EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA); Scientific Opinion on the substantiation of a health claim related to diacylglycerol (DAG) oil and reduction of body weight pursuant to Article 13(5) of Regulation (EC) No 1924/2006

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SCIENTIFIC OPINION

Scientific Opinion on the substantiation of a health claim related to diacylglycerol (DAG) oil and reduction of body weight pursuant to Article 13(5) of Regulation (EC) No 1924/2006[1]

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA)[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

Following an application from Kao Corporation, submitted pursuant to Article 13(5) of Regulation (EC) No 1924/2006 via the Competent Authority of the United Kingdom, the Panel on Dietetic Products, Nutrition and Allergies was asked to deliver an opinion on the scientific substantiation of a health claim related to diacylglycerol oil and reduction of body weight. The food constituent, diacylglycerol (DAG) oil, and the food constituents, vegetable oils of similar fatty acid composition containing mostly (>90%) triacylglycerol (TAG), which DAG oil should replace in order to obtain the claimed effect, are sufficiently characterised in relation to the claimed effect. Weight loss is a beneficial physiological effect for overweight subjects. Seven randomised controlled trials (RCTs) were identified by the applicant as being pertinent to the claim. One RCT which had major methodological limitations did not allow conclusions to be drawn for the scientific substantiation of the claim. The results from six RCTs with respect to the effect of DAG oil (as a replacement of TAG oils) on body weight are inconsistent and apparently unrelated to the DAG dose, study size or study duration, and the evidence provided in support of mechanisms by which DAG oil could exert the claimed effect in humans under the proposed conditions of use is not convincing. One unpublished meta-analysis on the effects of DAG oil (as compared to TAG oils) on body weight in humans which included data from all these RCTs was also provided. The meta-analysis had a number of potential sources of bias and did not provide additional information for the scientific substantiation of the claim. The Panel concludes that a cause and effect relationship has not been established between the consumption of DAG oil (as a replacement of TAG oils) and a reduction in body weight.

KEY WORDS

Diacylglycerol, triacylglycerol, body weight, fat oxidation, energy intake, health claims.

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[1] On request from the Competent Authority of the United Kingdom following an application by Kao Corporation, Question No EFSA-Q-2011-00751, adopted on 25 November 2011.

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[3] Acknowledgement: The Panel wishes to thank the members of the Working Group on Claims: Carlo Agostoni, Jean-Louis Bresson, Susan Fairweather-Tait, Albert Flynn, Ines Golly, Marina Heinonen, Hannu Korhonen, Martinus Løvik, Ambroise Martin, Hildegard Przyrembel, Seppo Salminen, Yolanda Sanz, Sean (J.J.) Strain, Inge Tetens, Hendrik van Loveren and Hans Verhagen for the preparatory work on this scientific opinion and Mette Kristensen for the support provided to this scientific opinion.

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SUMMARY

Following an application from Kao Corporation, submitted pursuant to Article 13(5) of Regulation (EC) No 1924/2006 via the Competent Authority of the United Kingdom, the Panel on Dietetic Products, Nutrition and Allergies was asked to deliver an opinion on the scientific substantiation of a health claim related to diacylglycerol oil and reduction of body weight.

The scope of the application was proposed to fall under a health claim based on newly developed scientific evidence.

The food constituent which is the subject of the health claim is diacylglycerol (DAG) oil of plant origin with ≥80 % diacylglycerols, as compared to vegetable oils containing mostly triacylglycerols (TAGs). The Panel considers that the food constituent, diacylglycerol (DAG) oil, and the food constituents, vegetable oils of similar fatty acid composition containing mostly (>90%) triacylglycerol (TAG), which DAG oil should replace in order to obtain the claimed effect, are sufficiently characterised in relation to the claimed effect.

The claimed effect is "helps in the management of body weight through weight loss". The target population proposed by the applicant is the general population. The Panel considers that weight loss is a beneficial physiological effect for overweight subjects.

Seven randomised controlled trials (RCTs) were identified by the applicant as being pertinent to the claim. In addition, one unpublished meta-analysis on the effects of DAG oil (as compared to TAG oils) on body weight in humans and which included data from all of these randomised controlled trials was provided.

The Panel notes that none of the RCTs provided were specifically designed to assess the effects of DAG oil on body weight (i.e. power calculations were not performed or were not preformed on this outcome), but rather had multiple outcomes or were designed to assess changes in body fat. The Panel also notes that none of these studies took multiplicity of outcomes into account in the statistical analyses, and that the results provided for changes in body weight as secondary outcome are at increased risk of bias.

