Food Standards Australia New Zealand

Meeting of the Epidemiology Scientific Advisory Group (EpiSAG) on Conjugated Linoleic Acid

Thursday 2 September, 2010

OUTCOME NOTES

EpiSAG Members in attendance:

Chairperson: Professor Neil Pearce, Massey University, New Zealand

- A/Prof Catherine Itsiopoulos, University of Canberra, Australian Capital Territory
- A/Prof Damien Jolley, Monash University, Victoria, Australia
- Prof Murray Skeaff, University of Otago, New Zealand
- Clinical A/Prof David Sullivan, Royal Prince Alfred Hospital The University of Sydney, NSW, Australia
- Prof Gary Wittert, University of Adelaide, South Australia

Apologies: Professor Graham Giles, Professor John McNeil, Professor Andrew Tonkin

The meeting opened at 9:30 am and closed at 3:30 pm.

Summary of key outcomes:

Issue	Agreed by EpiSAG Members in attendance
Effect of CLA on blood lipids	• The evidence that CLA lowers HDL-cholesterol (HDL) is convincing and raises health concerns at the population level. • The 95% confidence interval of the mean decrease in HDL is not the appropriate basis for considering the potential risk to individuals. Individual risk should be based on the mean minus two standard deviations; this number would cover 97.5% of the population. Thus, some individuals may experience a considerably larger fall in HDL which amounts to an added concern. • It is clear from the 95% confidence interval that CLA increases LDL-cholesterol (LDL) in the exposed population but the evidence is insufficient to estimate the magnitude of the effect. • It is appropriate for FSANZ to include data in its assessment that comes from studies where the isomers were not administered in a 1:1 form. • The 4:1 isomer study by Wanders *et al.*, (2010) supports concern around the effect of CLA on LDL in particular. • The fact that an adverse result from low doses of CLA is seen raises confidence in concluding that CLA has an adverse effect. • The adverse effects of CLA on blood lipids are especially important for population groups such as type 2 diabetics, pre-diabetics and those with metabolic syndrome. This is particularly relevant given the high burden of undiagnosed disease.
Trans fat intakes and population health	• The definition of *trans* fatty acids (TFA) is a separate issue to the safety of TFA and should not distract from the core issue of the safety profile of CLA. • CLA exerts effects on blood lipids that are similar but possibly not identical to the effects of TFA based on the limited data available.
Effect of CLA on glucose homeostasis	• Significant study design issues limit the ability to draw conclusions about the effect of CLA on glucose homeostasis in the general population. • Two well-conducted studies raise serious concern about potential harm from CLA for people with type 2 diabetes. • Long-term studies of CLA in pre-diabetic and diabetic patients are warranted; effects on glucose tolerance, micro-vascular complications and the full array of validated markers of CVD risk should be investigated.

Issue	Agreed by EpiSAG Members in attendance
Other risks of CLA	• Although other potential risks of CLA were noted in the agenda papers (such as those considered by EFSA); members' main concerns related to the unknown mechanism by which CLA potentially alters body composition and the unknown effects of CLA on pre-diabetic and diabetic populations referred to above. • Assessment of the effects of CLA on inflammatory markers, Apolipoprotein (Lp(a)) and liver function is also warranted.
Key uncertainties	• Importance of the food vehicle; in particular that real intakes might exceed 3.5 g CLA/day due to consumption of more than one food vehicle and the impracticality of limiting individual intake. • Insufficient data about the effect of background diets among the study participants. • The unknown effects of CLA in dyslipidaemic persons are a particular concern because half of the people with type 2 diabetes in Australia are undiagnosed. • There is also insufficient data about the effects of CLA on people with morbid obesity, overweight or obese children and adolescents, pre-diabetics, type 2 diabetics and gestational diabetics, subjects at high risk of CVD including those who have experienced a myocardial infarction, and older persons.
Effect of CLA on body composition	• CLA is having some kind of effect on body fat. • The available evidence is not conclusive of either overall positive or negative effects of the loss of body fat mass (BFM) in the general population. • Weight loss should improve glucose metabolism and HDL metabolism whereas this is not evident from available studies; although the effect on body weight is less conclusive. • There is neither clear nor substantial evidence of an effect of CLA on lean body mass. • The unknown mechanism of effect of CLA on BFM is an important safety concern because it is a false assumption that any fat loss is good. • Some mechanisms and patterns of fat loss may produce harmful effects. • Because weight loss is less apparent than fat loss, there is no clear evidence about what is happening to the redistribution of body fat when CLA is consumed. • Beneficial health effects from the small changes in BFM evident from these studies have not been demonstrated. There is also no evidence that the changes in body fat are sustained over a long period.

Issue	Agreed by EpiSAG Members in attendance
Effect of CLA on cardiovascular disease risk	• The overall increase in CVD risk is non-trivial and estimated to be up to 5% at the population level (limitations of the available evidence make it challenging to estimate the exact magnitude of the effect of CLA on risk of CVD). • The risk for some individuals could be substantially higher and the claimed benefits are unlikely to outweigh this risk.
Potential action arising	• It would be reasonable if FSANZ were to do an extra analysis that combined all CLA studies (1:1 and mixed isomer studies). • FSANZ could add value to Table 3 of SD2 by presenting results as a percentage of the original participants who completed the study to indicate dropout rates used in analysis of each study. This would more clearly identify studies analysed per protocol from studies analysed by intention to treat.

Agenda Item 1a: Welcome, apologies and introductions, confidentiality arrangements, outline of the day

All EpiSAG Members provided FSANZ with signed confidentiality agreements.

FSANZ organised the EpiSAG meeting to seek expert opinion relevant to its assessment of the 1:1 isomer ratio of conjugated linoleic acid (CLA) as a novel food ingredient. FSANZ emphasised that meeting discussions should: strive for robust decision-making; focus on scientific evidence from studies of the effect of CLA[1] in humans and achieve consensus as much as possible. The importance of avoiding, where possible, discussion of risk management options was also emphasised. If issues proved controversial, then the focus would be on seeking consensus firstly on high level issues and noting any differences as discussion progressed down to more detail.

