to enable its safe use; further, if FSANZ finds that it is safe for human consumption, then it cannot be listed as a novel food. Furthermore, the safety of DAG-oil is not an issue, because diglycerides are already approved as food additives and are by-products in edible oil.

Response:

FSANZ considers DAG-oil to be a non-traditional food because the proposed food uses would lead to a significant increase in consumption by the broad community in Australia or New Zealand. FSANZ acknowledges that there has been some consumption of DAG-oil as a food additive and as by-product in edible oils; however, currently DAG-oil is present at very low levels in the diet. FSANZ's evaluation therefore classifies it as a non-traditional food as there will be a significant increase in consumption in Australia and New Zealand if DAG-oil is permitted as a novel food in Standard 1.5.1.

The object of the Novel Food Standard is to assess the safety of non-traditional food for which 'there is insufficient knowledge to enable safe use' in the broader community. Prior to the application, DAG-oil had not undergone a safety assessment in the context of the Australian and New Zealand diets. There was therefore insufficient knowledge in the broad community to ensure safe use in the form in which it is presented. The safety of DAG-oil in the context of Australia and New Zealand has now been assessed by FSANZ as a consequence of DAG-oil being classified as a novel food. The safety assessment showed that DAG-oil is safe for human consumption.

Even though FSANZ's assessment has concluded that DAG-oil does not require specific conditions of use this does not mean that DAG-oil should no longer be considered as a novel food. Whether or not there are conditions of use is not a criterion for determining the novelty of the food under Standard 1.5.1, and the standard itself envisages approvals of novel foods without any restrictions on use.

8.1.2 Safety of DAG-oil

New Zealand Food Safety Authority and Dietitians Association of Australia required further information on the safety of DAG-oil before being able to support or reject the application.

A detailed report on the safety of DAG-oil is provided at **Attachment 2**.

The safety of DAG-oil has been evaluated in animals and well as in humans. The available animal studies on DAG-oil indicate that these substances are absorbed and metabolised similar to triglycerides, and have low toxicity.

There is no evidence of adverse health effects in the human studies.

8.1.3 Nutritional issues

New Zealand Food Safety Authority, Dietitians Association of Australia, Mr Patel and Mr Katvi made submissions on nutritional issues of DAG-oil.

The submissions received in response to the Initial Assessment Report were focused on two main nutritional issues.

- The contradiction between the reports of a similar energy content between DAG-oil and TAG-based oils, and the ability for DAG-oil intake to generate a loss in weight when compared to a similar intake of TAG-based oils. The second issue was the intake of DAG-oil by infants as components of infant formulas and foods. Because the Applicant has reported that consumption of DAG-oil resulted in a weight loss, submitters indicated that the consumption of these forms of fat by infants would not allow for their adequate growth and development.

A review of the available scientific literature, provided at **Attachment 3**, indicates that DAG-oil does not have any nutritional attributes that are substantially different from TAG-based oils.

An increased level of β-oxidation with DAG-oil intake is reported. However, the body's homeostatic processes appear to accommodate this change, resulting in the same level of energy expenditure, fat storage and fat excretion from the intake of DAG-oil as occurs with the intake of TAG-based oils. Thus the contradiction between a similar energy content and the ability to generate a loss in weight would seem justified, and the scientific literature remains inconclusive as to how DAG-oil can produce a loss in weight, or if it can produce this physiological change at all.

Because DAG-oil has been assessed as nutritionally equivalent to TAG-based oils, particularly in regard to the contribution to energy metabolism, their intake by infants and children will not pose any significant nutritional risk. Furthermore, there are energy requirements in Standard 2.9.1 – Infant Formula Products and Standard 2.9.2 – Foods for Infants, and specific fat requirements in Standard 2.9.1, which ensure that any contribution of DAG-oil to infant nutrition occurs within the boundaries of nutritional adequacy.

From a nutritional perspective, DAG-oil has no demonstrated nutritional benefits when compared to TAG-based oils. However, there is also no evidence to indicate an adverse impact on population nutrition, and therefore DAG-oil can be considered nutritionally adequate for general consumption.

8.1.4 Health claims

Submissions indicated that there must be supporting data if a 'claim' is being made that DAG-oil reduces body weight and body composition.

Health Claims are currently prohibited in the Code under the transitional Standard 1.1A.2 – Health Claims with an exception for a 'folate/neural tube defect' health claim on approved products. The prohibition on health claims prevents the label attached to a package of food or any advertising material for food from including:

- a claim or statement that food is a slimming food or has intrinsic weight-reducing properties;
- a claim for therapeutic or prophylactic action;
- the word 'health' or any other words of similar meaning as a part of or in conjunction with the name of the food;
- any word, statement, claim, express or implied, or design that directly or by implication could be interpreted as advice of a medical nature; and
- a reference to any disease or physiological condition.

On 12 December 2003, the Ministerial Council endorsed a nutrition, health and related claims policy guideline, which will allow health claims on food or in advertising provided they are true, scientifically substantiated and are not misleading. The policy aims to ensure that the health and safety of the public is protected, whilst allowing for food industry innovation and trade. For more information on the policy guideline please refer to the Food Regulation Secretariat website, www.foodsecretariat.health.gov.au.

The Ministerial Council has referred the policy guidelines to FSANZ to develop a Standard in the Code. However, it is anticipated that it will take at least 18 months before a new Standard is in operation. Until that time the prohibition on making health claims will remain.

FSANZ is now seeking public comment in order to assist in assessing this Application at Final Assessment.

Comments that would be useful could cover:

- scientific aspects of this application;
- other issues including labelling of foods and food products containing DAG-oil.
-

8.2 World Trade Organization (WTO)

As members of the World Trade Organization (WTO), Australia and New Zealand are obligated to notify WTO member nations where proposed mandatory regulatory measures are inconsistent with any existing or imminent international standards and the proposed measure may have a significant effect on trade.

Amending the Code to permit the use of DAG-oil as a novel food is likely to have a significant positive effect on trade. Therefore, notification will be made to the WTO as a TBT in accordance with the WTO Technical Barrier to Trade (TBT) agreements.

9. Conclusion and Recommendation

At Draft Assessment, FSANZ recommends the approval of the use of DAG-oil as a novel food for the following reasons.

- There are no public health and safety concern associated with the use of DAG-oil as proposed;
- from a nutritional perspective, DAG-oil is considered nutritionally adequate for general consumption;
- DAG-oil has similar uses to triacylglycerol-based oils;
- appropriate labelling requirements have been proposed;
- the proposed changes to the Code are consistent with the section 10 objectives of the FSANZ Act;
- the submissions in relation to this Application have been addressed;
- there are no more cost-effective alternatives available other than changing Standard 1.5.1; and
- the Regulatory Impact Statement indicates that, for the preferred option, namely, to approve the use of DAG-oil as a novel food, the benefits of the proposed amendment outweigh the costs.

The proposed drafting for amendment to Standard 1.2.4, Standard 1.5.1, and Standard 2.4.1 is at **Attachment 1**.

10. Implementation and review

FSANZ recommends that the draft variations come into effect on the date of gazettal.

ATTACHMENTS

1. Draft variations to the *Australia New Zealand Food Standards Code*
2. Safety Assessment Report on DAG-oil
3. Nutritional Assessment Report on DAG-oil
4. Dietary Exposure Assessment Report on DAG-oil
5. Food Technology Report on DAG-oil
- 6. Summary of issues raised in public submissions in the first round

Attachment 1

Draft variations to the *Australia New Zealand Food Standards Code*

To commence: on gazettal

[1] ***Standard 1.2.4*** *of the Australia New Zealand Food Standards Code is varied by inserting in the* Table to clause 4, *for the* Generic name *fats or oils, under the* Conditions for Use –

4. Must not be used for Diacylglycerol oil.

[2] ***Standard 1.5.1*** *of the Australia New Zealand Food Standards Code is varied by inserting in the* Table to clause 2 –

Diacylglycerol oil (DAG-Oil)	'Diacylglycerol oil' is a prescribed name. Notwithstanding clause 4 of Standard 1.2.4, diacylglycerol oil must be declared in the statement of ingredients using the prescribed name.

[3] ***Standard 2.4.1*** *of the Australia New Zealand Food Standards Code is varied by omitting from clause 1, the definition of* edible oils*, substituting* –

edible oils mean the triglycerides and/or diglycerides of fatty acids of plant or animal origin.

Attachment 2

Safety Assessment of Diacylglycerol-oil

SUMMARY

Introduction

The safety of DAG-oil is supported by several acute, short-term, and long-term studies in rats; a long-term study in dogs; *in vitro* mutagenicity studies on diglycerides, and a series of clinical studies conducted to determine the human tolerance and nutritional effects of DAG-oil. These studies were assessed in detail. Furthermore, The Joint FAO/WHO Expert Committee on Food Additives (JECFA) has evaluated monoglycerides and diglycerides as food additives in 1974 and the Federation of American Societies for Experimental Biology (FASEB) evaluated the health aspects of glycerine and glycerides as food ingredients in 1975. The applicant also submitted international assessments of DAG-oil by the Health Council of the Netherlands, and Generally Recognized As Safe Panel Critical Evaluation Report of DAG-Oil and Notification letters by the US-FDA.

Absorption, Distribution, Metabolism and Excretion (ADME) (See also the nutrition assessment, Attachment 3)

Following ingestion, triglycerides undergo initial metabolism in the gastrointestinal lumen. They are broken down mainly by pancreatic lipase with the formation of mono- and diglycerides. Pancreatic lipase removes fatty acids from the 1 and 3 positions preferentially, so that 1,2- diglycerides and 2-monoglycerides are the immediate products. Mono- and diglycerides are absorbed into the intestinal cells. In their passage through the intestinal mucosa they are largely converted back into triglycerides. Transesterification and isomerisation can occur under biological conditions. Diglycerides are readily converted in appropriate tissues either to triglycerides or to monoglycerides. There is no evidence that the presence of monoglycerides or diglycerides of food fats has any deleterious effect on cells or tissues. The composition of fatty acids will determine digestibility.
The metabolism of DAG-oil is comparable to that of partial glycerides (monoglycerides) and triglycerides. Depending upon their composition, and the overall diet composition, they are partially or completely hydrolysed in the intestinal lumen by lipases and the resulting products are absorbed for re-esterification to triglycerides and / or oxidised as a source of energy, to varying degrees.

Short-term animal studies

The acute toxicity of DAG-oil was studied in rats. No deaths were observed at 15 g/kg bw. No treatment related clinical effects were observed.

In a 4-week sub-acute study in rats, there was no evidence of toxicity following treatment with DAG-oil in the diet of rats up to doses of 5%. The NOEL was 3.25 g/kg bw/day in rats.

Long-term animal studies

A 105-week study in rats was conducted but was not in accordance with OECD guideline for chronic toxicological studies (Test Guideline 452). However, the information supplied was sufficient for the safety evaluation of DAG-oil during the first 77 weeks of exposure. There were no changes observed in clinical parameters or tissue histopathology following treatment with DAG-oil in the diet of rats up to doses of 5.3% (equivalent to 1.87 g/kg bw/day) for 77 weeks. The NOEL was the highest dose tested, namely, 1.87 g/kg bw/day.

In a 52-week long-term study in dogs, there was no evidence of toxicity following treatment with DAG-oil in the diet of rats up to doses of 9.5%, equivalent to 2.3 g/kg bw/day in female dogs. The NOEL was 2.3 g/kg bw/day in rats.

Genotoxicity studies

DAG-oil was negative in a bacterial genotoxicity test system at concentrations in vitro up to 5000 mg/ml, with and without metabolic activation.

Other animal studies

In a series of short-term efficacy studies in rats and a long-term efficacy study in mice physiological parameters were examined, which are relevant to the safety of DAG-oil. No effects were found in these studies that were relevant for the safety assessment.

Short-term human studies

In a series of human studies, the physiological effects of DAG-oil were examined in normal subjects and some patient groups. The DAG-oil was administered in different forms as vegetable oil, margarine, mayonnaise, bread, cookies, soup, shortbread, brioche, egg roll, milk shake, muffins, crackers, and granola bars.

Most studies were not considered relevant for the safety assessment of DAG-oil because of improper study design and / or measurement of relevant parameters were not performed, however a few studies were considered relevant for the safety assessment.

In a single double blind crossover test, ingestion of DAG-oil at 0.179 g/kg bw as mayonnaise did not result in any adverse effects on liver function.

In a double blind parallel study over a 8-week period in healthy female subjects, DAG-oil in bread, cookies, soup or shortbread was tolerated at 0.36 g/kg bw/day.

In a double blind parallel controlled clinical study for 12-weeks, DAG-oil was tolerated by healthy human subjects at a level of 0.5 g/kg bw/day.

In a double blind parallel study over a 24-week period in obese human subjects, DAG-oil in various food products was tolerated at 0.29 g/kg bw/day.

Safety related to changes in lipid profile

Experiments conducted in male rats with the DG diolein indicated an increase in serum fatty acids (oleic acid) in the portal blood following consumption (Watanabe *et al.*, 1997b) (See Attachment 3, Nutrition Assessment Report). This increased level of portal vein free fatty acids (FFA) after DAG-oil consumption, could result in increased insulin resistance (Bergman, 2000). To validate whether this could be of concern for humans consuming DAG-oil, all performed animal and human studies were verified whether the following parameters were measured: plasma/ portal vein FFA, plasma triglycerides, plasma insulin, plasma glucose and plasma HbA1C. Table 1 summarises the studies that measured some/all parameters.

Table 1: Effects of DAG-oil consumption on lipid parameters in animal and human studies.

	FFA	**TAG**	**Insulin**	**Glucose**	**HbA1c**
Animal Studies					
acute rats *Watanabe 97*	portal vein ↑ jugular vein -	nd	nd	nd	nd
2-3 wk rats *Murata 97*	nd	↓	nd	nd	nd
34 day rat *Hara 93*	nd	↓	nd	nd	nd
8-month mice *Murase 02*	-	-	↓	↓	nd
77-wk rats *Kimura 00*	-	-	-	-	nd
Humans					
acute *Tada 01*	nd	↓	nd	nd	nd
acute *Taguchi 00*	nd	↓	nd	nd	nd
acute *Takei 01*	-	nd	nd	nd	nd
8 wk females *Hasegawa 00*	↑ (20%)	-	-	-	nd
12 wk *Kobayashi 01*	-	-	nd	-	-
16 wk *Takei 01*	↑, however no difference compared to pre-treatment	-	-	↓	nd
16-wk *Nagao 00*	-	-	-	-	nd
24 wk obese *Maki 02*	nd	-	nd	-	nd
12 wk diabetics *Yamamoto 01*	nd	↓	nd	-	-

↑: means increased compared to control treatment
↓: means decrease compared to control treatment
-: not different compared to control treatment
nd: not determined

Animal studies did not reveal an increase in serum FFA and the chronic study in rats did not indicate any long-term effects on the lipid profile. In humans, two repeat studies resulted in an increase in serum free fatty acid levels compared to TAG-based oils treatment, while in two other studies no changes were observed. No effect on insulin levels was reported in any study. One study saw a decrease in glucose levels, while all other human studies did not reveal any changes. The HbA1C levels, which are an indication for long-term glucose levels did not show any changes in both healthy and diabetic volunteers.

In conclusion, the studies do not suggest that consumption of DAG-oil would result in an increased risk for insulin resistance.

Overall conclusion

The safety studies in animals and humans did not indicate any specific safety concerns with DAG-oil. There is no specific data available on allergenic potential of reproduction, however the available studies do not suggest that DAG-oil has allergenic potential or has any effects on reproduction and development. DAG-oil has no mutagenic or carcinogenic potential. Table 2 summarises the results of the acceptable studies for the safety assessment of DAG-oil. No specific adverse effects were observed in human studies that would indicate adverse health. Other studies, conducted in sub-population groups largely to examine efficacy, did also not indicate any major adverse health effects. These studies were conducted in individuals on haemodialysis, with type II diabetics and in obese and hyperlipidemic children.

Therefore, it is not necessary to set an upper limit for the use of DAG-oil.

Table 2: Summary of studies acceptable for the safety assessment of DAG-oil

Species	**Study**	**Study author**	**Test substance, dose**	**Limit/NOEL (g/kg bw/day)**	**Adverse effects**
rat	acute	Ishida, 1996a	DAG-oil	LD_{50} > 15 g/kg bw	-
rat	acute	Ishida, 1996b	DAG-oil	LD_{50} > 15 g/kg bw	-
rat	28-day	Serbian, 1991	DAG-oil, 0, 0.2, 1.0 and 5.0%	3.25	-
rat	77-week	Kimura, 2000	DAG-oil, 0, 2.65 and 5.3%	1.87	-
dog	1-year	Kirkpatrick, 2002	DAG-oil, 0, 1.5, 5.5, 9.5%	2.3	-
humans					
17 healthy males	acute	Takei, 2001	DAG-oil	0.179	-
28 females	8-week	Hasegawa, 2000	DAG-oil	0.36	-
45 males / females	12-week	Kobayashi, 2001	DAG-oil	0.59	-
43 obese males / females	24-week	Maki, 2002	DAG-oil	0.29	-

ANIMAL STUDIES

Acute studies

Single administration toxicity study of DAG-oil in rats (Ishida, 1996a)

Test material	DAG-oil preparation referred to as Kao Diglyceride (88.8% DAG, 11.2% TAG, 0.01-0.02% tocopherol)
Vehicle material	Natane triglyceride
Test Species	5 animals/sex/group Crj:CD Sprague Dawley rats; administration by gavage
Dose	15 000 mg/kg bw (1.5 mL/100 g body weight)
GLP/guidelines	Revision of Guidelines for Single and Repeated Dose Toxicity Studies Notification No. 88 of the Pharmaceutical Affairs Bureau, Ministry of Health and Welfare, Japan (1993)

Groups of 5 male and 5 female rats received single doses of DAG-oil or Natane triglyceride administered orally by gavage and were observed for mortality, morbidity, and clinical signs for 14 days post-dose. Body weights were measured prior dosing, at days 1, 2, 3, 7, 10 and 14. At day 15 the animals were sacrificed and necropsy was performed. Diarrhoea was observed in 3/5 males and 3/5 females in the control group and in 3/5 males and 2/5 females in the dosing group during the period from 1 hour to 6 hours after dosing. These signs were thought to be due to physical properties of the oil, which was administered in relatively large quantities. No further clinical signs and mortality was observed. Body weights and necropsy revealed no treatment related effects.

Conclusion
On the basis of this study, there was no evidence of acute toxicity of diglyceride at a dose of 15 g/kg bw.

Single administration toxicity study of DAG-oil in rats (Ishida, 1996b)

Test material	DAG oil preparation referred to as Diglyceride healthy oil (88.8% DAG, 11.2% TAG, 0.1% polyglycerine fatty acid ester, 0.075% tocopherol, 0.025% ascorbate palmitate)
Vehicle material	rapeseed oil
Test Species	5 animals/sex/group Crj:CD Sprague Dawley rats; administration by gavage
Dose	15 g/kg bw (1.5 mL/100 g body weight)
GLP/guidelines	Revision of Guidelines for Single and Repeated Dose Toxicity Studies Notification No. 88 of the Pharmaceutical Affairs Bureau, Ministry of Health and Welfare, Japan (1993)

Groups of 5 male and 5 female rats received single doses of DAG-oil (88.8% pure) or rapeseed oil administered orally by gavage and were observed for mortality, morbidity, and clinical signs for 14 days post-dose. Body weights were measured prior dosing, at days 1, 2, 3, 7, 10 and 14. At day 15 the animals were sacrificed and necropsy was performed.

Diarrhoea was observed in 4/5 males and 3/5 females in the control group and in 3/5 males and 3/5 females in the dosing group during the period from 1 hour to 6 hours after dosing. These signs were thought to be due to physical properties of the oil, which was administered in relatively large quantities. No further clinical signs and mortality was observed. Body weights and necropsy revealed no treatment related effects.

Conclusion

On the basis of this study, there was no evidence of acute toxicity of DAG-oil at a dose of 15 g/kg bw.

