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OFFICE OF
FOOD ADDITIVE SAFETY

GRN000521

May 12, 2013

Dr. Antonia Mattia
Office of Food Additive Safety (HFS-255)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5 100 Paint Branch Parkway
College Park, MD 20740

Subject: GRAS notice for Conjugated Linoleic Acids

Dear Dr. Antonia Mattia:

On behalf of InnoBio® Limited, we are submitting for FDA review a GRAS notification for conjugated linoleic acids-free fatty acids (CLA-FFA) and CLA-triglycerides (CLA-TG). The attached documentation contains the specific information that addresses the safe human food uses for the subject notified substance. We believe that this determination and notification are in compliance with proposed Sec. 170.36 of Part 21 of the Code of Federal Regulations (21 CFR section170.36) as published in the Federal Register, Vol. 62, No. 74, FR 18937, April 17, 1997.

We enclose an original and two copies of this notification for your review. Please feel free to contact me if additional information or clarification is needed as you proceed with the review. We would appreciate your kind attention to this matter.

Sincerely,

(b) (6)

5/12/2014

Susan Cho, Ph.D.
Susanscho1@yahoo.com
Agent for InnoBio

enclosure

GRAS Notifications for

Conjugated Linoleic Acids –Free Fatty Acids (CLA-FFA) and

CLA-Triglycerides (CLA-TG)

produced by

INNOBIO® Limited

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

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Abbreviations

8-iso-PGF2α = 8-iso-prostaglandin F2α
21CFR = Title 21 of the Code of Federal Redulations
ACE = angiotensin-converting enzyme
ACF = aberrant crypt foci
AI = Adequate Intake
ALP = alkaline phosphatase
ALT = alanine aminotransferase
AOM = azoxymethane
Apo B=apolipoprotein B
AQLQ = asthma quality of life questionnaire
ARH = airway hyper-responsiveness
AST= aspartate transaminase
AUC = area under the curve
BFM = body fat mass
BHB = beta hydroxylbutyrate
BMC = bone mineral content
BMI = body mass index
BP = blood pressure
BUN = blood urea nitrogen
BW = body weight
cGMP = current good manufacturing process
c9,t11=cis-9, trans-11 conjugated linoleic acid isomer
CAT = catalase activity
cGMP =current Good Manufacturing Process
CLA = conjugated linoleic acid(s)
CoA = coenzyme A
CO_2 = carbon dioxide
Conc. = concentration
CPT-1 = carnitine palmitoyltransferase 1
CRP = C-reactive protein
CSD = cortical spreading depression
CSFII = Continuing Survey of Food Intakes by Individuals
CVD = cardiovascular disease
CWD = Cold Water Dispersible
DBP = diastolic blood pressure
DHA = docosahexaenoic acid
DIM = days in milk
DM = dry matter
DMBA = 7,12-dimethylbenz [a]anthracene
ECP = enterocutaneous fistulas
EDI = estimated daily intake
EE = ethyl ester(s)
EPA = eicosapentaenoic acid

EFSA = European Food Safety Authority
FDA = Food and Drug Administration
FA = fatty acid(s)
FFA = free fatty acid(s)
FFM = fat free mass
FVII = factor VII
γ –GTP = γ - glytamyltranspeptidase
GRAS = Generally Recognized As Safe
GSH-Px = glutathione peroxidase activity
HACCP = Hazard Analysis and Critical Control Point
HbA1c = glycosylated hemoglobin
HDL-C = high density lipoprotein cholesterol
HGF = hepatocyte growth factor
HOMA-IR = Homeostasis Model of Assessment - Insulin Resistance
HR = heart rate
ICAM-1 = Intercellular adhesion molecule 1
Ig = immunoglobulin
IGF-1 = insulin-like growth factor 1
IL = interleukin
IDL = Intermediate-density lipoproteins
IFN-γ = interferon gamma
LA = linoleic acid
LBM = lean body mass
LDL-C = low density lipoprotein cholesterol
Lp(a) = lipoprotein a
LT = leukotrienes
MCHC = mean corpuscular hemoglobin concentration
MCP-1 = monocyte chemotactic protein-1
MCV = mean corpuscular volume
ME = methyl ester(s)
MUFA = monounsaturated fatty acid(s)
NA = not available
NADP = nicotinamide adenine dinucleotide phosphate
NEFA = non-esterified fatty acids
NHANES = National Health and Nutrition Examination Survey
NOAEL = no-observed-adverse-effect level
NS = not significant
OGTT = oral glucose tolerance test
P = parallel design
PAI-1 = plasminogen activator inhibitor-1
PAT = peripheral arterial tonometry
PBMC = peripheral blood mononuclear cells
PG = Prostaglandin
PGF2α = F2-isoprostane 8-iso-prostaglandin F
PPARα = peroxisome proliferators activated receptor alpha

PPARγ = peroxisome proliferators activated receptor gamma
PUFA = polyunsaturated fatty acids
RA = rumenic acid
RACC = reference amounts customarily consumed
RCT = randomized clinical trial
RMR = resting metabolic rate
SBP = systolic blood pressure
SFA = saturated fatty acids
SHBG = sex hormone-binding globulin
SI = stimulation index
sICAM-1 =soluble Intercellular adhesion molecule 1
SOD = superoxide dismutase
t10,c12 = trans-10, cis-12 conjugated linoleic acid isomer
TB = total bilirubin
TC = total cholesterol
TG = triglycerides
TGF-β = transforming growth factor-beta
TNFα = tumor necrosis factor alpha
TRAP = total radical-trapping antioxidant potential
TVA = trans vaccenic acid
UL=Tolerable Upper Intake Levels
VA = vaccenic acid
VCAM-1 = vascular cell adhesion molecule-1
VO_2 max = maximal oxygen consumptionWC = waist circumference
WDP – Water Dispersible PowderWHR = waist-to-hip ratio
X = cross-over design

I. GRAS EXEMPTION CLAIM

I.A. Claim of exemption from the requirement for premarket approval pursuant to Proposed Title 21 of the Code of Federal Regulations (21 CFR) 170.36(1)

INNOBIO® Limited. (INNOBIO®; the notifier) has determined that its conjugated linoleic acids (CLA; both CLA-Free fatty acids [FFA] and CLA-Triglycerides [TG]) are Generally Recognized As Safe (GRAS). Consistent with Section 201(s) of the *Federal Food, Drug, and Cosmetic Act*, this determination is based on scientific procedures described in the following sections. Since these procedures specify the conditions of its intended use in food, the use of CLA is exempt from the requirement of premarket approval.

Signed

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Agent for INNOBIO® Limited (INNOBIO®)

I.B. Notifier's Name and Address

Notifier's name: INNOBIO® Limited (INNOBIO®)
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Fax number: 86-411-87406801
Physical address: No.49, DDA, Dalian, Liaoning, 116600, China.
E mail address: sunx@innobio.com

I.C. Name of GRAS substance

Conjugated linoleic acids (CLA), manufactured by INNOBIO®.
INNOBIO® CLA 80 TG (oil form),
INNOBIO® CLA TG 40 Powder CWD,
INNOBIO® CLA TG 40 Powder WDP,
INNOBIO® CLA TG 60 Powder WDP,
INNOBIO® CLA 80 FFA (oil form),
INNOBIO® CLA 95 FFA (oil form),
INNOBIO® CLA FFA 40 Powder CWD, and
INNOBIO® CLA FFA 40 Powder WDP.

I.D. Product description

Conjugated linoleic acids (CLAs) comprise a group of positional and geometric isomers of octadecadienoic acid (linoleic acid, C18:2) characterized by conjugated double bonds. The conjugation of the double bond results in conformational properties different from the unconjugated linoleic acid forms. These changes might have a marked effect on the biological activities of CLA, depending on the position of the double bonds within the C18 backbone and on the *cis* and *trans* conformation. Generally, 2 isomers are of special importance: cis-9, trans-11 (c9,t11) CLA and trans-10, cis-12 (t10,c12) CLA. In processed vegetable oils, the t10,c12-isomer is found in approximately equal amounts as the c9,t11-isomer. Heated frying fats and partially

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5/12/2014

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INNOBIO® CLA TG 40 Powder WDP,
INNOBIO® CLA TG 60 Powder WDP,
INNOBIO® CLA 80 FFA (oil form),
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hydrogenated vegetable oils also contain a mixture of several CLA isomers. Until 2003, most CLA studies were done on FFA form since CLA-TG form was not available prior to 2003. Since 2003, studies on both CLA-FFA and CLA-TG were found from the literature.

From a nutritional and labeling perspective, CLA is not considered a trans fatty acid. The United States Food and Drug Administration (FDA) has stated, "Under FDA's definition, CLA would be excluded from the definition of trans fat" (68 Fed. Reg. 41433, 41462, [July 11, 2003, FDA 2003]). According to the FDA, synthetically produced CLA is a component of certain foods, such as processed vegetable oils.

INNOBIO® CLAs are available in 2 chemical forms—free fatty acids (FFA) and triglycerides (TG). Conjugated linoleic acid oils (in a chemical form of either FFA or TG) are food-grade preparations derived from processed safflower oil. They consist of approximately 1:1 mixture of c9,t11 and t10,c12 CLA isomers (″1:1 mixture"). INNOBIO® CLAs are available in different physical forms as either powder or liquid and these are available in different concentrations. INNOBIO® CLA liquid oils are CLA 80 TG, CLA 95 FFA, and CLA 80 FFA. INNOBIO® CLA powdered products are CLA TG 40 Powder CWD, CLA TG 40 Powder WDP, CLA TG 60 Powder WDP, CLA FFA 40 Powder CWD, and CLA FFA 40 Powder WDP. CLA-FFA and CLA-TG oils are processed in a manner similar to current methods used for food oils. CLA-FFAs are processed using current food grade oil.

INNOBIO® CLA 80 TG has a 1:1 ratio of the c9,t11 and t10,c12 isomers in TG form with a concentration of approximately 80% (78-84%) CLA, derived from 100% pure, natural safflower seed oil.

INNOBIO® CLA 80 FFA has a 1:1 ratio of the c9,t11 and t10,c12 isomers in FFA form with approximately 80% (78-84%) CLA, derived from 100% pure, natural safflower seed oil with molecular distillation process.

INNOBIO® CLA 95 FFA is the highest concentration INNOBIO® CLA with approximately 95% (93-97%) CLA in FFA form. It has a 1:1 ratio of the c9,t11 and t10,c12 isomers, derived from 100% pure, natural safflower seed oil with molecular distillation process.

INNOBIO® CLA powder products include CLA FFA and TG chemical forms. These powders are available with two different shell compositions for the WDP and CWD. The WDP (water dispersible powder) is protein based. The CWD (cold water dispersible) is starch based.

I.E. Applicable conditions for use of the notified substance

Conjugated linoleic acids are intended to be used in the same categories of foods as those described in GRN 232 (FDA, 2008) at 1.5 g CLA per serving (or reference amounts customarily consumed; RACC) for specific food categories. The maximum proposed uses of CLA by weight percent in each food category are as follows: 0.625% in specific soy milk beverages, meal replacement beverages, milk (filled), flavored milk products, and fruit juice products; 3.75% in meal replacement bars; and 0.667% in specific yogurt products.

Based on the 2009-2010 National Health and Nutrition Examination Survey (NHANES) dataset

(USDA, 2013), mean intake of users is estimated to be 0.84 g/person/day (16.5 mg/kg BW/day) and the 90th percentile intake of users is 2.64 g/person/day (47.8 mg/kg BW/day) for Americans aged 2 years and older. Among specific groups, children aged 2-12 years and male teens aged 13-18 years consumed the highest intakes with a 90th percentile value of 3.12 g/person/day which corresponds to 134.0 and 48.2 mg/kg BW/day for children and male teens, respectively.

I.F. Basis for GRAS determination

In accordance with 21CFR 170.30, the intended use of CLA is considered GRAS based on scientific procedures. The GRAS determination is based on the following factors:

1. There is sufficient qualitative and quantitative scientific literature in the public domain, including human and animal data that show no indications of significant adverse CLA-related effects.