FSANZ provided a brief overview of the use of CLA internationally including:

- its status as 'Generally Recognized as Safe' in the United States via an industry self-assessed process
- widespread use in supplement form in several countries in North America and Europe
- consideration as a novel food ingredient by the European Commission, after receipt of a scientific opinion from the European Food Safety Authority (EFSA).

A CD of reference material was provided to Members before the meeting.

Agenda Item 1b: Background from FSANZ

The agenda papers provided detail on the chemical structure of the two isomers of CLA (*c9,t11* and *t10,c12*) under discussion. FSANZ explained that the two Applicants sought permission to add synthetic CLA, derived from safflower oil, as a novel ingredient to a range of foods to achieve a daily intake 3-3.5 g/day. The synthetic CLA has the two isomers in a 1:1 ratio.

Members commented on the importance of the food vehicle and noted that regulatory permission to add CLA could result in CLA being present in many foods over time. This raises uncertainty about how intakes could be restricted to 3-3.5 g/day. An unintended consequence of promoting foods containing CLA to help weight maintenance could lead consumers to eat more of those foods and thus mitigate the Applicants' intended effect. Members therefore noted that although they were requested to assess a dose of 3-3.5 g/day in achieving the stated purpose (weight maintenance), no practical method existed to reliably restrict intakes from CLA in food, and higher intakes in at least some consumers were therefore likely.

[1] In this document, unless stated otherwise, CLA means a synthetic preparation containing the CLA isomers *c9,t11* and *t10,c12* in a 1:1 ratio.

Members also noted that the available literature did not provide enough information about background diets of test subjects and doubted that effects of CLA would be reproduced consistently if background diets differed considerably. For example, CLA as part of a high fibre diet might have a very different effect from CLA as part of a high fat, low fibre diet.

Agenda Item 2a: Effect of CLA (1:1 isomers) on markers for cardiovascular health (blood lipids)

FSANZ introduced this item by noting that its assessment showed that an intake of approximately 3 g/day of CLA equates to approximately 1% of dietary energy. The available evidence shows that the effects on blood lipids of replacing approximately 3 g of cis-unsaturated fatty acids with CLA are similar to replacing 1% energy from *cis*-unsaturated fatty acids with 1% energy from *trans* fatty acids (TFA).

Members commented that the evidence for an effect of CLA on HDL is based on studies skewed towards a lower age range with a disproportionate number of females and overweight and mildly obese rather than the more morbidly obese people.

The available evidence also seems to be based largely on subjects with normal baseline blood lipid levels. Subjects of special interest such as those with dyslipidaemia have not been studied extensively to date and the effect of CLA in those subjects is important for a full risk assessment. Apolipoprotein$_a$ (Lp(a)) has emerged as an important risk factor for cardiovascular disease (CVD). Lp(a) levels are inversely related to the risk of CVD and data on the effect of CLA on Lp(a) is very limited at this time. This was identified as an important deficiency in the safety data set for CLA.

Members noted that a meta-analysis by Mensink *et al*. 2003[2] of effect of fatty acids on blood lipids included the ratio of total:HDL-cholesterol, and then noted that FSANZ had assessed the effect of CLA on HDL and LDL separately. Members noted that the available CLA studies generally did not report total:HDL ratios and that Brouwer *et al.,* (2010) had calculated LDL:HDL ratios and reported an adverse dose response relationship for the effect of CLA on LDL:HDL.

Approximately 31% of the Australian population has one or more elements of the metabolic syndrome. Members noted that the implications of giving CLA to this population might be more adverse than the measured effects on HDL and LDL in the study populations because the starting point is a large population with a degree of dyslipidaemia.

Members agreed that despite the limitations of the available evidence noted above, the evidence that CLA lowers HDL is convincing and is a health concern at the population level, particularly as HDL is inversely related to CVD risk. In considering the effect on individuals of a reduction of HDL, members agreed that the appropriate estimate was the mean minus 2

[2] Mensink *et al*. 2003 conclude from their meta-analysis that "the effects of dietary fats on total:HDL cholesterol may differ markedly from their effects on LDL".

standard deviations (SD). Members commented that the 95% confidence interval (CI) describes the uncertainty around the location of the mean. The spread of risk in the population is wider than the CI and is related to the SD, not the standard error.

Members agreed that some individuals could experience a considerably larger fall in HDL than suggested by the 95% CI, which amounts to an added concern in the overall assessment. With regard to the assessment of effect of CLA on LDL, it is clear from the 95% CI (-0.008 to 0.106) that CLA increases LDL, but the evidence is insufficient to estimate the magnitude of the effect on LDL.

The 0.036 mmol/L decrease in HDL can be roughly estimated as a 1-2% decrease in HDL and this effect on HDL is small. It has been estimated that each % reduction in HDL corresponds to an increased risk of CVD by <5%. However, if the increase in LDL from CLA is considered at the same time (which corresponds to about a 1% increase in CVD risk), Members estimated that the overall increase in CVD is non-trivial and up to 5%. This is a population estimate based on measures of the mean effect of CLA, and 95% CI data indicate that the risk for some individuals could be substantially higher. While Members estimated a small overall effect on CVD risk, it is a non-trivial, adverse effect and so it is a legitimate public health concern. The concern is increased by the observation that intakes of CLA in food cannot be reliably limited to the 3.5 g/day recommended by the Applicants. Limitations of the available evidence make it challenging to estimate the exact magnitude of the effect of CLA on risk of CVD.

The EpiSAG concluded that an estimate of the effect of CLA on total:HDL-cholesterol, while desirable, was not essential because HDL and LDL are both independent risk factors for CVD. However, an estimate of the effect of CLA on Lp(a) would have provided useful and important information had the data been available. If the effect of CLA on Lp(a) were known, Members suspected the overall conclusion about an adverse effect of CLA in terms of CVD risk would be strengthened. Members also predicted that the effect of CLA on total:HDL ratios would be adverse.

Agenda Item 2b: Effect of CLA (other isomer ratios) on markers of cardiovascular health (blood lipids)

FSANZ introduced this item by highlighting that the key consideration was whether the available evidence provided a basis for concluding that the two isomers (*c9,t11* and *t10,c12*) affect lipids differently and in a manner that might justify excluding studies where the isomers were not delivered in a 1:1 ratio.