Short-term studies

4-week subacute oral toxicity study of DAG-oil in rats (Serbian, 1991)

Test material:	DAG-oil referred to as Kao Diglyceride (76.6% DAG, 18.2% TAG, 4.4% monoglycerides, 0.01-0.02% mix tocopherol)
Test Species:	Crl:CD®BR rats
Vehicle	corn oil
Dose:	0, 0.2, 1.0, 5.0% in diet for 28 days, equivalent to 0.14, 0.71, 3.25 g/kg bw/day in males and 0.15, 0.74, 3.55 g/kg bw/day in females.
GLP:	USA GLP Regulations
Guidelines:	Japanese Ministry of Health and Welfare Guidelines (Notification No 118, 1984)

Study conduct

After acclimatisation for at least fourteen days, three groups of rats (10/sex/group) were treated with DAG-oil in the diet at 0.2, 1.0 or 5.0% (equivalent to 0.14, 0.71, 3.25 g/kg bw/day in males and 0.15, 0.74, 3.55 g/kg bw/day in females) for four weeks. Two control groups were included. The first control group received 4.3% dietary fat through the diet and 5.7% corn oil (Purina Certified Rodent Chow). The second control group received 5.0% rapeseed oil and 5.0% corn oil (Purina Basal Purified Diet). The treated animals were also fed the Purina Basal Purified Diet, and the total dietary fat intake was 10 % in all groups. To receive the amount of 10% dietary fat, the treated groups were also fed corn oil.

Clinical observations were recorded twice daily, and bodyweight and food consumption were recorded weekly. Haematology, clinical chemistry, and urinalysis were performed prior to necroscopy. For a list of parameters measured see Appendix 1. Ophthalmology of all animals was performed before the study and near termination. At the end of the study, all animals were sacrificed and a complete necroscopy was performed (gross examination, organ weights and tissue sampling). Histopathology was performed on all tissues from the two control and high dose groups. Appendix 1 lists the histopathological parameters measured.

Results

On day 8 stability analyses performed on the fat extracted from the diets of Control group 2 and the low and high dose groups revealed an increase in peroxide values relative to the Day 0 stability analysis. This increase in peroxide values was not considered to have influenced the results of the study.
All animals survived until the end of the treatment. There were no treatment related effects in clinical signs, body weights and body weight gain. Significantly decreased mean total food consumption was observed in control group 2, the low and high dose treatment group males during week 1 to 4 when compared to control group 1. No effects were observed in females.

The reporting ophthalmologist concluded that there were no ocular abnormalities associated with the test material.

There were no treatment related differences in haematology, clinical chemistry or urinalysis at any dose. Serum cholesterol and triglyceride levels in the treated groups were not significantly different to controls. Incidental but statistically significant decreases in blood urea nitrogen were noted in the females of control group 2 and all treatment groups compared to the control group 1 females. These differences are not considered treatment related, since a dose response relation was not observed.

In general organ weights, organ morphology and microscopic features were unaffected by treatment at up to 5% in the diet. Exceptions were increase in absolute mandibular salivary gland weight value for the females at 0.2% compared to the control groups. In addition, significantly decreased relative kidney (females of control group 2 and all treatment groups) and liver weight values (low, medium and high treatment male groups) were observed compared to the first control group. However, these increases were small, confined to one sex and there were no accompanying histopathological or enzyme findings.

Conclusion

There was no evidence of toxicity following treatment with DAG-oil in the diet of rats up to doses of 5% (equivalent to 3.25 g/kg bw/day) for 28 days. The NOAEL was the highest dose tested, namely, 3.25 g/kg bw/day for 28 days in male rats.

Chronic studies

Long-term nutritional toxicity study of DAG-oil in rats (Kimura, 2000)

Test material:	DAG-oil preparation referred as KA-1 (90.3% DAG, 7.0% TAG, 1.2% monoglycerides, and 0.01-0.02% mix tocopherol. The fatty acid composition was 3.7% C16, 3.7% C18, 45.4% C18:1, 39.5% C18:2, 4.5% C18:3)
Control Diet 1	5.3 % vegetable oils whose fatty acid composition was comparable to that of DAG-oil (1.5% DAG, 96.5% TAG, and 0.01-0.02% mix tocopherol. The fatty acid composition was 5.6% C16, 2.0% C18, 47.0% C18:1, 36% C18:2, 6.5% C18:3)

Control Diet 2	rapeseed oil and soybean oil, which had a total fat content of 5.3% (1.0% DAG, 97.4% TAG and 0.01-0.02% mix tocopherol. The fatty acid composition was 5.4% C16, 2.3% C18, 51.6% C18:1, 27.9% C18:2, 9.8% C18:3)
Test Species:	Crl:CD Sprague Dawley rats
Dose:	0, 2.65, 5.3 % in diet for 105 weeks.
GLP:	Not reported
Guidelines:	Not reported

Study conduct

After acclimatisation for at least twelve days, two groups of rats (60/sex/group) were treated with DAG-oil in the diet at 2.65 or 5.3% (equivalent to 0.94 and 1.87 g/kg bw/day in males and 1.12 and 2.29 g/kg bw/day in females). Two control groups were included.
The first control group (Con1) received a diet containing with 5.3 % vegetable oils whose fatty acid composition was comparable to that of DAG-oil. A second control group (Con2) received feed mixed with rapeseed oil and soybean oil, which had a total fat content of 5.3%.

Clinical observations were recorded daily, and bodyweight and food consumption were recorded weekly until week 30 and once every two weeks thereafter. Ten animals/sex/group were sacrificed after 30 and 77 weeks and complete necropsy was performed (gross examination, organ weights and tissue sampling). Haematology, clinical chemistry, and urinalysis were performed prior to necropsy at week 30 and 77. For a list of parameters measured see Appendix 1. Dead animals were necropsied immediately after discovery. At the end of the study (week 105), all remaining animals were sacrificed and gross examination, and organ weights were performed, however histopathology was not performed. Appendix 1 lists the histopathological parameters measured.

Results

No mortality was observed before week 30. The mortality rates between week 30 and 77 were 10/50, 10/50, 6/50, 7/50 (males) and 3/50, 11/50, 12/50, 4/50 (females) of Con1, Con2, 2.65 and 5.3% groups respectively. The survival until week 105 was 12/40, 13/40, 16/40, 11/40 (males) and 18/40, 9/40, 10/40, 11/40 (females) of Con1, Con2, 2.65 and 5.3% groups respectively. No treatment related effects on clinical signs and body weight were observed. Food consumption was significantly lower at various times in the 2.65% males group, however this reduction was small and not dose related and therefore not considered relevant. No treatment related effects were observed on urinalysis. During treatment various haematological parameters were occasionally different in the treated groups (prothrombin time, number of platelets), clinical chemical parameters (several aminotransferases, lactate dehydrogenase, HDL cholesterol) and organ weights (pituitary, thyroids and spleen). However, these changes were observed at one point in time only and they showed no dose dependent effects. Furthermore, none of these changes were associated with histopathological changes. The occurrence of malignant mammary gland tumours in dead female rats exposed to 5.3% DAG-oil was significantly greater than in the control group (see Table 3). However, this increased incidence is not considered to be related to DAG-oil treatment, because there was no dose-related increase, the increase was not statistically significant, the increase was only significant in females which were found dead or in moribund condition, and the incidence was within the range of tumours in historical controls.

Table 3: Summary of proliferative lesions of the mammary gland and skin in female rats

	Control 1	Control 2	2.65% DAG-oil	5.3% DAG-oil
77-weeks				
Total benign tumours	2/9	3/10	1/10	4/9
Total malignant epithelial tumours	1/9	2/10	2/10	2/9
Total rats with epithelial tumours (benign and malignant)	2/9	4/10	2/10	5/9
105-weeks				
Total benign tumours	12/18	4/10	7/10	9/12
Total malignant epithelial tumours	6/18	3/10	4/10	2/12
Total rats with epithelial tumours (benign and malignant)	14/18	5/10	9/10	11/12
Early death (found dead and moribund sacrifice)				
Total benign tumours	10/23	12/30	12/30	17/29
Total malignant epithelial tumours	4/23	1/23	4/30	7/29*
Total rats with epithelial tumours (benign and malignant)	12/23	13/23	14/30	20/29

* significant differently compared to Control 1

Conclusion

The long-term study in rats was not carried out in accordance with OECD guideline for chronic toxicological studies (Test Guideline 452). However, the information supplied is sufficient for the safety evaluation of DAG-oil during 77 weeks of exposure. This is because full toxicological assessments, including histopathology, were carried out after 30 and 77 weeks of exposure. There was no evidence of toxicity or carcinogenicity following treatment with DAG-oil in the diet of rats up to doses of 5.3% (equivalent to 1.87 g/kg bw/day) for 77 weeks. The no observed effect level (NOEL) was the highest dose tested, namely, 1.87 g/kg bw/day for 77 weeks in male rats.

One-year dietary toxicity study of DAG-oil in Beagle dogs (Kirkpatrick, 2002)

Test material:	DAG-oil (>80% diglycerides, <20% triglycerides, <5% monoglycerides
Control group 1	9.5 % TAG-based oil (>80% triglycerides, <20% diglycerides, <5% monoglycerides, similar fatty acid contents as DAG-oil)
Control group 2	Standard basal diet (fat content of 9.5%)
Test Species:	Beagle dogs, 4/sex/dose
Dose:	0, 1.5, 5.5, and 9.5 % DAG-oil for 52 weeks, equivalent to 0.33, 1.23, 2.54 g/kg bw/day in males and 0.35, 1.49 and 2.3 g/kg bw/day for females.
GLP:	In compliance with FDA and OECD
Guidelines:	In general accordance with the FDA's 1993 Redbook II, similar to OECD Guideline 452 (Chronic Toxicity Study).

Study conduct

Beagle dogs (4/sex/dose) were given DAG-oil in the diet at concentrations of 0% DAG-oil/9.5% TAG-based oil (TAG-based oil control), 1.5% DAG-oil/8.0% TAG-based oil, 5.5% DAG-oil/4.0% TAG-based oil, and 9.5% DAG-oil/0% TAG-based oil daily, seven days per week, for 52 weeks (equivalent to 0.33, 1.23, 2.54 g/kg bw/day in males and 0.35, 1.49 and 2.3 g/kg bw/day for females). A second concurrent control group received the standard basal diet (fat content of 9.5%). Treatment was initiated in prejuvenile (2.5 months old) dogs.

Clinical observations were recorded daily, and bodyweight and food consumption were recorded twice weekly until week 6 and weekly thereafter. Haematology, clinical chemistry, and urinalysis were performed in week –1, 13, 26, 39 and 52 weeks. For a list of parameters measured, see Appendix 1. Ophthalmology of all animals was performed at week –1, 12, 25, 38 and 51. Electrocardiograms were recorded for all animals in week –1, 25 and 51. At the end of the study, all animals were sacrificed and a complete necroscopy was performed (gross examination, organ weights, tissue sampling and histopathology. Appendix 1 lists the histopathological parameters measured.

Results

All animals survived until the end of the treatment. There were no treatment related effects in clinical signs. The food consumption in the control group with standard basal diet was significantly higher compared to all other groups. This control group is therefore less relevant than the TAG-based oil control group and the results compared to this group will not be further considered. The body weights and body weight gain were dose related decreased in males with DAG-oil treatment during the study, however this effect was not statistically significant (12% decease at 9.5% DAG). There were no treatment related effects on food consumption between the TAG-based oil control group and treatment groups.

The reporting ophthalmologist and veterinary cardiologist concluded that there were no ocular or electro cardiac abnormalities associated with the test material.

There were no treatment related differences in haematology, or urinalysis at any dose and any time. Alkaline phosphatase activity was elevated in both males and females at various times at 9.5% DAG-oil compared to the TAG-based oil group, however dose responsive was not observed, the effects were not statistically significant and not correlated to histopathological observations and therefore not toxicological relevant. Glucose concentrations tended to be higher in the DAG-oil treated groups during the study, however a dose response was not apparent. Therefore, these changes are not considered toxicological relevant.

In general organ weights, organ morphology and microscopic features were unaffected by treatment at up to 9.5% in the diet.

Conclusion

There was no evidence of toxicity following treatment with DAG-oil in the diet of dogs up to doses of 9.5% (equivalent to 2.3 g/kg bw/day) for one year. The NOAEL was the highest dose tested, namely, 2.3 g/kg bw/day for one year in female dogs.

Genotoxicity studies

Bacterial mutation assay on DAG-oil (Jones, 1992)

Test article
The test article, referred to as diglyceride, batch 1231 was used. DAG-oil consisted of more than 80% DAG, less than 20% TAG, less than 5% monoglycerides and 0.01-0/02% mix tocopherol.

Study conduct

Diglyceride was examined for mutagenic activity in four strains of *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537) and one strain of *Escherichia coli* (WP2 urvA). Experiments were performed with or without metabolic activation using liver S9 fraction from chemically pre-treated rats. The study design resembles OECD guideline 471 (adopted 1997), the study was conducted under GLP guidelines.
A preliminary toxicity test was performed to select the concentrations of the test article to be used in the main assays. The study comprised of negative and positive controls with or without S9 metabolising system. Ethanol, the solvent used in the study, was employed as a negative control in the main study. Experiments for survival determination and estimation of mutant numbers were carried out in triplicates at each test point. Five doses of test substance were applied with 5 mg/plate as the highest dose level. The sensitivity of the individual bacterial strains was confirmed by significant increases in the number of revertant colonies induced by diagnostic mutagens.

Results

Test	*Test material*	*Concentration*	*Test object*	*Result*
Reverse mutation (*In vitro*)	diglyceride	0, 313, 625, 1250, 2500, 5000 µg/plate, with and without S9 mix	*S. typhimurium* TA98, TA100, TA1535, TA1537. *E. coli* WP2 urvA	-ve

Conclusion

Based on the results of these tests, it is concluded that DAG-oil is not mutagenic in these *Salmonella* and *E Coli* strains.

Other animal studies

Comment: It is noted that the primary purpose of these studies was to examine efficacy rather than to examine potential toxicity and as such are deficient in reporting of any features normally associated with toxicology studies. However, these studies are reported here since it provides some limited information relevant to the toxicity of DAG-oil.

5-week DAG-oil intake study in genetically obese female rats (Sugimoto, 2003a)

Test material:	DAG-oil (53.8% 1,3-DAG, 27.6% 1,2-DAG and 18.6% TAG. The fatty acid composition for DAG-oil was 16:0, 4.57%; 18:0, 1.62%; 18:1 39.8%; 18:2, 49.9%; 18:3, 4.19%)
Control:	TAG-based oil (fatty acid composition was 16:0, 8.46%; 18:0, 2.59%; 18:1, 49.3%; 18:2, 34.5%; 18:3, 5.15%)
Test Species:	female genetically obese Wistar fatty rats (fa/fa) and their lean littermates (Fa/Fa, fa/Fa), 7 animals/group.
Dose:	10% test oil in the diet, pair-fed for the genotype control (equivalent to 5 g/kg bw/day).
GLP:	unknown
Guidelines:	unknown

Study conduct

Female genetically obese Wistar fatty rats and their lean litter mates were given equal energy-containing diets (56% sucrose) per body weight per day in the DAG-oil and TAG-based oil groups for each genotype for 5 weeks. The fatty rats were hypertriglycemidemic and hyperinsulinemic, but normoglycaemic. The spontaneous food consumption was similar in the 10% (by weight) DAG-oil and TAG-based oil groups. After 4 weeks an oral glucose tolerance test was performed in 6 animals per group. For this, rats were given a 400 g/L glucose solution (3 g glucose/kg bw) by gavage after 20 h food deprivation. After 5 weeks rat were sacrificed and non-fasting blood samples from the portal vein and inferior vena cava vein were obtained for glucose, insulin, free fatty acids and ketone bodies determination. Liver and abdominal white adipose tissue was assessed for TG levels, glucose content and insulin area.

Results

No deaths were reported. In this study no effects were observed on body weight and abdominal fat weight. After 5 weeks, the free fatty acid (~15-40%) and glucose levels (~13%) were elevated in the DAG-oil group in both groups as compared to their respective TAG-based oil control group, particularly in the obese group. The triglyceride levels were not different between the treatment groups. In the glucose tolerance test, the obese rats fed DAG-oil showed higher glucose levels for 2 h after the glucose treatment compared to TAG-based oil (200% increase in area under the curve).

The potential variation of feeding status of the rats when blood samples were taken will have impact on the levels of glucose, insulin, free fatty acids, ketone bodies and triglycerides. Differences in these levels between the treated groups might therefore not be the result of feeding status, but of differences in feeding status. Therefore, the data found in this study where the animals were non-fasted are discarded.

Conclusion

This study is not acceptable for the safety assessment of DAG-oil, since the study was not performed according to guidelines. For instance, blood parameters were tested under non-fasting conditions, the number of animals per group was low, and only one dose of DAG-oil was tested.

1 to 12-week DAG-oil intake study in young and old rats (Sugimoto, 2003b)

Test material:	DAG-oil (53.8% 1,3-DAG, 27.6% 1,2-DAG and 18.6% TAG. The fatty acid composition for DAG-oil was 16:0, 4.57%; 18:0, 1.62%; 18:1 39.8%; 18:2, 49.9%; 18:3, 4.19%)
Control group	TAG-based oil (the fatty acid composition was 16:0, 8.46%; 18:0, 2.59%; 18:1, 49.3%; 18:2, 34.5%; 18:3, 5.15%)
Test Species:	7-week old (young) and 8 month old (old) Wistar male rats, 7 animals/group.
Dose:	10% test oil in the diet, pair-fed (equivalent to 5 g/kg bw/day) for 1-12 weeks.
GLP:	unknown
Guidelines:	unknown

Study conduct

Male 7-week or 8-months old Wistar rats were given a diet containing 10% TAG-based oil or DAG-oil for 1, 4, 8, and 12 weeks. An oral glucose tolerance test was performed in the 3rd and 7th week of feeding, one week before the animals were sacrificed. Rats were given a 400 g/L glucose solution (3 g glucose/ kg bw) after been deprived of food for 16 h. After 1, 4, 8 or 12 weeks of treatment, rats were sacrificed (not food deprived) and blood samples from the portal vein and inferior vena cava vein were obtained for glucose, insulin, free fatty acids, triacylglycerol and ketone bodies determination. In a separate group blood samples were taken from the tail vein after rats were food-deprived for 16 h. Livers and white adipose tissue were assayed for binding capacity and affinity constant of purified insulin.

Results

No deaths were reported. In this study no effects were observed on body weight, liver weight and adipose tissue weight.
In non-fasted rats, plasma glucose levels were elevated in both portal vein and inferior vena cava after DAG-oil treatment in both young and old rats. In old rats treated with DAG-oil, insulin concentrations in the portal vein were higher compared to TAG-based oil. Plasma free fatty acids were elevated in all groups at various times, while triacylglycerol levels were unchanged.

After the acute glucose dose, glucose increase was significantly greater in the DAG-oil group compared to TAG-based oil in both young and old rats. The insulin increase was higher in the DAG-oil group compared to TAG-based oil only in young rats. The basal values for glucose and insulin levels in the animals before the acute glucose dose were not reported. In fasted rats, fatty acid and triacylglycerol levels were unchanged by treatment, but plasma glucose (~9 vs.~7.5 mmol/L) and plasma insulin (~0.18 vs. ~0.12 nmol/L) concentrations were higher in DAG-oil group compared to TAG-based oil in old rats.

The potential variation of feeding status of the rats when blood samples were taken will have impact on the levels of glucose, insulin, free fatty acids, ketone bodies and triglycerides. Differences in these levels between the treated groups might therefore not be the result of feeding status, but of differences in feeding status. Therefore, the data found in this study where the animals were non-fasted are discarded.

Conclusion

This study is not acceptable for the safety assessment of DAG-oil, since the study was not performed according to guidelines. For instance, the number of animals per group was low, and only one dose of DAG-oil was tested.