2. Numerous reviews conducted by experts in the field unanimously support the safety of CLA. Findings from a review of recently published literature continue to support the previous assessment of the safety of CLA as used. Numerous clinical trials have evaluated the effects of the 1:1 mixture (c9,t11 plus t10,c12) as well as a number of other isomers on similar parameters. Numerous reviews conducted by experts in the field unanimously support the safety of CLA. Human studies show that administration of daily dose of 3.4-3.6 g CLA (either FFA or TG form) for 1-2 years resulted in no serious adverse effects. Also, a daily dose 6 g CLA (either FFA or TG form) for 6 months -1 year was not associated with any adverse effects (Larsen et al., 2006; Tarnopolsky et al., 2007; Whigham et al., 2004). Short term studies (3-8 weeks) showed that daily doses of 6.8 g CLA-FFA and 19 g CLA-TG were well tolerated with no serious side effects (Blankson et al., 2000; Wanders 2010a, 2010b). In addition, a daily dose of 6.4 g for 16 weeks did not cause adverse effects on glucose metabolism in subjects with type 2 diabetes (Asp et al., 2011). For these “pivotal” studies, the levels of consumption represent the maximum dose consumed, rather than absolute safety endpoints.

3. A comprehensive review of the clinical data demonstrates that consumption of 1:1 CLA isomers (in a form of CLA oil) at doses of up to 6 g/day for up to 1 year and 3.4 g/day for up to 2 years is safe and has no significant effects on cardiovascular parameters (lipid metabolism, markers of inflammation, or markers of oxidative stress), insulin sensitivity and glucose, or maternal milk yield and milk fat. The levels of consumption in these “pivotal” studies represent the maximum doses consumed rather than absolute safety endpoints. A 3-week human study that used a daily dose of 19 g of CLA demonstrated no adverse events in healthy subjects.

4. The U.S. FDA has not raised questions on the safety of CLA-TG (GRN 232 [FDA, 2008]). Since the FDA’s last review of CLA in 2007-2008 (GRN 232 [FDA, 2008]), no published literature has contradicted the agency’s prior decision.

5. The proposed food use results in estimated daily intake (EDI) of users (mean of 0.84 g/person/day or 16.5 mg/kg BW/day and the 90th percentile intake of CLA at 2.64

g/person/day or 47.8 mg/kg BW/day). These EDIs are well within safe intake levels: consumption up to 6 g/day for 6-12 months is known to be safe for both CLA-TG and CLA-FFA in humans.

6. The compositional data and product properties are consistent with carefully controlled current good manufacturing process (cGMP) production and purification. INNOBIO® CLA preparations contain no impurities or contaminants of concern for human health.

I.G. Availability of information

The data that serve as the basis for this GRAS determination will be sent to the FDA upon request. They are also available for the FDA's review and copying at reasonable times at the office of INNOBIO® or NutraSource, Inc.

II. INFORMATION ABOUT THE IDENTITY OF THE NOTIFIED SUBSTANCE

II.A. Common or trade name

Conjugated linoleic acids (CLA) mostly consist of two isomers: cis-9, trans 11 (c9,t11) and trans-10, cis-12 (t10,c12) CLA. They are available in two forms: FFA and TG.
INNOBIO® CLA 80 TG (oil form),
INNOBIO® CLA TG 40 Powder CWD,
INNOBIO® CLA TG 40 Powder WDP,
INNOBIO® CLA TG 60 Powder WDP,
INNOBIO® CLA 80 FFA (oil form),
INNOBIO® CLA 95 FFA (oil form),
INNOBIO® CLA FFA 40 Powder CWD, and
INNOBIO® CLA FFA 40 Powder WDP.

II.B. Standards of identity

INNOBIO® intends to use CLAs in several food categories, including those that have standards of identity located in 21 CFR. We note that an ingredient that is lawfully added to food products may be used in a standardized food only if permitted by the applicable standard of identity in 21 CFR.

II.C. Background

In 1979, researchers from the University of Wisconsin applied a beef extract to mice skin. The mice were then exposed to a strong carcinogen, 7,12-dimethylbenz [a]anthracene (DMBA). Sixteen weeks later, the researchers found that the mice exposed to the beef extract had 20% fewer tumors (Ha et al., 1987). After 8 years, this anti-carcinogen was identitied as CLA (Ha et al., 1987). Although CLA is known for its anticancer properties, researchers have also found that the c9, t11 form of CLA can reduce the risk for cardiovascular disease (Tricon et al., 2004) and inflammation (Zulet et al., 2005).

Conjugated linoleic acids refer to a mixture of positional and geometric isomers of octadecadienoic acid (linoleic acid, c9,c12-C18:2, LA) characterized by conjugated double bonds (Danicke et al., 2012). The conjugation of the double bond results in conformational properties different from the unconjugated linoleic acid forms. It is a natural compound mainly found in ruminant products, such as meat, milk, and other dairy foods. These represent the main source of CLA for humans. Of the two biologically important isomers, c9,t11 (rumenic acid, RA) is the most prevalent one in foods. It comprises about 80 to 90% of total CLA in ruminant products. In processed vegetable oils, the t10,c12-isomer is found in approximately equal amounts with the c9,t11-isomer. In addition, heated frying fats and partially hydrogenated vegetable oils contain a mixture of several CLA isomers. The conjugated double bonds can be present in either the cis or trans configuration and are present predominantly in positions 8 and 10, 9 and 11, 10 and 12, or 11 and 13.

Since RA (c9, t11-CLA isomer) is bioactive when supplied as a natural food component in the form of CLA-enriched milk fat (Anadon et al., 2010, 2011), it confers a number of beneficial biological effects that include anti-carcinogenesis, immunomodulation, and anti-atherosclerosis. It is formed as an intermediate product of the biohydrogenation process that occurs in rumen; a

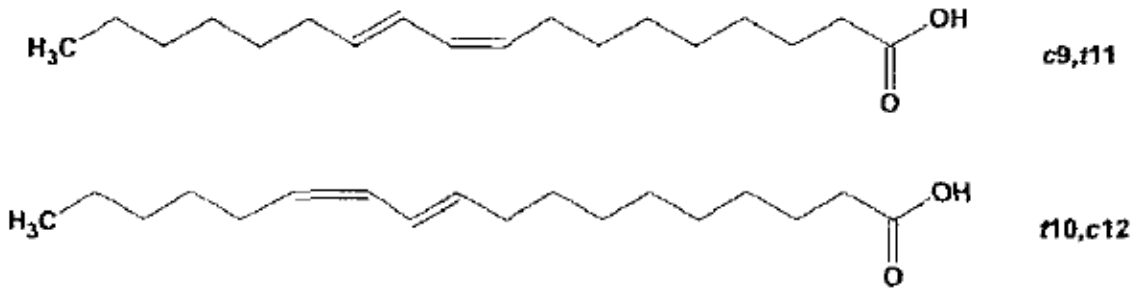
multistep mechanism carried out by different microorganisms on unsaturated fatty acids (FA) to produce stearic acid (C18:0). It can also be produced by desaturation of trans vaccenic acid (TVA; t11-C18:1). This process occurs in different tissues, such as mammary glands (Anadon et al., 2010; Turpeinen et al., 2002).

From a nutritional and labeling perspective, CLA are not trans fatty acids. The U.S. FDA has stated, "Under FDA's definition, conjugated linoleic acid would be excluded from the definition of trans fat" (68 Fed. Reg. 41433, 41462 [July 11, 2003; FDA, 2003]). The FDA has also concluded that synthetically produced CLA is a component of certain foods, such as processed vegetable oils (FDA letter to I.S. Bass, Mar. 12, 2003).

II.D. Chemistry, physico-chemical properties, and structure

The molecular weight of CLA is 280.4, and its molecular formula is $C_{18}H_{32}O_2$. The CAS numbers of c9,t11 and t10,c12 CLA isomers are 872-23-1 and 2420-44-2, respectively.
The structural formula of CLA is shown in Figure 1.

Figure 1. Structure of CLA



INNOBIO® CLA preparations are derived from processed safflower oil; Each CLA-FFA and CLA-TG is processed by following standard procedures common to the current fat and oil industry.
INNOBIO® CLA 80 TG consist of approximately 80% (78-84%) total CLA isomers in TG form and at least 75% of a 1:1 mixture of c9,t11 and t10,c12 CLA isomers. The remaining oil consists of other fatty acids, such as linoleic, oleic, palmitic, and stearic.

INNOBIO® CLA TG 40 Powder CWD is a free flowing and water dispersible powder. It contains approximately 40% (38-43%) of CLA in TG form derived from 100% natural safflower seed oil and is spray dried with gum arabic, maltodextrin, modified starch, silicon dioxide, mono- and di-glycerides, sodium ascorbate, natural mixed tocopherols and sunflower seed oil derived lecithin.

INNOBIO® CLA TG 40 Powder WDP is a free flowing and water dispersible powder. It contains approximately 40% (38-43%) of CLA in TG form derived from 100% natural safflower seed oil and is spray dried with sodium caseinate, maltodextrin, silicon dioxide, mono- and di-glycerides, sodium tripolyphosphate, natural mixed tocopherols and sunflower

seed oil derived lecithin.

INNOBIO® CLA TG 60 Powder WDP is a free flowing and water dispersible powder. It contains approximately 60% (57-64%) of CLA in TG form derived from 100% natural safflower seed oil and is spray dried with sodium caseinate, maltodextrin, silicon dioxide, mono- and di-glycerides, sodium tripolyphosphate, natural mixed tocopherols and sunflower seed oil derived lecithin.

INNOBIO® CLA 80 FFA has approximately 80% (78-84%) CLA in FFA form, derived from 100% pure, natural safflower seed oil with molecular distillation process. It has at least 75% of a 1:1 mixture of c9,t11 and t10,c12 CLA isomers.

INNOBIO® CLA 95 FFA consists of approximately 95% (93-97%) total CLA isomers and at least 88% of a 1:1 mixture of c9,t11 and t10,c12 CLA isomers. The remaining oil consists of other fatty acids, such as linoleic, oleic, palmitic, and stearic.

INNOBIO® CLA FFA 40 Powder CWD is a free flowing and water dispersible powder. It contains approximately 40% (38-44%) of CLA in FFA form derived from 100% natural safflower seed oil. It is spray dried with gum arabic, modified starch, maltodextrin, sodium ascorbate, silicon dioxide, mono- and di-glycerides, natural mixed tocopherols and sunflower seed oil derived lecithin.

INNOBIO® CLA FFA 40 Powder WDP is a free flowing and water dispersible powder. It contains approximately 40% (38-44%) of CLA in FFA form derived from 100% natural safflower seed oil. It is spray dried with sodium caseinate, maltodextrin, silicon dioxide, mono- and di-glycerides, sodium tripolyphosphate, natural mixed tocopherols, and sunflower seed oil derived lecithin.INNOBIO® CLA 80 TG has a 1:1 ratio of the c9, t11 and t10, c12 isomers in TG form with a concentration of approximately 80% (78-84%) CLA, derived from 100% pure, natural safflower seed oil.

II.E. Manufacturing process

Current Good Manufacturing Practice (cGMP), which includes Hazard Analysis and Critical Control Point (HACCP) risk analysis as well as hygiene and transport standards, is applied throughout the manufacturing process; continuous monitoring is used for control. Sampling schemes and analysis schedules are applied to raw materials, products in all stages of processing, and end products. The finished product is routinely tested to ensure that it meets specifications. Highly modern production facilities and electronic monitoring systems enable precise process control at all stages. Every step in the production of CLA is carried out under nitrogen, eliminating contact with air. The finished products are routinely tested to assure they meet specifications. CLA-FFA and CLA-TG oils are processed in a manner similar to current methods used for food oils. CLA-FFAs are processed using current food grade oil.

II.E.1. CLA 80 FFA

CLA 80 FFA is prepared using commercially available food grade safflower oil derived from safflower seeds rich with linoleic acid (LA). Safflower oil is trans-esterified with methanol to

generate fatty acid (FA) methyl esters (ME) that are rich in LA ME using sodium hydroxide as a catalyst. Then the sodium hydroxide is neutralized by adding sulfuric acid. The FA ME become CLA isomers by an isomerization process using sodium hydroxide and methanol under high pH conditions and controlled temperature. The product of above step, CLA sodium salt and excess sodium hydroxide, are neutralized by adding sulfuric acid. Solvent is removed by exsolution. After acidification, the CLA isomers are decolorized, distilled and purified. Activated carbon, a processing aid, is used as an adsorbent to remove natural pigments and trace impurities from CLA during the decolorization process. The used activated carbon is effectively removed by filtration and thus is not present in the finished product. Food grade mixed tocopherols are added to the product as an antioxidant before packaging. Main difference between manufacturing process of CLA-FFA and CLA-TG is that CLA-FFA does not employ trans-esterification of CLA ME/EE isomers with triacetate to form CLA-TG.