Members noted there is no clear evidence to show that the two isomers behave differently. This can be determined from studies where the individual isomers were compared head-to-head with the 1:1 manufactured mix or with mixtures of major CLA isomers in other ratios.

Members pointed out that the higher dose study by Wanders *et al*. (2010), which used a 4:1 ratio, gives confidence about the assessment of risk of higher dietary intakes of CLA. Therefore, inclusion of the higher dose study by Wanders *et al*. (2010) is an important part of the assessment. In particular, it supports the conclusion that CLA does elevate LDL.

In real life, fatty acids are always grouped together so it is not always possible to know what happens when individual fatty acids are grouped in different combinations. The study by Wanders *et al*. (2010), strengthens the Members' concern around the effect of CLA on LDL.

Members advised that it would be reasonable for FSANZ to conduct an extra analysis in which all CLA studies were combined to compare: the estimated effect of CLA isomers in the 1:1 form; the estimated effect from mixed isomer studies; and the estimated effect when all CLA studies are combined.

Members also noted that studies on the 4:1 isomer ratio clearly demonstrate that the conjugation of a *cis* with a *trans* bond does not of itself negate the trans fat like effects on blood lipids.

Agenda Item 3: Trans fat intakes and population health

FSANZ staff introduced this item by explaining that TFAs are defined differently in different parts of the world for labelling purposes. Australia and New Zealand have been working to reduce dietary intakes of TFA by non-regulatory approaches. The definition of TFA for nutrition information panel labelling purposes in the *Australia New Zealand Food Standards Code* does not exclude CLA as a TFA. As such, consideration of permission to add CLA to foods in Australia and New Zealand raises the possibility of increasing dietary intakes of TFA in both countries.

Members indicated that it would not be productive to attempt to classify CLA as a particular type of fatty acid. The definition of TFA is a separate issue to the safety of TFA. With regard to safety, discussion of earlier Agenda Items had clearly raised concerns about the effect of CLA on blood lipids. CLA exerts effects on blood lipids that are similar to the effects of TFA. However, while expressing some reservations about the evidence suggesting differences between CLA and TFA, some effects of CLA do not appear from the available data to be entirely identical to those expected from current knowledge about TFA. Members noted for example that TFA does not promote weight loss in consumers.

Thus, CLA has an effect on blood lipids and blood glucose much like that expected of TFA, but the effect of CLA on body weight is not what is expected of a TFA.

It was noted by Members that it is somewhat surprising that any effect of CLA has been measured from the low doses of CLA used in most studies. If studies on the effect of mono- or polyunsaturated fatty acids were undertaken at such low doses, effects would not be seen in studies of the size and design available for CLA. But it is known from higher dose studies

that a real effect of mono- and polyunsaturated fatty acids exists. The fact that adverse effects on parameters associated with adverse CVD outcomes are observed with low doses of CLA, consistent with observations from a better designed study with higher doses of the 4:1 isomer, provides confidence that the overall effect of CLA at potential intakes resulting from addition to food is likely to be adverse.

Members concluded that the available evidence is sufficient to confidently conclude that CLA has adverse effects on blood lipids that are comparable to the effects of TFAs.

Agenda Item 4a: Other potential risks – effect of CLA on glucose homeostasis

FSANZ staff introduced this item by summarising that FSANZ's assessment of the available evidence found that there is insufficient information to draw a conclusion about the effect of CLA on glucose homeostasis. As such, FSANZ is seeking guidance and comment on the strength and interpretation of the available data. The FSANZ assessment is underpinned by variable study design and methodological issues as well as, for the most part, statistically non-significant findings of outcome measures with changes being reported in both directions. FSANZ noted two studies in which CLA was given to people with type 2 diabetes did produce statistically significant adverse findings. FSANZ asked Members for their opinion of the available evidence in light of EFSA's scientific opinion that the safety of CLA has not been established for people with type 2 diabetes.

Members advised that the concerns about data quality discussed in regard to the assessment of CLA's effect on blood lipids are the same for all aspects of the CLA data set.

Members further advised that the results of Norris *et al.* (2009), where no beneficial effect on fasting blood glucose or insulin was found despite a statistically significant change in body weight in the CLA group, adds to concern about the effect of consuming added CLA, and in particular the nature and distribution of the reported weight loss. The high rate of dropouts from this study (20 dropouts from a recruitment of 55) was also noted which lead to a discussion of the crucial nature of undertaking analyses by intention to treat in weight loss trials.

Lean body mass (LBM) is a major determinant of glucose disposal. The available data do not show a clear effect of CLA on LBM. A reduction in body fat would be expected to produce a favourable effect on fasting blood glucose and fasting insulin, and lipid metabolism, in particular triglycerides and HDL. However, FSANZ's assessment has not identified such an effect from the available literature. Members agreed that beneficial physiological effects of reduced body fat have not been demonstrated in the available studies of CLA.

The results of Moloney *et al.* (2004) were also discussed. Members noted the small sample size in this study but were of the opinion that this is a well-conducted study, and together with the study by Norris *et al.* (2010) raises safety concerns in diabetic populations.

Members were not unanimous in their view that HOMA is a validated measure of glucose homeostasis. Instead, it was suggested that assessment of fasting glucose and insulin measures, without conversion to a surrogate index, is more appropriate. Members stressed that control of blood glucose levels (BGL) is not essential for estimating the risk of CVD. For example, the ACCORD trial[3] showed that reducing BGL did not decrease cardiovascular events (Skyler *et al*., 2009). Worsening glycaemic control has adverse impacts other than increasing CVD risk; i.e. a reduction in glycaemic control *per se* is an independent adverse finding. However, the adverse effects of CLA on blood lipids are highly likely to be hazardous for populations, particularly in groups such as type 2 diabetics.

There is a spectrum of micro-vascular complications in diabetic patients (e.g. retinopathy, renal failure, peripheral neuropathy) that are also of concern. Members advised that long-term studies of CLA in diabetic and pre-diabetic patients would be desirable to explore potential effects on outcome measures such as these.

It has been estimated that the number of people in Australia with undiagnosed type 2 diabetes is equal to the number of people with diagnosed type 2 diabetes (Colaguiri *et al*., 2004). To understand the potential harm of CLA, there is a need to ask what CLA does to the full range of CVD markers, particularly in pre-diabetic or undiagnosed type 2 diabetic populations.