One RCT which had major methodological limitations did not allow conclusions to be drawn for the scientific substantiation of the claim.

Three RCTs, two of which were at high risk of bias, reported a small effect of DAG oil on body weight at doses of 10 g/day, whereas three RCTs did not show an effect at doses ranging from 10 to 45 g/day. The Panel notes that the results from these RCTs with respect to the effect of DAG oil (as compared to TAG oils) on body weight are inconsistent and apparently unrelated to the dose of DAG used, to study size, or to study duration. The Panel also notes that no significant differences in energy intake were observed between the DAG and TAG groups during the intervention period in any of the RCTs provided.

The Panel notes that the unpublished meta-analysis had a number of potential sources of bias, that no sensitivity analyses were performed and that publication bias was not assessed. The Panel considers that this meta-analysis does not provide additional information for the scientific substantiation of the claim.

A total of 17 human studies and 22 non-human studies which investigated the energy value, bioavailability and metabolism of DAG oil, as well as the mechanisms by which DAG oil could exert the claimed effect, were provided by the applicant. The Panel considers that the evidence provided in support of mechanisms by which DAG oil (as a replacement of TAG oils) could induce a reduction in body weight in humans under the proposed conditions of use is not convincing.

In weighing the evidence, the Panel took into account that the results from six RCTs with respect to the effect of DAG oil (as a replacement of TAG oils) on body weight are inconsistent and apparently unrelated to the DAG dose, study size or study duration, and that the evidence provided in support of mechanisms by which DAG oil could exert the claimed effect in humans under the proposed conditions of use is not convincing.

The Panel concludes that a cause and effect relationship has not been established between the consumption of DAG oil (as a replacement of TAG oils) and a reduction in body weight.

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BACKGROUND

Regulation (EC) No 1924/2006[4] harmonises the provisions that relate to nutrition and health claims, and establishes rules governing the Community authorisation of health claims made on foods. As a rule, health claims are prohibited unless they comply with the general and specific requirements of this Regulation, are authorised in accordance with this Regulation and are included in the lists of authorised claims provided for in Articles 13 and 14 thereof. In particular, Article 13(5) of this Regulation lays down provisions for the addition of claims (other than those referring to the reduction of disease risk and to children's development and health) which are based on newly developed scientific evidence, or which include a request for the protection of proprietary data, to the Community list of permitted claims referred to in Article 13(3).

According to Article 18 of this Regulation, an application for inclusion in the Community list of permitted claims referred to in Art 13(3) shall be submitted by the applicant to the national competent authority of a Member State, which will make the application and any supplementary information supplied by the applicant available to the European Food Safety Authority (EFSA).

STEPS TAKEN BY EFSA:

- The application was received on 17/05/2011.
- The scope of the application was proposed to fall under a health claim based on newly developed scientific evidence.
- The scientific evaluation procedure started on 30/05/2011.
- On 15/07/2011, the Working Group on Claims of the NDA Panel agreed on a list of questions for the applicant to provide additional information to accompany the application and the clock was stopped on 25/07/2011 in compliance with Art. 18(3) of Regulation (EC) No 1924/2006.
- On 08/08/2011, EFSA received the requested information as submitted by the applicant and the clock was restarted.
- During the meeting on 25/11/2011, the NDA Panel, having evaluated the data submitted, adopted an opinion on the scientific substantiation of a health claim related to diacylglycerols and reduction of body weight.

TERMS OF REFERENCE

EFSA is requested to evaluate the scientific data submitted by the applicant in accordance with Article 16(3) of Regulation (EC) No 1924/2006. On the basis of that evaluation, EFSA will issue an opinion on the scientific substantiation of a health claim related to diacylglycerol oil and reduction of body weight.

EFSA DISCLAIMER

The present opinion does not constitute, and cannot be construed as, an authorisation to the marketing of diacylglycerol oil, a positive assessment of its safety, nor a decision on whether diacylglycerol oil is, or is not, classified as a foodstuff. It should be noted that such an assessment is not foreseen in the framework of Regulation (EC) No 1924/2006.

[4] European Parliament and Council (2006). Regulation (EC) No 1924/2006 of the European Parliament and of the Council of 20 December 2006 on nutrition and health claims made on foods. Official Journal of the European Union OJ L 404, 30.12.2006. Corrigendum OJ L 12, 18.1.2007, p. 3–18.

It should also be highlighted that the scope, the proposed wording of the claim, and the conditions of use as proposed by the applicant may be subject to changes, pending the outcome of the authorisation procedure foreseen in Article 18(4) of Regulation (EC) No 1924/2006.