8-month DAG-oil intake study in male mice (Murase, 2002)

Test material:	DAG-oil (90% diglycerides, 10% triglycerides)
Control	triglyceride oil
Test Species:	C57BL/6J male mice (10-20/group)
Dose:	Low TAG-based oil (5% TAG-based oil in diet), High TAG-based oil (25% TAG-based oil in diet), high DAG-oil (15% DAG-oil and 10% TAG-based oil in diet), for eight months. This DAG-oil dose is equivalent to 22.5 g/kg bw/day.
GLP:	unknown
Guidelines:	unknown

Study conduct

One group of 10 male mice was treated with diglyceride in the diet at 15% combined with 10% triglycerides. Two control groups were included. The first control group (10 males) received a low triglyceride diet (rapeseed, 5%), while the second group (20 males) received a high triglyceride diet (25%) dietary fat through the diet.

Food intake was measured weekly on a per-cage basis (5 mice/cage). On the final day, blood was collected for lipid analysis both with and without fasting. Fat tissue was weighed and beta-oxidation activity was measured in the liver.

Results

No deaths were reported. Dietary DAG-oil significantly reduced the body fat accumulation induced by a high-fat diet. Compared with the low-TAG-based oil diet, feeding with the high-TAG-based oil diet for 8 months produced significant increases in body weight and adipose tissue weight. High DAG-oil-diet reduced body weight gain and adipose tissue weight compared to the high TAG-based oil diet.

There were no treatment related effects on plasma parameters (TAG, cholesterol, free fatty acids, glucose, insulin, and leptin) under non-fasting conditions, however glucose, insulin and leptin concentrations under fasting conditions in the DAG-oil group were significantly lower compared to the high TAG-based oil group. There were no differences in average energy intake between the high-TAG-based oil and DAG-oil group.

Conclusion

Dietary DAG-oil (22.5 g/kg bw/day) reduced body weight gain compared to high TAG-based oil diet in mice, however the body weight was still significantly higher compared to low TAG-based oil diet.

With regard to safety, blood glucose, insulin and leptin were significantly lower after DAG-oil treatment as compared to a high TAG-based oil diet. The blood parameters measured did not indicate any changes indicative of an adverse effect.

HUMAN STUDIES

In a series of human studies, provided by the Applicant physiological effects of DAG-oil were examined in normal subjects and some patient groups. The DAG-oil was administered in different forms, vegetable oil, margarine, mayonnaise, bread, cookies, soup, shortbread, brioche, egg roll, milk shake, muffins, crackers, and granola bars.

Comment: It is noted that the primary purpose of these studies was to examine efficacy rather than to examine potential toxicity and as such they are deficient in reporting may of the parameters normally associated with toxicology studies. However, they are reported here since they provide some limited information relevant to the toxicity of DAG-oil.

Single dose studies

Acute DAG-oil intake study in healthy male volunteers (Takei, 2001)

Test material:	DAG-oil
Control material	TAG-based oil
Test groups:	17 healthy Japanese males (double blind crossover)
Dose:	10.39 g TAG-based oil or DAG-oil/60 kg bw in 15 g mayonnaise
GLP:	Not stated.

Study conduct

A single ingestion test of DAG-oil and TAG-based oil were performed at a one-week interval by double blind cross over method, after overnight fasting. Prior to consumption a blood sample was taken and 2, 3, 4 hours after consumption of 15 g mayonnaise/ 60 kg bw with 10 g lettuce. The lipid profile was determined and GOT, GPT, gamma-GTP, total ketone body, acetoacetic acid and 3-hydroxybutyric acid was measured.

Results

The percent increase in serum triglyceride levels 3 hr after ingestion was significantly lower in the DAG-oil group compared to TAG-based oil (32 vs. 56%, respectively). The increase in 3-hydroxybutyric acid was diminished in the DAG-oil group compared to TAG-based oil after 1 hour, but similar after two hours. No treatment related effects were observed in liver function (GOT, GPT, gamma-GTP).

Conclusion

Based on the limited parameters investigated, an acute dose of DAG-oil at a dose level of 0.179 g/kg bw did not result in any adverse effects on liver function.

Acute DAG-oil intake study in healthy males (Taguchi, 2000)

Test material:	DAG-oil
Control material	TAG-based oil (DAG-oil and TAG-based oil were prepared in order to have a similar fatty acid content)
Test groups:	healthy males (double blind cross over, 7 days apart)
Dose:	10, 20, and 44 g fat/person in a 100 g emulsion
GLP:	Not stated.

Study protocol

Two test emulsions (100 g) were given randomised so that half the subjects received the DAG-oil emulsion first and the other half received the TAG-based oil after overnight fasting. The test emulsions contained one of the test oils at different doses: 10 g (n=13), 20 g (n=10) or 44 g (n=17) per 60 kg body weight, this equals to 0.17, 0.33 and 0.73 g/kg bw. Blood sampling was performed before, and after 2, 4, and 6 h in the 10 g fat group, after 2, 4, 6, 8 h in the 44 g fat group and after 4 and 6 h in the 20 g fat group. The blood was analysed for lipid content.

Results

The ingestion of DAG-oil, as compared to TAG-based oil, caused smaller increases in serum TAG concentrations (23% lower after 6 h when 44 g fat was consumed). No safety parameters were examined.

Conclusion

Since only the lipid profile was evaluated, this study does not address the safety of DAG-oil.

Acute DAG-oil intake study in healthy male volunteers (Tada, 2001)

Test material:	DAG-oil (>90% of diglycerides (1,3-DAG: 1,2-DAG in a ratio of 7:3), <10% triglycerides)
Control material	TAG-based oil (prepared by mixing rapeseed, soybean and safflower oil to make a fatty acid composition similar to DAG-oil)

Test groups:	6 healthy Japanese males (double blind crossover, with one month interval)
Dose:	30 g lipid/m^2 of body surface area in the morning after overnight fasting (consumed as creamed 35% creamed test oil)
GLP:	Not stated.

Study conduct

The subjects were comprised of six male volunteers who orally took creamed test meals prepared with either DAG-oil or TAG-based oil at a dose of 30 g lipid/m^2 of body surface area in the early morning after overnight fasting. The study was of a double blind cross over design. Blood was taken before and 2, 3, 4, 6, and 8 h after lipid loading for measurement of serum lipids and remnants.

Results

Serum triglycerol concentrations at 2, 3 and 8 h after loading of DAG-oil were significantly lower than those after loading of TAG-based oil. The serum remnant-like lipoprotein concentration of cholesterol was significantly lower after DAG-oil than TAG-based oil at various time-points.

Conclusion

This study provides no information relevant to the safety assessment of DAG-oil, since the dosage per kg bw was not presented and the limited parameters evaluated.

Repeat dose studies

4-week DAG-oil intake study in healthy adult male volunteers (Watanabe, 1997a)

Test material:	DAG-oil (91.8% diglycerides, 8.2% triglycerides; the fatty acid composition was comparable between TAG-based oil and DAG-oil. DG mixture 1,2-DG: 1,3-DG in a ratio of 30.3: 69.7)
Control material:	TAG-based oil (amount of triglycerides, diglycerides, and monoglycerides unknown)
Test groups:	8 healthy adult males, BMI 24.3 (double blind cross-over).
Dose:	44 g/ 60 kg body weight/day for 4-weeks, in brioche, egg roll, milk shake and mayonnaise (equal to 0.73 g/kg bw/day).
GLP:	Not stated.

Study conduct

Prior to treatment, subjects received four weeks of TAG-based oil. After the controlled intake period, measurement by CT scan and blood test was conducted. Afterwards, double blind crossover tests with DAG-oil and TAG-based oil as test oils were conducted for 4 weeks each. On the end of each test period blood samples were taken and a CT scan was performed after 14-15 hour fasting.

The blood samples were used to measure haematology parameters (WBC, erythrocyte count, Hb, Hct, thrombocytes), lipids (TG, beta-lipoprotein, total CHOL, serum free CHOL, HDL CHOL, phospholipids, free fatty acids, glucose, blood insulin, apolipoproteins), and clinical chemistry parameters (GPT, GOT).
Data were only presented as change from initial values (i.e. variation compared before and after the test period).

Results

No treatment related effects on vital signs, body weight, body fat ratio, BMI, and hip circumferences were observed. The increase in waist size was attenuated by DAG-oil intake compared with TAG-based oil intake according to the study author, however when the data from the graphs (difference from pre-treatment) were calculated to actual numbers, a 6 cm increase in waist circumference would have been occurred after treatment with TAG-based oil, compared to a 2 cm decrease after DAG-oil. After only a 4-week treatment period these results are very questionable, particularly as there is no corresponding effect on body weight.

No adverse clinical effects in subjects were reported. The analysis of blood parameters (e.g. to determine liver and kidney function) did not indicate any treatment-related changes, but data was inadequately presented.

Conclusion

This study is not acceptable for the assessment of effectiveness and safety of DAG-oil, since data was not presented in an adequate manner.

8-week DAG-oil intake study in young female volunteers (Hasegawa, 2000)

Test material:	DAG-oil (1,2-DAG: 1,3-DAG = 3: 7; the fatty acid composition of TAG-based oil was almost similar to DAG-oil)
Control material:	TAG-based oil
Test groups:	28 healthy Japanese females/group (double blind parallel, average age 20 y)
Dose:	20 gram oil in the form of bread, cookie, soup and shortbread for 8 weeks, equal to 0.36 g/kg bw/day
GLP:	Not stated.

Study conduct

Two groups of 28 women, with an average age of 20 years, were either administered 20 g DAG-oil or TAG-based oil per day in the form of bread, cookies, soup and shortbread for 8 weeks. The total daily intake of fats and oils was restricted to 60 g/day.
Body size measurement and blood sampling were conducted at 0, 4 and 8 weeks in the test period after overnight fasting. Body fat measurement was conducted at 0 and 8 weeks. The following biochemical parameters were measured: serum triglyceride, total cholesterol, HDL cholesterol, Free fatty acid, total ketone body, acetoacetic acid, 3-hydroxybutyric acid, insulin, glucose, GPT, GOT, and γ-GPT.

Results

Mean total energy intake for both groups was approximately 1900 kcal/day and mean total fat intake, including test oils was 58 g/day. The average consumption of DAG-oil in the study was 0.36 g/kg bw/day (0.26-0.45 g/kg bw/day) and for TAG-based oil was 0.36 g/kg bw/day (0.30-0.42 g/kg bw/day).

No adverse effects in subjects were reported, although an extensive analysis of blood chemistry parameters (e.g. to determine liver and kidney function) was not undertaken.

No changes occurred in body weight between groups and during treatment. Serum triglyceride levels increased in both groups over time, however no differences were observed between treatments. Cholesterol levels differed between the groups prior to treatment, with the DAG-oil group having higher initial levels. In both groups the cholesterol levels decreased during treatment. Free fatty acids were significantly increased in both groups during treatment and were higher in the DAG-oil group in week 8. Total ketone, acetoacetic acid and 3-HBA levels generally increased over initial values during the course of the study in both groups, with no differences between treatments. Fasting blood sugar, serum GOT, GPT, γ-GPT did not change during the test period.

Conclusion

With regard to effectiveness, treatment of healthy female volunteers with DAG-oil at 0.36 g/kg bw/day for 8 weeks did not reduce body weight.

With regard to safety, this study demonstrated that normal healthy women appeared to tolerate doses of DAG-oil in bread, cookies, soup or shortbread at 0.36 g/kg bw/day over an 8-week period, without adverse effects.

12-week DAG-oil intake study in healthy male and female volunteers (Kobayashi, 2001)

Test material:	DAG-oil (0.77% monoglycerides, 84.21% diglycerides, 12.76 triglycerides)
Control material:	TAG-based oil (0.0 % monoglycerides, 4.76% diglycerides, 90.68% triglycerides; the fatty acid composition was comparable between TAG-based oil and DAG-oil)
Test groups:	45 subjects per group (working at the KAO plant).
Dose:	randomised double blind controlled parallel trial for 12 weeks. 0.5 g of test oil/kg body weight/day (consumed as a meal substitute from prepared box lunch, mayonnaise, bread and shortbread.
GLP:	Not stated.

Study conduct

In a double blind parallel controlled clinical study, 90 healthy subjects (45 males and 45 females) ranged 23-50 years were randomly assigned to TAG-based oil or DAG-oil treatment. Each group consumed approximately 0.5 g/kg bw/day of the test oil, consumed as a meal substitute from prepared box lunch, mayonnaise, bread and shortbread.

Prior to treatments, subjects received a complete physical examination, and fasting blood was collected for blood chemistry, haematology, and lipid profile. Blood was also collected after week 4, 8, 12 of treatment to measure blood chemistry (GOT, GPT, and gamma GTP, total ketone, acetoacetate, 3-HBA, total protein, albumin, T-bilirubin, LDH, alkaline phosphatase, uric acid, blood urea nitrogen, Na, K, Cl, Ca, IP, M, creatinine and CPK), haematology (WBC, RBC, hematocrit, haemoglobin, platelet) and lipid profile (total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides).

Furthermore, vital signs, anthropometrics, physical examination dietary analysis was performed throughout the study.

Results

Total caloric and fat intake was increased over the study period in both groups. This increase was considered due to the higher intake of test oil. Protein and carbohydrate intake remained constant during the test period, except for a decrease in total protein intake in female TAG-based oil group at the later half of the test period. No treatment related effects on vital signs, body weight, body fat ratio, BMI, waist and hip circumferences and blood pressure. Skin fold thickness decreased in both groups during treatment, with a slightly larger percentage decrease in the DAG-oil group (22 vs. 14 % decrease in males for DAG-oil and TAG-based oil respectively and 13 vs. 8% decrease in females for DAG-oil and TAG-based oil respectively). No treatment related effects on lipid profile were observed. No treatment related effects on liver function, production of ketosis were observed. Other blood chemistry parameters and haematology did not show any treatment related effects.

Conclusion

With regard to the effectiveness of the DAG-oil diet, there were no significant beneficial effects related to DAG-oil diet, except slight difference in skin fold thickness after 12 weeks between DAG-oil and TAG-based oil in both males and females, when the data were analysed as decrease in skin fold thickness. The study authors suggested that the reason for failure to detect significant difference to be attributed to the initial healthy conditions of the subjects.

With regard to safety, this study demonstrated that human subjects tolerated doses of DAG-oil in various products at a level of 0.5g/kg bw/day over a 12-week period.

12-week DAG-oil intake study in healthy male volunteers: effects on fat-soluble vitamin status (Watanabe, 2001)

Test material:	DAG-oil (<10% triglycerides (1,3-DAG and 1,2-DAG isomers at a ratio of 7:3); the fatty acid composition was comparable between TAG-based oil and DAG-oil)
Control material:	TAG-based oil
Test groups:	27 males aged 27-47. DAG-oil group n=15, TAG-based oil group n=12. (Randomised double blind controlled parallel trial).
Dose:	The subjects consumed 20 g of test oil/day, equal to 0.3 g/kg bw/day for 12 weeks, consumed as mayonnaise or an emulsion drink, once a day.
GLP:	Not stated.

Study conduct

The subjects were in good health as determined by medical history, physical examination, and clinical laboratory data. Men using vitamin supplements were excluded from the study. After sampling fasted blood for an initial value, subjects were randomised and 15 subjects ingested 20 g DAG-oil/day (equal to 0.3 g/kg bw/day) and 12 subjects ingested 20 g TAG-based oil/day (equal to 0.3 g/kg bw/day) for 12 weeks. All subjects ingested the mayonnaise or emulsion drink during lunch. At 4, 8 and 12 weeks fasting blood samples were drawn and serum concentrations of vitamins A, E and D were determined.

Furthermore, vital signs, anthropometrics, physical examination, and dietary analysis was performed throughout the study.

Results

Body weight, waist circumference, hip circumference and serum parameter changes indicative of test-food related effects were not observed during the study. There were no treatment related effects on vitamin A, D and E concentrations throughout the study period. The researches concluded that the absorption of fat-soluble vitamins had not been adversely affected.

Conclusion

With regard to effectiveness, treatment of healthy male volunteers with 0.3 g DAG-oil/kg bw/day for 12 weeks did not have any effects on body weight, waist and hip circumferences.

With regard to safety, this study showed that healthy males treated for 12 weeks with 0.3 g/kg bw/day did not have a changed vitamin A, D and E status compared to the control group. This study scanned only a limited set of variables, however no mention was made of carotenoids (pro-vitamins A), for example. It is not expected that ingestion of diacylglycerols will change the uptake of other fat-soluble vitamins.

16-week DAG-oil intake study in healthy male volunteers (Takei, 2001)

Test material:	DAG-oil (consisting 80-83% diglycerides)
Control material	TAG-based oil
Test groups:	20 or 23 healthy Japanese males per group (double blind parallel)
Dose:	9.95 g DAG or TAG-based oil/ day, equivalent to 0.15 g test oil/kg bw/day) for 16 weeks in 15 g mayonnaise*.
GLP:	Not stated

Study conduct

A double blind parallel trial with DAG-oil and TAG-based oil in the diet in the form of 15 g mayonnaise/day for 16 weeks was performed. The total oil intake/day including test food was determined to be 50 g ± 5 g. After a control period of 4 weeks, where TAG-based oil was ingested, the persons were randomly divided into 2 groups, using the visceral fat area at the initiation of the control period as an index. Somatometry, blood collection and abdominal CT scanning were performed at the time of the initiation of the test and after 0, 4, 8, 12, 16 weeks.

No parameters for liver and kidney function were examined. During the study exercise was not monitored, however, it was told to maintain the normal amount of exercise during the study period.

Results

When statistics were performed in changes compared to initial values, then there was a small decrease in body fat parameters in the DAG-oil group as compared to the TAG-based oil group. However, the absolute data did not indicate any improvement in body fat parameters. The free fatty acids decreased significantly during treatment of TAG-based oil as compared to the DAG-oil group (~12% vs. 0% decrease). Blood glucose was lower in the DAG-oil group compared to the TAG-based oil group (~2 vs. 0% respectively) and 3-hydroxybutyric acid did not change in the DAG-oil group during treatment, while decreased in the TAG-based oil group (+20 vs. –3% after 16 weeks, respectively).

Conclusion

With regard to effectiveness, treatment of 0.15 g/kg bw/day to healthy males did not improve body fat parameters.

With regard to safety, this study does not provide any information relevant to the safety assessment of DAG-oil, since no toxicity parameters were examined

16-week DAG-oil intake study in healthy male volunteers (Nagao, 2000)

Test material:	DAG-oil (1,2 diglycerides: 1,3 diglycerides 32:68, 83% diglycerides and 17% triglycerides)
Control material:	TAG-based oil (The fatty acid composition of TAG-based oil and DAG-oil were comparable)
Test groups:	38 subjects healthy men aged 27 to 49 years (BMI 21.7-27.1) (double blind controlled study).
Dose:	10 g/day over 16 weeks (equal to 0.14 g DAG-oil/kg bw/day and 0.15 g TAG-based oil/kg bw/day), consumed as bread, mayonnaise or short bread.
GLP:	Not stated.

Study conduct

Before treatment subjects were asked to consume 50 g of fat daily. After a control period of 4 weeks, all subjects underwent baseline measurements and were divided in the two groups. During the 16-week test period 10 g of the test oil/day was consumed as bread, mayonnaise or shortbread, while the total fat intake was aimed to be 50 g/day. During the testing period a diet diary was recorded; anthropometric measurements, body fat content and blood samples (lipids) were performed on week 0 and 16.

Results

The actual daily fat intake was 43 g. Body weight, BMI and waist circumference decreased in both groups. The change in body weight was significant greater in the DAG-oil group compared to TAG-based oil (2.6 vs. 1.1 kilo), however, since the initial body weight was higher in the DAG-oil group, this group was still heavier after the treatment period.

No treatment related effects were observed on blood parameters (TG, free fatty acids, total cholesterol, glucose, insulin, total ketone bodies).

Conclusion

With regard to effectiveness, very small decreases in body weight were observed during DAG-oil treatment.

With regard to safety, this study does not provide any information relevant to the safety assessment of DAG-oil, since toxicity parameters were not measured

12-month DAG-oil intake study in male and female volunteers (Katsuragi, 1999)

Test material:	DAG-oil (>80% diglycerides)
Test groups:	114 employees of Kao Corporation (average age 39.3 y, 95 males and 19 females)
Dose:	consumed in place of edible oils for 12 months.
GLP:	Not stated.