II.E.2. CLA 95 FFA

CLA 95 FFA is prepared using commercially available food grade safflower oil derived from safflower seeds rich with linoleic acid. Safflower oil is trans-esterified with methanol to generate FA ME that are rich in LA ME by using sodium hydroxide as a catalyst. The sodium hydroxide is neutralized by adding sulfuric acid. The FA ME become CLA isomers by an isomerization process using sodium hydroxide and methanol under high pH conditions and controlled temperature. The products of above step, CLA sodium salt and excess sodium hydroxide, are neutralized by adding sulfuric acid. Solvent is removed by exsolution. After acidification, the CLA isomers go through decolorization, vacuum rectification and molecular distillation stages.One additional vacuum rectification step is incorporated into the process to remove the saturated or monounsaturated fatty acid (SFA; MUFA) for concentration of the CLA FFA from 80% up to 95-97%. Activated carbon, a processing aid, is used as an adsorbent to remove natural pigments and trace impurities from CLA during the decolorization process. The used activated carbon is effectively removed by filtration and thus is not present in the finished product. Food grade mixed tocopherols are added to the product as an antioxidant before packaging.

II.E.3. CLA 80 TG

CLA 80 TG is prepared using commercially available food grade safflower oil derived from safflower seeds rich with LA. Safflower oil trans-esterifies with methanol/ethanol to generate FA ME or ethyl ester (EE) that are rich in LA ME/EE, using sodium hydroxide as a catalyst. Methanol or ethanol is removed by exsolution and then washed from FA ME or EE using water. The FA ME/EE become CLA ME/EE isomers by an isomerization process using potassium methylate methanol / potassium ethylate ethanol under high pH conditions and controlled temperature. The CLA ME/EE isomers are washed with methanol/ethanol aqueous solution to remove the potassium methylate/potassium ethylate and some small amount of fatty acid salt. The CLA ME/EE isomers will trans-esterify with triacetate using sodium methylate methanol or sodium ethylate ethanol to generate CLA TG. The CLA TG are then decolorized, distilled, and purified. Activated carbon is used in the manufacturing process as an adsorbent to remove natural pigments and trace impurities from CLA TG. Diatomite is used as a processing aid for removing impurities from CLA TG. The activated carbon and diatomite are effectively removed by filtration and are not present in the finished product. Mixed tocopherol is added to the product as an antioxidant before packaging. Main difference between manufacturing process of CLA FFA

and CLA-TG is that CLA-TG employs trans-esterification of CLA ME/EE isomers with triacetate to form CLA-TG and CLA-FFA skips this procedure.

II.E.4. CLA FFA 40 Powder CWD, CLA FFA 40 Powder WDP, CLA TG 40 Powder CWD, CLA TG 40 Powder WDP, and CLA TG 60 Powder WDP

The CWD (cold water dispersible) and the WDP (water dispersible powder) powders have different shell material only and the manufacturing process is identical. Food grade CLA FFA or CLA TG is combined with a food grade emulsifier and an antioxidant (food grade mixed tocopherols) then mixed at a set temperature until all ingredients combine to form an oil phase. The CWD powders are starch based and the WDP powders are protein based. Water, base ingredients (either protein or starch), and a food grade antioxidant are combined and mixed together to form an aqueous phase. Both the oil phase and the water phase mixtures are combined and mixed to form an initial emulsion. This initial emulsion becomes a homogenous emulsion under a high pressure homogenization process. The resulting homogenous emulsion is dried to powder as a fine grain by spraying. For CWD varieties, excipients (gum arabic, maltodextrin, modified starch, mono- and di-glycerides, and lecithin), food grade SiO_2 (less than 1.5%), and antioxidants (sodium ascorbate and natural mixed tocopherols) are added to the final powder. For WDP varieties, sodium caseinate, maltodextrin, silicon dioxide (<1.5%), mono- and di-glycerides, sodium tripolyphosphate, lecithin, and natural mixed tocopherols are added before spray drying. The final steps include blending, sieving, and packaging.

II. F. Specifications of INNOBIO® CLA

Tables 1-1 - 1-8 show specifications of various CLA preparations produced by INNOBIO®.

Table 1-1. Specifications of INNOBIO® CLA 80 TG

Tests	Limits	Methods
Appearance	Clear colorless or light yellow oil	Visual
CLA (%)	78.0-84.0	ISO 15304
-c9, t11 CLA+ t10, c12 CLA	75.0 Min.	
-c9, t11 CLA (%)	37.5 Min.	ISO 15304
-t10, c12 CLA (%)	37.5 Min.	ISO 15304
Palmitic acid (%)	6.0 Max.	ISO 15304
Stearic acid (%)	4.0 Max.	ISO 15304
Linoleic acid (%)	2.5 Max.	ISO 15304
Oleic acid (%)	15.0 Max.	ISO 15304
Color (LOVIBOND 5 1/4’’)	Y10.0, R1.0 Max.	AOCS Cc 13e-92
Acid value (mg KOH/g)	195.0-204.0	AOCS Cd 3d-63
Peroxide value (meq/kg)	5.0 Max.	AOCS Cd 8-53
Moisture (%)	0.1 Max.	AOCS Td 2-64
Saponification value (mg KOH/g)	195.0-204.0	AOCS Cd 3-25
Unsaponifiable matter (%)	1.0 Max.	AOCS Ca 6a-40
Lead (ppm)	0.2 Max.	EN ISO 17294
Arsenic (ppm)	0.2 Max.	EN ISO 17294
Cadmium (ppm)	0.2 Max.	EN ISO 17294
Mercury (ppm)	0.1 Max.	BS EN 13806
Mixed tocopherols (ppm)	250 ppm Min.	

Table 1-2. Specifications of INNOBIO® CLA TG 40 Powder CWD

Tests	Limits	Methods
Appearance	White or off white, free-flowing powder	Visual
Pure CLA Content (%)	38.0-44.0	AOAC 996.06
-c9, t11 CLA+ t10, c12 CLA	34.0 Min.	
-c9, t11 CLA (%)	17.0 Min.	ISO 15304
-t10, c12 CLA (%)	17.0 Min.	ISO 15304
Peroxide value (meq/kg)	10.0 Max.	AOCS Cd 8-53
Loss on drying (%)	4.0 Max.	USP 731
Bulk density (g/mL)	0.40-0.60	USP 616
Particle size	95% through 40 mesh	USP 786
Lead (ppm)	1.0 Max.	EN ISO 17294
Arsenic (ppm)	1.0 Max.	EN ISO 17294
Cadmium (ppm)	1.0 Max.	EN ISO 17294
Mercury (ppm)	0.1 Max.	BS EN 13806
Total plate count (CFU/g)	3,000 Max.	ISO 4833
Yeasts and molds (CFU/g)	300 Max.	ISO 7954
Coliform (CFU/g)	10 Max.	ISO 4832
E. Coli	Negative	ISO 16649
Salmonella	Negative/25g	ISO 6579
Mixed tocopherols (ppm)	250 ppm Min.	

Table 1-3. Specifications of INNOBIO® CLA TG 40 Powder WDP

Tests	Limits	Methods
Appearance	White or off white, free-flowing powder	Visual
Pure CLA Content (%)	38.0-43.0	AOAC 996.06
-c9, t11 CLA+ t10, c12 CLA	34.0 Min.	
-c9, t11 CLA (%)	17.0 Min.	ISO 15304
-t10, c12 CLA (%)	17.0 Min.	ISO 15304
Peroxide value (meq/kg)	10.0 Max.	AOCS Cd 8-53
Loss on drying (%)	4.0 Max.	USP 731
Bulk density (g/mL)	0.40-0.60	USP 616
Particle size	95% through 40 mesh	USP 786
Lead (ppm)	1.0 Max.	EN ISO 17294
Arsenic (ppm)	1.0 Max.	EN ISO 17294
Cadmium (ppm)	1.0 Max.	EN ISO 17294
Mercury (ppm)	0.1 Max.	BS EN 13806
Total plate count (CFU/g)	3,000 Max.	ISO 4833
Yeasts and molds (CFU/g)	300 Max.	ISO 7954
Coliform (CFU/g)	10 Max.	ISO 4832
E. Coli	Negative	ISO 16649
Salmonella	Negative/25g	ISO 6579
Mixed tocopherols (ppm)	250 ppm Min.	

Table 1-4. Specifications of INNOBIO® CLA TG 60 Powder WDP

Tests	Limits	Methods
Appearance	White or off white, free-flowing powder	Visual
Pure CLA content (%)	57.0-64.0	AOAC 996.06
-c9, t11 CLA+ t10, c12 CLA	54.0 Min.	
-c9, t11 CLA (%)	27.0 Min.	ISO 15304
-t10, c12 CLA (%)	27.0 Min.	ISO 15304
Peroxide value (meq/kg)	10.0 Max.	AOCS Cd 8-53
Loss on drying (%)	4.0 Max.	USP 731
Bulk density (g/mL)	0.20-0.50	USP 616
Particle size	95% through 40 mesh	USP 786
Lead (ppm)	1.0 Max.	EN ISO 17294
Arsenic (ppm)	1.0 Max.	EN ISO 17294
Cadmium (ppm)	1.0 Max.	EN ISO 17294
Mercury (ppm)	0.1 Max.	BS EN 13806
Total plate count (CFU/g)	3,000 Max.	ISO 4833
Yeasts and molds (CFU/g)	300 Max.	ISO 7954
Coliform (CFU/g)	10 Max.	ISO 4832
E. Coli	Negative	ISO 16649
Salmonella	Negative/25g	ISO 6579
Mixed tocopherols (ppm)	250 ppm Min.	

Table 1-5. Specifications of INNOBIO® CLA 80 FFA

Tests	Limits	Methods
Appearance	Clear colorless or light yellow oil	Visual
CLA (%)	78.0-84.0	ISO 15304
-c9, t11+ t10,c12 CLA (%)	75.0 Min.	ISO 15304
-c9, t11 CLA (%)	37.5Min.	ISO 15304
-t10, c12 CLA (%)	37.5 Min.	ISO 15304
Palmitic acid (%)	6.0 Max.	ISO 15304
Stearic acid (%)	4.0 Max.	ISO 15304
Linoleic acid (%)	2.5 Max.	ISO 15304
Oleic acid (%)	15.0 Max.	ISO 15304
Color (LOVIBOND 5 1/4’’)	Y5.0, R0.5 Max.	AOCS Cc 13e-92
Acid value (mg KOH/g)	195.0-204.0	AOCS Cd 3d-63
Peroxide value (meq/kg)	5.0 Max.	AOCS Cd 8-53
Moisture (%)	0.1 Max.	AOCS Td 2-64
Saponification value (mg KOH/g)	195.0-204.0	AOCS Cd 3-25
Unsaponifiable matter (%)	1.0 Max.	AOCS Ca 6a-40
Lead (ppm)	0.2 Max.	EN ISO 17294
Arsenic (ppm)	0.2 Max.	EN ISO 17294
Cadmium (ppm)	0.2 Max.	EN ISO 17294
Mercury (ppm)	0.1 Max.	BS EN 13806
Mixed tocopherols (ppm)	250 ppm Min.	