Members concluded that significant design issues limit conclusions that can be drawn with regard to the effect of CLA on glucose homeostasis in the general population. However, Members were confident to conclude that there is good evidence that CLA is likely to be hazardous for people with type 2 diabetes, based upon the results of two well-conducted studies (Moloney *et al*., 2004; Norris *et al*., 2009) and the adverse effects of CLA on blood lipids.

Agenda Item 4b: Other potential risks of CLA

During discussion of previous Agenda Items, uncertainties regarding the mechanism of the effect of CLA on body composition and the unknown effects of CLA on pre-diabetic and diabetic populations emerged as common themes. To further explore this Item, FSANZ drew attention to the study by Sluijs *et al.* (2010), with 401 subjects, a 4 g/day dose of CLA and six months' duration, which reported no differences in cardiovascular risk factors. Members were asked their opinion on how repeating a study design of this type might add to the assessment.

Members suggested the following characteristics as key elements of a useful study design to improve the level of evidence regarding effects of CLA in human populations:

- longer study duration (most reversal studies on metabolic syndrome are 1-2 years duration)

[3] The ACCORD (Action to Control Cardiovascular Risk in Diabetes) trial sought to determine the effect of lowering glucose to near-normal levels on cardiovascular risk. It was a large multi-centre study (n=10,251) involving participants with pre-existing diabetes.

- sufficiently powered design to show a beneficial effect on fasting glucose and fasting insulin, if it exists
- assessment of the effect of CLA on inflammatory markers, Lp(a) and liver function
- focus on assessment of CLA's effect on pre-diabetic subjects with central obesity
- investigation of the pattern/location of fat loss.

More data on natural tissue distribution of CLA in humans and animal models are also recommended.

A good model for confirming an adverse effect would be a short-term dose escalation study using a hyperinsulinaemic euglycaemic clamp. If there is an effect, it would be detected in about four weeks using the clamp technique.

Because the available evidence indicates that CLA adversely affects HDL and LDL, how CLA affects Lp(a) is an important risk assessment question to be answered. Members did not believe that value would be added to the assessment by investigating the effect of CLA on triglyceride levels, even in diabetics, since triglycerides are a reciprocal indicator of HDL levels. However, one member considered that triglycerides were a useful and easily measured blood parameter. In particular, it would be important to confirm that the expected reciprocal relationship is preserved during CLA use. This is important because the inverse relationship between HDL and weight loss is not preserved.

If CLA changes the distribution of body fat, this raises interest in effects on liver function. The available evidence is not clear but it would be important to know which compartment of body fat is affected by CLA and the mechanism of the effect because there can be physiological consequences of lipid metabolism that are not beneficial.

The EpiSAG noted that the bone findings of Racine *et al*. (2010) are difficult to interpret owing to the children being peri-pubertal and so small differences in puberty between the groups might lead to differences in bone density. Of lesser interest is the effect of CLA on bone health which is largely unknown, although the prevalence of osteoporosis in Australia and New Zealand's ageing populations is such that this should not be completely ignored.

Agenda Item 5: Characterise the uncertainties about safety of CLA

Members affirmed that the safety of adding manufactured CLA to a range of foods has not been established. At the dose recommended by the Applicants to achieve 3-3.5 g/day CLA (1:1), the available evidence indicates potential harm, such that there is:

- an adverse effect on blood lipids and no apparent benefit to lipid metabolism
- evidence of an adverse effect on glucose homeostasis for people with type 2 diabetes and no evidence of a favourable effect on glucose or insulin metabolism
- these effects were often observed in the same studies and subjects where weight loss was being investigated.

The major uncertainty about safety of CLA, in the opinion of EpiSAG Members, was with regard to redistribution of body fat. CLA appears to produce a small reduction in body fat stores, but there is insufficient evidence of the mechanism and pattern of this effect and insufficient evidence to confirm that the effect leads to beneficial physiological outcomes.

Lost body fat may be metabolised or redistributed in a way that results in harm, not benefit. More detail about the effects of CLA on liver function would help to elucidate the mechanism by which CLA affects body fat stores.

Members also discussed the importance of the unknown effects of CLA in pre-diabetic, dyslipidaemic populations, particularly given the large proportion of the Australian population that, according to Ausdiab, unknowingly fit this category (Colaguiri *et al*., 2004).

There is currently insufficient data about the effects of CLA on the following groups:

- pre-diabetic populations
- type 2 diabetics, including evidence relating to effects of CLA on micro-vascular complications and longer term study of the effects of CLA on glycosylated haemoglobin
- women with gestational diabetes
- subjects who have experienced a cardiac infarction
- subjects at high risk of CVD
- obese children and adolescents
- older persons

More information is also needed on the effects of CLA on inflammatory markers and the full range of markers of CVD risk in these populations.

FSANZ also tabled a one-page graphic that summarises the quality of studies captured in SD2 with a focus on sample size and bias (see Appendix to Outcome Notes). The graphic shows poor reporting of important design features generally among the trials. Members suggested that ‘allocation concealment’ is an important design feature missing from the appended summary. Members noted the four criteria that are used by the Cochrane

Collaboration in assessing study quality to deal with bias. These are: 1. randomisation; 2. allocation concealment; 3. blinding; and 4. follow-up. The Cochrane Collaboration considers study quality against those four criteria and classifies studies as either 'at high risk of bias' or 'not at risk of bias'.

Members advised that given the novel nature of the Applications, and given the uncertainty about how CLA intakes could be restricted to 3-3.5 g/day if CLA was added to various foods, it was reasonable to expect the Applicants to have a greater responsibility to demonstrate safety.

Agenda Item 6a: The effect of CLA on body composition

FSANZ introduced this item by walking Members through the results of its assessment and reviewing the data summarised in Table 3 of SD2 (FSANZ, 2010). The results in this table, which capture three studies conducted over 6-12 months on participants with body mass index (BMI) 25-32 kgm^{-2} , are the main basis for FSANZ's conclusion that there is limited evidence that 3.2-3.6 g CLA/day taken for 6-12 months reduces body fat mass (BFM) by an average of 1.2-2.6 kg[4] in overweight and mildly obese individuals.