Study conduct

Subjects were employees of Kao Corporation and were taking DAG-oil as a replacement for edible oils for 12 months. Blood sampling (lipid, liver function etc) and anthropometric measurements were performed at 0, 3, 6, 9 and 12 months. At the same time diet diary were kept over three consecutive days every 3 months. This article was an English translation of a Japanese journal, and the applicant did not supply the figures.

Conclusions

The study is not acceptable for assessment of the safety and efficacy of DAG-oil, since this study did not include a control group.

Note: submission of figures would not change the conclusion.

12-week DAG-oil intake study in type II diabetic patients with hypertriglyceridemia (Yamamoto, 2001)

Test material:	DAG-oil (the commercially available Healthy Econa Cooking Oil (~80% diglycerides)
Control material:	TAG-based oil (the fatty acid were similar between TAG-based oil and DAG-oil)
Test groups:	16 diabetic patients, BMI 26.3. (Randomised single blind controlled parallel study)
Dose:	target dose 10 g diglycerides/day, consumed as cooking oil, for 12 weeks (equal to 0.22 g DAG-oil/kg bw/day)
GLP:	Not stated.

Study conduct

Sixteen diabetic patients with a mean body mass index of 26.3 kg/m^2 were randomly assigned to the DAG-oil or TAG-based oil group in a randomised single blind controlled parallel study for 12 weeks. The target dose of the DAG-oil was 10 g/day. The DAG-oil group consisted of 3 males and 5 females, while the TAG-based oil group consisted of 4 males and 4 females.

Dietary records, blood sampling and anthropometric measurements were performed during week 0 and 12. Clinical effects were not recorded.

Results

No significant changes from the initial values in body weight, body mass index, and energy intake was observed in either group. The serum triglyceride level in the DAG-oil group decreased during treatment and differed significantly compared to the control group (1.52 vs. 3.59 mmol/L in the DAG-oil and control group, respectively). There were no treatment related changes in blood sugar levels. In the DAG-oil group the glycohaemoglobin A_{1c} levels significantly decreased (9.7%) during treatment, however there was no significant difference between the control and DAG-oil group after 12 weeks.

Conclusion

With regard to the effectiveness, serum triglyceride levels were significantly decreased in 8 diabetic patients after 12 weeks of treatment with 0.22 g DAG-oil/kg bw/day.

With regard to safety, since toxicity parameters were not measured, this study does not provide any information relevant to the safety assessment of DAG-oil.

3-month DAG-oil intake study in patients with haemodialysis treatment (Teramoto, 2000)

Test material:	DAG-oil
Test groups:	10 outpatients with regular haemodialysis, with IIb or IV type of hyperlipidemia.
Dose	9 g DAG-oil/ day, as cooking oil for 3 months in place of the conventional oil, with a 3 month was out period..
GLP:	Not stated.

Study conduct

Ten outpatients with regular haemodialysis with type IIb or IV hyperlipidemia received DAG-oil as cooking oil for 3 months in place of conventional oil. Three months after DAG-oil treatment was finished, the recovery was measured. Subjects were instructed not to change dietary habits.

Fasting blood samples (lipids) were collected prior to haemodialysis treatment every month until 2 month after DAG-oil treatment finished. Body fat was measured before, and the end of DAG-oil treatment and 3 months after DAG-oil treatment finished.

Results

A decrease in visceral fat and improvement of lipid profile was observed during DAG-oil treatment, compared to pre-treatment.

Conclusion

This study did not provide any information relevant to the safety assessment of DAG-oil.

5-month DAG-oil intake study in children with obesity and / or hyperlipidemia (Matsuyama, 2000)

Test material:	DAG-oil (17% triglycerides, 24.9% 1,2-diglycerides, 58.1% 1,3-diglycerides)
Test groups:	13 obese and / or hyperlipidemia children aged 7-17 years
Dose:	ad libitum in the form of cooking oil, average dose 11.3 g DAG-oil/day for 5 months,
GLP:	Not stated.

Study conduct

DAG-oil was administered as cooking oil to male and female children outpatients with hyperlipidemia or obesity for 5 months. Diet contents, blood sampling (lipids) and body size measurements were determined before testing and monthly during the study period. A control group was not included. Clinical effects of DAG-oil treatment were not reported.

Results

Ten of the thirteen children gained body weight; while in two children a small body weight decrease was observed. There was a high correlation between body weight and total fat amount.

Conclusion
This study did not provide any information relevant to the safety assessment of DAG-oil.

24-week DAG-oil intake study in overweight male and female volunteers (Maki, 2002)

Test material:	DAG-oil (~90% diglycerides, ration of 1,2 DG: 1,3-DG was 3:7)
Control material:	TAG-based oil (prepared from a mixture of rapeseed, soybean and safflower oils; the fatty acid content was comparable between DAG and TAG-based oil)
Test groups:	Overweight or obese men (waist circumference >90 cm) and women (waist circumference >87 cm), 65 persons (25 m/40 f) started DAG-oil and 66 persons (27 m/39 f) started TAG-based oil treatment.

Dose:	The test oil was consumed as muffins, crackers, soup, cookies, and granola bars into a reduced energy diet (2100-3350 kJ/day deficit) for 24 weeks. It was aimed to achieve 15% of the total energy in the diet from test oil. The intake of DAG-oil was 0.29 g/kg bw/day. .
GLP:	Not stated.

Study conduct

In a 1-4 week period before treatment overweight or obese men (waist circumference >90 cm) and women (waist circumference >87 cm). underwent a physical examination and a detailed medical history was taken and anthropometric measurements were assessed. Various criteria for inclusion / exclusion in the study were ascertained. Subjects incorporated various food products containing either DAG-oil or TAG-based oil into their diets during the trial (muffins, crackers, instant soup mix, cookies and granola bars) for a 24-week period. Test foods were substituted for normal foods in such a way that ~15% of the energy intake was from the test oil. Based on the estimate of initial energy needs, a diet was prescribed aiming to induce an energy deficit of 2100-3350 kJ/day. The DAG-oil intake was 0.29 g/kg bw/day.

A physical examination and blood chemistry analysis was performed pre-treatment and at various times during treatment.

One hundred and thirty-one subjects human volunteers (males and females aged 19 to 71 years old) with a body mass index of ~34 kg/m^2 started the study. 79 subjects completed the study. Reasons for non-completion included withdrawal of consent, adverse events or other reasons.

Study periods consisted of a screening/run in phase (1-4 weeks), and active treatment (24 weeks). At 2, 4, 6, 8, 12, 16, 20 and 24 weeks patients underwent a clinical assessment and 3-day diet records were collected. Fasting serum samples were collected at week 24 for serum clinical chemistry (sodium, potassium, chloride, CO2, glucose, BUN, creatinine, uric acid, calcium, phosphorus, alkaline phosphatase, albumin, total bilirubin, SGOT, SGPT, GGT, LDH, creatine kinase, magnesium) and haematology analyses (WBC, RBC, haemoglobin, haematocrit, MCV, MCH, MCHC, platelets, neutrophils, lymphocytes, monocytes, eosinophils, basophils). At weeks 12 and 24 samples were collected for urinalysis (specific gravity and pH) and a urine pregnancy test for women with childbearing potential. At week 4, 12 and 24 a 7-day physical activity recall questionnaire was completed.

Results

A thorough critical evaluation of the efficacy of treatment was not undertaken by FSANZ. However, as an overall conclusion at 0.29 g DAG-oil/kg bw/day, the change in body weight after 24 week treatment was 3.5% and 2.5% for DAG-oil and TAG-based oil, respectively (~3.4 and ~2.4 kg decrease in body weight for DAG-oil and TAG-based oil, respectively). The change in fat mass decreased over time in both treatment groups, which was more pronounced in the DAG-oil group (8 vs. 5.5% or 35.4 to 32.6 kg vs. 34.7 to 32.8 kg in DAG-oil vs. TAG-based oil, respectively).

In the DAG-oil group 22 persons did not complete the study and in the TAG-based oil group 30 persons did not finish the study, reasons included withdrawal of consent, non-compliance, adverse events and other reasons.

Therefore, 43 persons finished DAG-oil treatment and 36 persons finished TAG-based oil treatment. During the study adverse events were generally few and the most common adverse events were upper respiratory infections (n=29), headaches (n=22), and gastrointestinal complaints (n=54). There were no significant differences between the groups in any adverse events reported. Most events were regarded as unrelated to treatment, however some gastrointestinal system complaints (n=26) and one instance each of headache, upper respiratory infection, acne, and rash were judged to be possibly related. Only 2 subjects reported serious adverse events during the trial. One subject had a cholecystectomy and dropped out of the study. The other subject had a urinary tract infection, which was treated, but remained in the study.

No significant differences were noted in any of the clinical chemistry, haematology and urinalysis parameters between placebo and treatment groups.

Conclusion

With regard to effectiveness, DAG-oil reduced body weight and fat mass slightly more than control treatment.

With regard to safety, this study demonstrated that obese males and females tolerated doses of DAG-oil in muffins, crackers, soup, cookies, and granola bars at 0.29 g/kg bw/day over a 24-week period, with no treatment-related changes to clinical parameters.

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APPENDIX 1

LABORATORY INVESTIGATION PARAMETERS
28 study in rats

Haematology	**Clinical Chemistry**	**Urinalysis**
Activated partial thromboplastin time (APTT) Erythrocyte count (RBC) Haematocrit (Hct) Haemoglobin (Hb) Leucocyte count (WBC) Leucocyte differential Mean corpuscular haemoglobin (MCH) Mean corpuscular haemoglobin concentration (MCHC) Mean corpuscular volume (MCV) Platelet count Prothrombin time Reticulocyte count (incl. absolute)	Alanine aminotransferase (ALT, GPT) Albumin Albumin/globulin ratio Alkaline phosphatase (ALK P) Aspartate aminotransferase (AST) Bilirubin (total) Calcium Chloride Cholesterol Creatinine Gamma glutamyltransferase Globulin Glucose Inorganic phosphorus Potassium Protein (total) Sodium Triglycerides Urea nitrogen	Appearance Bilirubin Glucose Ketones Occult blood Osmolality pH Protein Reducing substances Sediment Specific gravity Urobilinogin Volume
Organs Weighed	**Tissues Examined Microscopically**	
Adrenals Brain Heart Kidneys Liver Lungs Ovaries Pituitary Prostate Seminal vesicles Spleen Submaxillary gland Testes (with epididymides) Thymus Thyroid (with parathyroids) Uterus	Adrenals Bone marrow (femur) Brain (3 levels) Caecum Colon Duodenum Epididymes Eyes with optic nerve Harderian gland Heart Ileum Jejunum Kidneys Liver Lungs and bronchi Mammary gland Mesenteric lymph node Oesophagus Ovaries	Pancreas Pituitary Prostate Rectum Seminal vesicle Spinal cord Spleen Stomach Submaxillary gland Testes Thymus Thyroid with parathyroids Tongue Trachea Urinary bladder Uterus Vagina Tissues with gross lesions

long-term study in rats

Haematology	**Clinical Chemistry**	**Urinalysis**
Activated partial thromboplastin time (APTT) Erythrocyte count (RBC) Fibrogen concentration Haematocrit (Hct) Haemoglobin (Hb) Leucocyte count (WBC) Leucocyte differential Mean corpuscular haemoglobin (MCH) Mean corpuscular haemoglobin concentration (MCHC) Mean corpuscular volume (MCV) Platelet count Prothrombin time Reticulocyte count	Alanine aminotransferase (ALT, GPT) Bilirubin (total) Calcium Chloride Cholesterol (free and total) Creatinine Free fatty acids Glucose GOT Insulin Lactate dehydrogenase Lipoprotein fraction Phospholipids Potassium Protein (total) Sodium Triglycerides Urea nitrogen	Appearance Bilirubin Chloride Glucose Ketones Occult blood pH Potassium Protein Sediment Sodium Specific gravity Urobilinogin Volume

Organs Weighed	**Tissues Examined Microscopically**	
Adrenals Brain Heart Kidneys Liver Lungs Ovaries Pituitary Prostate Salivary gland Spleen Testes (with epididymides) Thymus Thyroid Uterus	Adrenals Aorta Bone marrow (femur) Brain (3 levels) Caecum Clitoral gland Coagulating gland Colon Duodenum Epididymes External ear Eyes with optic nerve Femoral muscles Harderian gland Heart Ileum Jejunum Kidneys Larynx Liver Lungs and bronchi Lymph nodes Mammary gland Nasal cavity	Oesophagus Ovaries Pancreas Pharynx Pituitary Preputial gland Prostate Rectum Salivary glands Seminal vesicle Skin Spinal cord Spleen Stomach Submaxillary gland Testes Thymus Thyroid with parathyroids Tongue Trachea Urinary bladder Uterus Vagina Tissues with gross lesions

one-year study in dogs

Haematology	**Clinical Chemistry**	**Urinalysis**
Activated partial thromboplastin time (APTT) Erythrocyte count (RBC) Haematocrit (Hct) Haemoglobin (Hb) Leucocyte count (WBC) Leucocyte differential Mean corpuscular haemoglobin (MCH) Mean corpuscular haemoglobin concentration (MCHC) Mean corpuscular volume (MCV) Platelet count Prothrombin time Red cell morphology	Alanine aminotransferase (ALT, GPT) Albumin Albumin/globulin ratio Alkaline phosphatase (ALK P) Aspartate aminotransferase (AST) Beta hydroxyl butyrate Bilirubin (total) Calcium Chloride Cholesterol Creatine kinase Creatinine Gamma glutamyltransferase Globulin Glucose Inorganic phosphorus Potassium Protein (total) Sodium Triglycerides Urea nitrogen	Appearance Bilirubin Color Glucose Ketones Leucocytes Nitrates Occult blood Osmolality pH Protein Reducing substances Sediment Specific gravity Urobilinogin Volume
Organs Weighed	**Tissues Examined Microscopically**	
Adrenals Brain Kidneys Liver Ovaries Testes (with epididymides) Thyroid (with parathyroids)	Adrenals Aorta Bone marrow (femur) Brain (3 levels) Caecum Colon Duodenum Epididymes Eyes with optic nerve Gall bladder Heart Ileum Jejunum Kidneys Liver Lungs and bronchi Mammary gland Lymph node (mandibular and mesenteric) Oesophagus Ovaries	Pancreas Pituitary Prostate Rectum Salivary gland Sciatic nerve Seminal vesicle Skeletal muscle Skin Spinal cord Spleen Stomach Testes Thymus Thyroid with parathyroids Tongue Trachea Urinary bladder Uterus Vagina Tissues with gross lesions

Attachment 3

Nutrition Assessment of Diacylglycerol Oil

EXECUTIVE SUMMARY

This nutrition assessment has been undertaken with the aim of addressing the nutritional issues of relevance to the inclusion of DAG-oil in foods. Therefore, this assessment reviews:

- the nutritional impact of DAG-oil by assessing the physiology of DAG-oil digestion;
- the contribution of DAG-oil to energy metabolism;
- the consequences for developing chronic lifestyle illnesses; and
- the potential changes to micronutrient bioavailabilities.

The findings of the nutrition assessment demonstrate that DAG-oil does not have any nutritional attributes that are substantially different from TAG-based oil. There is evidence of an increased level of β-oxidation associated with DAG-oil intake; however, the body's homeostatic processes appear to accommodate this change, resulting in the same level of energy expenditure, fat storage and fat excretion from the intake of DAG-oil as occurs with the intake of TAG-based oil.

The nutrition assessment also identifies a decrease serum TAG levels over time with DAG-oil consumption. However, the influence of DAG-oil on other risk factors linked to the development of chronic diseases appears to be limited, and at best, only marginally greater than the influence provided by TAG-based oil of similar fatty acid composition.

It is concluded that although DAG-oil has no demonstrated nutritional benefits, there is also no evidence to indicate an adverse impact on population nutrition, thus DAG-oil can be considered nutritionally adequate for general consumption.

INTRODUCTION

Application A505 has been raised to assess whether diacylglycerol/s (DAG) should be permitted as novel ingredients in the food supply. The DAG-oils of primary focus are those composed of 1,3-diacylglycerols (1,3-DAG) and 1,2-diacylglycerols (1,2-DAG) in the ratio of 7:3.

Because DAG are a form of fat, a comparison with the main form of dietary fat – triacylglycerol/s (TAG) – is required to determine whether the dietary substitution of TAG-based oils with DAG-oil will significantly alter the health and nutritional status of Australian and New Zealand populations. This assessment will review:

- the nutritional impact of DAG-oil by assessing the physiology of DAG digestion;
- the contribution of DAG to energy metabolism;
- the consequences for developing chronic lifestyle illnesses; and
- the potential changes to micronutrient bioavailabilities.

Some details on scientific studies cited in this report is located in Appendices 1, 2 and 3 of this Attachment, where the volume of information made it impractical for placement in the main body of Attachment 3.

The Scope of Application A505

- The Applicant has requested that claims should be permitted on the labels of foods containing DAG-oil, specifically for foods containing the ENOVA oil product. These claims relate to the promotion of DAG-oil as contributing to weight loss, and to improving blood lipid profiles. However, as health claims are prohibited within the Code, the Applicant's request to make such claims will not be considered in this nutrition assessment. The information relating to weight loss and blood lipids will, however, be incorporated into assessments of the various nutritional issues.

PHYSIOLOGY OF DIACYLGYCEROL DIGESTION

The Applicant reports that the physiology of DAG digestion is comparable to that of monoacylglycerol/s (MAG) and TAG, with energy values and digestibility coefficients equivalent to TAG of similar fatty acid composition. It is also mentioned that because DAG-oil produces a high level of free fatty acids in the portal vein following a meal when compared to TAG-based oils, they are used more as a direct source of energy by the body and therefore contribute less to fat stores.

To verify and assess the Applicant's position, scientific literature on the intestinal processes of digestion and absorption of DAG, and fat in general, has been reviewed.

Breakdown of Fat in the Intestinal Lumen

TAG and DAG that are found naturally in food are digested predominantly into MAG containing a single fatty acid bound to the sn-2 position[1]; TAG and DAG are not absorbed by the intestineas digestive processes cannot transport these forms of fat through the intestinal wall[2].

Usually, a small proportion of the total digested fat consists of MAG that do not have a fatty acid bound to the sn-2 position. However, discussion in available literature[3,4] and results from a rat study[5] indicate that because DAG lack a fatty acid at the sn-2 position, a meal incorporating DAG-oil will instead produce digestive by-products that predominantly consist of MAG with a fatty acid attached to the sn-1 position (1-MAG). The Applicant has argued that the increased production of 1-MAG by DAG-oil compared to TAG-based oil has significant physiological and metabolic implications, including an impact on blood lipids, and energy and weight balance.

Intestinal Absorption

Upon absorption into the cells that line the intestinal wall, fatty acids of length C>12 (either as part of MAG or in free form) are resynthesised into TAG. These TAG are then combined with lipoproteins and other lipid substances to form chylomicrons, which are placed into the lymph for circulation around the body. Fatty acids of length C<12 are released into the portal vein as MAG or free fatty acids bound to albumin, and travel directly to the liver[6]. It is well established that the different absorption pathways for fatty acids of various lengths is due to their water solubility; fatty acids of lengths C<12 are more water soluble and can be readily placed into the blood, while other fatty acids need to be included in lipoprotein structures before they can be transported[1].

The Applicant has provided some data, which indicates that DAG-oil consumption alters the typical passage of fats through the intestinal wall. As detailed in Table 1 below, a rat study by Watanabe *et al*[4] has found that following a dose of 10g DAG-oil comprised solely of fatty acids with a length C>12, a greater proportion of absorbed fat was placed into the portal vein as MAG and free fatty acids when compared to the intake of TAG-based oils at a similar dose and fatty acid composition. Watanabe *et al* did not indicate if these results were statistically significant, however the large variation between DAG-oil and TAG-based oil results suggest that this is the case.