Table 1-6. Specifications of INNOBIO® CLA 95 FFA

Tests	Limits	Methods
Appearance	Clear colorless or light yellow oil	Visual
CLA (%)	93.0-97.0	ISO 15304
-c9, t11 CLA+ t10, c12 CLA	88.0 Min.	
-c9, t11 CLA (%)	44.0 Min.	ISO 15304
-t10, c12 CLA (%)	44.0 Min.	ISO 15304
Palmitic acid (%)	1.0 Max.	ISO 15304
Stearic acid (%)	1.0 Max.	ISO 15304
Linoleic acid (%)	2.5 Max.	ISO 15304
Oleic acid (%)	5.0 Max.	ISO 15304
Color (LOVIBOND 5 1/4’’)	Y10.0, R1.0 Max.	AOCS Cc 13e-92
Acid value (mg KOH/g)	195.0-204.0	AOCS Cd 3d-63
Peroxide value (meq/kg)	5.0 Max.	AOCS Cd 8-53
Moisture (%)	0.1 Max.	AOCS Td 2-64
Saponification value (mg KOH/g)	195.0-204.0	AOCS Cd 3-25
Unsaponifiable matter (%)	1.0 Max.	AOCS Ca 6a-40
Lead (ppm)	0.2 Max.	EN ISO 17294
Arsenic (ppm)	0.2 Max.	EN ISO 17294
Cadmium (ppm)	0.2 Max.	EN ISO 17294
Mercury (ppm)	0.1 Max.	BS EN 13806
Mixed tocopherols (ppm)	250 ppm Min.	

Table 1-7. Specifications of INNOBIO® CLA FFA 40 Powder CWD

Tests	Limits	Methods
Appearance	White or off white, free-flowing powder	Visual
Pure CLA Content (%)	38.0-44.0	AOAC 996.06
-c9, t11 CLA+ t10, c12 CLA	34.0 Min.	
-c9, t11 CLA (%)	17.0 Min.	ISO 15304
-t10, c12 CLA (%)	17.0 Min.	ISO 15304
Peroxide value (meq/kg)	10.0 Max.	AOCS Cd 8-53
Loss on drying (%)	4.0 Max.	USP 731
Bulk density (g/mL)	0.40-0.60	USP 616
Particle size	95% through 40 mesh	USP 786
Lead (ppm)	1.0 Max.	EN ISO 17294
Arsenic (ppm)	1.0 Max.	EN ISO 17294
Cadmium (ppm)	1.0 Max.	EN ISO 17294
Mercury (ppm)	0.1 Max.	BS EN 13806
Total plate count (CFU/g)	3,000 Max.	ISO 4833
Yeasts and molds (CFU/g)	300 Max.	ISO 7954
Coliform (CFU/g)	10 Max.	ISO 4832
E. Coli	Negative	ISO 16649
Salmonella	Negative/25g	ISO 6579
Mixed tocopherols (ppm)	250 ppm Min.	

Table 1-8. Specifications of INNOBIO® CLA FFA 40 Powder WDP

Tests	Limits	Methods
Appearance	White or off white, free-flowing powder	Visual
Pure CLA Content (%)	38.0-44.0	AOAC 996.06
-c9, t11 CLA+ t10, c12 CLA	34.0 Min.	
-c9, t11 CLA (%)	17.0 Min.	ISO 15304
-t10, c12 CLA (%)	17.0 Min.	ISO 15304
Peroxide value (meq/kg)	10.0 Max.	AOCS Cd 8-53
Loss on drying (%)	4.0 Max.	USP 731
Bulk density (g/mL)	0.40-0.60	USP 616
Particle size	95% through 40 mesh	USP 786
Lead (ppm)	1.0 Max.	EN ISO 17294
Arsenic (ppm)	1.0 Max.	EN ISO 17294
Cadmium (ppm)	1.0 Max.	EN ISO 17294
Mercury (ppm)	0.1 Max.	BS EN 13806
Total plate count (CFU/g)	3,000 Max.	ISO 4833
Yeasts and molds (CFU/g)	300 Max.	ISO 7954
Coliform (CFU/g)	10 Max.	ISO 4832
E. Coli	Negative	ISO 16649
Salmonella	Negative/25g	ISO 6579
Mixed tocopherols (ppm)	250 ppm Min.	

II.F.1. Stability of CLA preparations

Various CLA preparations are stabilized with approximately 250-300 ppm of mixed tocopherols and are stable at ambient temperature for up to 2 years.

III. INTENDED USES AND EXPOSURE ESTIMATES

III.A. Food sources of CLA

Conjugated linoleic acids naturally occur in ruminant products, such as beef, lamb, and dairy goods. This is due to the bacterial biohydrogenation of linoleic acid in the rumen, with the c9,t11 isomer accounting for more than 90% of CLA intake in the diet (Bhattacharya et al., 2006). Production of CLA in ruminants is subject to seasonal variation and depends on animal feed type. Total CLA content in milk or dairy products ranges from 0.34 to 1.07% of total fat (Dhiman et al., 2005). Total CLA content in raw or processed beef ranges from 0.12 to 0.68% of total fat. It is currently estimated that the average adult consumes only one third to one half of the amount of CLA that has been shown to reduce cancer in animal studies (Dhiman et al., 2005).

III.B. Intended use

Conjugated linoleic acids are intended to be used in the same categories of foods as those described in GRN 232 (FDA, 2008) at 1.5 g CLA per serving (or reference amounts customarily consumed; RACC) for specific food categories as shown in Table 2. The maximum proposed uses of CLA by weight percent in each food category are as follows: 0.625% in specific soy milk beverages, meal replacement beverages, milk (filled), flavored milk products, and fruit juice products; 3.75% in meal replacement bars; and 0.667% in specific yogurt products.

Table 2. Summary of maximum use-levels for CLA under the intended use

Food Category	Intended Food-Use	RACC, g or mL	Maximum CLA, g/serving	Maximum use level, %
Beverages and beverage bases	Specific soy milk beverages	240	1.5	0.625
	Specific meal replacement beverages	240	1.5	0.625
Grain products and Pasta	Meal replacement bars	40	1.5	3.75
Milk and milk Products	Specific flavored milk products	240	1.5	0.625
	Milk (Filled)	240	1.5	0.625
	Specific yogurt products	225	1.5	0.667
Processed fruit and fruit Juices	Specific fruit juice products	240	1.5	0.625

To deliver 1.5 g CLA, it requires 1.58 g of CLA 95 FFA, 1.875 g CLA 80 FFA, 1.875 g CLA 80 TG, 3.75 g CLA FFA 40 Powder, 3.75 g CLA TG 40 Powder, or 2.5 g CLA TG 60 Powder.

III.C. Exposure estimates

III.C.1. Estimated Daily Intakes (EDI) from before the intended use

The mean daily dietary CLA intake from the background diet is an estimated 212 mg/day for men and 151 mg/day for women in the general U.S. population, with subgroups consuming up to 300 mg/day (Ritzenthaler et al., 2001). The Ritzenthaler et al. (2001) study, with 51 men and 51 women between the ages of 18 and 60 years, compared 3-day food records and food frequency questionnaires. It was well-designed, fully reported, and representative of the general population—a very relevant report for this GRAS determination.

A prior study showed that the EDI of college-aged males and females were estimated to be 137 and 52 mg/day, respectively (Ritzenthaler et al., 1998). The EDI of lactating women with high- and low-dairy diets were 291 and 15 mg/day, respectively (Park et al., 1999). Studies in the German population show an estimated daily CLA intake of 350 to 430 mg for men and women, respectively. In other countries, the figures were 120 to 140 mg/day (Van Nieuwenhove et al., 2012).

III.C.2. EDI under the intended use

Using the NHANES (2009-2010) dataset, mean intake of users is estimated to be 0.84 g/person/day (16.5 mg/kg BW/day) and the 90th percentile intake of users is 2.64 g/person/day (47.8 mg/kg BW/day) for Americans aged 2 years and older. Among specific groups, children aged 2-12 years and male teens aged 13-18 years consumed the highest EDIs with a 90th percentile value of 3.12 g/person/day which corresponds to 134.0 and 48.2 mg/kg BW/day for children and male teens, respectively. Table 3 shows EDI of users of CLA under the intended use. The NHANES food codes are listed in the Appendix D.

Table 3. EDI of users of CLA under the intended use (NHANES 2009-2010)

Age, yr /gender	N, users+ nonusers	N of users	EDI, g/day		EDI, mg/kg BW/day	
			Mean	90th percentile	Mean	90th percentile
2-12, all	2,166	1,157	1.19±0.06	3.12±0.06	49.6± 3.3	134.0±6.2
2-12, M	1,111	585	1.18±0.08	3.12±0.09	50.6± 4.6	137.0±11.3
2-12, F	1,055	572	1.21±0.07	3.12±0.15	48.6± 2.6	133.3±6.2
13-18, all	958	331	0.85±0.10	2.72±0.27	13.6±1.6	43.3±5.3
13-18, M	510	181	1.00±0.15	3.12±0.35	15.3± 2.3	48.2±7.4
13-18, F	448	150	0.71±0.10	2.24±0.23	12.0±1.7	38.5±4.6
19-50, all	3,123	966	0.77±0.04	2.55±0.14	10.1±0.61	33.5±1.6
19-50, M	1,530	441	0.86±0.06	2.90±0.16	10.6± 0.78	34.1±1.8
19-50, F	1,593	525	0.68±0.04	2.25±0.16	9.6±0.60	32.6±2.0
51-99, all	2,697	949	0.76±0.05	2.34±0.11	9.9±0.62	30.7±1.6
51-99, M	1,350	429	0.77±0.07	2.39±0.21	8.9±0.75	27.8±2.2
51-99, F	1,347	520	0.75±0.06	2.31±0.14	10.8±0.85	32.8±1.6
2-99, all	8,944	3,403	0.84±0.03	2.64±0.09	16.5±0.76	47.8±2.8
3-99, M	4,501	1,636	0.90±0.03	2.87±0.08	16.9±0.93	50.1±3.4
2-99, F	4,443	1,767	0.79±0.03	2.40±0.10	16.1 0.80	45.7±3.0

M=males; F=females

IV. BASIS FOR GRAS DETERMINATION

IV.A. Current regulatory status

In 2004, two GRAS notices were individually filed with the FDA (GRN 148 [FDA, 2004] by Loders Croklaan in March, 2004, and GRN 153 [FDA, 2004] by Cognis Corp. in June, 2004). Reviewers noted several issues that led to "no basis" response letters. Safety concerns from published literature included: 1) CLA may induce insulin resistance in humans; 2) it may increase the risk of CVD as indicated by inflammatory biomarkers including C-reactive protein (CRP), oxidative stress (isoprostanes), and impaired endothelial function; and 3) may decrease maternal milk yield and/or milk fat production.

In August, 2007, the two companies, Lipid Nutrition and Cognis, submitted a joint GRAS notice (GRN 232 [FDA, 2008]) with revised chemistry and toxicology sections that addressed each of the FDA's concerns. In 2008, the FDA issued a no question letter related to the safety of CLA.

In 2010, the EFSA stated that consumption of CLA up to 3.0 g/day for up to 6 months is safe, and that the safety of CLA consumption for periods longer than six months has not been established. In 2012, the EFSA Panel re-assured that the safety of CLA consumption for periods longer than six months has not been established under the proposed conditions of use. In addition, the Panel further concluded that the safety of CLA consumption by type-2 diabetic subjects has not been established.

Food Standards Australia and New Zealand (FSANZ, 2010) concluded that it was not considered appropriate to establish a health standard for CLA. It was due to the following facts: 1) the clinical studies were primarily designed to assess efficacy, very little relevant toxicological information could be derived from them; 2) given the nature of these fatty acids, their presence already in the diet and their known metabolic pathways, it was considered that hazards would not have been identified from the measured parameters, and 3) the majority of animal studies with CLA have been conducted in mice and rats, with a smaller number of studies in pigs, hamsters and rabbits also being available. FSANZ (2010) states that ' These studies were of variable duration, e.g. ⩽8 weeks in mice; 2 to 36 weeks in rats; ⩽14 weeks in pigs; and 8 weeks in hamsters, with key data coming from two 90-day studies in rats.' It appears that the FSANZ review did not include a 18 month rat study of Park et al. (2005).

IV.B. Intended technical effects

Conjugated linoleic acids can be used as food ingredients at concentrations consistent with cGMP.

IV.C. Review of safety data

Since the FDA's last review of CLA in 2007-2008 (GRN232), no literature contradicting the agency's review (leading to 'no question') has been published. To find out if there are any recent reports that may not be consistent with the FDA's 2008 review, we reviewed literature published since 2007 on the safety of CLA from 3 perspectives: 1) glucose metabolism, 2) CVD risk factors, including BW measures, inflammatory biomarkers, and fat oxidation, and 3) human milk

yield and/or milk fat production.