INFORMATION PROVIDED BY THE APPLICANT

Applicant's name and address: Kao Corporation, 1-4-10 Kayaba-cho, Nihonbashi, Chuo-ku, Tokyo 103-8210, Japan.

Food/constituent as stated by the applicant

Diacylglycerol (DAG) oil of plant origin with ≥ 80 % diacylglycerols.

Health relationship as claimed by the applicant

According to the applicant, the current health claim application is for DAG oil and its effect on body weight.

Wording of the health claim as proposed by the applicant

The applicant has proposed the following wording for the health claim: "Substituting your usual vegetable oil with DAG oil helps in the management of body weight through weight loss".

Specific conditions of use as proposed by the applicant

According to the applicant, the target population is the general population. DAG oil is recommended for use as a total or partial replacement of traditional edible oils, which are composed primarily of triglycerides. The minimum effective quantity is 10 g/day of DAG oil, which can be reasonably consumed as part of a balanced diet.

ASSESSMENT

1. Characterisation of the food/constituent

The food constituent which is the subject of the health claim is diacylglycerol (DAG) oil of plant origin with ≥80 % diacylglycerols, as compared to vegetable oils containing mostly triacylglycerols (TAGs).

Diacylglycerol (DAG) oil of plant origin was authorised in 2006[5] as a novel food ingredient for use in cooking oils, fat spreads, salad dressings, mayonnaise, drinks presented as a replacement for one or more meals of the daily diet, bakery products and yoghurt-type products. The oil is derived from the esterification of fatty acids (mainly soybean and rapeseed oil) with either monoacylglycerol (MAG) or glycerol in the presence of an immobilised specific 1,3 lipase produced by *Rhizomucor miehei*. The final product contains ≥80 % DAG of which 70 % is 1,3-DAG and 30 % is 1,2-DAG. Besides DAG, the oil also contains some triacylglycerol (up to 20 %) and some monoacylglycerol (up to 5 %). The fatty acid composition differs depending on the source, but the main fatty acids are oleic acid, linoleic acid and linolenic acid. Saturated fatty acids and *trans* fatty acids should not exceed 10 % and 1 % of total fatty acids, respectively.

From the information provided, the Panel notes that the food constituent for which the claim is made is DAG oil, which should replace vegetable oils of similar fatty acid composition (e.g. rapeseed oil, soybean oil and safflower oil) containing mostly (>90%) TAG, in order to obtain the claimed effect.

The Panel considers that the food constituent, diacylglycerol (DAG) oil, and the food constituents, vegetable oils of similar fatty acid composition containing mostly (>90%) triacylglycerol (TAG),

[5] 2006/720/EC: Commission Decision of 23 October 2006 authorising the placing on the market of diacylglycerol oil of plant origin as a novel food under Regulation (EC) No 258/97 of the European Parliament and of the Council OJ L 296, 26.10.2006.

which DAG oil should replace in order to obtain the claimed effect, are sufficiently characterised in relation to the claimed effect.

2. Relevance of the claimed effect to human health

The claimed effect is "helps in the management of body weight through weight loss". The target population proposed by the applicant is the general population.

Management of body weight through weight loss can be interpreted as a reduction of body weight in overweight subjects. Even a moderate weight loss without achieving a normal body weight in this population sub-group is considered a beneficial physiological effect. However, a reduction of body weight in normal-weight individuals may not be considered a beneficial physiological effect.

The Panel considers that weight loss is a beneficial physiological effect for overweight subjects.

3. Scientific substantiation of the claimed effect

The applicant performed a literature search in 15 databases to identify randomised controlled trials (RCTs) in humans which reported on the effects of DAG oil (as compared to TAG oils) on body weight. Inclusion and exclusion criteria are clearly specified in the application. Two studies conducted in subjects with type 2 diabetes who were on antidiabetic medications which could affect body weight (Li et al., 2008; Yamamoto et al., 2006) and two studies of two weeks duration (Hibi et al., 2007; Hibi et al., 2008a) were excluded by the applicant.

Seven RCTs were identified by the applicant as being pertinent to the claim. In addition, one unpublished meta-analysis on the effects of DAG oil (as compared to TAG oils) on body weight in humans and which included data from all of these RCTs was provided.

Human intervention studies

Seven RCTs in humans on the effects of DAG oil, which reported on body weight changes and had a duration of 4-52 weeks, were considered by the applicant as being pertinent to the claim (Kawashima et al., 2008; Maki et al., 2002; Nagao et al., 2000; Shoji et al., 2008; Takeshita et al., 2007; Tomonobu et al., 2004; Yuan et al., 2010). All the studies except one (Yuan et al., 2010) were conducted for 12 weeks or longer.