Table 1: Changes in Portal Vein TAG and free fatty acid levels following ingestion of either DAG-oil or TAG-based oil (study by Watanabe et al[4])

Study Period	Serum TAG Levels		Serum Free Fatty Acids	
	DAG Group	TAG Group	DAG Group	TAG Group
30 min	0.18 mg/mL	0.30 mg/mL	62 μg/mL	22 μg/mL
42 min	0.16 mg/mL	0.40 mg/mL	50 μg/mL	18 μg/mL
60 min	0.22 mg/mL	0.60 mg/mL	41 μg/mL	19 μg/mL
102 min	0.40 mg/mL	0.42 mg/mL	24 μg/mL	17 μg/mL
198 min	0.50 mg/mL	0.85 mg/mL	26 μg/mL	19 μg/mL

1. Values in this table are estimates, as results were only supplied in a column graph format.
2. Each group contained 4 rats (13 weeks old).

There is some debate in the scientific literature as to whether an increased intake of DAG-oil directly results in a postprandial increase of fatty acid and MAG levels in the portal vein, and if so, the means by which this increase is achieved. There are hypotheses stating that a reason for the observed changes in portal vein levels may be due to the unusual 1-MAG digestive by-product entering an unidentified metabolic pathway after absorption through the intestinal wall[8,9]. Other commentators have alternatively suggested that chylomicron assembly is impaired because DAG-oil consumption reduces the amount of 2-MAG available for absorption through the intestinal wall.

With a lower level of available 2-MAG, it is argued that there is a reduction in the intestinal wall's metabolic activity that places TAG into chylomicrons, and thus inadvertently results in more free fatty acids entering the portal vein. This argument is supported by several studies which indicate that chylomicron activity may indeed be influenced by DAG-oil intake[3,10,11] (see Tables 2 and 3 below). However, the results in these studies were obtained from indirect measurements of chylomicron activity (e.g. analysis of remnant-like proteins, liver TAG levels or chylomicron TAG fractions); no studies have been identified that directly measure chylomicron levels following DAG-oil consumption.

Several authors have alternatively suggested that a postprandial reduction in the availability of 2-MAG may precipitate a higher level of *de novo* TAG production in the intestinal wall from absorbed free fatty acids, with their subsequent placement into chylomicrons for transport[12,13]. It has been also been argued that while there is a good understanding of the crucial role 2-MAG plays in triglyceride resynthesis and chylomicron assembly, the actual contribution of 1-MAG to these metabolic processes remains unclear[14].

Table 2: Changes to chylomicron levels following the ingestion of DAG-oil or TAG-based oil

Study Variable	Test Oil	Time of Blood Sampling (hours)						Area under the Curve (AUC) (mmol/L/hour)	Significant Difference ($p<0.05$)?
		0	2	3	4	6	8		
Tada *et al* (2001)[3]									
Cholesterol in serum remnant-like proteins (mg/dL)	DAG	0.14 ±0.1	0.19 ±0.1	0.23 ±0.1	0.27 ±0.2	0.23 ±0.2	0.12 ±0.1	1.63±1.1	Yes – at hours 2,3 and 8 No - AUC
	TAG	0.13 ±0.1	0.25 ±0.1	0.30 ±0.1	0.32 ±0.2	0.27 ±0.2	0.14 ±0.1	1.97±1.0	
TAG in serum remnant-like proteins (mg/dL)	DAG	0.24 ±0.2	0.61 ±0.5	1.03 ±0.8	1.16 ±0.8	0.96 ±1.1	0.26 ±0.3	0.76±0.6	Yes – AUC No – for each time sample
	TAG	0.22 ±0.1	1.10 ±0.6	1.57 ±1.1	1.70 ±1.4	1.08 ±0.9	0.30 ±0.3	1.06±0.6	
Taguchi *et al* (2000)[11]									
Chylomicron cholesterol fraction (mmol/L)	DAG	0.001	-	-	0.02	-	0.001	-	No
	TAG	0.001	-	-	0.07	-	0.1	-	
Chylomicron TAG fraction (mmol/L)	DAG	0.01	-	-	0.3	-	0.01	-	Yes at 4-hour sample time
	TAG	0.01	-	-	0.7	-	0.03	-	
Chylomicron phospholipid fraction (mmol/L)	DAG	0.001	-	-	0.03	-	0.003	-	No
	TAG	0.001	-	-	0.08	-	0.003	-	

Table 3: Serum and Liver TAG results used to indirectly assess changes to chylomicron levels from ingestion of DAG-oil or TAG-based oil (Experiment 1 of Hara et al, 1993[10])

Study diet	Serum TAG, Day 17 (mg/mL)	Serum TAG, Day 34 (mg/mL)	Liver TAG, Day 34 (mg/g)	Significant Difference ($p<0.05$)?
TAG	1.61±0.08	2.48±0.3	24.3±0.9	Yes – between DAG and TAG groups for TAG at days 17 and 34 No – liver TAG
DAG	1.13±0.07	1.77±0.13	23.5±0.0	

Summary

The scientific literature provides support for the Applicant's argument that DAG-oil intake results in the digestive production of 1-MAG instead of the typical 2-MAG that occurs from the digestion of TAG-based oils.

From a study on rats, an increase in 1-MAG has been shown to produce a subsequent postprandial increase in free fatty acid and MAG portal vein levels. Although there are some conflicting views within the scientific community as to the validity of this outcome, none of the alternative arguments have been thoroughly tested, and there is additional indirect evidence in support of the observed results.

Therefore, at the present time, it can be established that the intake of DAG-oil results in an increased placement of free fatty acids and MAG into the portal vein, with subsequent implications for further metabolic processes.

THE CONTRIBUTION OF DIACYLGLYCEROLS TO ENERGY METABOLISM

It is well documented that the liver directly takes up MAG and fatty acids that enter the portal vein after intestinal absorption. In times of energy expenditure, the liver can use these substances for β-oxidation, a process that allows fat to be converted into acetyl-CoA for use as an energy source[15].

The Applicant has mentioned that by increasing the postprandial placement of free fatty acids and MAG into the portal vein, DAG-oil intake compared to TAG-based oil intake result in more fatty acids bypassing the lipoprotein transport processes resulting in an increased level of β-oxidation. It is stated that an increase in the rate of β-oxidation increases the amount of energy expended by the body with the result of a decrease in the amount of fat stored.

In considering the argument made by the Applicant, several key aspects of fat and energy metabolism have been reviewed:

- the amount of energy available to the body from DAG-oil (its energy value);
- energy loss, through energy expenditure and the production of ketones; and
- overall energy balance and weight management.

The Energy Value of Diacylglcerols

The Applicant has cited a rat study by Taguchi *et al*[8] as the basis for the argument that DAG-oil contributes the same amount of energy to the human body as TAG-based oils, and that the energy value for DAG-oil is therefore consistent with the generic 37 kJ/g energy value attributed to fat. This study has also been used to support the position that the digestibility of DAG-oil is comparable to TAG-based oils, and therefore the amount of fat excreted into the faeces does not increase with DAG-oil consumption.

Although the Applicant is not seeking the calculation of an energy value specific to DAG-oil, the guidelines[16] established by FSANZ for undertaking such calculations can be used to assess the quality of the cited study.

In applying these guidelines, the study met all of the criteria for an acceptable piece of scientific literature, except for the elimination of coprophagy in rats, and the type of funding arrangements that were made.

The study by Taguchi *et al* involved the feeding of DAG-oil to rats at a dose of 20g/100g of feed, and included the use of a bomb calorimeter for energy content assessments. The study indicates that DAG:

- have a similar energy content as TAG (38.9 kJ/g for DAG and 39.6 kJ/g for TAG),
- do not significantly alter the mean body weight of rats when compared to TAG (DAG group = 173.9$\pm$2.1g and TAG group = 174.5$\pm$2.2g as initial weights; DAG = 289.1$\pm$4.4g and TAG = 283.8$\pm$3.9g as final weights),
- do not significantly increase the excretion of fat into the faeces of rats over that of TAG (3.7% for both DAG and TAG).

A detailed review of the study by Taguchi *et al* can be found in Appendix 1 to this Attachment. The Applicant has supplied no other studies on the energy value of DAG-oil, nor has FSANZ identified any additional studies in this area. Therefore on the basis of this material, it is assessed that the energy value and digestibility of DAG-oil is comparable to that of TAG-based oils.

Energy Expenditure

Only one study by Kamphuis *et al*[9] has directly assessed the impact of DAG-oil intake on energy expenditure in humans. This study was conducted as a single-blind, controlled, crossover trial using a respiratory chamber managed under strict metabolic conditions, and was designed to observe the contribution of fat and carbohydrate to the body's energy expenditure following the consumption of DAG-oil versus TAG-based oils. The results showed a significant increase in the β-oxidation of fat with a substitution of TAG-based oils by DAG-oil (substitution at 40% of fat intake over 36 hours). The results also show that the overall expenditure of energy by the body is the same for this amount of DAG-oil intake as that observed with a similar TAG-based oil intake. A detailed review of Kamphuis *et al* can be found in Appendix 1 to this Attachment.

The results of Kamphuis *et al* are supported by another trial conducted on rats[17], which has demonstrated that β-oxidation rates increase with DAG-oil intake compared to TAG-based oil intake, although this study did not assess the impact of DAG-oil intake on overall energy expenditure *per se*.

On the basis that overall energy expenditure did not alter in the study by Kamphuis *et al*, it can be concluded that DAG-oil does not influence the level at which energy is utilised by the body any differently than TAG-based oils. Instead, it is likely that they produced a change in the body's system of managing its various energy sources. The liver is capable of using an increase in portal vein MAG and free fatty acids resulting from DAG-oil consumption for direct energy expenditure, while directing other energy sources – such as carbohydrate – into fat synthesis and fat storage to maintain an energy balance within the body.

Ketone Production

It is possible for fatty acids to be converted into ketones – an alternative source of energy – instead of being stored as fat or used in β-oxidation. Ketones may also be excreted into the urine, a process that represents the main method by which fat can be removed from the human body instead of contributing to overall energy expenditure or storage[18].

The important role that ketones play in the energy metabolism of fat has encouraged a number of human and animal DAG-oil studies to include an evaluation of circulating blood and urinary ketones levels[4,19-22]. A summary of the results from all of these studies can be found in Table 4 of Appendix 2 to this Attachment. The majority of these studies indicate that with the consumption of DAG-oil, ketone levels remain comparable to those observed when fat intake is comprised solely of TAG-based oils. The only study to report a significant change in ketone levels is that conducted by Watanabe *et al*[4], where an increase in urinary ketone levels was observed following the consumption of DAG-oil by rats at a dose of 20g/100g feed for six days.

Although Watanabe *et al* reported a change in ketone levels; the totality of evidence, particularly in humans, indicates that these levels remain unaltered following the intake of DAG-oil. It is therefore surmised that DAG-oil consumption does not produce a significantly higher excretion of energy via ketone production when compared to the same amount of TAG-based oil included in the diet.

Energy and Weight Balance

A simple yet well-supported model of energy and weight balance[23,] can be viewed as:

Change in stored energy (weight) = Energy Intake – Energy Expenditure

This balance is controlled within the human body to a remarkably accurate degree, with evidence showing that the balance is maintained within a 2% margin of error[18]. Clearly then, if there is no change in the energy intake from DAG-oil, energy intake from other food sources, and the body's expenditure of energy; there is unlikely to be a difference in the rate at which body weight is lost or gained. However, the Applicant has taken both the position that DAG-oil supplies an equivalent amount of energy to TAG-based oils, and that DAG-oil can also contribute to a reduction in weight and fat mass.

To clarify the exact relationship of DAG-oil to weight balance, five studies have been identified that discuss the influence of DAG-oil on weight and fat storage in humans[19-22,24]. A summary of the results of these studies can be found in Table 5 of Appendix 2 to this Attachment. Three of the five studies indicate that the replacement of TAG-based oils with DAG-oil can promote a decrease in body weight[21,22,24], while two studies reported no alteration in weight as a result of such a dietary change[19,20].

However, none of the five studies have been assessed as producing reliable results because either:

- there was no of monitoring of any change in physical activity levels, or
- an assessment of dietary energy intake was incorrectly administered or did not occur altogether, or

- there was no assessment of changes to the body mass index (BMI) or to the waist-to-hip ratio (WHR), which would ensure that the physical variations of the subject populations did not influence the reported changes in weight.

One animal study[5] has also been identified that mentions a decrease in weight and fat mass in mice resulting from DAG-oil consumption. However as with human trials, this study did not control for the level of physical activity, and did not therefore control for changes in energy expenditure.

By assessing the available scientific studies on DAG-oil and weight, it would appear that there is insufficient evidence to support or refute the argument that DAG-oil intake promotes a change in the weight balance of humans when there is control over the energy intake from other food sources or control of energy expenditure patterns.

Summary

The contribution of DAG-oil to energy metabolism is very similar to that made by TAG-based oils. There is insufficient evidence to indicate whether DAG-oil consumption can actually produce a change in the body weight of either animals or humans, however other studies indicate that DAG-oil consumption results in a similar intake of energy, loss of energy, and maintenance of energy balance in the human body as occurs with TAG-based oil consumption. There does not appear, therefore, to be adequate support for the Applicant's argument that DAG-oil consumption results in changes to overall energy and weight balance through an increase in the rate of β-oxidation.

The Impact on Risk Factors for Chronic Disease

As discussed earlier, the production of 1-MAG from DAG-oil consumption has the potential to alter blood lipid profiles, particularly those in the portal vein. Such haematological changes could have ramifications for population health, as changes in blood lipids (and the associated impacts on blood glucose management) can influence the risk of developing chronic diseases such as diabetes and cardiovascular disease.

Ten studies[3,4,5,7,10,17,19,20,25] have been identified that assess the impact of DAG-oil consumption on serum blood lipids at doses ranging from 2.82-15% of feed intake (rats), 0.22 g/kg body weight/day (humans), and 30 g/m^2 of body surface area (humans). The lipid results from two of the 10 studies[5,7] were excluded from further consideration due to the use of non-fasted subjects in blood sampling. A summary of the results from the remaining eight studies can be found in Tables 6-1 and 6-2 of Appendix 2 to this Attachment.

Of the eight viable studies measuring serum lipid levels, all included an observation of changes in serum TAG levels, with five of these studies reporting a significant decrease over the course of the relevant study periods (198-480 minutes in acute studies, and 14-84 days in long-term studies). The reported outcomes for other blood lipid biomarkers are not, however, as consistent between the seven studies; both significant and non-significant outcomes are reported for decreases in serum cholesterol, serum low-density lipoprotein (LDL), liver TAG levels and for an increase in serum high-density lipoprotein (HDL) levels.

Four studies[5,7,20,25] have been identified that observed changes in blood glucose variables. The results of these studies (summarised in Tables 7-1 and 7-2 of Appendix 2 to this Attachment) reported conflicting results over long-term intervention periods ranging from seven weeks to eight months. One study reported a decrease in blood glucose and insulin levels from DAG-oil consumption compared to TAG-based oil consumption[5], two studies reported no difference between intervention groups[20,25], and one study reported a significant increase in blood glucose levels following DAG-oil intake compared to TAG-based oil intake[7]. Two of the four studies[20,25] also assessed the impact of DAG-oil intake on glycosylated haemoglobin (HbA1c) levels and – consistent with their results on blood glucose levels – reported no significant difference between DAG and TAG groups.

Except for the study conducted by Kobayashi *et al*[20], all of the studies that examine the impact of DAG-oil on blood cholesterol levels and/or blood glucose levels do not discuss the reasons as to why their results may be inconsistent with each other. Kobayashi *et al* attributed the lack of any significant change in their study variables to the initial healthy condition of the subject populations.

Summary

There is some evidence indicating that DAG-oil intake can reduce blood TAG levels over a period of time. However, due to the high degree of inconsistency within the available literature for results on other blood lipid and blood glucose parameters, there is little support for the view that DAG-oil intake can influence (either positively or negatively) the main haematological risk factors identified with the development of major chronic diseases. Overall, the influence of DAG-oil intake on the risk factors for chronic disease appears to be similar to that attributed with the intake of TAG-based oils of similar fatty acid composition.

The Impact on Micronutrient Bioavailability

Several substances have been previously developed that were capable of reducing the contribution of fat intake to energy metabolism. However, the consumption of these substances in the diet has also been associated with a significant impairment in the intestinal absorption of fat-soluble vitamins[26,27].

There is limited evidence in one study by Watanabe *et al*[28] suggesting that the absorption of fat-soluble vitamins is not impaired by the consumption of DAG-oil. This study was conducted over a 12-week intervention period using a dose of 20 g of test oil/day, and reported that serum levels of vitamins A, D, and E did not significantly vary with the consumption of either DAG-oil or TAG-based oil. This outcome is likely to be due to the minimal influence that DAG-oil has on gastrointestinal absorption processes, while the opposite effect has been reported with the consumption of other low energy contributing fat substances. A detailed review of this study can be found in Appendix 2 to this Attachment.

CONCLUSION

The currently available evidence provides strong support for the argument that DAG-oil does not have any nutritional attributes that are substantially different from TAG-based oils. There is literature reporting an increased level of β-oxidation with DAG-oil intake; however, the body's homeostatic processes appear to accommodate this change, resulting in the same level of energy expenditure, fat storage and fat excretion from the intake of DAG-oil as occurs with the intake of TAG-based oils.

DAG-oil consumption has been observed to decrease serum TAG levels over time. Overall though, the influence of DAG-oil on the development of chronic disease appears to be limited, and at best, only marginally greater than the influence provided by TAG-based oils of similar fatty acid composition.

From a nutritional perspective, although DAG-oil has no demonstrated nutritional benefits, there is also no evidence to indicate an adverse impact on population nutrition, and therefore DAG-oil can be considered nutritionally adequate for general consumption.

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APPENDIX 2 TO ATTACHMENT 3

Key Studies Cited Within the Nutrition Assessment Report

Of the references cited in this nutrition assessment, two are of particular significance. Because of the relevance of the studies' findings, both have are summarised in detail below.

For the purposes of this Appendix, the term 'DAG-oil' refers to oil consisting of >90% of DAG (1,3-DAG: 1,2-DAG in a ratio of 7:3) and <10% TAG, with a similar fatty acid profile to any TAG-based oil used in the study.

Study 1: Taguchi H, Nagao T, Watanabe H, Onizawa K, Matsuo N, Tokimitsu I, Itakura H (2001); *'Energy value and digestibility of dietary oil containing mainly 1,3-Diacylglycerol are similar to those of triacylglycerol'*; Lipids, 36(4): 379-382.

Test material:	TAG-based oil (control), DAG-oil (87% DAG, 13% TAG; fatty acid profile similar to TAG-based oil).
Test subjects:	16 male Sprague-Dawley rats, 5 weeks old.
Dose:	Feed contained 20g/100g of either TAG-based oil or DAG-oil.

Study Conduct

Paired groupings of rats (n=8) were fed test diets over 15 days following an acclimatisation period of five days. Each rat was housed in a metal cage with free access to food and water. Food intake was recorded each day, and weights were recorded every 3-4 days. Faeces were collected on days 13, 14 and 15, whereby lipids were extracted for faecal fat content analysis by gas chromatography.

Each of the test materials used in this study was tested for their energy content (heat of combustion). Energy content was measured from two samples of each oil using a bomb calorimeter, with the two results averaged.

Results

Food consumption and body weights (DAG group = 173.9±2.1 g and TAG group = 174.5±2.2 g as initial weights; DAG = 289.1±4.4 g and TAG = 283.8±3.9 g as final weights) of the two study groups did not vary significantly over the course of the study period. Faecal fat content did not vary significantly between the two groups; TAG-based oil group results were 10.49±0.38 g lipids/100 g faeces (3.7% of dietary fat absorbed), and DAG group results were 11.18±1.03 g lipids/100 g faeces (3.7% of dietary fat absorbed).

The energy content of the DAG-oil was measured at 98% of the energy content of TAG-based oil; the values were calculated as 38.9 kJ/g and 40.0 kJ/g respectively.