IV.C.1. Metabolic fate of CLA

Overview

The metabolism of CLA (in a form of either FFA or TG) has been widely studied and reported, and follows the standard pathway of dietary FFA or TG (GRN 232; FDA, 2008). Thus, no systematic toxicity is expected after ingestion and metabolism of 1.5 g/serving in selected foods. Triglycerides is hydrolyzed into FFA before being metabolized. CLA-TG is expected to be hydrolyzed into CLA-FFA. Thus, the data found from human and animal toxicity studies of CLA-TG may be applicable to CLA-FFA, or vice versa.

Regulation of lipid metabolism is controlled by the liver and involves catabolism of TG for use as energy, desaturation, elongation of FA, and synthesis of TG and phospholipids (Krummel, 1996). TAG molecules are present in the small intestine are broken down by pancreatic lipase. Lipase mainly attacks the primary ester bonds, resulting in the production of free fatty acids and monoacylglycerol. These form mixed micelles in the presence of bile acids, in which form they are absorbed by intestinal epithelial cells for re-esterification to triglycerides and / or oxidised as a source of energy.

Upon absorption into the cells that line the intestinal wall, fatty acids of length C>12 (either as part of monoacylglycerol or in free form) are resynthesised into TAG. These TAG are then combined with lipoproteins and other lipid substances to form chylomicrons, which are released into the lymph vessels, from where they enter the bloodstream. The metabolism of absorbed FAs depends, in part, upon their chemical structure. Medium-chain length FAs are used mainly as energy sources (β-oxidation); longer-chain SFA and MUFA are generally stored (i.e., in adipose tissue as TG); and polyunsaturated fatty acids (PUFAs) are typically taken up by cells for incorporation into membrane phospholipids (Linder, 1991).

Energy production from long-chain FAs generally occurs in the mitochondria via β-oxidation, and is initiated by the lipase-dependent hydrolysis of TG to FFA. Fatty acids are transported across the mitochondrial membrane in the form of acyl-carnitine, and subsequently undergo sequential removal of 2-carbon units until the final product, acetyl Coenzyme A (CoA), is formed and enters the citric acid cycle for energy production (Linscheer and Vergroesen, 1994).

The metabolism of CLA isomers is very similar to that of linoleic acid (Banni, 2002). Conjugated linoleic acids are metabolized by 2 distinct pathways—desaturation, elongation, and oxidation. Desaturation of CLA has been investigated more extensively than oxidation; however, desaturation and oxidation are both well-recognized metabolic pathways for CLA (Sebedio et al., 2003). In animal and human tissues, the t10,c12 and c9,t11 CLA isomers undergo Δ6 desaturation, elongation, and further Δ5 desaturation that forms 18:3, 20:3, and 20:4 FA. Both isomers may also be oxidized in the β-oxidation pathway. Studies investigating the metabolism of different CLA preparations are discussed below.

CLA-FFA metabolism

CLAs are desaturated by Δ6 desaturase and Δ5 desaturase (Banni et al., 1996; Belury and

Kempa-Steczko, 1997; Sebedio et al., 1997). The metabolites produced depend on the type of FAs in the diet (Sebedio et al., 1997). For instance, when CLA are fed to rats deficient in dietary linoleic acids, the c9,t11 and c10,t12 CLA isomers are converted into conjugated isomers of arachidonic acids (i.e., 8,11,13-20:3 and 8,12,14-20:3, respectively; Banni et al., 2002). These conjugated arachidonic acid-type metabolites are further desaturated into c5,c8,c11,t13-20:4 and c5,c8,t12,c14-20:4 for c9,t11 and c10,t12 CLA isomers, respectively. When rats were fed a FA-rich diet, the c9,t11 CLA isomer was converted into c8,c11,t13-20:3 as the final end product and the t10,c12 CLA isomer was converted into c6,t10,c12-18:3 and t8,c10-16:2 (Banni et al., 2001). Metabolites of CLA were incorporated into the lipid component(s) of adipose tissue, liver, heart, and kidney (Sebedio et al., 2003).

CLAs are also metabolized to carbon dioxide (CO_2) by β-oxidation *in vivo* in rats (Sergiel et al., 2001). Sergiel et al. (2001) demonstrated that 70% of the total oral dose of c9,t11 CLA or t10,c12 CLA was converted into CO_2 over a 24-hour period.

In the serum of human subjects taking supplements of CLA (6 g/day, containing ~37% c9,t11 CLA and ~39% t10,c12 CLA), accumulation of the Δ6 desaturase metabolite of the t10,c12 CLA isomer was reported to be higher than the c9,t11 CLA isomer. This outcome indicates more rapid metabolism of the t10,c12 isomer (Belury, 2002; Noone et al., 2002).

<u>Comparison of CLA-TG and CLA-FFA</u>

Terpstra et al. (2003) found that male BALB/cOlaHsd mice fed CLA in either TG or FFA (0.5 g CLA/100 g diet; control diet with high oleic sunflower oil) did not show differences in body weight and total body energy based on the type of CLA received. Both groups had 50% lower body fat and a 30% reduction in total body energy compared to the high oleic sunflower oil in mice (Terpstra et al., 2003).

Hoye et al. (2004) evaluated the relative bioavailability of CLA from CLA-TG or in FFA form in healthy male volunteers (n=12). Each subject received two treatments as single oral administrations. The first was CLA-TG (8.9 g CLA) administered on visit 2. After a washout period of 9 to 14 days, CLA-FFA (11.4 g -with a balanced isomer ratio) was administered on visit 4. The bioavailability of CLA was measured in the serum as area under the curve (AUC). The authors reported that CLA in the form of FFA was 15-19% greater than CLA in the TG form. However they noted that this might be due to the high load of the FFA form, changing the rate of absorption and disposition. The CLA reached a peak concentration at 2 to 8 hours (depending on the isomer and form) and after 24 hours there was only a small fraction left. Half-lives of the isomers in the two forms ranged between 16 and 29 hours. No adverse events were noted during the trial period of 10 to 15 days.

Park and Pariza (2007) studied effects of CLA-FFA, CLA-diacylglyceride (DG-CLA), and CLA-TG on body composition. Four-week old ICR male mice were fed semipurified diet containing 0.7% of CLA (w/w, equivalent to 0.5% CLA at final) with different preparations for 4 weeks. All CLA preparations contained about 73% CLA (cis-9,trans-11, 33.4–33.9; trans-10,cis-12, 34.4–35.5; cis, cis, 2.3-2.4; trans, trans, 1.6–2.0). Comapred to the control group, body protein and water composition were significantly higher in CLA groups, but no differernces were noted among the 3 CLA groups. Body fat composition was significantly lower in CLA groups, but the

3 CLA groups had comparable body fat content. The data suggest that CLA-FFA and CLA-TG have comparable metabolic effects.

IV.C.2. Mutagenicity and genotoxicity studies of CLA

IV.C.2.1. Summary of mutagenicity and genotoxicity studies done in or before 2007 (GRN 232)

Preclinical data demonstrate an absence of significant toxicological, mutagenic or reproductive and developmental effects of CLA-TG (O'Hagan and Menzel, 2003). Similarly, there was no mutagenic potential of CLA-TG in two *in vitro* assays (reverse mutation and chromosomal aberration in human lymphocytes; O'Hagan and Menzel, 2003).

IV.C.2.2. Studies published since 2007

No studies on the mutagenicity and genotoxicity of CLA have been published since 2007. A recent report by EFSA (2010) states that available data from non-human studies do not indicate risk for genotoxicity, reproductive toxicity, carcinogenicity, or allergenicity.

IV.C.3. Animal toxicity studies of CLA

IV.C.3.1. Summary of animal toxicity studies done in or before mid-2007 (adopted from GRN 232)

"Preclinical data demonstrate an absence of significant toxicological, mutagenic, or reproductive and developmental effects. Based on a 13-week feeding study, the reported no-observed-adverse-effect level (NOAEL) for the 1:1 CLA-TG isomer mixture in rats was 5% in the diet, the highest level fed. This is equivalent to 2,433 and 2,728 mg/kg BW/day for males and females, respectively (O'Hagan and Menzel, 2003). Such observations are further supported by studies conducted by Park et al. (2005). They examined the effects of long-term feeding of male Fischer 344 rats with a diet containing 1% CLA (41.9% c9,t11 and 43.5% t10,c12; 1,000 mg/kg BW/day) for a period of 18 months."

"Reproductive and developmental toxicity studies in rats and pigs have also shown a lack of adverse effects on maternal food consumption and BW, litter size, and offspring growth and development following exposure to CLA (0.25 to 2% in the diet) throughout gestation, lactation, and/or during a post-weaning period (Chin et al., 1994; Bee, 2000a, 2000b; Poulos et al., 2001)."

"In summary, the data indicate that daily consumption of CLA mixture in FFA and EE forms up to 1-1.25 g/kg BW/day was considered safe." (Berven et al., 2002)

It is noteworthy that these animal toxicity studies were conducted with both CLA-TG and CLA-FFA. Since CLA-TG was thought to have a similar metabolic pathway to that of CLA-FFA, GRN 232 included the animal toxicity data from CLA-FFA studies to justify the safety of CLA-TG. The subchronic toxicity study of O'Hagan and Menzel (2003) was based on CLA-TG, but the reproductive and developmental toxicity studies of Bee (2000a, 2000b), Chin et al. (1994), and Poulos et al. (2001) as well as a 18 month chronic toxicity study of Park et al. (2005) employed CLA-FFA.

IV.C.3.2. Studies published since 2007

The available data from animal toxicity studies indicate that dietary administration of CLA is generally well tolerated without adverse effects (Tables 4-8). Animal efficacy studies reporting health benefits of CLA, such as Park et al. (2007, 2008, 2013), are not summarized in this section.

IV.C.3.2.1. Animal toxicity studies

No new literature on animal toxicity studies have been published since 2007, indicating that conclusions from a previous review that the no-observed-adverse-effect level (NOAEL) for the 1:1 CLA isomer mixture is 5% in the diet of rats are still valid. This was the highest level fed, which is equivalent to 2,433 and 2,728 mg/kg BW/day for males and females, respectively (O'Hagan and Menzel, 2003; GRN 232).

IV.C.3.2.2. Animal studies evaluating reproductive performance of CLA (Table 4)

No recent literature contradicted the 2007 review of reproductive and developmental toxicity studies, which reported on the NOAEL of 0.25 to 2% CLA-FFA in the diets of rats and pigs (Chin et al., 1994; Bee, 2000a, 2000b; Poulos et al., 2001; GRN 232).

Recent studies (Danicke et al., 2012; de Veth et al., 2009; Hayashi et al., 2007; Peng et al., 2010; Veaute et al., 2007) also showed that CLA supplementation did not adversely impact reproductive performance or fetus weight in mice and dairy cows. In these recent studies, a CLA form was not specified. Danicke et al. (2012) tested supplementation of 8 g CLA/day (1:1 mixture of c9, t11 and t10, c12 CLA) from day 190 to 262 of pregnancy in cows, or fetal exposure times of day 19 to 102. De Veth et al. (2009) administered 18.3 g of t10,c12 CLA/day during early lactation (from day 37 to day 154) in dairy cows to show that reproductive performance of dairy cows may be improved by feeding of CLA supplements during early lactation. In the study of Peng et al. (2010), CLA was administered in pigs at 1% of diet (containing 28.02% c9,t11 CLA and 30.07% t10,c12 CLA) during the last 50 day of gestation and throughout a 26 day lactation to demonstrate no adverse effects of CLA on reproductive performance and piglet growth.

In a two-generation mouse study, Veaute et al. (2007) found no differences in BW gain, fetus number, and estrus cycle among groups of animals when fed one of 4 diets; control (7% total fat) or one of 3 experimental high fat (20% total fat) diets (cis FA, trans FA, or CLA). The CLA diet contained 3% CLA-rich oil (purity not specified). In both first and second generations, females receiving high levels of dietary fat tended to have higher rates of resorptions irrespective of the type of dietary fat. No CLA-specific effects were noted. In the first and second generation, CLA had lower values of placental interleukin (IL)-4 (~80-85%) and transforming growth factor-β (TGF-β) expressions (by 60-80%). Second generation males fed trans FA and CLA had lower rates of induced sperm acrosome reaction (~15% at 90 min and 40% at 30 min). Whether the specific changes are of clinical importance is uncertain; sperm acrosome reaction is not an established biomarker of reproductive performance. It is noteworthy that tumor necrosis factor alpha (TNF-α), sperm number, and motility were not different among groups.