The Panel notes that none of the RCTs provided were specifically designed to assess the effects of DAG oil on body weight (i.e. power calculations were not performed or were not preformed on this outcome), but rather had multiple outcomes or were designed to assess changes in body fat (Maki et al., 2002; Yuan et al., 2010). The Panel also notes that none of these studies took multiplicity of outcomes into account in the statistical analyses, and that the results provided for changes in body weight as secondary outcome are at increased risk of bias (Schulz and Grimes, 2005).

Takeshita et al. (2007) conducted a randomised, double-blind, controlled, parallel intervention study in 70 Japanese men (mean age = 37 years; mean body mass index (BMI) = 24.5 kg/m^2), who were allocated to consume either TAG oil, DAG oil or DAG oil with phytosterol esters for 16 weeks after a two-week run-in period, followed by a wash-out period of eight weeks. The subjects were provided with 10 g/day of the test oils as mayonnaise. Upon EFSA's request, the applicant states that randomisation was stratified by "serum lipid level", BMI, waist circumference and age, but no information on the methods used was provided. Anthropometry (body weight, waist and hip circumferences) and blood lipids were assessed before the run-in phase, at baseline and every four weeks. Computed tomography (CT) scans were performed at baseline and at weeks 8, 12 and 16 to measure the total fat area, the visceral fat area and the subcutaneous fat area. Compliance was assessed using 24-h dietary records over a seven-day period prior to each visit. The effect of treatment

on body weight using absolute body weight values and body weight changes as percentage from baseline at different time points was assessed by two-way analysis of variance (ANOVA), and differences between groups at a given time point by one-way ANOVA. The Panel notes that repeated measures were not taken into account in data analyses. A total of 57 subjects (TAG oil n=18; DAG oil n=21; DAG oil with phytosterol esters n=18) completed the study and entered the per-protocol (PP) data analysis. Thirteen subjects were excluded from analysis owing to "unstable" cholesterol concentrations during run-in (n=3), diagnosis of type 2 diabetes (n=1), "changes in living environment" (n=5), and compliance < 75% (n=4). The Panel notes that no intention-to-treat (ITT) analysis was performed, and that the PP analysis excluded subjects for reasons unrelated to body weight changes (e.g. unstable cholesterol concentrations during the run-in period). The Panel considers that owing to major methodological limitations (i.e. limited information on the method of randomisation, repeated measures not being taken into account in data analysis, lack of correction for multiple comparisons, lack of ITT analysis, PP analysis unsuitable for body weight outcomes), no conclusions can be drawn from this study for the scientific substantiation of the claim.

In a one-year randomised, double-blind, controlled, parallel intervention study, Kawashima et al. (2008) investigated the effect of a DAG oil (approximately 85.5 % DAG and 12.5 % TAG) *vs*. TAG oil (approximately 94 % TAG and 3 % DAG) in a group of 312 (n=174 males) Japanese adults with BMI ≥ 25 kg/m^2 and age between 22 and 73. Inclusion criteria were BMI ≥ 25 kg/m^2 and/or hypertriglyceridaemia (fasting serum triglyceride concentration $\geq$ 150 mg/dL (1.70mmol/L)). The oils were provided in 600 mL containers and the participants were instructed to replace their usual cooking oil with the assigned test oils and use them freely. Body weight, waist and hip circumference, skinfold thicknesses, blood lipids, dietary records (three arbitrarily selected consecutive days) and a lifestyle questionnaire were assessed every three months during the study. Compliance with the treatment was assessed by mean daily test oil intake during the study. Mean test oil intake was estimated using vegetable oil intake from the dietary records and frequency of meals eaten away from home as indicated on the lifestyle questionnaire. Statistical analyses were performed in the sample of subjects who returned at least one dietary record by carrying forward the last observation. The Panel notes that the analyses were not carried out on the ITT population and that the last observation carried forward is not an appropriate method of dealing with missing data. Repeated measures analysis of variance (RM-ANOVA) was used to assess the effects of treatment on body weight expressed as absolute changes from baseline (in kg). Out of the 312 subjects randomised (n=155 to DAG and n=157 to TAG), 150 in the DAG and 153 in the TAG groups were considered for analyses, whereas 21 subjects in the DAG and 14 in the TAG groups did not complete the study. Dietary records showed that mean consumption of test oils was 7.6 g/day in the DAG group and 8.2 g/day in the TAG group (p=0.22), and also that energy intake did not differ between the two groups. A treatment x time interaction (p<0.01) for body weight was reported using RM-ANOVA. After one year, the DAG group had a small weight loss (mean±SD = -0.6±2.6 kg) compared to a small weight gain (mean±SD = +0.3±2.2 kg) in the TAG group. The Panel considers that this study reports an effect of DAG oil when replacing TAG oil on body weight (0.9 kg weight difference) at doses of about 8 g/day consumed for 52 weeks. However, the Panel considers that the results of this study are at high risk of bias owing to the important limitations of the statistical analyses performed.