Members complimented FSANZ on the rigour of its assessment and agreed that CLA is having some kind of effect on body fat. Members advised that the available evidence is not conclusive of either positive or negative effects of loss of BFM in the general population. Members stressed that it is a flawed assumption to conclude that any fat loss is good. Some mechanisms and patterns of weight loss may produce harmful effects.

Members noted that the issues around quality of evidence for the studies captured in SD2 are the same as the issues previously discussed around the studies captured by FSANZ in SD1 and SD3.

There is neither clear nor substantial evidence of an effect of CLA on LBM (based on discussion of data in Table 3 of SD2) and only a trend toward decreasing total body weight. Methods of measuring body composition were discussed. Dual energy x-ray absorptiometry (DEXA) provides a reasonable measure of fat mass and lean body mass as well as the distribution of body fat. The amount of intra-abdominal fat can be specifically estimated. Accordingly, DEXA is capable of determining redistribution of body mass. However, DEXA cannot differentiate the presence of intramyocellular fat from fat that is located around the muscle. Bioelectrical impedance is by far a cruder method of estimating total body fat and a measure of lean body mass can be derived. While a relative change between the amount of fat mass and lean body mass can theoretically be determined, regional changes in fat mass cannot be determined with any degree of reliability, if at all.

[4] FSANZ staff made note of a typographical error in the agenda papers under item 6a (page 15), regarding the report of a 95% CI. It should read… "Results from two sub-group analyses investigating the effects of duration and dose of CLA on body fat mass indicated a loss of fat of 1.1 kg (95% CI: -1.9 to -0.4 kg; p=0.003)".

It was a shared opinion that there is no clear evidence about what happened to body fat after taking CLA – which fat depots were affected, did it go into other tissues or did oxidation increase? Not knowing this considerably limits a consideration of the potential risks or benefits of CLA. Complexity is added in that some studies found less weight gain whereas other studies found greater reduction in body weight, and these are not the comparable outcomes. The evidence for CLA preventing weight gain is not convincing.

It is very difficult to look at one effect of CLA in isolation. Ideally, a study of the effect of CLA on body composition needs to clearly separate the effect of CLA on weight loss from the effect of CLA on different compartments of body fat. If subcutaneous body fat stores are being reduced, but visceral body fat stores are increasing at the same time, the overall effect is bad. A 0.5 cm change in waist circumference, as indicated in the evidence included in SD2, could be from a change in visceral or subcutaneous fat stores and the available evidence cannot distinguish which change is taking place. A small change in BFM could therefore be harmful.

To assume that all populations of overweight and obese people are the same is overly simplistic; the heterogeneity of these populations is an important consideration. There are overweight populations that are metabolically fit, and others that have metabolic measures indicating that they are at considerably greater risk of CVD. Another example is sarcopenic obesity, which is problematic in older persons because they have reduced lean body mass and increased body fat.

The size of the change in HDL in a population at risk of CVD is more important than the size of the same change in a healthy population. A log linear relation shows increasing CVD risk with increasing LDL, which suggests a law of diminishing returns. Trials on dietary fatty acids and blood lipids are of 3-8 weeks duration as a general rule, and a sustained pattern of change on blood lipids is typically observed through such studies. Longer term studies are required to demonstrate sustained changes in body composition.

The available evidence is much more convincing with regard to how CLA affects blood lipids than with regard to how it affects body composition. The available evidence of effects of CLA suggests a marginal change in body weight (the duration of which remains uncertain) with prolonged adverse effects on blood lipids, which is of concern. In metabolically fit but overweight or obese subjects, a 2 kg reduction in body weight would produce no health benefit. The same could be said for a group such as this in terms of the clinical significance of a small change in HDL. From the available evidence, the permanency of a loss of body weight (up to 2kg after 12 months, as indicated in Table 3 of SD2) is difficult to attribute to CLA and there is no evidence of health benefit. If subjects were morbidly obese however, the metabolic benefits of weight loss occur only with significant weight loss, not with a weight loss as small as 2 kg. To confer a health benefit, approximately 4-5% loss of initial body weight is required.

The FSANZ assessment grouped BFM data from all methods of measuring body fat. It is difficult to assess changes in BFM without examining the concurrent change in body weight. Studies of weight loss should always be analysed according to intention to treat. For example, the placebo group in weight loss trials almost always lose 2-3 kg in 6 months (and then regain the weight afterwards) but this expected effect is not generally seen in the placebo groups of the CLA trials. FSANZ could add value to Table 3 of SD2 by presenting results as a percentage of the original participants who completed the study to indicate dropout rates used in analysis of each study, so to more clearly identify studies analysed per protocol from studies analysed by intention to treat (ITT).

Trials on body weight should also report their results showing mean changes in body weight in the control group from baseline, mean changes in body weight in the treatment group from baseline and the comparison of changes between control and intervention groups. The quality of available CLA studies is brought into question by lack of clarity regarding analysis by ITT, and absence of graphical data.

The clinical significance of FSANZ's estimate of lost body fat from CLA is unclear because:

- there is insufficient evidence to confidently explain where the body fat comes from or goes to; data in the trials about liver function might provide some insight
- data on energy intake in these studies are rudimentary
- measures of effect of CLA on energy expenditure are sparse
- improved glucose homeostasis would usually be seen in studies with weight loss, but this is not seen in these studies of CLA
- angiogenesis (development of blood vessels) is needed to maintain fat mass; it could be speculated that CLA is acting as an anti-metabolite but there is little data to explore this concept.

Because CLA seems to affect lipids and glucose homeostasis in the same way as TFA, it might be expected to also affect body weight like TFAs do, but this is not seen. The inconsistency therefore raises important questions about the mechanism by which CLA is affecting body fat.

The small changes in BFM evident from these studies might be good for a person's self esteem but beneficial health effects of CLA via changes to body composition have not been demonstrated. Similarly, there is no evidence that the changes in body fat are sustained over a long period.

Agenda Item 6b: Mechanism of the observed effects of CLA

FSANZ discussed three postulated mechanisms about the proposed mode of action of CLA as described by the Applicants. FSANZ sought to understand the proposed mode of action of CLA in achieving the stated purpose to determine whether safety concerns other than its effect on blood lipids were apparent.