In a study by Hayashi et al. (2007), 1.35% CLA in diets (containing 24% c9,t11; 35% t10,c12; 15% c8,t10; 17% c11,t13; and 9% of others) was administered to Wistar rats from day 1 of

parturition (considered day 1 of lactation) to day 15 of lactation. On day 15 post-partum, CLA supplementation reduced pup growth by 21%. Conjugated linoleic acids treatment in the mammary glands and liver, reduced FA synthase, glucose 6-phosphate dehydrogenase, and 6-phosphogluconate dehydrogenase. The activity of nicotinamide adenine dinucleotide phosphate (NADP)-malate dehydrogenase was unchanged. CLA supplementation during lactation (0, 0.5, or 1.0% CLA in diet during the last 50 d of gestation and throughout a 26-d lactation) did not affect the dam's food intake or BW. In the study of Peng et al. (2010), feeding sows diets supplemented with CLA resulted in increases (linear and quadratic, $P < 0.01$) in the CLA content of plasma, backfat, and the LM in their offspring with no adverse effects. Overall, CLA supplementation, regardless of CLA form (TG or FFA), did not adversely impact reproductive performance in various animals.

IV.C.3.2.3. Animal studies evaluating metabolic effects of CLA (Table 5)

Supplementation with CLA did not induce major metabolic changes, including risk factors of CVD, diabetes, and obesity and immune responses, in dairy cows, rats, and pigs (Akter et al., 2011; Corino et al., 2009; Fernandez-Figares et al., 2012; Soares et al., 2012). Two studies specified CLA source in FFA form (Atker et al., 2011; Corino et al., 2009). Other studies did not specify the chemical form of CLA.

Atker et al. (2011) reported that CLA-FFA induced decreases in adipocyte size indicated lipolytic or anti-lipogenic effects of CLA on adipose tissue in primiparous dairy cows. Corino et al. (2009) reported that CLA-FFA supplementation improved BW at weaning, and immune responses in piglets, with no changes in TG content in the liver, serum low density lipoprotein cholesterol (LDL-C), non-esterified fatty acids (NEFAs), and histology.

Soares et al. (2012) found no intergroup differences in body and brain weights, body length, body mass index (BMI), abdominal and thoracic circumferences, and the abdominal to thoracic circumference ratio in progeny rats at 35-45 days of life. A pig study of Fernandez-Figares et al. (2012) determined possible mechanisms of action that might explain the nutrient partitioning effect of betaine and CLA in pigs. Twenty gilts were restrictively fed from 20 to 50 kg BW control, 0.5% betaine, 1% CLA or 0.5% betaine plus 1% CLA diets. Conjugated linoleic acids and CLA plus betaine did not influence serum concentrations of glucose, LDL-C, and NEFA, indices of insulin resistance, liver TG conc. or histopathology. However, increases in serum insulin (28% and 83%, respectively; $P=0.0001$) and serum TG (58% and 28%, respectively; $P<0.01$) were noted in 1% CLA and in 0.5% betaine + 1% CLA pigs. The increase in serum TG indicated that CLA and betaine + CLA could have reduced adipose tissue TG synthesis from preformed FA.

Overall, none of these studies report data that contradict the prior review, which concluded that supplementation with CLA-FFA or CLA-TG did not impair glucose metabolism and did not increase CVD factors.

IV.C.3.2.4. Animal studies evaluating milk yield or milk fat production (Table 6)

Most studies evaluating milk yield or milk fat production employed encapsulated CLA-FFA (Castaneda-Gutierrez et al., 2007; Hutchinson et al., 2012;Sigl et al., 2010) or calcium salt of CLA-FFA (Medeiros et al., 2010). Castaneda-Gutierrez et al. (2007) reported that in Holstein cows, supplementation with 9.5-14.2 g/day of CLA-FFA isomer mixtures had no significant

effect on milk fat yield or content over the entire supplementation period. Milk fat content, however, decreased by week 5 by 14.1 and 6.1% for 14.2 g CLA (c9,t11:t10,c12=:1:1) and 9.5 g CLA (c9,t11:t10,c12=75:25), respectively (P =0.005) compared with the controls. After termination of supplementation, fat content became similar to that of controls. No difference was observed in milk protein or milk lactose content or yield. Hayashi et al. (2007) administered 1.35% CLA (CLA form, not specified) in the diet of Wistar rats (containing 24% c9,t11; 35% t10,c12; 15% c8,t10; 17% c11,t13; and 9% others) from day 1 of parturition (considered day 1 of lactation) to day 15 of lactation. On day 15 post-partum, CLA supplementation during lactation reduced milk fat content by 33% without affecting the dam's food intake or BW. Total milk production was not measured in this study.

Energy spared from milk fat depression is repartitioned for milk production to increase yield from dairy cows (Hutchinson et al., 2012; Medeiros et al., 2010). Milk energy output and energy balances were similar for CLA and controls in dairy cows (Medeiros et al., 2010). Also, Sigl et al. (2010) reported no significant effects of CLA-FFA on milk composition and yield in brown Swiss cows. Overall, due to increased milk yield, there were no adverse effects of CLA on milk energy output despite depressed milk fat production which were found in some studies.

IV.C.3.2.5. Animal studies evaluating anti-carcinogenicity of CLA (Table 7)

Several studies reported anticarcinogenic activity of CLA in rats and mice (Kim et al., 2010; Shiraishi et al., 2010). In a study by Shiraishi et al. (2010), CLA-TG and CLA-FFA attenuated the increase in aberrant crypt foci (ACFs) induced by 10% beef tallow and azoxymethane (AOM) pretreatment at week 12 in Sprague-Dawley rats. At 44 weeks, both forms of CLA attenuated multiple colon cancers, although CLA-FFA reduced the incidence of colon cancer to 50% of that seen with CLA-TG. These results suggested that both CLA-TG and CLA-FFA suppressed colon carcinogenesis in rats with long-term feeding of a 10% beef tallow diet through several mechanisms. The authors concluded that results of this rat study might be applicable to humans.

The study of Kim et al. (2010) employed CLA-FFA to report anticarcinogenic potential of CLA in a mouse forestomach carcinogenesis regimen induced by benzo(a)pyrene.

In summary, a subchronic toxicity study reporting the NOAEL for the 1:1 CLA-TG isomer mixture as 5% in the diet of rats is still valid. This was the highest level fed, which is equivalent to 2,433 and 2,728 mg/kg BW/day for males and females, respectively (O'Hagan and Menzel, 2003; GRN 232). The GRN 232 included the NOAEL of CLA-FFA found from developmental and reproductive toxicity studies to justify the safety of CLA-TG: the NOAELs of 0.25 to 2% CLA-FFA in the diets of rats and pigs (Chin et al., 1994; Bee, 2000a, 2000b; Poulos et al., 2001; GRN 232) are still valid.

Table 4. Animal reproductive performance studies reporting no adverse effects of CLA

Species	Dose	Duration	Measured outcome	Results	Reference
German Holstein cows (n=17, pregnant/lactating, 100% F and their respective calves)	3 Groups: 1) control fat supplement 2) CLA50 (50 g/day CLA-containing fat supplement; or 4 g CLA/day) 3) CLA100 (100 g/day CLA-containing fat supplement or 8 g CLA/day); the ratio of c9, t11 and t10,c12 isomers; CLA form not specified	First generation study, 182 day after calving; Depletion time (i.e., the individual period elapsed between the day of terminating the feeding of the expt. diet in the preceding lactation period and the day of calving) ranged from 190 to 262 days, or fetal exposure times of 19 to 102 days.	Stimulation ability of PBMC expressed as SI, dietary consumption, milk yield, BW, FA profile of erythrocyte lipids and milk fat, depletion time, plasma metabolites, and hematological parameters	The SI was influenced by days in all groups, but not by diet; SI was increased significantly by 55-77 % within 21 day after calving. However, there were no differences in SI, plasma metabolites and hematological parameters among groups, the FA pattern of erythrocyte lipids and colostrum of cows among the groups. The only exception was the lower SI value of CLA50 group before and after colostrums intake. No dose-response relationship was noted: CLA100 and control groups had comparable SI values in all treatment days. CLA100 had a lower erythrocyte conc. of C18:1 trans FA.	Danicke et al., 2012
Dairy cows (n=212, pregnant primiparous and multiparous, 100% F and their calves)	Across the 5 studies, the CLA dose ranged from 0 to 63.2 g/day and this provided 0 to 18.3 g of t10,c12 CLA/day; CLA form not specified	Multi-study analysis compiling 5 controlled studies, CLA supplementation was initiated between 16 and 21 DIM and terminated between 58 and 140 DIM (overall supplementation period ranged from 37 to 154 days in total).	Time to first ovulation, conception, and success of pregnancy	The probability of cows becoming pregnant increased in a nonlinear manner as t10,c12 CLA dose increased. At the optimal dose (10.1 g/day), the probability of pregnancy was increased by 26% compared with the control group. Similarly, the log-normal model predicted that time to conception was decreased in a nonlinear manner with increasing t10,c12 CLA dose. The predicted optimal dose was 10.5 g t10,c12 CLA/d and at this dose the median time to conception was decreased by 34 days from 151 DIM when compared with no CLA group. Overall, CLA improved reproductive performance with no adverse effects.	de Veth et al., 2009

F=females; PBMC=peripheral blood mononuclear cells; SI=stimulation index; DIM=days in milk.

Table 4. Animal reproductive performance studies reporting no adverse effects of CLA, continued

Species	Dose	Duration	Measured outcome	Results	Reference
First generation: CFI mice (n=87, M+F); Second generation: CFI mice (n=70, M+F)	Control diet (7% total fat) or one of 3 experimental high fat (20% total fat) diets (cis FA, trans FA, or CLA); CLA form not specified	1st generation: after 30 days of feeding, mice fed the same diet were mated and part of females sacrificed at day 18 of pregnancy. 2nd generation: after 30 days, mice were mated. At weaning litters were fed the same diet as the dams for 30 days. Then they were mated. At day 18 of pregnancy, the females were killed.	Resorption, number of fetuses, placental cytokine expression, and sperm acrosome reaction.	No differences in BW gain, fetus number, and estrus cycle among groups. In both first and second generations, females receiving high levels of dietary fat presented or tended to have higher rates of resorptions irrespective of the type of dietary fat. In the first and second generation, CLA had lower values of placental IL-4 (~80-85%) and TGF-β expressions (by 60-80%) Second generation males fed trans FA and CLA showed lower rates of induced sperm acrosome reaction (~15% at 90 min and 40% at 30 min). However, TNFα, sperm number and motility were not different among groups.	Veaute et al., 2007
Dalland sows and their offspring (n=18, pregnant, mixed parity, F sows, M piglets)	3 Groups: 1) control (soybean oil) diet 2) 0.5 % CLA diet 3) 1.0% CLA diet; CLA contained 28.02% c9,t11-18:2 and 30.07% t10,c12-18:2; CLA form not specified	Last 50 days of gestation and throughout a 26 day lactation; Randomized-block design	Transfer pattern of CLA via umbilical cord blood and milk, FA profiles of colostrum, milk, umbilical cord plasma, and plasma in neonatal and weanling piglets, backfat and lean muscle in neonatal and weanling piglets, and sow reproductive performance.	Supplementation with CLA during late gestation (last 50 days) and lactation increased the CLA isomer amounts in sow colostrum and milk, but did not alter sow reproductive performance or the birth weight and BW gain of piglets. Dietary CLA increased the conc. of total SFA, but reduced the total MUFA in the colostrum (both-linear and quadratic, $P<0.01$). Dietary CLA increased total SFA conc. (quadratic, $P<0.01$), but had no influence on total MUFA conc. in the milk. CLA increased (linear and quadratic, $P<0.01$) the CLA content of plasma, backfat, and the lean muscle in the offspring. However, t10,c12 isomer, rather than c9,t11 isomer, was detected in the umbilical cord blood, indicating CLA transport from the sow to the fetus in an isomer-specific manner.	Peng et al., 2010

M=male; F=female; IL= interleukin; MUFA = monounsaturated fatty acid; SFA=saturated fatty acid; TGF-β =transforming growth factor-β; TNFα=tumor necrosis factor alpha.