In a double-blind, controlled, parallel study, Nagao et al. (2000) investigated the effect of 10 g/day of DAG oil *vs*. the same amount of TAG oil as part of food products for breakfast (i.e. bread, mayonnaise or shortbread) given for 16 weeks in 38 apparently healthy Japanese men with a mean BMI (mean±SEM) = 24.1±0.4 kg/m^2 after a run-in period of four weeks in which all subjects consumed the TAG products. Anthropometry (body weight, hip and waist circumferences), blood sampling and CT scans at the umbilical level (for the assessment of total abdominal fat, visceral fat area, subcutaneous fat area and hepatic fat content) were assessed before the run-in phase, at baseline and every four weeks. Total body fat was assessed by air-replacement method using a body densitometer at baseline and at weeks 12 and 16. Energy and nutrient intake, and compliance with the study oils, were assessed by means of food diaries for the entire run-in period and the last four weeks

of the intervention. Subjects were randomly allocated to the DAG or TAG group (n=19 each) using block randomisation (block size of six) stratifying by BMI, percentage body fat and hepatic fat content. Differences between groups at baseline, changes within groups from baseline, and between-group differences at different time points were assessed by Student's *t* test (two-tailed) in the sample of completers. The Panel notes that repeated measures were not taken into account, and that no correction for multiple comparisons was applied. No information on completers or drop outs is provided in the publication. Upon EFSA's request, the applicant states that two subjects dropped out of the study at month two, one from each study group. Body weight loss was reported to be greater in the DAG group (mean±SEM = -2.6±0.3 kg) than in the TAG group (mean±SEM = -1.1±0.4 kg; $p<0.01$). The Panel notes that this study reports a greater effect of DAG oil when replacing TAG oil on body weight (1.5 kg body weight difference) at doses of about 10 g/day when consumed for 16 weeks. However, the Panel considers that the results of this study are at high risk of bias owing to the limitations of the statistical analyses performed.

Shoji et al. (2008) conducted a multicentre, double-blind, controlled, parallel intervention study in 179 Japanese males (35-65 years) and postmenopausal females (< 65 years) with a BMI ≥ 25 kg/m^2 who were randomly allocated to consume either DAG oil or TAG oil for 12 weeks after two to four weeks run-in period. The subjects were provided with 10 g/day of the test oils as mayonnaise and shortbread. Anthropometry (body weight, waist and hip circumferences), blood lipids and blood pressure were assessed at baseline and every four weeks. CT scans were performed at baseline and at week 12 to measure the total fat area, the visceral fat area and the subcutaneous fat area. Subjects received dietary counselling to keep fat intake below 25 % of energy intake. Compliance with the study oils was assessed by means of three-day food records, eight times during the study. Changes between groups over time were assessed by two-way RM-ANCOVA using baseline values as covariate. A total of 155 subjects (DAG oil n=78; TAG oil n=77) completed the study and entered the PP data analysis. No ITT analysis was provided. Sixteen subjects did not meet the inclusion criteria at baseline, three dropped out before baseline, and five did not adhere to the study protocol. A significant effect of treatment ($p=0.030$) and time ($p=0.024$), but no time x treatment interaction ($p=0.086$) on body weight (RM-ANCOVA) was observed. This suggests that the main effect of treatment took place during the first observation period (four weeks). Mean weight difference from baseline was about -0.9±0.2 kg (mean±SEM) in the DAG group and -0.3±0.1 kg (mean±SEM) in the TAG group. The Panel notes that this study shows a small but statistically significant effect of DAG oil when replacing TAG oil on body weight (about 0.6 kg weight difference) at doses of about 10 g/day consumed for 12 weeks.

The Panel notes that three RCTs (Kawashima et al., 2008; Nagao et al., 2000; Shoji et al., 2008) reported an effect of DAG oil consumed as a replacement of TAG oils at doses of about 10 g/day on body weight. However, the Panel considers that the results of two (Kawashima et al., 2008; Nagao et al., 2000) out of these three RCTs are at high risk of bias.