EpiSAG Members noted that the mechanisms proposed by the Applicants have not been adequately demonstrated, are highly speculative and unconvincing, if not biologically implausible.

The proposed modes of action are flawed and not consistent with current knowledge of intermediary metabolism. They are also inconsistent with the results of Herrmann *et al*. (2009); for example, a decrease in lipoprotein lipase would produce an increase in triglycerides.

As discussed under previous items, the EpiSAG noted that potential mechanisms for weight loss could produce adverse physiological effects. The unknown mechanism of effect of CLA on body fat is an important safety concern because it is a false assumption that any weight loss is good.

Robust data on the mechanism of CLA weight loss was concluded to be an essential prerequisite to a sound assessment of the potential risks or benefits of CLA addition to food.

Agenda Item 7: Overall effect of CLA on cardiovascular disease risk

It was suggested that a composite tool such as the New Zealand Cardiovascular Risk Calculator (New Zealand Guidelines Group, 2009) for estimation of overall CVD risk might be referred to, and that the New Zealand Cardiovascular Risk Calculator would be preferable to the Framingham Calculator (Anderson *et al*., 2010). However the assumption that weight loss has a beneficial effect should not be included in any risk estimations owing to the adverse findings on lipids and glucose metabolism.

Members unanimously agreed that the evidence for adverse effects of CLA on blood lipids is much stronger than evidence for an effect of CLA on body composition.

It is extremely likely that the effect of CLA on blood lipids is adverse and the available evidence is sufficient to have confidence in this conclusion. The adverse effects of CLA on blood lipids are especially important for population groups such as type 2 diabetics, undiagnosed and pre-diabetics and those with metabolic syndrome.

Potential benefit from taking CLA has not been demonstrated primarily because the evidence does not adequately demonstrate which compartment of body fat is changing or by what mechanism the changes in body fat are occurring. Beneficial effects of CLA could operate

through a range of factors, e.g. improved insulin sensitivity, but there is no evidence that these factors are favourably altered by CLA.

At the population level, a small increase in risk of CVD of up to 5% is estimated. Although small, this effect is non-trivial and definitely adverse.

Table 1: Summary of EpiSAG Members' responses to specific questions posed by FSANZ

The Members of EpiSAG suggested some minor amendments to the specific questions listed in the agenda papers. Where this occurred, text in the specific questions has been deleted (~~thus~~), or added accordingly (**underlined**).

Specific Questions	EpiSAG Members' Responses
Agenda Item 2a: Effect of CLA (1:1 isomers) on markers for cardiovascular health (blood lipids)	
i. What is the clinical significance of a fall in HDL-cholesterol of 0.036 mmol/L: a) At an individual level?, and b) At a population level?	A reduction of HDL by 0.036 mmol/L is a small but adverse effect on HDL. However, individual variation will be larger than the 95% confidence interval (-0.069 to -0.002 mmol/L). The 95% confidence interval is not the appropriate basis for considering the potential risk to individuals, which should be based on the mean minus two standard deviations; this number would cover 97.5% of the population. This indicates the risk may be much greater for some individuals and this is an additional concern. At the population level, this change in HDL is estimated to result in a small increase in risk of CVD of <5%. The exact magnitude of the increased risk is difficult to estimate. However, though small, this adverse effect is non-trivial and a legitimate health concern at the population level.
ii. What potential clinical relevance should be attributed to the non-significant rise in LDL-cholesterol of 0.049 mmol/L from trials comparing the 1:1 ratio of CLA isomers?	The confidence interval (CLA increases LDL 0.049 mmol/L; 95% CI -0.008 to 0.106) is indicating that it is highly likely CLA increases LDL. The direction of the effect is clear from the 1:1 isomer studies but the evidence in isolation is insufficient to estimate the magnitude of the effect.
Agenda Item 2b: Effect of CLA (other isomer ratios) on markers of cardiovascular health (blood lipids)	
iii. To what extent can data from studies where the *c9,t11* and *t10,c12* CLA isomers were administered in other ratios be used to inform the assessment of the effects of consuming these isomers in a 1:1 preparation?	The available evidence does not show that the *c9,t11* and *t10,c12* isomers behave differently from one another on lipids. Therefore, it is appropriate for FSANZ to include data in its assessment that comes from studies where the isomers were not administered in a 1:1 form.

Specific Questions		EpiSAG Members' Responses
iv.	Would you modify your response to question ii. (above) in light of the significant rise in LDL-cholesterol that was reported from a study using the same CLA isomers in a 4:1 ratio (Wanders *et al.*, (2010)?	The results of Wanders *et al.* (2010) strengthen the Member's concern about the effect of CLA on LDL.
Agenda Item 3: Trans fat intakes and population health		
v.	Is it scientifically reasonable (given the results of the meta-analyses on the 1:1 ratio and adequately powered study on the 4:1 ratio of CLA isomers, re effects of CLA on HDL- and LDL-cholesterol) to a) Conclude that CLA behaves like a TFA, and therefore to b) Combine the CLA consumption (at the levels proposed by the Applicants) with estimates of current TFA consumption?	CLA behaves in a way that is similar but not necessarily identical to TFA. While there may be room for debate about how to classify CLA, the main concern is that the available evidence is sufficient to confidently conclude that CLA behaves with similar adverse effects to TFA on blood lipids.
vi.	How much of an increase in dietary intakes of TFA in Australia and New Zealand would be necessary to raise health concerns ~~in~~ **about the effects on** the consuming population?	A consensus on this issue was not pursued as Members did not believe it was necessary to get drawn into a definitional debate in order to assess the potential risks of CLA.
Agenda Item 4a: Other potential risks – effect of CLA on glucose homeostasis		
vii.	Do Members agree with the FSANZ assessment of the effects of CLA (*c9,t11* and *t10,c12* in 1:1 ratio) on glucose homeostasis (SD3)?	Yes, the evidence is inconclusive regarding an effect of CLA on glucose homeostasis in the general population.
viii.	What questions or concerns are raised by the current limited evidence on the effect of CLA on glucose homeostasis? And a) What data would be required to permit a conclusion about the effect of CLA on glucose homeostasis?	There is very limited evidence about the effect of CLA in pre-diabetic populations, especially those with central obesity. What is the long-term effect of exposure of CLA in people with metabolic syndrome? Studies in relevant populations would include the obese, and insulin resistant and pre-diabetic persons.