Table 4. Animal reproductive performance studies reporting no adverse effects of CLA, continued

Species	Dose	Duration	Measured outcome	Results	Reference
Wistar rats (n=16, pregnant dams, 100% F (pups were also assessed)	1) control diet (253.7 g crude protein/kg, 50.5 g crude fiber/kg, 38.6 g fat/kg, 82.9 g ash/kg, 13 g Ca/kg, and 78.3 g P/kg on a DM basis) 2) 1.35% CLA in diet (containing 24% c9,t11; 35% t10,c12, 15% c8,t10, 17% c11,t13; and 9% others); CLA form not specified	Intervention-controlled design, from day 1 of parturition (considered day 1 of lactation) to day 15 of lactation	Food intake, plasma lipid and glucose conc. in dams, milk composition and BW of dams and pups. FA profile in mammary gland, liver, adipose tissue, and milk of lactating dams, and enzyme activities in the mammary gland, liver, and adipose tissue	On day 15 post partum, CLA supplementation reduced pup growth by 21% without affecting dam's food intake or BW. FA synthase, glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase were reduced by CLA treatment in the mammary gland and liver. The activity of NADP-malate dehydrogenase was unchanged.	Hayashi et al., 2007

DM=dry matter; FA=fatty acid; NADP=nicotinamide adenine dinucleotide phosphate

Table 5. Animal studies of CLA evaluating metabolic effects, adipocytes, or immune responses

Species	Dose	Duration	Measured outcome	Results	Reference
German Holstein Heifers (n=25, primiparous and pregnant, 100% F); age no specified	2 Groups: 1) control (fed 100 g/day of a CLA analygous FA mixture) 2) 100 g/day CLA (1:1 mixture of CLA c9,t11 and t10,c12 isomers), the pelleted CLA-concentrate contained about 6% each of the CLA isomers; CLA-FFA	105 DIM; slaughtered at day 42 and 105; control , slaughtered at day 0, 42, and 105 (n=5/group)	Mean adipocyte areas of 3 subcutaneous (tailhead, withers, and sternum) and 3 visceral (omental, mesenteric, and retroperitoneal) depots to characterize 6 different fat depots in terms of adipocyte size	CLA-induced decreases in adipocyte size indicate lipolytic or antilipogenic effects of CLA, or both effects, on adipose tissue in primiparous dairy cows. Adipocyte sizes were decreased in subcutaneous depots from the tailhead at 105 DIM and from the sternum at 42 DIM in CLA cows, whereas visceral depots, adipocyte sizes were decreased in mesenteric fat at 42 and 105 DIM in CLA versus control cows. Within the CLA group, adipocyte sizes were smaller in the subcutaneous depot from the tailhead at 105 DIM than at 42 DIM. No adverse effects of CLA on measured outcome were reported.	Akter et al., 2011
Iberian (Silvela strain) gilts (pigs) (n=20, from the same farrowing group, M+F)	1)control 2) betaine, 0.5% 3) CLA (1% CLA, 60% CLA isomers, 1:1 mixture of CLA c9,t11 and t10,c12 isomers in ME), or 4) 1% CLA + 0.5% betaine Note: the background diet was composted of barley and soybean meal based and formulated to contain 12.0% crude protein, 0.81% lysine and 14.8 MJ of ME/kg DM; CLA form not specified	Randomized intervention-controlled design, until pigs reached 50 kg BW	Biochemical parameters (glucose, urea, creatinine, lipid profiles, NEFA, liver fat), hormones (growth hormone, IGF-1, insulin, leptin, insulin sensitivity), and OGTT	No changes in TG content in liver, serum glucose, LDL-C and NEFA as well as histology. The increase in serum TG (58% and 28%, respectively; P=0.0098) indicated that CLA and betaine + CLA could have reduced adipose tissue TG synthesis from preformed FA. Decreased serum urea in pigs fed betaine and betaine+ CLA diets (11%; P=0.0001) indicated a more efficient N utilization. CLA and betaine + CLA decreased serum growth hormone (20% to 23%; P=0.0209). Although serum insulin increased in CLA pigs, and especially in betaine + CLA pigs (28% and 83%; P=0.0001), serum glucose and indices of insulin resistance were unaffected. No adverse effects of CLA on measured outcome were reported.	Fernandez-Figares et al., 2012

M=male; F=female; DIM=days in milk; IGF-1=insulin-like growth factor 1; LDL-C=low density lipoprotein cholesterol; ME=methyl esters; NEFA=non-esterified fatty acids; OGTT=oral glucose tolerance test; TG=triglyceride.

Table 5. Animal studies of CLA evaluating metabolic effects, adipocytes, and immune responses, continued

Species	Dose	Duration	Measured outcome	Results	Reference
Goland pigs (n=21, multiparous pregnant sows, 100% F) Goland piglets (n=168, average BW of 6.9 kg, 50% M + 50% F)	1) control (0.5% soybean oil) 2) 0.5% CLA from 7 d before partrition until 7 day postpartum (T1) 3) 0.5% CLA from 7 days before partrition until weaning (T2) Note: the CLA powder contained 32% c9, t1 and 31% t10, c12 CLA isomers; CLA-FFA.	7 days before parturition to day 7 postpartum (Group 1) or until weaning (Group 2)	BW, serum glucose, NEFA, hormones: insulin, IGF-1, thyroxine, leptin; serum and colostrum concentrations of IgG, IgA, and IgM.	Piglet BW at weaning were greater in the CLA groups compared with the control. Dietary CLA supplementation increased (by 15%) serum thyroxine concentration in sows, but had no effects on serum insulin, glucose, NEFA, IGF-1, and leptin concentrations. Colostral IgG, IgA, and IgM titers were greater in sows fed CLA than in control sows (P<0.05). At weaning (day 21), serum IgG titer of the piglets was greater (by 50%, P<0.05) in the 2 CLA groups than the control group; at day13 post weaning, a difference (45%, P<0.05) was observed between the control and the 0.5% CLA group. CLA supplementation improved BW at weaning and immune responses in piglets. No adverse effects of CLA on measured outcome were reported.	Corino et al., 2009
Wister rats (120-150 days of life for the dams, F; 35-45 days of life for the progeny)	1) control (standard diet - 7% fat from soybean oil, no CLA) 2) 7% fat from goat milk (1.2% CLA) during gestation 3) 7% fat from goat milk (1.2% CLA) during lactation 4)7% fat from goat milk (1.2% CLA) during gestation plus gestation; CLA form not specified	From gestation day 0 to 35-45 day of pups life. After weaning all pups were kept on the control diet until the day of the CSD recording session.	BW, body length, abdominal and thoracic circumferences, BMI, abdominal to thoracic circumference ratio, brain weight, and electrophysiol-ogical CSD effects of the male progeny	Compared to a control group, BW increased at 14, 21, and 28 days, but not at 35-45 days, in the experimental diet received during lactation and the gestation and lactation groups (P<0.05). No intergroup difference was found of body and brain weights, body length, BMI, abdominal and thoracic circumferences, and abdominal to thoracic circumference ratio at day 35-45. At day 35-45, the CSD velocities of propagation were significantly higher (P<0.05) in the lactation group as compared with the control and gestation groups, and in the lactation and gestation, as compared with other 3 groups, suggesting a long-lasting brain effect. No adverse effects of CLA on measured outcome were reported.	Soares et al., 2012

BMI=body mass index; CSD=cortical spreading depression; Ig=immunoglobulin; IGF-1=insulin-like growth factor 1; NEFA=non-esterified fatty acids.

Table 6. Animal studies of CLA on milk fat and/or milk yield

Species	Dose	Duration	Measured outcome	Results	Reference
Holstein cows (n=45, lactating dairy cows, 100% F)	1) control (70 g/day of Ca salts of tallow) 2) 63 g/day CLA 75:25 (lipid-encapsulated CLA mixture that provided 7.1 g/day of c9, t11 CLA and 2.4 g/day of t10, c12 CLA) 3) 76 g/day CLA 1:1 (lipid-encapsulated CLA mixture that provided 7.1 g/day each of c9, t11 and t10, c12 CLA; lipid-encapsulated CLA-FFA	Randomized, block-design, From 20±1 DIM to 56 ± 1 DIM plus 4 weeks post-treatment period	Reproductive markers (uterine PGF2α, response to oxytocin challenge, steroideogenesis in ovarian follicles and corpus luteum, and effects on circulating concentrations of reproductive and metabolic hormones). NEFA concentrations, IGF-1, progesterone, estradiol.	Supplementation with mixtures of CLA did not result in differences in estimated net energy balance, or in plasma concentrations of non esterified FA, milk fat yield, and milk yield. The CLA isomers were not detectable in endometrium, and there was no difference in the prostaglandin response to an oxytocin challenge. Cows supplemented with CLA 50:50 had 14% higher Plasma IGF-I conc. and tended to have elevated plasma progesterone (by 15%; NS) during the early luteal phase. CLA may improve endocrine signals that can be beneficial to reproduction. No adverse effects of CLA on measured outcome were reported.	Castaneda-Gutierrez et al., 2007
Dairy Cow [n=48 early lactation Holstein-Friesian (21 primiparous, 27 multiparous), F]	1) Palmitic acid (Control), (2) flaxseed (rich in 18:3 n-3 FA; Flax), (3) CLA (a mixture of c9, t11 and t10, c12 isomers; CLA), and (4) fish oil. Total lipid supplement- 500 g/day; lipid encapsulated CLA-FFA	70 days; Randomized-block design,	Follicle development, plasma steroid hormone concentrations and prostaglandin synthesis.	Fat supplementation had little effect on follicle development. Milk yield was greater for CLA-supplemented cows compared with all other treatments. Milk protein concentration was reduced for CLA-supplemented cows compared with cows receiving control and flax treatments. No adverse effects of CLA on measured outcome were reported.	Hutchinson et al., 2012

IGF-1 = insulin-like growth factor 1; NEFA=non-esterified fatty acids; PGF2α =F2-isoprostane 8-iso-prostaglandin F; NS=not significant; FA=fatty acid.

Table 6. Animal studies of CLA on milk fat and/or milk yield, continued

Species	Dose	Duration	Measured outcome	Results	Reference
Dairy cows(n=30, crossbred Holstein x Zebu cows in early lactation, F)	150 g of Ca-salts of CLA (providing 15 g/day c9,t11 and 22 g/day t10,c12) (test) or 150 g of Ca-salts of palm oil (control); Ca salts of CLA-FFA	Block design, 28 to 84 DIM	Milk production, milk composition, and FA profile of cows grazing tropical forages.	CLA treatment decreased milk fat and energy conc. and increased milk volume and milk protein conc., resulting in unchanged energy output. No adverse effects of CLA on measured outcome were reported.	Medeiros et al., 2010
Brown Swiss cows (n=10, Primiparous , F)	2 Groups: 1)7.5 g CLA/day (1:1 ratio of c9,t11 and t10,c12-CLA isomers) starting 2 weeks before expected calving and 20 g CLA/day throughout day 1 to 28 of lactation, or 2) control diet (basal diet during the prepartum period, and then switched to a lactational diet); encapsulated CLA-FFA.	Intervention-control design, began 2 weeks prior to calving and continued throughout 28 days of lactation.	Milk composition (FA, protein, fat, lactose, urea, and pH), blood conc. of glucose, NEFA, BHB, and TB, and gene expression (PPARα, PPARγ, and TNFα)	No significant effects of both CLA isomers on milk yield and composition or on metabolic key parameters in blood as well as on gene expression of PPARα, PPARγ, sterol regulatory element-binding protein-1 and TNFα in liver tissue. The CLA supplement was insufficiently accepted by the animals: only 61.5% of the intended amount was ingested. On average over the entire treatment period, there was a decrease of saturated FA in milk fat of CLA supplemented cows, combined with a higher content of MUFA and trans fat. No adverse effects of CLA on measured outcome were reported.	Sigl et al., 2010

BHB= beta hydroxylbutyrate; MUFA= monounsaturated fatty acid; NEFA=non-esterified fatty acids; PPAR=peroxisome proliferator-activated receptor; TB= total bilirubin; TNFα = tumor necrosis factor alpha.