In a randomised, double-blind, controlled, parallel intervention study, Maki et al. (2002) investigated the effect of DAG *vs.* TAG oils consumed as part of food products (muffins, crackers, soup, cookies and granola bars) with the same fatty acid composition for 24 weeks in 131 overweight and obese North American men and women aged 19 to 71 years with a mean BMI of ~34 kg/m^2 and a waist circumference ≥ 87 cm (women) or ≥ 90 cm (men). DAG or TAG-rich products were designed to supply 15 E% as either DAG or TAG as part of an energy-restricted diet (energy deficit = 2100-3500 kJ/day, 16-45 g of either oil/day). Anthropometric measurements (body weight, waist, thigh and mid-arm circumferences) were assessed at baseline and at weeks 2, 4, 6, 8, 12, 16, 20 and 24 of the study, whereas dual-energy X-ray absorptiometry (DEXA) and CT scans were performed at baseline and at weeks 12 and 24 of the study for the assessment of body composition and intra-abdominal fat (IAF) area, respectively. It is reported in the publication that primary outcomes of the study were body weight, body fat and IAF area. Upon EFSA's request, the applicant clarified that power calculations were performed using IAF area as the primary outcome of the study. Compliance was assessed by asking subjects to bring back the entire study product supply, including empty and full packages.

Three-day diet records and study-product diaries were also collected eight times during the study. Statistical analyses were performed in the ITT and PP populations. ITT analyses included data from all subjects who had at least one clinic visit after receiving at least one dose of the study product. For those subjects who did not have efficacy measurements at the planned analysis time points, the most recent available value was carried forward to the rest of the analysis time points. The Panel notes that this is not an appropriate method for dealing with missing data. PP analyses included data from all subjects who completed ≥ 12 weeks of the study and complied with the study protocol by consuming ≥ 75 % of the study products. Only measures for the time points that were available were considered. RM-ANOVA was used to assess the effects of treatment on body weight expressed as percentage of change from baseline. Upon EFSA's request, the applicant also provided this analysis using absolute body weight values and body weight changes from baseline (in kg) at different time points. A total of 79 subjects (n=43 in DAG, 22 (34 %) drop-outs; n=36 in TAG, 30 (45 %) drop-outs) of the 131 subjects randomised completed the study. The Panel notes the high drop-out rate (around 40 %) in this study. A significant effect of time ($p<0.001$) but no significant effect of treatment or time x treatment interaction was reported on body weight (absolute values) or body weight changes (in kg) by RM-ANOVA on ITT. When body weight changes were expressed as percentage of changes from baseline, a significant effect of treatment ($p=0.025$) and time ($p<0.001$), but no significant effect of treatment x time interaction ($p=0.123$), on body weight in favour of the DAG group compared to the TAG group was reported. The Panel notes that this transformed variable (body weight as percentage of changes from baseline) may not meet the normality assumption of the RM-ANOVA model. No "goodness of fit" measure was provided. It was reported that PP analyses were not significantly different from ITT (data not provided). The Panel considers that this study does not show an effect on body weight of DAG oil when replacing TAG oil at doses of about 16-45 g/day consumed for 24 weeks.

Tomonobu et al. (2004) conducted a double-blind, controlled, parallel intervention study in 210 Japanese men (n=103) and women (age 25-65 years; BMI=24-30 kg/m^2), who were allocated after a four-week run-in period to consume either TAG oil (n=68), DAG oil (n=70) or DAG oil with phytosterol esters (n=72) for 16 weeks followed by a wash-out period of eight weeks. The subjects were provided with 10 g of the test oils daily as mayonnaise-like food. Details on subject allocation (other than aiming for the groups to be comparable for baseline BMI, abdominal fat, and serum cholesterol concentrations) were not provided. Randomisation is not mentioned. Upon EFSA's request, the applicant states that randomisation was performed using computer-generated numbers, and was stratified by BMI, blood total cholesterol and IAF. Anthropometry (body weight, waist and hip circumferences) and blood lipids were assessed at baseline and every four weeks. CT scans were performed at baseline, at week 16 and at week eight of wash-out to measure the total fat area, the visceral fat area and the subcutaneous fat area. Compliance with the study oils was assessed by means of five-day food diaries at each study visit. The effect of treatment on body weight was assessed by two-way ANOVA, and differences between groups at a given time point by one-way ANOVA. The Panel notes that repeated measures were not taken into account in data analyses. A total of 184 subjects (TAG oil n=62; DAG oil n=61; DAG oil with phytosterol esters n=61) completed the study and entered data analysis, which was performed in the sample of completers only. No significant effect of treatment on body weight was observed by two-way ANOVA ($p=0.4712$). No significant differences between groups in body weight at the end of the study were observed by one-way ANOVA ($p=0.6377$). The Panel considers that this study does not show an effect on body weight of DAG oil when replacing TAG oil at doses of about 10 g/day consumed for 16 weeks.