Conclusion

The results of the bomb calorimetry and the weight assessments of test subjects indicate that DAG-oil has a very similar energy content to that of TAG-based oils of the same fatty acid composition. Digestibility of DAG-oil as measured in this study was also assessed as being similar to that of TAG-based oils.

Study 2: Kamphuis M, Mela D, Westerterp-Plantenga M (2003);* 'Diacylglycerols affect substrate oxidation and appetite in humans'*; AJCN, 77: 1133-1139.

Test material:	TAG-based oil (control), DAG-oil (80% DAG, 20% TAG; fatty acid profile similar to TAG-based oil).
Test subjects:	Twelve healthy non-smoking women with a BMI of 23-30 (weight stable in the preceding three months) and a mean age of 34.5±9.4.
Dose:	40% of fat intake as either TAG-based oil or DAG-oil. Oil was provided in yoghurt given at main meals on day 1 of the study, and in a snack provided at 1500 hours on day 2.

Study Conduct

The purpose of this study was to examine the energy expenditure patterns associated with the consumption of DAG-oil versus TAG-based oils. The study was conducted as two 36-hour sessions in a single blinded, randomised crossover format for each subject. The test oils given during the first session were either TAG-based oil or DAG-oil, with the other oil provided at the following session. Sessions were spaced four months apart and designed to coincide with the menstrual cycle of each subject.

Subjects were housed in a respiratory chamber over the 36-hour period, with dietary energy intake provided in such a way that they would be in energy balance. A low intensity exercise program was allocated in the form of bench stepping twice a day for 30 minutes.

Oxygen consumption, carbon dioxide production and temperature variations were measured for the purposes of undertaking calculations on energy expenditure. Twenty-four hour energy expenditure was calculated as sleeping metabolic rate + diet-induced thermogenesis + activity induced energy expenditure. The respiratory quotient for each subject was calculated as carbon dioxide production divided by oxygen consumption, and was further used for the determination of fat and carbohydrate oxidation values. Blood samples were taken at baseline, 1130 and 1600 hours on each day via the placement of the subject's arm in an airlock. Serum glucose, insulin, glycerol, TAG, free fatty acid, and beta-hydroxybutarone (BHB) plasma levels were assessed.

Results

Subjects were observed as being in an equivalent state of positive energy balance during both the DAG and TAG sessions (0.7±0.7 MJ and 0.8±0.5 MJ respectively). Mean 24-hour energy expenditure did not significantly vary between the DAG and TAG sessions. Fat oxidation was significantly higher in the DAG session than the TAG session on both days, while carbohydrate oxidation did not vary significantly between the two sessions. Respiratory quotient values were significantly lower in the DAG session, which also indicates a greater level of fat oxidation was occurring compared to the TAG session. Energy expenditure results are summarised in the table below.

Table: Results from the metabolic assessment of DAG-oil intake versus TAG-based oil intake

Study Intervention (n=12)	Fat Oxidation (g/day)		Carbohydrate Oxidation (g/day)		Respiratory Quotient (36 hour mean)	Energy Expenditure (MJ/day)
	Day 1	Day 2	Day 1	Day 2		
DAG session	60.7±15.8**	64.6±16.1*	150.8±23.8	158.0±24.1	0.849±0.018*	9.5
TAG session	55.8±14.4**	60.6±13.7*	164.3±20.0	171.2±19.7	0.855±0.017*	9.4

* Results statistically significant between sessions ($P<0.05$).

** Results statistically significant between sessions ($P<0.004$).

Analyses of blood variables indicated that there were no significant differences except for the analysis of BHB, where readings at 1130 hours on day 2 were significantly higher ($P<0.05$) in DAG sessions than in TAG sessions.

Conclusion

This study demonstrates that although fat oxidation may increase with the partial replacement of dietary TAG-based oils by dietary DAG-oil, the overall energy expenditure by the human body remains the same. As blood variables were also concurrently measured, the reported increase in fat oxidation does not appear to be influenced by these parameters.

Study 3: Watanabe H, Onizawa K, Naito S, Taguchi H, Goto N, Nagao T, Matsuo N, Tokimitsu I, Yasukawa T, Tsushima R, Shimasaki H, Itakura H (2001); '*Fat-soluble Vitamin Status is not Affected by Diacylglycerol Consumption*'; Ann Nutr Metab, 45: 259-264

Test Material: TAG-based oil (controls), DAG-oil
Test Subjects: 27 males aged 27-47. DAG group n=15, TAG group n=12.
Dose: 20 g of test oil/day, equal to 0.3 g/kg bw/day, consumed as mayonnaise or an emulsion drink, once a day.

Study conduct

This study was carried out as a double-blind randomised controlled trial as a means of determining whether DAG consumption affects the serum levels of fat-soluble vitamin biomarkers.

The subjects were in good health as determined by medical history, physical examination, and clinical laboratory data. Subjects who were identified as using vitamin supplements were excluded from the study. Subjects consumed the test oil as part of the test foods at lunch for 12 weeks. At 4, 8 and 12 weeks, fasting blood samples were taken and serum concentrations of vitamins A, E and D were determined. Anthropometrics measurements (weight, WHR), and an analysis of physical activity and dietary records, were performed every four weeks throughout the study period.

Results

Body weight, waist circumference, hip circumference and serum parameter changes were not observed during the study. There were no significant treatment related effects on vitamin A, D and E concentrations throughout the study period.

Conclusion

The researches concluded that the absorption of fat-soluble vitamins had not been adversely affected by the consumption of DAG. Although a limited set of dietary variables was assessed (there was no mention of carotenoids for example), the assessment of serum vitamin biomarkers ensures that the impact on the end-points of vitamin intake is known.

Results from Studies Cited Within the Nutrition Assessment Report

Table 4: Studies Assessing the Impact of DAG-oil Consumption on Ketone Levels

Study	Study Design	Study Period	Study Groups				Results		Significant difference between groups? (P<0.05)
			Group name	No. Subjects	Dose	Subgroup	Serum Ketones (μmol/L)	Urinary Ketones (mmol/100g bw/day)	
Watanabe *et al*, 1997[4], rat study, (day 6 results)	RCT	6 days	DAG diet	5	20 g DAG-oil /100 g feed/day		-	8.5	Yes
			TAG diet	5	20 g TAG-based oil /100 g feed/day		-	7.0	
Hasegawa, 2000[19], human study	Double blind RCT	8 weeks	DAG diet	14 females	0.36 g DAG-oil/kg bw/day in food	Initial reading	67.9±18.6	-	No significant differences were reported at weeks 0, 4 and 8.
						Week 4 reading	113.3±28.6	-	
						Week 8 reading	121.1±21.5	-	
			TAG diet	14 females	0.36 g TAG-based oil/kg bw/day in food	Initial reading	65.6±11.9	-	
						Week 4 reading	115.3±21.5	-	
						Week 8 reading	86.6±12.8	-	
Koboyashi 2001[20], human study (results as changes over the study period)	RCT	12 weeks	Male	45	0.5 g DAG-oil/kg bw/day in food	DAG diet	-30	-	No
					0.5 g TAG-based oil/kg bw/day in food	TAG diet	+10	-	
	RCT	12 weeks	Female	45	0.5 g TAG-based oil/kg bw/day in food	DAG diet	-5	-	No
					0.5 g DAG-oil/kg bw/day in food	TAG diet	-3	-	
Matsuyama, 2000[21], human study	Single administration	20 weeks	Obese children	13	11.3 g DAG-oil/day	Initial reading	60	-	No
						Final reading	55	-	
Nagao *et al*, 2000[22], human study (results as changes over the study period)	Double blind RCT	16 weeks	DAG diet	14	0.14 g DAG-oil/kg bw/day		+2.8±5.8	-	No
			TAG diet	14	0.15 g TAG-based oil/kg bw/day		-12.7±18.2	-	

- = variable not assessed as part of the study

Table 5: Human Studies Assessing the Impact of DAG-oil Consumption on Anthropometry

Study	Study Design	Study Period	Subject Groups				Results (changes over the study period)				Significant difference between groups? (P<0.05)
			Group name	No. Subjects	Dose	Subgroup	Wt (kg)	BMI (wt/ht^2)	WHR	Fat Mass (% body wt)	
Hasegawa, 2000[19]	Double blind RCT	8 weeks	DAG diet	14 females	0.36g DAG-oil/kg bw/day in food		-0.9	-	-0.006	-	No
			TAG diet	14 females	0.36g TAG-based oil/kg bw/day in food		-1.3	-	-0.006	-	
Koboyashi 2001[20]	RCT	12 weeks	Males	45	0.5g DAG-oil/kg bw/day in food	DAG diet	+0.2	+0.1	-0.011	-	No
					0.5g TAG-based oil/kg bw/day in food	TAG diet	+0.6	+0.2	0.000	-	
			Females	45	0.5g TAG-based oil/kg bw/day in food	DAG diet	-0.2	+0.1	+0.001	-	
					0.5g DAG-oil/kg bw/day in food	TAG diet	-0.1	-0.3	-0.009	-	
Nagao *et al*, 2000[22]	Double blind RCT	16 weeks	DAG diet	14	0.14g DAG-oil/kg bw/day		-2.6±0.3	-0.9±0.1	-0.04±0.0	-1.1±0.3	Yes for weight and BMI only
			TAG diet	14	0.15g TAG-based oil/kg bw/day		-1.1±0.4	-0.4±0.1	-0.02±0.0	-1.5±0.7	
Maki *et al*[24]	Double blind RCT	24 weeks	DAG diet	43	DAG-oil added to food at 15% of subject's energy requirements		-3.43	-	-	-2.8	Yes for weight and fat mass
			TAG diet	36	TAG-based oil added to food at 15% of subject's energy requirements		-2.15	-	-	-1.7	

- = variable not assessed as part of the study
RCT = randomised controlled trial

Table 6-1: Impact of DAG-Oil on Blood and Liver Lipids - Rat Studies

Study	Study Design	Study Period	Study Groups				Results		Significant Difference between groups? (P<0.05)
			Group name	No. Subjects	Dose	Subgroup	Serum TAG (mg/mL)	Liver TAG (mg/g)	
Watanabe *et al*, 1997[4]	RCT	198 minutes	DAG diet	4	10g/100g body weight	Initial reading	0.18	-	Not reported
						Final reading	0.50	-	
			TAG diet	4	10g/100g body weight	Initial reading	0.30	-	Not reported
						Final reading	0.85	-	
Hara *et al*, 1993[10] Readings on day 34	RCT	34 days	DAG diet	8	10.5g DAG-oil/100g feed		1.8±0.1	23.5±0.0	Yes, for both TAG variables between groups
			TAG diet	8	10.0g TAG-based oil/100g feed		2.5±0.3	24.3±0.9	
Murata *et al*, 1997[17] Results collected at the end of the study period	Experiment 1 - RCT	14 days	DAG diet	8	105g DAG-oil/kg feed		1.9±0.5	34.0±6.3	Yes, for both TAG variables between groups
			TAG diet	8	100g TAG-based oil /kg feed		3.1±1.3	44.7±5.3	
	Experiment 2 - RCT	21 days	TAG diet	7	93.9g TAG-based oil/100g feed		3.7±0.5	31.4±5.2	Results for DAG diets 2 and 3 are significantly lower than the TAG results. There is no significant difference between DAG diet 1 and TAG results.
			DAG diet 1	7	28.2g DAG-oil/100g feed		3.6±0.3	33.2±4.5	
			DAG diet 2	7	65.7g DAG-oil/100g feed		2.9±0.2	27.5±3.6	
			DAG diet 3	7	93.9g DAG-oil/100g feed		2.1±0.1	25.5±3.0	

- = variable not assessed as part of the study

Table 6-2: Impact of DAG-oil on Blood Lipids - Human Studies

Study	Study Design	Study Period	Study Groups				Results				Significant difference between groups? (P<0.05)
			Group Name	No. Subjects	Dose	Subgroup	Serum TAG	Serum HDL	Serum LDL	Serum Chol	
Tada *et al*, 2001[3]	Double blind, cross-over	8 hours (1 month washout)	DAG diet	6	30 g lipid/m^2 as bolus	Initial reading	1.48±0.5 mmol/L	1.09±0.22 mmol/L	-	1.20±0.2 mmol/L	Yes - Serum TAG and serum HDL No – serum cholesterol.
						Final reading	1.32±0.8 mmol/L	1.12±0.21 mmol/L	-	1.20±0.2 mmol/L	
		8 hours (1 month washout)	TAG diet	6	30 g lipid/m^2 as bolus	Initial reading	1.4±0.3 mmol/L	1.11±0.2 mmol/L	-	1.17±0.2 mmol/L	No – for all variables measured
						Final reading	1.38±0.2 mmol/L	1.19±0.2 mmol/L	-	1.19±0.2 mmol/L	
Taguchi *et al*, 2000[11] (results assessed as an area under the curve)	double blind cross-over	8 hours (7 days washout)	Group 1	17	44 g/60 kg bw	DAG-oil bolus	6.5±5.1 mmol.h/L	-	-	graph	Yes between DAG and TAG
					44 g/60 kg bw	TAG-based oil bolus	8.45±7.5 mmol.h/L	-	-	graph	
	double blind cross-over	8 hours (7 days washout)	Group 2	10	20g/60 kg bw	DAG-oil bolus	graph	-	-	graph	Yes between DAG and TAG
					20 g/60 kg bw	TAG-based oil bolus	graph	-	-	graph	
	double blind cross-over	8 hours (7 days washout)	Group 3	13	10 g/60 kg bw	DAG-oil bolus	graph	-	-	graph	Yes between DAG and TAG
					10 g/60kg bw	TAG-based oil bolus	graph	-	-	graph	
Hasegawa 2000[19]	Double blind, RCT	8 weeks	DAG group	14	0.36 g/kg bw/day in food	Initial reading	54.9 mg/dL	63.2 mg/dL	-	173.0 mg/dL	No significant differences for Serum TAG, Serum, HDL or Serum cholesterol were observed between the DAG and TAG groups.
						Week 4 reading	63.9 mg/dL	62.5 mg/dL	-	161.2 mg/dL	
						Week 8 reading	67.8 mg/dL	59.1 mg/dL	-	158.0 mg/dL	
		8 weeks	TAG group	14	0.36 g/kg bw/day in food	Initial reading	45.7 mg/dL	64.0 mg/dL	-	158.5 mg/dL	
						Week 4 reading	51.0 mg/dL	63.7 mg/dL	-	152.5 mg/dL	
						Week 8 reading	62.2 mg/dL	60.5 mg/dL	-	150.7 mg/dL	

Study	Study Design	Study Period	Study Groups				Results				Significant difference between groups? (P<0.05)
			Group Name	No. Subjects	Dose	Subgroup	Serum TAG	Serum HDL	Serum LDL	Serum Chol	
Koboyashi 2001[20], (results as changes over the study period)	RCT	12 weeks	Male	45	0.5 g DAG-oil/kg bw/day in food	DAG diet	+4 mg/dL	+1 mg/dL	+2 mg/dL	+7 mg/dL	No – for all variables measured
					0.5 g TAG-based oil/kg bw/day in food	TAG diet	+5 mg/dL	+2 mg/dL	-4 mg/dL	+1 mg/dL	
	RCT	12 weeks	Female	45	0.5 g TAG-based oil/kg bw/day in food	DAG diet	-5 mg/dL	+1 mg/dL	+3 mg/dL	+5 mg/dL	
					0.5 g DAG-oil/kg bw/day in food	TAG diet	-9 mg/dL	-3 mg/dL	-7 mg/dL	-8 mg/dL	
Yamamoto *et al*, 2001[25]	RCT	12 weeks	DAG diet	8	0.22 g DAG-oil/kg bw/day	Initial reading	2.51±0.8 mmol/L	1.27±0.2 mmol/L	-	5.82±1.3 mmol/L	Yes for Serum TAG in DAG group, No for other variables
					Maintained current TAG-based oil use	Final reading	1.52±0.3 mmol/L	1.34±0.4 mmol/L	-	5.87±0.8 mmol/L	
	RCT	12 weeks	TAG diet	8	0.22 g DAG-oil/kg bw/day	Initial reading	3.22±2.1 mmol/L	1.09±0.2 mmol/L	-	6.00±1.0 mmol/L	
					Maintained current TAG-based oil use	Final reading	3.59±1.7 mmol/L	1.22±0.4 mmol/L	-	5.74±0.7 mmol/L	

- = variable not assessed as part of the study graph = results were displayed in graph format only RCT = randomised controlled trial

Table 7-1: Impact of DAG-oil on Blood Glucose and Insulin Levels – Animal Studies

Study	Study Design	Study Period	Study Groups				Results			Significant Difference between groups? (P<0.05)
			Group Name	No. Subjects	Dose	Subgroup	Fasting Blood Glucose	HbA1c	Fasting Insulin	
Murase *et al* 2002[5], Rat study	RCT	8 months	Low TAG diet	20	5 g TAG-based oil / 100 g feed		67.6±12.7 mg/dL	-	0.6±0.1 ng/mL	Yes – the high DAG diet had significantly lower values than the high TAG diet, and the high TAG diet had significantly higher values than the low TAG diet
			High TAG diet	10	25 g TAG-based oil / 100 g feed		111.6±25.2 mg/dL	-	1.5±1.0 ng/mL	
			High DAG diet	10	15 g DAG-oil / 100 g feed and 10 g TAG-based oil / 100 g feed		83.3±13.2 mg/dL	-	0.6±0.6 ng/mL	
Sugimoto *et al*, 2003[7], Rat study	RCT	12 months	DAG diet	7	5 g DAG-oil/kg bw/day	Young rats (7 weeks) - reading at week 4	7.0 mmol/L	-	0.11 nmol/L	There was no significant difference between TAG and DAG groups except for the readings (glucose and insulin) of old rats at week 8.
						Young rats (7 weeks) - Reading at week 8	7.0 mmol/L	-	0.10 nmol/L	
						Old rats (8 months) - reading at week 4	7.5 mmol/L	-	1.80 nmol/L	
						Old rats (8 months) - reading at week 8	9.5 mmol/L	-	1.70 nmol/L	
			TAG diet	7	5 g TAG-based oil/kg bw/day	Young rats (7 weeks) - reading at week 4	7.0 mmol/L	-	0.90 nmol/L	
						Young rats (7 weeks) - Reading at week 8	7.0 mmol/L	-	1.20 nmol/L	
						Old rats (8 months) - reading at week 4	7.5 mmol/L	-	1.50 nmol/L	
						Old rats (8 months) - reading at week 8	8.5 mmol/L	-	1.30 nmol/L	

- = variable not assessed as part of the study RCT = randomised controlled trial

Table 7-2: Impact of DAG-oil on Blood Glucose and Insulin Levels – Human Studies

Study	Study Design	Study Period	Study Groups				Results			Significant Difference between groups? (P<0.05)
			Group name	No. Subjects	Dose	Subgroup	Fasting Blood Glucose	HbA1c	Fasting Insulin	
Kobayashi, 2001[20], Human study (results expressed as changes over the study period)	RCT	12 weeks	Male	45	0.5 g DAG-oil/kg bw/day	DAG diet	-2 mg/dL	-0.15%	-	No – for all variables measured
					0.5 g TAG-based oil/kg bw/day	TAG diet	-1 mg/dL	-0.14%	-	
	RCT	12 weeks	Female	45	0.5 g TAG-based oil/kg bw/day	DAG diet	0 mg/dL	-0.26%	-	
					0.5 g DAG-oil/kg bw/day	TAG diet	-1 mg/dL	-0.30%	-	
Yamamoto *et al*, 2001[25], Human study	RCT	12 weeks	DAG diet	8	0.22 g/kg bw/day of TAG-based oil intake replaced by DAG-oil	Initial reading	6.72±0.7 mg/dL	6.4±1.2%	-	No significant differences between the DAG-oil and TAG-based oil groups were reported. HbA1c levels decreased significantly from baseline levels for the DAG-oil group.
						Final reading	6.94±0.9 mg/dL	5.8±0.9%	-	
	RCT	12 weeks	TAG diet	8	Maintained current TAG-based oil use	Initial reading	7.83±1.8 mg/dL	6.9±0.5%	-	
						Final reading	7.77±2.5 mg/dL	6.7±0.7%	-	

- = variable not assessed as part of the study RCT = randomised controlled trial

APPENDIX 3 TO ATTACHMENT 3

Material Excluded from the Nutrition Assessment

Study 1: Watanabe H, Goto N, Fujimori N, Toi T, Kobori M, Onizawa T, Taguchi H, Naito S, Hattori M, Shimasaki H, Miyazawa Y, Yashiro N, Matsuzawa Y, Itakura H (1997); *'Kao Corporation Office report: effect of DAG on human long term intake'*; (Unpublished)

Test Material:	TAG-based oil (control), DAG-oil (91.8% DAG, 8.2% TAG; the fatty acid composition was comparable between TAG-based oil and DAG-oil).
Test Subjects:	8 healthy adult males, 30-47 years old, BMI 24.3.
Dose:	44 g/ 60 kg body weight/day for 4-weeks in the form of brioche, pancake, mayonnaise.