Specific Questions		EpiSAG Members' Responses
	b) How important is a conclusion about the effect of CLA on glucose homeostasis to the question of likely change in CVD risk from CLA?	The adverse effects of CLA on glucose homeostasis in persons with diabetes are an additional concern. The particular concern is the measures of adverse effects of CLA on blood lipids, being an added risk for people with type 2 diabetes. A longer-term study designed with sufficient power to show a beneficial effect on fasting glucose and fasting insulin measures (if it exists), while measuring changes to body composition and CVD risk factors, is desirable. Worsening glycaemic control is an independent adverse finding.
ix.	Is there sufficient evidence to reach conclusions about the safety of CLA (*c9,t11* and *t10,c12* in 1:1 ratio) for adults with type 2 diabetes? If so, what do you conclude?	Two well conducted studies raise serious concern about potential harm from CLA for people with type 2 diabetes through results of altered glucose homeostasis, which adds to concern about the adverse effects of CLA on lipid metabolism. The absence of a beneficial effect on glucose homeostasis in groups that experience reduced body weight when consuming added CLA provides further concern. While potential harm for diabetics is likely, further research is required for definitive conclusions to be drawn. A prospective, long-term (1-2 year) study measuring glucose tolerance and effects on the full spectrum of micro-vascular complications in diabetes is desirable.
Agenda Item 4b: Other potential risks of CLA		
x.	Is there sufficient evidence to assess the effect of CLA on other lipoproteins, liver function, bone health or any other outcome measure?	Members were particularly interested in the physiological consequence of redistribution of body fat stores. If the change in body fat mass is from redistribution, this would raise interest in effects of CLA on liver function. A key question that goes to potential risk from CLA is which compartment of body fat is being affected, and how? Members also wanted to know how CLA affects apolipoprotein$_a$ (Lp(a)) given the similarity of CLA's behaviour to TFA in respect of its effect on blood lipids.

Specific Questions	EpiSAG Members' Responses
Agenda Item 5: Characterise the uncertainties about safety of CLA	
xi. Has the safety of adding manufactured CLA (*c9,t11* and *t10,c12* isomers in 1:1 ratio) to a range of foods at the amounts recommended by the Applicants to achieve 3 - 3.5 g CLA intake per day been established? If not: a) What questions are raised by current evidence, or lack of evidence, about the safety of CLA added to foods? b) What data would be required to permit a conclusion about the safety of CLA added to foods?	No, safety has not been established. Where does the body fat come from and go to and what is the mechanism by which this occurs? What is the effect of CLA on Lp(a)? What does CLA do to the full range of CVD markers? Of lesser interest is the effect of CLA on bone health, though the prevalence of osteoporosis in Australia and New Zealand's ageing populations is such that this should not be completely ignored.
xii. Should the risk assessment focus attention on any sub-population groups?	To adequately assess the risk to potentially vulnerable sub-populations, data are required on • pre-diabetic populations • type 2 diabetics (longer term; effects on glycosylated haemoglobin being of particular interest), including evidence relating to effects of CLA on micro-vascular complications • women with gestational diabetes • subjects who have experienced a myocardial infarction • obese children and adolescents • subjects at high risk of CVD • older persons
Agenda Item 6a: The effect of CLA on body composition	
xiii. Has FSANZ adequately assessed the effect of CLA consumption on body composition?	Yes, but only in so far as the assessment has explored the effect on changes in total body fat mass. Curves showing mean changes in body weight in treatment and control groups would add value. And it is not clear how changes in body weight compare alongside recorded changes in body fat mass in the same studies.

Specific Questions		EpiSAG Members' Responses
xiv.	Are the conclusions FSANZ has drawn sufficiently robust to indicate a ~~positive~~ **significant** effect on body fat mass loss **in adipose tissue**?	Yes. However, direct evidence of health benefits from CLA has not been demonstrated in the available data. There are significant potential concerns through the unknown mechanism of CLA's effect on body fat, and uncertainty about where the body fat comes from and goes to. It is unclear if physiological benefits (e.g. improved insulin sensitivity) result from the small measure of reduction in total body fat mass. There is no evidence of favourable redistribution of body fat. The estimated changes in body fat may be meaningful for an individual's self esteem, but there is sufficient and significant concern from uncertainties to counter this small benefit.
Agenda Item 6b: Mechanism of the observed effects of CLA		
xv.	Do Members have any knowledge of the mechanism of action of CLA to achieve the stated purpose? If not, a) Does lack of information about the mechanism that might cause fat mass loss raise any potential safety concerns?	Members are not aware of any mechanism of action of CLA in terms of reducing fat mass. Yes, because some mechanisms of weight loss can produce adverse physiological outcomes. It is a flawed assumption that any weight loss is good.
Agenda Item 7: Overall effect of CLA on cardiovascular disease risk		
xvi.	Can the effect of CLA on LDL-cholesterol be included in the assessment of overall effect of CLA on CVD risk?	Yes.

Specific Questions	EpiSAG Members' Responses
xvii. What is the likely change in CVD risk from a concurrent decrease in HDL-cholesterol and body fat and any other factors that the EpiSAG think should be included: a) At an individual level?, and b) At a population level?	There is no direct evidence of overall benefit of consuming CLA in the available literature. Specifically, weight loss affects health through a variety of mechanisms, including favourable changes in lipids and glucose. As the effects on lipids and glucose metabolism in diabetics change in an adverse direction, it is not possible to say whether weight loss following use of CLA has a health benefit. EpiSAG Members concluded that the effect of CLA is likely to be adverse. It is likely that the effect of CLA on blood lipids is hazardous, particularly in groups such as type 2 diabetics.
xviii. Would a population decrease of HDL-cholesterol of 0.036 mmol/L be a significant increase in population health risk? If this decrease occurred at the same time as a 1.1 kg decrease in body fat after 3-4 months, how would this change your opinion?	When combined with the adverse effect on LDL, the change in HDL from consuming CLA can be estimated to increase the risk of CVD by up to 5%. This is a small but non-trivial adverse finding and a legitimate health concern at the population level. Potential benefit from taking CLA has not been demonstrated.
xix. Do the potential health benefits of consuming novel foods with added CLA outweigh the potential health risks?	No. There is a lack of convincing evidence for benefits of CLA and convincing evidence of potential harm through adverse effects on blood lipids and adverse effects on glucose homeostasis in type 2 diabetics.