Yuan et al. (2010) conducted a randomised, single-blind, controlled, cross-over intervention study in 29 North American females (age 18-65 years, BMI=24.5-36 kg/m^2) who consumed 40 g/day of either DAG oil or TAG oil for four weeks each with at least four weeks of wash-out in between. Half of the oil dose (20 g/day) was consumed at breakfast under supervision, whereas 20 g/day was given to participants to be consumed with the remaining daily meals. The primary outcome of the study was changes in body fat measured by DEXA, which was used for power calculations. Secondary outcomes included serum lipid profiles, *de novo* lipogenesis, energy expenditure, and body weight. All outcome

measures were assessed at the beginning and end of each intervention. The initial recruitment goal was 33 allowing for an estimated drop-out rate of 20–25 %. Baseline, endpoint, and percentage change from baseline data were compared using paired Student's *t* tests. The Panel considers that this test is not an appropriate analysis for cross-over designs. A total of 26 subjects completed the study and entered statistical analyses (on completers only). Changes in body weight were not significantly different between interventions (+0.2±0.3 % in the DAG group compared to +0.6±0.3 % in the TAG group). The Panel considers that this study did not show an effect on body weight of DAG oil, when replacing TAG oil at doses of 40 g/day consumed for four weeks.

The Panel notes that three RCTs (Maki et al., 2002; Tomonobu et al., 2004; Yuan et al., 2010) did not show an effect of DAG oil consumed as a replacement of TAG oils at doses ranging from 10 to 45 g/day on body weight.

The Panel also notes that no significant differences in energy intake were observed between the DAG and TAG groups during the intervention period in any of the RCTs provided.

An unpublished meta-analysis, which considered the seven RCTs discussed above, was also provided. The Panel notes that this meta-analysis had a number of potential sources of bias (e.g. inadequate statistical analyses in most of the studies included, and inadequate assumptions for deriving summary statistics for the cross-over study), that no sensitivity analyses were performed, and that publication bias was not assessed. The Panel considers that this meta-analysis does not provide additional information for the scientific substantiation of the claim.

Summary of human intervention studies

Three RCTs (Kawashima et al., 2008; Nagao et al., 2000; Shoji et al., 2008), two of which were at high risk of bias (Kawashima et al., 2008; Nagao et al., 2000), reported a small effect of DAG oil on body weight at doses of 10 g/day, whereas three RCTs (Maki et al., 2002; Tomonobu et al., 2004; Yuan et al., 2010) did not show an effect at doses ranging from 10 to 45 g/day. The Panel notes that the results from these RCTs with respect to the effect of DAG oil (as compared to TAG oils) on body weight are inconsistent and apparently unrelated to the dose of DAG used, to study size, or to study duration.

Mechanistic studies

A total of 17 human studies and 22 non-human studies which investigated the energy value, bioavailability and metabolism of DAG oil, as well as the mechanisms by which DAG oil could exert the claimed effect, were provided by the applicant.

The applicant proposed that the main mechanism by which DAG oil could exert the claimed effect is by inducing an increase in fat oxidation. The applicant proposed that, despite a comparable energy value, digestibility and metabolism of DAG oil and TAG oils, the consumption of DAG oil could induce an increase in fat oxidation compared to TAG oils because 1-MAG and 3-MAG (the main products of 1-3 DAG hydrolysis) preferentially undergo β-oxidation for energy generation rather than re-esterification and deposition in adipose tissue, which is proposed to be the main fate of absorbed 2-MAG (the main product of TAG hydrolysis). Such an increase in fat oxidation would prevent body fat accumulation and induce body weight loss in the long-term. The applicant argues that lower rates of fat oxidation in overweight and obese individuals have been associated with increased body weight gain in the long term independently of energy expenditure in cross-sectional and prospective cohort studies.