Study Conduct

This study was conducted as a double blind crossover trial. Four weeks prior to the study, dietary patterns were controlled (in an unspecified manner) to provide a consistent intake of TAG-based oils. Subjects were randomly provided with either the DAG-oil or TAG-based oil in the test foods for consumption over the period of four weeks, and then the other test oil for the following four-week period. The authors did not specify whether a washout period had been used.

At the end of each test period blood samples were taken and a CT scan was performed after 14-15 hour fasting. The blood samples were used to measure haematology parameters, and blood chemistry (TG, beta-lipoprotein, total cholesterol, serum free cholesterol, HDL cholesterol, phospholipids, free fatty acids, glucose, blood insulin, apolipoproteins). Data was only presented as a change from initial values.

Results

No treatment related effects on vital signs, body weight, body fat ratio, BMI, and hip circumferences were observed. The increase in waist size was attenuated by DAG-oil intake compared with TAG-based oil intake according to the study author. However, when the data from the graphs (difference from pre-treatment) was calculated to actual numbers, a 6 cm increase in waist circumference would have been occurred after treatment with TAG-based oil, compared to a 2 cm decrease after DAG-oil. After only a 4-week treatment period these results are very questionable, particularly as there is no corresponding effect on body weight.

No adverse clinical effects were reported. The analysis of blood parameters (e.g. to determine liver and kidney function) did not indicate any treatment-related changes, although data was presented as graphs only and therefore did not allow for a more detailed analysis.

Conclusion

This study is not acceptable for use in the nutritional assessment, due to the ambiguous presentation of data.

Study 2: Teramoto T, Nagao T, Watanabe H, Itoh K, Omata Y, Hurukawa T, Shimoda K, Hoshino M (2000); 'Effects of the diacylglycerol diet on lipid metabolism in the patient with hemodialysis'; Kao Corporation report, Japan (Unpublished)

Test Material:	DAG-oil
Test Subjects:	10 haemodialysis outpatients with Type IIb or IV hyperlipidemia.
Dose:	9 g DAG-oil/ day in place of TAG cooking oil for 3 months, with a 3-month washout period.

Study conduct

Very little detail was provided on the study conduct within this article. It was evident however, that no controls had been used. Therefore, this study has not been included within the deliberations of this nutritional assessment.

Conclusion

The lack of detail and exclusion of controls renders this as inadequate for the purposes of the nutritional assessment.

Study 3: Katsuragi Y, Toi T, Yasukawa T (1999); *'Effects of dietary diacylglycerol on obesity and hyperlipidaemia'*; J Jpn Soc Human Dry Dock (1999); 14:258-262.

Test material:	DAG-oil (>80% diglycerides)
Test groups:	114 employees of Kao Corporation (average age 39.3 years, 95 males and 19 females)
Dose:	Unknown - Cooking oils substituted for DAG-oil, subjects requested to eat at Kao Corporation cafeteria where oil was used in meal preparations.

Study Conduct

Subjects were employees of Kao Corporation and were taking DAG-oil as a replacement for edible oils for 12 months. Blood sampling (lipid, liver function etc) and anthropometric measurements were performed at 0, 3, 6, 9 and 12 months. At the same time diet diaries were kept over three consecutive days every three months. This article was an English translation of a Japanese journal supplied by the Applicant that did not contain all figures and graphs of results.

Conclusion

This study has been assessed as unsuitable for use in this nutrition assessment, as it did not include a control group and did not control the dose of DAG-oil oil consumed by test subjects. It should be noted that the submission of figures with the study would not have changed this conclusion.

Attachment 4

Dietary exposure assessment report

A505 – Diacylglycerol oil (DAG-oil)

SUMMARY

An application was received by FSANZ requesting the Code be amended to allow the use of DAG-oil as a novel food ingredient, under Standard 1.5.1- Novel Foods, for use in cooking oil, salad dressings, mayonnaise, viscous dressings, fat spreads/margarines, baked products (including bread, biscuits, cakes, crackers and cookies, croissants, pastries, pizza), health bars and health drinks (meal replacements). A dietary exposure assessment was undertaken to determine the potential dietary impact of allowing DAG-oil to be added to the above foods.

The Applicant proposes to use DAG-oil as a 1:1 (w/w) replacement for conventional triglyceride (TG) in edible vegetable cooking oil. A dietary exposure assessment was conducted for the general Australian and New Zealand populations (2+ and 15+ years respectively), and for the population considered at potential risk from higher exposures; children (2-12 years, Australia only). Food consumption data were derived from the 1995 Australian National Nutrition Survey (NNS) and the 1997 New Zealand NNS. DAG-oil concentration data were derived from levels proposed in the Application.

Estimated mean and 95th percentile dietary exposures for consumers of DAG-oil in the Australian population (2+ years) from all proposed foods were 0.4 and 1.3 grams per kilogram of body weight per day (g/kg BW/day) respectively. Estimated mean and 95th percentile dietary exposures for consumers of DAG-oil in the New Zealand population (15+ years) from all proposed foods were 0.3 and 1.0 g/kg BW/day respectively. Australian children (2-12 years) had estimated dietary exposures of 0.7 g/kg BW/day (mean) and 2.4 g/kg BW/day (95th percentile). The highest percentage contribution to dietary exposure was from oil and oil emulsions for all age groups.

Estimated mean and 95th percentile dietary exposures for consumers of DAG-oil in the Australian population (2+ years) from oil and oil emulsions only were 0.4 and 1.3 g/kg BW/day respectively. Estimated mean and 95th percentile dietary exposures for consumers of DAG-oil in the New Zealand population (15+ years) from oil and oil emulsions only were 0.2 and 0.7 g/kg BW/day respectively. Australian children (2-12 years) had estimated dietary exposures of 0.7 g/kg BW/day (mean) and 2.3 g/kg BW/day (95th percentile) from oil and oil emulsions only.

It is recognised that the estimated exposures to DAG-oil from all foods versus just oil and oil emulsions are similar. This is due to both methodological reasons and DAG-oil concentrations in the foods.

BACKGROUND

DAG-oil is manufactured from natural edible oils such as soybean, canola (rapeseed) or corn oil by enzymatic esterification. DAG-oil is composed largely of randomised diacylglycerols and contains approximately 80% diacylglycerol, 20% triacylglycerol, 5% monoacylglycerol and <0.2% emulsifiers (polyglycerol esters of fatty acids) and antioxidants (ascorbyl palmitate and tocopherol). These constituents of DAG-oil are already present in the Australian and New Zealand diets as components of conventional dietary oils.

DAG-oil has the same general physical properties as mono- and diglycerides, i.e. all are practically insoluble in water and soluble in ethanol, chloroform, and benzene, and all are metabolised via the same general metabolic pathways. Therefore DAG-oil is intended to replace conventional triglycerides (TG) in edible vegetable cooking oil and as an ingredient in various products.

The applicant provided information that DAG-oil has been commercially available and consumed in Japan for over five years. During this time no at risk individuals or groups have been identified and all available evidence indicates that both children and adults can consume it safely.

DIETARY EXPOSURE ASSESSMENT PROVIDED BY THE APPLICANT

The applicant provided a detailed dietary exposure assessment for DAG-oil, based on food groups similar to those being proposed for Australia and New Zealand. The dietary exposure assessment provided by the applicant was based on the Australian and New Zealand NNS data along with United States food consumption data (US Department of Agriculture Continuing Survey of Food Intakes by Individuals, 1994-96). The Applicant indicated that US data was used because the Australian summarised published data lacked particular details including recipes in which vegetable oil is used. However, this assessment was not considered to be sufficient for assessing the safety of potential exposure to DAG-oil in Australia and New Zealand. Therefore, FSANZ conducted a dietary exposure assessment using the Australian and New Zealand consumption data for raw commodities from the NNSs, and recipe data files in DIAMOND, to estimate the potential exposure to DAG-oil if it was permitted to be used in the foods requested in the application.

The exposure assessment submitted by the applicant indicated that mean daily exposure to DAG-oil for Australian 90th percentile consumers of foods containing DAG-oil were 0.233 g/kg bw/day and 0.521 g/kg bw/day for adults (65 years and over) and children (2-3 years) respectively.

The exposure assessment submitted by the applicant indicated that mean daily exposure to DAG-oil for New Zealand 90th percentile consumers of foods containing DAG-oil were 0.290 g/kg bw/day and 0.365 g/kg bw/day for male adults (65 years and over) and 12-15 year olds respectively.

Female adults (65 years and over) and 12-15 year olds had lower 90th percentile daily exposures to DAG-oil (0.233 and 0.318 g/kg bw/day respectively).

The applicant indicated that these estimated exposure levels are likely to be an overestimate since it was presumed that DAG-oil would replace all the various components of vegetable oil.

DIETARY MODELLING

The FSANZ dietary exposure assessment was conducted using dietary modelling techniques that combine food consumption data with food chemical concentration data to estimate the exposure to the food chemical from the diet. The dietary exposure assessment was conducted using FSANZ's dietary modelling computer program, DIAMOND.

Dietary exposure = food chemical concentration x food consumption

The exposure was estimated by combining usual patterns of food consumption, as derived from NNS data, with proposed levels of use of DAG-oil in foods.

Dietary Survey Data

DIAMOND contains dietary survey data for both Australia and New Zealand; the 1995 NNS from Australia that surveyed 13 858 people aged 2 years and above, and the 1997 New Zealand NNS that surveyed 4 636 people aged 15 years and above. Both of the NNSs used a 24-hour food recall methodology.

The dietary exposure assessment was conducted for both Australian and New Zealand populations. An assessment was conducted for the whole population, as well as for children aged 2-12 years (Australia only). An exposure assessment was conducted for children because children generally have higher dietary exposures due to their smaller body weight, and greater consumption of food per kilogram of body weight compared to adults. A particular concern is the metabolic effects of DAG-oil (weight loss and increased fat oxidation) because children need to gain weight as part of their normal growth. They are also likely to consume the types of products that are proposed to have added DAG-oil, such as biscuits, cakes, bread and breakfast cereal bars.

Additional Food Consumption Data or Other Relevant Data

No further information was required or identified for the purpose of refining the dietary exposure estimates for this Application.

DAG-oil concentration levels

The levels of DAG-oil in foods that were used in the exposure assessment were derived from the Application. The foods and proposed levels of use are shown below in Table 1. DAG-oil is proposed to be a 1:1 substitution with vegetable fats/oils in foods.

The percentage of DAG-oil in foods was based on the percent of fat/oil in the product (AUSNUT, 1999). For mixed foods such as biscuits, it was calculated as the percent of added fat/oil, not total fat/oil, which could also come from other ingredients (eg, eggs).

Table 1: Proposed use of DAG-oil in foods and levels of use

Food Code	Food Name	Assumed level of DAG-oil in product (%)	Concentration Level used in modelling (mg/kg)
2	Edible oils and oil emulsions	100	1 000 000
2.2.2	Spreads	50	500 000
7.1	Bread	1	10 000
7.2	Biscuits, cakes and Pastries	1	10 000
13.3	Health drinks (meal replacements)	3	30 000
20.2.3	Health (cereal) bars	5	50 000
20.2.4	Mayonnaise & salad dressings	50	500 000
21.1.5	Pizza	5	50 000

Scenarios used in the exposure assessment

Two scenarios were modelled in the exposure assessment. The first assuming that all of the proposed foods from the Application contained DAG-oil. This represents the likely exposure should all proposed foods be granted approval to contain DAG-oil. A second scenario was modelled with only oil and oil emulsions containing DAG-oil. This represents the likely exposure should only these products be approved for the Australian and New Zealand market.

Estimating Risk

Estimated dietary exposures are usually compared to a reference health standard in order to determine the potential risk to health of the population or its subgroups. However, the metabolism of DAG-oil is comparable to that of partial glycerides (monoglycerides) and triglycerides. Furthermore, no specific adverse effects were observed in both animal and human studies that would indicate adverse health effects if DAG-oil were to be allowed in Australia and New Zealand. Therefore, the estimated exposures reported below are simply reported in grams per kilogram body weight per day.

How were the estimated dietary exposures calculated

The DIAMOND program allows DAG-oil concentrations to be assigned to food groups. All foods in this group are assigned the concentration of DAG-oil shown in Table 1.

Each individual's exposure to DAG-oil was calculated using his or her individual food records from the dietary survey. The DIAMOND program multiplies the specified concentration of DAG-oil by the amount of food that an individual consumed from that group in order to estimate the exposure from each food.

Once this has been completed for all of the foods specified to contain DAG-oil, the total DAG-oil exposure from all foods is summed for each individual. Population statistics (mean and high percentile exposures) are then derived from the individuals' ranked exposures.

Where estimated dietary exposures are expressed per kilogram of body weight, each individuals' total dietary exposure is divided by their own body weight, the results ranked, and population statistics derived.

Percentage contributions of each food group to total estimated exposures are calculated by dividing the sum of consumers' exposures from a food group by the sum of all consumers' exposures from all foods, and multiplying this by 100.

Food consumption amounts for each individual take into account where each food in a classification code is consumed alone and as an ingredient in mixed foods. For example, where edible oils are used in cooking.

ASSUMPTIONS IN THE DIETARY MODELLING

Assumptions made in the dietary modelling include:

- where a permission is given to a food group classification, all foods in that group contain DAG-oil at the proposed levels;
- where a group of foods may contain a variety of products with different fat/oil percentages, the higher value was taken to assume a worst case;
- all the products are assumed to contain a percentage of DAG-oil that is equivalent to the percentage of vegetable fat/oil in the product;
- consumption of foods as recorded in the Australian and New Zealand NNSs represent current food consumption patterns; and
- consumers always selected the products containing DAG-oil.

These assumptions are likely to lead to a conservative estimate for DAG-oil dietary exposure.

LIMITATIONS OF THE DIETARY MODELLING

A limitation of estimating dietary exposure over a period of time associated with the dietary modelling is that only 24-hour dietary survey data were available, and these tend to over-estimate habitual food consumption amounts for high consumers. Therefore, predicted high percentile exposures are likely to be higher than actual high percentile exposures over a lifetime. However in the case of foods such as margarines, spreads and breads the majority of consumers will be daily consumers of these foods, therefore 24 hour dietary data will more closely represent habitual exposures.

RESULTS

Estimated dietary exposures to DAG-oil

Detailed results tables showing exposures to DAG-oil are shown in Appendix A1.1 for all proposed foods and A1.2 for oil and oil emulsions only.

The estimated dietary exposures for DAG-oil for all proposed foods are shown in Figure 1. Estimated mean exposures from all proposed foods for all Australian consumers of DAG-oil are 0.38 g/kg BW/day, and 0.30 g/kg BW/day for New Zealand. Estimated 95th percentile exposures for consumers of DAG-oil from all proposed foods are 1.3 and 1.0 g/kg BW/day for Australia and New Zealand respectively. Australian children 2-12 years had estimated mean dietary exposures of 0.73 g/kg BW/day and estimated 95th percentile exposures of 2.4 g/kg BW/day.

Figure 2 shows estimated mean exposures for consumers of DAG-oil, if it was permitted for use only in oil and oil emulsions. Estimated mean exposure for consumers of DAG-oil from oil and oil emulsions are 0.4 and 0.2 g/kg BW/day for Australia and New Zealand respectively. Estimated 95th percentile exposures for consumers of DAG-oil from oils and oil emulsions are 1.3 and 0.7 g/kg BW/day for Australia and New Zealand respectively. Australian children 2-12 years had estimated mean dietary exposures of 0.7 g/kg BW/day and estimated 95th percentile exposures of 2.3 g/kg BW/day.

Figure 1: Estimated mean and 95th percentile dietary exposures for consumers DAG-oils from all proposed foods for Australia and New Zealand