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Appendix

Summary of study report quality – consideration of sample size and bias in studies in SD2 (FSANZ 2010)

Refer to SD2 for reference citations

Author, Year	Power calculation given[1]	Recruitment, randomisation and blinding details reported[2]	Inclusion/ exclusion criteria reported[3]	Similarity between groups at baseline[4]	Compliance defined and reported to be greater than 90%[5]	Dropouts reported and reasons given[6]	Analysed and reported per ITT or compared for the characteristics of those who completed with those who dropped out[7]
Berven *et al*., 2000	☐	■	■	■‡	■	■	■
Blankson *et al*., 2000	☐	■	■	■	☐ ☐	■	■
Bonet Serra *et al*., 2008	☐	☐	■	◨	☐#	■	■
Close *et al*., 2007	☐	☐	■	■‡	☐#	■	☐
Eyjolfson *et al*., 2004	☐	☐	■	◨	◨	■	■
Gaullier *et al*., 2004	◨	■	■	■	☐ ☐	■	■
Gaullier *et al*., 2007	◨	☐	■	■	■	■	■
Kamphuis *et al*., 2003	☐	☐	■	■‡	☐#	■	☐
Lambert *et al*. 2007	■†	☐	■	■‡	■	■	☐
Larsen *et al*., 2006	■	■	■	■	■	■	■
Laso *et al*., 2007	☐	☐	■	☐	■	■	☐
Mougios *et al*., 2001	☐	☐	■	■‡	◨	■	☐
Nazare *et al.* 2007	■	☐	■	■	■	■	■
Noone *et al.* 2002	☐	☐	■	■	■	■	■
Norris *et al*., 2009‡	☐	☐	■	■	■	■	■
Park *et al*., 2008	☐	☐	■	■	☐#	■	■
Petridou *et al*., 2003‡ (crossover study)	☐	☐	■	■‡	■	■	☐
Pinkoski *et al*., 2006 (parallel study)	☐	☐	■	■‡	■	■	☐

Author, Year	Power calculation given[1]	Recruitment, randomisation and blinding details reported[2]	Inclusion/ exclusion criteria reported[3]	Similarity between groups at baseline[4]	Compliance defined and reported to be greater than 90%[5]	Dropouts reported and reasons given[6]	Analysed and reported per ITT or compared for the characteristics of those who completed with those who dropped out[7]
Pinkoski *et al*., 2006‡ (crossover study)	☐	☐	☐	■‡	■	■	☐
Racine *et al*., 2010	◨	☐	■	■‡	☐₣	■	■
Risérus *et al*., 2001	☐	☐	■	■	■	■	■
Risérus *et al*., 2002a	☐^	■	■	■‡	☐†	■	■
Smedman and Vessby, 2001	☐^	☐	☐	■‡	■	■	☐
Steck *et al*. 2007	■	☐	■	■‡	■	■	☐
Taylor *et al*., 2006	☐	☐	■	■	☐#	■	■
Watras *et al*., 2006	◨	☐	■	■‡	■	■	☐

Legend: ■ Criterion met ◨ Criterion partially met ☐ Criterion not met

[1] Studies met this criterion if a sample size calculation was done and the required number of participants in treated groups completed the study.

† Sample size calculation done however data not available as to number of participants in each group. It was assumed the study was sufficiently powered from the limited information available, as they had more in total than the required sample size.

Studies partially met this criterion if they undertook a sample size calculation however failed to state the required number of participants thus it was unable to be determined if study adequately powered post subject withdrawal/exclusion.

Studies did not meet this criterion if data was not reported, thus it was assumed to be insufficiently powered.

^ Sample size calculation undertaken using a variable unrelated to this investigation. It was assumed the study was not sufficiently powered.

[2] Studies did not meet this criterion if all three components were not specifically detailed. Stating 'the study was a randomised, double-blind placebo-controlled trial' was not sufficient to meet this criterion.

[3] Studies met this criterion if either inclusion or exclusion criteria were specified. If only one was specified, the other was assumed by means of default.

[4] Studies met this criterion if statistical tests of baseline demographics were undertaken with non-significant differences.

‡ Studies reported non-significant differences between baseline variables only among those who completed the study and did not compare the characteristics of those who withdrew/ were excluded

Studies partially met this criterion if a range of values or limited information was provided to make a full assessment.

Studies did not meet this criterion if significant differences between groups at baseline were observed.

[5] Studies met this criterion if compliance was defined and reported to be greater than 90%.

Studies partially met this criterion if compliance was not defined but was reported to be greater than 90%.

Studies did not meet this criterion if:

- Compliance was defined but did not exceed 90%. Nb: Gaullier *et al.* (2004) achieved 89% compliance and Risérus *et al*. (2002a) achieved 89.5% compliance;
- # Compliance was neither defined nor reported; or
- Ŧ Compliance was defined but not reported.

[6] Studies met this criterion if there drop-outs were reported and reasons given or if there were no drop-outs and thus reporting of reasons was not required. Where no drop-outs were reported, however it could be determined from the results that there were none, it was assumed they met this criterion.

[7] Studies met this criterion if:

It was reported an ITT analysis was undertaken;

Where ITT analysis was not reported, studies met this criterion if:

There were no drop-outs reported or it was apparent from the results that all subjects were analysed it was assumed this criterion was satisfied;

A modified ITT was undertaken; or

A per protocol analysis was undertaken however comparison of subject characteristics of those completed with those withdrawn revealed no significant differences.

Studies did not meet this criterion if:

A per protocol analysis was undertaken and comparison of subject characteristics of those completed with those withdrawn was not undertaken;

No values for n were reported in the results, thus it was assumed ITT analysis was not undertaken; or

Not specifically reported, it was assumed ITT analysis was not undertaken if it was apparent from the results that not all subjects were analysed.