Eight controlled human intervention studies which investigated the effect of DAG *vs.* TAG oils on energy expenditure, fat oxidation, and respiratory quotient were provided by the applicant to support this mechanism of action. Two studies used a single dose of DAG oil (Saito et al., 2006; Saito et al., 2009), whereas in the remaining studies DAG oil was administered daily for 3-4 days (Hibi et al.,

2008b; Hibi et al., 2011; Kamphuis et al., 2003), 14 days (Hibi et al., 2007; Hibi et al., 2008a) and 28 days (Yuan et al., 2010), respectively. The majority of these studies reported an acute increase in post-prandial or 24-h fat oxidation (and/or a decrease in the respiratory quotient as a proxy for fat oxidation). However, results from the longer-term studies (14 to 28 days) are inconsistent. The study of longer duration (28 days) and using the highest dose of DAG oil (40 g/day) reported no effect on post-prandial energy expenditure, fat oxidation or 24-h *de novo* lipogenesis (Yuan et al., 2010). None of these studies reported a significant effect of DAG oil on energy expenditure. The Panel notes that the evidence provided does not establish a sustained effect of DAG oil on fat oxidation, or that an increase in fat oxidation independent of changes in energy expenditure (as in the mechanistic studies above) would affect body weight in the long term, particularly in the absence of significant changes in energy intake (as reported in the RCTs provided for substantiation of the claimed effect which have been discussed in the previous section).

The animal studies submitted aimed to investigate the acute effects of DAG oil intake (as compared to TAG oil) on fat oxidation, and the effects of longer-term (three weeks to eight months) DAG oil consumption on body weight, body fat, and gene expression of enzymes involved in β-oxidation of fatty acids. A significant increase in fat oxidation was reported in two rat studies after a single dose of DAG oil (Kimura et al., 2006; Watanabe et al., 1997), whereas only one (Meng et al., 2004) out of eight (Hara et al., 1993; Harris and Sherman, 1954; Mattson et al., 1951; Meng et al., 2004; Mori et al., 2005; Murata et al., 1997; Taguchi et al., 2002; Watanabe et al., 1997) studies in rats reported a significant effect of DAG on body weight when consumed for periods of three to 22 weeks. Conversely, all the three studies provided and which were conducted in mice (study duration 15 weeks to eight months, Murase et al., 2001; Murase et al., 2002; Saito et al., 2007) reported a significant effect of DAG oil intake on body weight, but no data were provided with respect to the effects of DAG oil intake on fat oxidation in mice. Energy intake was not significantly different between the DAG and TAG groups in any of these animal studies. The Panel notes that the results from the animal studies provided are inconsistent, and that they do not provide evidence that an increase in fat oxidation will induce changes in energy balance or body weight. The Panel considers that the animal studies submitted do not provide additional insight on a plausible mechanism by which consumption of DAG oil (as a replacement of TAG oils) could have an effect on body weight in humans.

The Panel considers that the evidence provided in support of mechanisms by which DAG oil (as a replacement of TAG oils) could induce a reduction in body weight in humans (under the proposed conditions of use is not convincing.

In weighing the evidence, the Panel took into account that the results from six RCTs with respect to the effect of DAG oil (as a replacement of TAG oils) on body weight are inconsistent and apparently unrelated to the DAG dose, study size or study duration, and that the evidence provided in support of mechanisms by which DAG oil could exert the claimed effect in humans under the proposed conditions of use is not convincing.

The Panel concludes that a cause and effect relationship has not been established between the consumption of DAG oil (as a replacement of TAG oils) and a reduction in body weight.

CONCLUSIONS

On the basis of the data presented, the Panel concludes that:

- The food constituent, diacylglycerol (DAG) oil, and the food constituents, vegetable oils of similar fatty acid composition containing mostly (>90%) triacylglycerol (TAG), which DAG oil should replace in order to obtain the claimed effect, are sufficiently characterised in relation to the claimed effect.

- The claimed effect is "helps in the management of body weight through weight loss". The target population proposed by the applicant is the general population. Weight loss is a beneficial physiological effect for overweight subjects.

- A cause and effect relationship has not been established between the consumption of DAG oil (as a replacement of TAG oils) and a reduction in body weight.

DOCUMENTATION PROVIDED TO EFSA

Health claim application on diacylglycerols and reduction of body weight pursuant to Article 13(5) of Regulation (EC) No 1924/2006 (Claim serial No: 0301_UK). May 2011. Submitted by Kao Corporation.

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GLOSSARY / ABBREVIATIONS

ANOVA	Analysis of variance
BMI	Body Mass Index
CT	Computed tomography
DAG	Diacylglycerol
DEXA	Dual energy X-ray absorptiometry
IAF	Intra-abdominal fat
ITT	Intention-to-treat
MAG	Monoacylglycerol
PP	Per-protocol
RCT	Randomised controlled trial
RM-ANCOVA	Repeated measures analysis of covariance
RM-ANOVA	Repeated measures analysis of variance
SEM	Standard error of the mean
TAG	Triacylglycerols