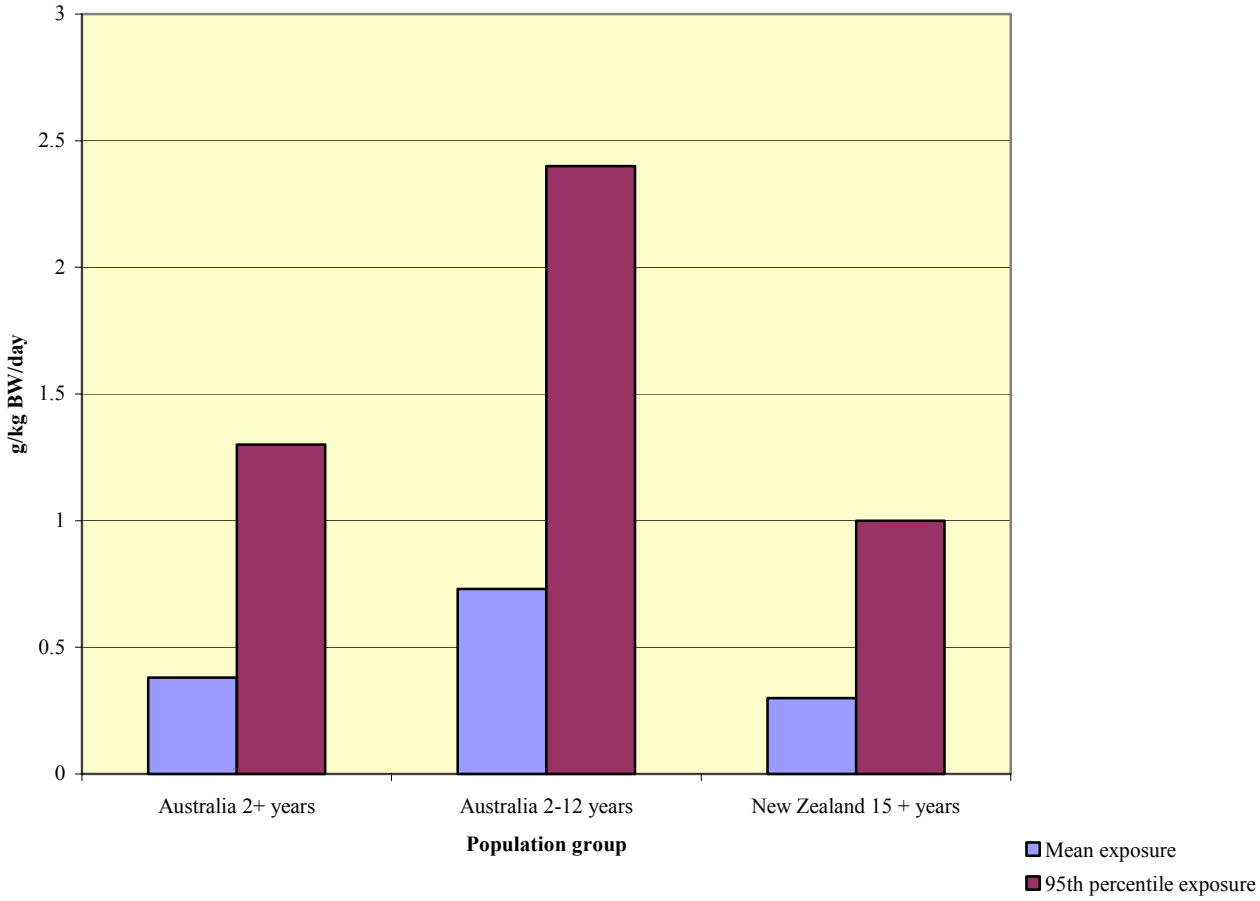
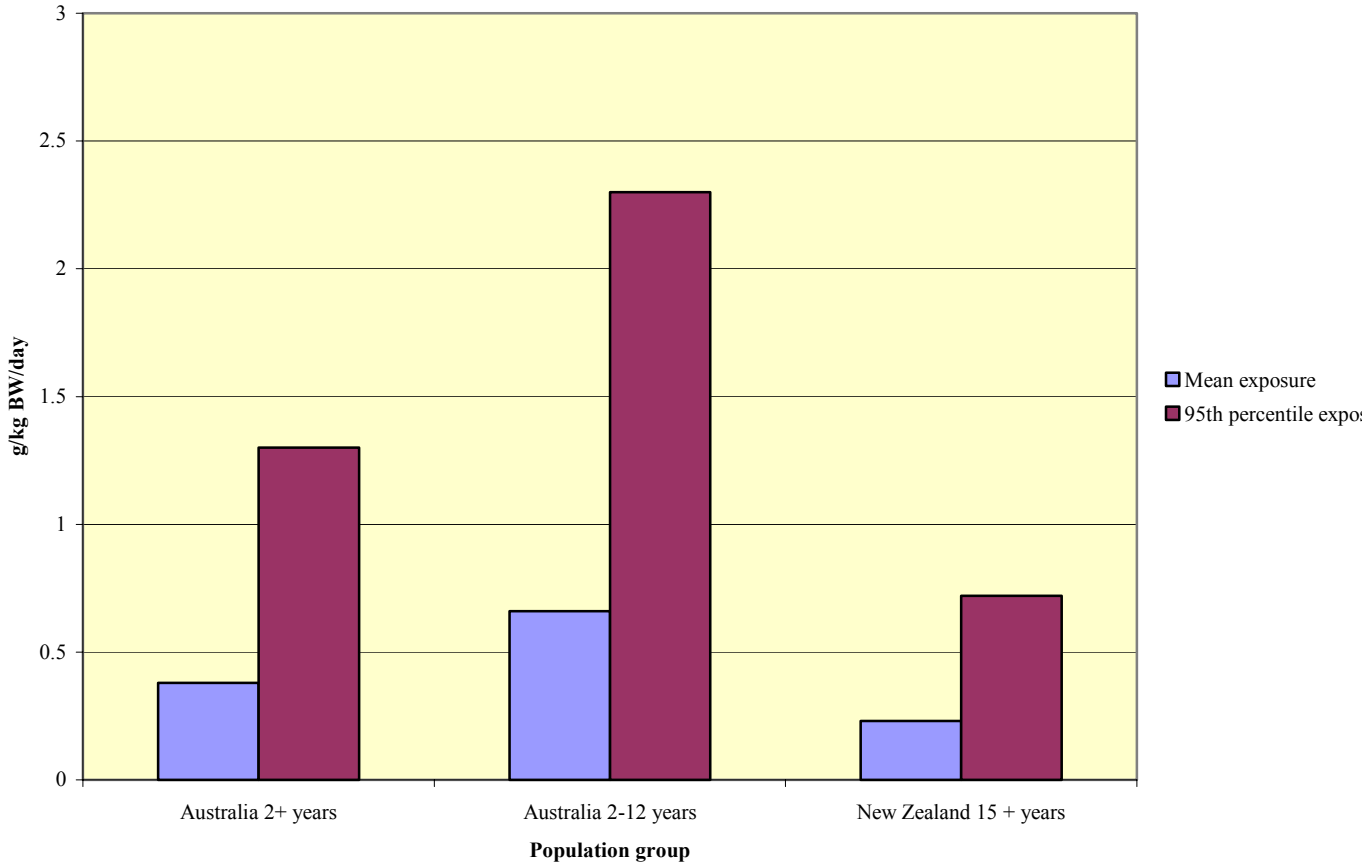


Figure 2: Estimated mean and 95th percentile dietary exposures for consumers of DAG-oils from oil and oil emulsions for Australia and New Zealand



Foods contributing to total estimated dietary exposures

Detailed information regarding the percent contribution for each food group to total DAG-oil exposures are shown in Appendix 2.

The percentage contributions of different food groups to total estimated dietary exposures to DAG-oil are displayed in Figure 3 (Australia 2+ years), figure 4 (Australia 2-12 years) and figure 5 (New Zealand 15+ years). These contributions are calculated assuming all the proposed foods contain DAG-oil. Edible oils and oil emulsions were the major contributors for each population group, contributing 47% – 64%. Sauces, mayonnaises, salad dressings (18% - 35%), margarines and spreads (8% - 10%) and bread and related products (5% - 6%) were the other major contributors for each population group.

Figure 3: Percent contribution to estimated DAG-oil dietary exposure for Australians aged 2+ years

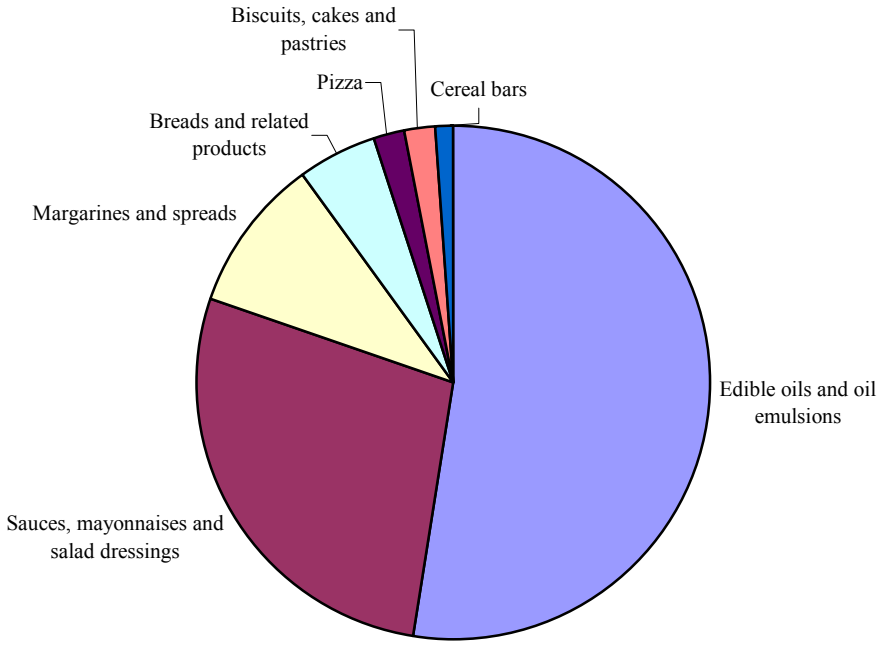


Figure 4: Percent contribution to estimated DAG-oil dietary exposure for Australians aged 2-12 years

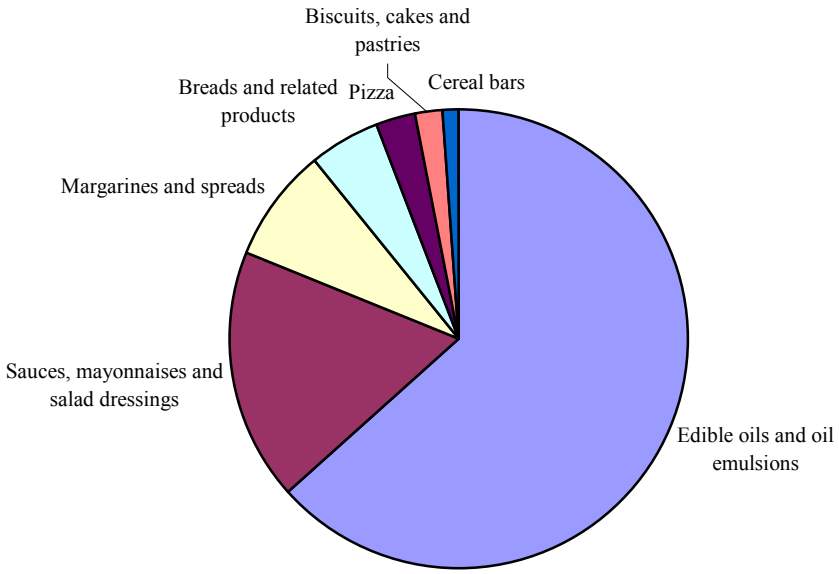
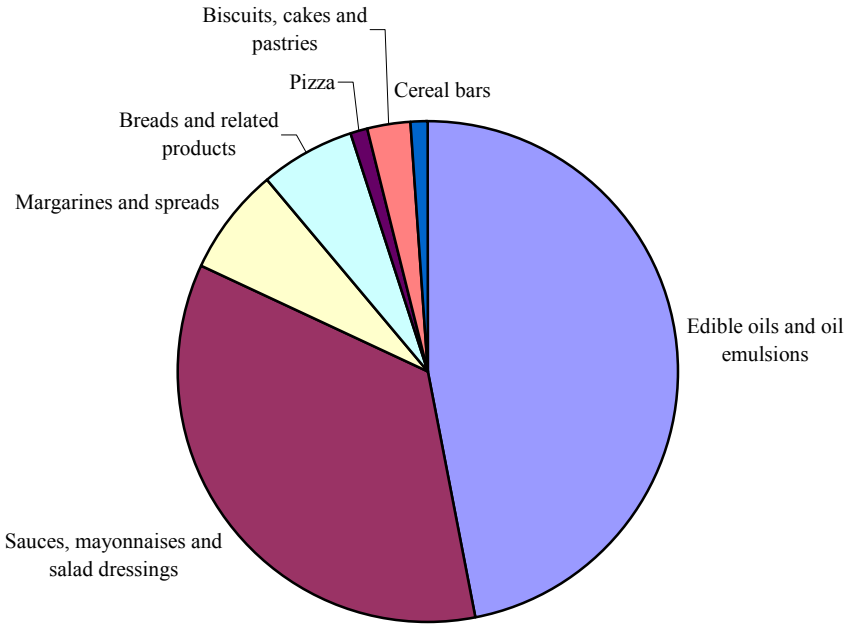


Figure 5: Percent contribution to estimated DAG-oil dietary exposure for New Zealanders aged 15+ years



At first inspection, it may appear surprising that the exposure to DAG-oil from only oil and oil emulsions results in the same predicted exposures as adding it to all proposed foods for all Australians and Australians 2-12years, and only slightly different for all foods for New Zealand. These findings reflect:

- the way DIAMOND is programmed;
- the higher contribution that oils and oil emulsions make in the all foods model;
- the greater number of serves of oil and oil emulsions consumed over the day than some of the other foods DAG-oil is added to;
- the much larger proportion of consumers of oil and oil emulsions than for other proposed products (As noted earlier, the DIAMOND program derives results from each individual's food consumption patterns);
- when DAG-oils and oil emulsions are eaten as products on their own they are 100% DAG-oil, where as the other proposed foods contain DAG-oil at a much lower percentage.

REFERENCES

Australian Food and Nutrient Database, 1999, Explanatory notes, Australian New Zealand Food Authority, Canberra.

Appendix 1

ESTIMATED DIETARY EXPOSURES TO DAG-OILS FOR AUSTRALIA AND NEW ZEALAND

Table A1.1: Estimated dietary exposures for consumers of DAG-oil from all proposed foods for Australia and New Zealand and for different age groups

Country	Age group	Number of consumers of DAG-oil	Consumers as a % of total respondents[#]	Mean consumers g/kg BW/day	95th percentile consumers g/kg BW/day
Australia	Whole population (2 years+)	13655	99	0.4	1.3
	2-12 years	2061	99	0.7	2.4
New Zealand	Whole population (15 years+)	4591	99	0.3	1.0

Total number of respondents for Australia: whole population = 13 858, 2-12 years = 2 079, New Zealand: whole population = 4 636.

Table A1.2: Estimated dietary exposures for consumers of DAG-oil from oil and oil emulsions only for Australia and New Zealand, and for different age groups

Country	Age group	Number of consumers of DAG-oil	Consumers as a % of total respondents[#]	Mean consumers g/kg BW/day	95th percentile consumers g/kg BW/day
Australia	Whole population (2 years+)	8564	62	0.4	1.3
	2-12 years	1546	74	0.7	2.3
New Zealand	Whole population (15 years+)	4489	97	0.2	0.7

Total number of respondents for Australia: whole population = 13 858, 2-12 years = 2 079, New Zealand: whole population = 4 636.

Appendix 2

Contribution of each food group to total DAG-oil dietary exposures for Australia and New Zealand, and for different age groups

Country	Age group	*Major contributing foods to DAG-oil exposures*	*Percent contribution to DAG-oil dietary exposure*
Australia	Whole population (2+ years)	Edible oils and oil emulsions	53
		Sauces, mayonnaises, salad dressings	28
		Margarines and spreads	10
		Breads and related products	5
		Pizza	2
		Biscuits, cakes and pastries	2
		Cereal bars	1
		Formula dietary foods	0
	2-12 years	Edible oils and oil emulsions	64
		Sauces, mayonnaises, salad dressings	18
		Margarines and spreads	8
		Breads and related products	5
		Pizza	3
		Biscuits, cakes and pastries	2
		Cereal bars	1
		Formula dietary foods	0
New Zealand	Whole population (15+ years)	Edible oils and oil emulsions	47
		Sauces, mayonnaises, salad dressings	35
		Margarines and spreads	8
		Breads and related products	6
		Biscuits, cakes and pastries	3
		Pizza	1
		Cereal bars	1
		Formula dietary foods	0

Attachment 5

Food technology report

DIACYLGLYCEROL OIL

Introduction

Acylglycerols are esters of glycerol and fatty acids. Diacylglycerols (diglycerides) are fatty acid diesters and occur in two isomeric forms – 1,2-Diacyl-*sn*-glycerol and – 1,3-Diacyl-*sn*-glycerol. Partial glycerides are important intermediates of metabolism, and triacylglycerols are the major constituents of edible oils.

In order to designate the stereochemistry of glycerol-containing components, the carbon atoms of glycerol are numbered stereospecifically. When the glycerol molecule is drawn in a Fischer projection with the secondary hydroxyl group to the left of the central prochiral carbon atom, the carbons are numbered 1, 2 and 3 from top to bottom. Molecules that are stereospecifically numbered in this fashion have the prefix "*sn*" immediately preceding the term "glycerol" in the name of the compound to distinguish them from compounds that are numbered in conventional fashion.

Description

Diacylglycerol-oil (DAG-oil) oil looks and tastes like a vegetable oil – light yellow in colour with a mild flavour. DAG is a naturally occurring component of vegetable oil. DAG-oil is made from the vegetable oil sources such as soy and canola. The vegetable oils are processed to raise the concentrations of diacylglycerol to about 80% by weight.

DAG-oil contains predominantly diglycerides, which contain two fatty acids on the end of the fat molecule instead of three fatty acids in triacylglycerols (TAG). The remainder of DAG-oil is comprised of TAG, monoglyceride (MAG), and free fatty acids. In comparison, a vegetable oil contains closer to 95% TAG and 1-5% DAG by weight.

Manufacture and Uses:

DAG-oil is manufactured through a process that begins with glycerol and fatty acids prepared from soy and canola. The fatty acids are esterified, or linked to glycerol to form diacylglycerols in the presence of an enzyme, a lipase, which is specific for the 1 and 3 positions of the glycerol molecule. Then, the oil is refined into DAG-oil.

DAG-oil is used as a food ingredient and has similar uses to triacylglycerol oils. It may be used in foods including spreads, salad dressings, mayonnaise, bakery products, fried foods, beverages, soups, sauces, and gravies.

DAG is approved in Australia and New Zealand as a food additive with the technological function of an emulsifier. The application A505 is for DAG-oil is as a food not as a food additive.

History of Enova oil

DAGs have been used as emulsifiers for many years but not as main components of food products. In addition, DAG is naturally present in all vegetable oils in smaller quantities, from 1 to 5 percent by weight.

The Kao Corporation introduced DAG-oil (Healthy Econa™ Cooking Oil as if is known in Japan) as cooking oil in Japan in 1999. It has been approved by Japan's Ministry of Health Labor and Welfare for labelling as a "Food for Specified Health Use" (FOSHU).

Since then, DAG-oil has been introduced in other products such as salad dressings, margarine and canned tuna – in which the DAG-oil is substituted for vegetable oil in the product. In August 2002, Kao introduced DAG mayonnaise into the Japanese market. Archer Daniels Midland Company and Kao formed a joint venture, ADM Kao LLC, to manufacture and market Econa oil as Enova oil. In 2000, Kao notified the US FDA of the GRAS (generally recognized as safe) status of Enova oil for use in home cooking oil and vegetable oil spreads.

Conclusion

DAG-oil may be used as a food ingredient and has similar uses to triacylglycerol oils. It may be used in foods including spreads, salad dressings, mayonnaise, bakery products, fried foods, beverages, soups, sauces, and gravies.

Attachment 6

Summary of public submissions

A505 – Diacylglycerol oil

Food Technology Association of Victoria

- **Agreed** with option 2- to amend the Code, but questioned whether the Novel Food status was necessary. FTA suggested that it would be more appropriate to approve DAG-oil as an edible oil, which would require change to Standard 2.4.1.

Queensland Health

- Did not accept or reject the application.
- Expressed concern about how DAG-oil can contribute to decreasing body weight and fat mass, if it is equivalent in caloric value to conventional oils. Furthermore, concern was expressed that the application being used as a "marketing tool for the use of health claims which might be misleading".
- If this would occur, costs would be on government as additional education programs would be required to allay consumer confusion about this product.

Dadhich Patel, student Food Science, University of Auckland

- **Agre**ed with amending the Code in approving DAG-oil. The following suggestions were submitted:
- Not to be included in infant food, because fat is essential for their growth.
- The oil should not be mixed with other oils.
- Specifications should be made clear.

Tony Tsang, student Food Science, University of Auckland

- **Agreed** with amending the Code in approving DAG-oil as a novel food in New Zealand.

Sagar Katvi, student Food Science, University of Auckland

- **Agreed** with amending the Code in approving DAG-oil as a novel food in Australia and New Zealand.
- The following suggestions were also submitted:
- The use of DAG-oil in infant food should be avoided, as it may not be beneficial for them
- Appropriate labelling is needed, in order that consumers know what they buy.
- Specifications about the maximum allowable consumption should be mentioned.
- The cost of DAG-oil should be reasonable as compared to other edible oils.

AQIS

- AQIS will assess the regulatory impact of any proposed amendment to the Code on AQIS operations after the Draft Assessment.

New Zealand Food Safety Authority

- Labelling needs to be considered further during Draft Assessment. The consideration should include clear direction on the naming of oil and on claims that go beyond nutritional claims, and may be considered health claims.
- The NZFSA will consider the safety data and nutritional implication at the Draft Assessment Stage.
- They noted that there is an application for this novel food before the United Kingdom's Advisory Committee on Novel Foods and Processes. It would be useful if any progress from this committee were reported on in the Draft Assessment Report.

Australian Food and Grocery Council

- The AFGC considers that FSANZ has failed to comply with the requirements imposed on it by Section 13 of the FSANZ Act.
- Does not consider DAG-oil a novel food, because DAG-oil is not considered non-traditional. There has been significant human consumption of the components of DAG-oil by the broad community of Australia and New Zealand, through their intake as approved food additives and occurrence as metabolites of normal lipid metabolism following the consumption of dietary fat.
- Considers that FSANZ has already carried out safety assessment on mono- and diglycerides and the emulsifiers and antioxidants used in DAG-oil.
- Regarding labelling, AFGC considers that DAG-oil and products containing DAG-oil would automatically be subject to the full requirements of the Code. The AFGC also considers that unless FSANZ intends imposing labelling restrictions (advisory/warning statement) or the Applicant is seeking permission for specific labelling claims, labelling is not an issue for consideration in this Application.

Dietitians Association of Australia

- DAA cannot support either of the options at this stage, as they require further information on the safety and efficacy of DAG-oil.
- Modelling to determine the effects of higher DAG-oil intakes on infants, children, pregnant and lactating women, as well as people with disorders of fat absorption/digestion should be taken into consideration.