Table 6. Animal studies of CLA on milk fat and/or milk yield, continued

Species	Dose	Duration	Measured outcome	Results	Reference
Wistar rats (n=16, pregnant dams, 100% F (pups were also assessed)	1) control diet (253.7 g crude protein/kg, 50.5 g crude fiber/kg, 38.6 g fat/kg, 82.9 g ash/kg, 13 g Ca/kg, and 78.3 g P/kg on a DM basis) 2) 1.35% CLA in diet containing 24% c9,t11; 35% t10,c12, 15% c8,t10, 17% c11,t13; and 9% others; CLA form not specified	Intervention-controlled design, From day 1 of parturition (considered day 1 of lactation) to day 15 of lactation	Food intake, milk composition and BW of dams and pups, FA profile in mammary gland, liver, adipose tissue, and milk of lactating dams	On day 15 post partum, CLA supplementation reduced milk fat content by 33% without affecting dam's food intake or BW. Total milk production was not measured. No adverse effects of CLA on measured outcome were reported.	Hayashi et al., 2007

DM=dry matter; FA=fatty acid.

Table 7. Animal studies of CLA intake on cancer

Species	Dose	Duration	Measured outcome	Results	Reference
Sprague-Dawley rats (n=NA, 6 weeks old, M)	3 groups: 1) 10% beef tallow 2) 10% beef tallow + 1% CLA-TG (80%-1:1 ratio of c9,t11 and t10,c12-CLA isomers) 3) 10% beef tallow + 1% CLA-FFA	44 weeks after first injection of AOM (to induce colon carcinogenesis) or saline.	Aberrant crypt foci (ACFs), cancer, cell proliferation, apoptosis, Wnt signaling, and arachidonic acid cascade	At 12 weeks, CLA-TG and CLA-FFA attenuated the increase in ACFs induced by 10% beef tallow and AOM pretreatment. At 44 weeks, both forms of CLA attenuated multiple colon cancers, and CLA-FFA reduced the incidence of colon cancer to 50% of that seen with CLA-TG. CLA-TG and CLA-FFA decreased the number of 5-bromo-2'-deoxyuridine-positive cells in AOM-pretreated rats fed with 10% beef tallow. CLA-FFA increased the number of apoptotic cells and the activity of caspase-3 in the colon mucosa, and CLA-TG enhanced the activity of caspase-3. Both forms of CLA suppressed Wnt signaling and the arachidonic acid cascade in rats treated with beef tallow and AOM. No adverse effects of CLA on measured outcome were reported.	Shiraishi et al., 2010
ICR Mice (n=180; 30 mice/group, 6 groups, 6-7 weeks old; F)	1) saline control 2) 0.1 ml LA 3) 0.1 ml CLA, 97% 4) 0.1 ml c9,t11-CLA, 89.5%, 5) 0.1 ml t10,c12-CLA, 99% 6) 0.1 ml t,t-CLA mixed isomers, 99%; CLA-FFA	For 4 weeks, each animal was orally given 0.1 mL of sample mixed with 0.1 mL of olive oil on Mon. and Wed. and 2 mg of benzo-(a)pyrene in 0.2 mL olive oil on Fri. Sacrificed 23 weeks after first dose of benzo-(a)pyrene	Forestomach neoplasia	CLA (c9,t11 CLA and t10,c12 CLA) significantly reduced tumor incidence ($p<0.05$) relative to 100% LA and control. No adverse effects of CLA on measured outcome were reported.	Kim et al., 2010

ACF=aberrant crypt foci; AOM=azoxymethane; BW=body weight; FFA= free fatty acid(s); LA = linoleic acid; TG= triglycerides.

IV.C.4. Human clinical studies of CLA

IV.C.4.1. Summary of human clinical studies published in or before mid-2007 (adopted from GRN 232)

Numerous clinical trials have evaluated the effects of a 1:1 mixture of c9,t11 and t10,c12-CLA isomers and a number of other isomers on similar parameters. A comprehensive review of clinical data demonstrated that consumption of CLA isomers at doses of up to 6 g/day (or 7.5 g/day CLA-rich oil containing 80% CLA) for up to 1 year (Whigham et al., 2004; Larsen et al., 2006) and 3.4 g/day (or 4.5 g/day CLA-rich oil) for up to 2 years (Gaullier et al., 2004, 2005) was safe and had no significant effects on cardiovascular parameters (lipid metabolism, markers of inflammation, and markers of oxidative stress), insulin sensitivity and glucose metabolism, or maternal milk fat. In these pivotal studies, the levels of consumption represented the maximum dose consumed rather than absolute safety endpoints. In bioavailability studies, no adverse effects were found in a single oral dose of approximately 15 g of CLA-rich oil (approximately 9 g of CLA isomers). Below are the summaries for each health parameter from GRN 232.

<u>IV.C.4.1.1. Cardiovascular disease risk (CVD)</u> "Lipid Metabolism: A number of studies in humans and animals of CLA (50:50 mixture) report no evidence of adverse effects on blood lipid parameters. Certain studies report statistically significant changes from baseline or control values, but these effects are not considered to be clinically significant. We conclude, based on the weight of the evidence, that CLA (50:50 mixture) intake does not adversely affect lipid parameters associated with CVD risk. In terms of markers of inflammation: Human studies using the 1:1 mixture show no effect on markers of inflammation related to cardiovascular disease risk. Several studies report that the 1:1 mixture does not affect C-reactive protein (CRP) levels. One study suggests that a single CLA isomer (t10,c12) may increase CRP levels, but not with the 1:1 mixture. Neither animal studies nor in-vitro studies support suppression of relevant inflammatory markers. Results relating to a single isomer are not relevant in assessing the safety of CLA-Rich Oil. We conclude, based on the weight of the evidence, that CLA (50:50 mixture) intake does not adversely affect markers of inflammation."

"Markers of Oxidative Stress: Some increase in isoprostanes following CLA intake has been observed in humans. The studies demonstrate that this effect was not due to oxidative stress because no markers of such stress other than isoprostanes increase with CLA consumption. The observed effect on isoprostanes appears to be metabolic: the evidence indicates that CLA competes with F2-isoprostanes for the same metabolic pathway, with the increase in F2-isoprostane levels being the result of a decrease in F2-isoprostane catabolism. We conclude, based on the weight of the evidence, that the observed effects on isoprostane levels do not represent a harmful effect of CLA (50:50 mixture)."

"Endothelial Function: Based on the weight of the evidence representing studies in various human population groups, we conclude that CLA (50:50 mixture) demonstrates no significant adverse effects on endothelial function."

<u>IV.C.4.1.2. Insulin sensitivity and glucose metabolism</u>

"A number of human studies demonstrate no adverse effects of CLA (50:50 mixture) on glucose and insulin after periods of up to 6 months of CLA consumption at levels of 4 g/day. CLA does

not affect insulin sensitivity in healthy, in overweight, or in obese or sedentary individuals. In one human study, an increase in insulin resistance was observed, but this was associated with the administration of only the t10,c12 isomer. We conclude, based on the weight of the evidence, that the 1:1 mixture of CLA isomers does not increase insulin resistance."

IV.C.4.1.3. Maternal milk fat

"The effect of CLA on milk fat production has been studied in cows, rodents and pigs, but these data cannot be relied on as evidence of the effect of CLA on lipogenesis in humans due to a different mechanism of mobilization of fat stores during lactation in humans. One study in humans showed reduction of milk fat associated with CLA, whereas a more recent study from the same authors using the same protocol except for the use of higher doses and a larger cohort showed no effect. Based on the limited available data, there is no evidence to suggest that the consumption of CLA containing foods by lactating women affects milk fat levels beyond the range of normal biological variation. We conclude, based on the weight of the evidence, that CLA (50:50 mixture) would not be harmful with respect to effects on milk fat levels."

IV.C.4.1.4. Human clinical studies presented in GRN 232

Human clinical studies on CLA-TG first appeared in the literature in 2003 (Albers et al., 2003) since CLA-TG has been available since early 2000. In GRN 232, CLA-FFA studies were included to justify the safety of CLA-TG since CLA-TG and CLA-FFA follow similar metabolic fates in human bodies, Comparative studies of Gaullier et al. (2004, 2005) and a single dose metabolic study of Hoye et al. (2004) confirmed that CLA-TG and CLA-FFA have similar metabolic effects.

Tables 8 and 9 summarizes repeated dose human clinical studies presented in GRN 232.
The studies on CLA-FFA only are found from Atkinson et al., 1999; Basu et al., 2000a (Riserus et al., 2001); Belury et al., 2003; Berven et al., 2000; Blankson et al., 2000; Eyjolfson et al., 2004; Kamphuis et al., 2003a,b ; Masters et al., 2002; Mougios et al., 2001; Noone et al., 2002 (Nugent et al., 2005); Smedman and Vessby, 2001(Basu et al., 2000b); Smedman et al., 2004 (Basu et al., 2000a), 2005; Taylor et al., 2006; Thom et al., 2001. Data for CLA-TG only are available from the studies of Doyle et al., 2005; Gaullier et al., 2005, 2007; Lambert et al**.,** 2007; Larsen et al., 2006; Moloney et al., 2004; Nazare et al., 2007; Pinkoski et al., 2006; Raff et al., 2006; Song et al., 2005; Steck et al**.,** 2007; Syvertsen et al., 2007; Watras et al., 2007; Whigham et al., 2004. A comparative study results for CLA-FFA and CLA-TG were reported in Albers et al. (2003) and Gaullier et al., (2004).

The highest dose of CLA-FFA was 6.1 g/day for 8 weeks (Belury et al., 2003). The highest chronic dose tested on CLA-TG was 6 g/day for 1 year, which did not produce adverse effects (Larsen et al., 2006; Whigham et al., 2004). In a comparative study of Gaullier et al. (2004), administration of both CLA-FFA (3.6 g/day for 1 year) and CLA-TG (3.4 g/day for 1 year) produced no adverse effects when body composition, glucose and lipid metabolism, serum biochemistry, and adverse events were compared. A single dose study demonstrated that administration of CLA-FFA at 11.2 g and CLA-TG at 8.9 g was not associated with adverse effects (Hoye et al., 2004).

Table 8. Studies on CLA-FFA presented in GRN 232

Studies on CLA-FFA	Daily dose, g/day	Duration
Atkinson et al., 1999	2.7	26 weeks
Thom et al., 2001	1.8	12 weeks
Mougios et al., 2001	0.7, and1.4	4 weeks
Blankson et al., 2000	1.7, 3.4, 5.1, and 6.8	12 weeks
Berven et al., 2000	3.4	12 weeks
Basu et al., 2000a: Riserus et al., 2001	4.2	4 weeks
Smedman and Vessby, 2001, Basu et al., 2000b	3.2	12 weeks
Noone et al., 2002: Nugent et al., 2005	2.0 mix/1.8 (1.3 as c9,t11)	8 weeks
Kamphuis et al., 2003a,b	1.8, and 3.6	13 weeks
Eyjolfson et al., 2004	3.0	8 weeks
Smedman et al., 2004: Basu et al., 2000a	3.5 as mix/4.0 as t10,c12	6 weeks
Masters et al., 2002	1.2	5 days
Belury et al., 2003	6.1	8 weeks
Smedman et al., 2005	3.2	12 weeks
Taylor et al., 2006	4.5	12 weeks

Table 9. Studies on CLA-TG presented in GRN 232

	Daily dose, g/day	Duration
CLA-TG alone		
Whigham et al., 2004	6.0	1 y
Moloney et al., 2004	2.2	8 weeks
Song et al., 2005	3.0	12 weeks
Doyle et al., 2005	2.2	8 weeks
Raff et al., 2006	4.4 g CLA/3.6 g Vaccenic acid	5 weeks
Pinkoski et al., 2006	5.1	7 weeks
Syvertsen et al., 2007	3.4	6 mo
Nazare et al., 2007	2.8	14 weeks
Larsen et al., 2006	3.4	1 y
Watras et al., 2007	3.2	6 mo
Steck et al., 2007	3.2 and 6.4	12 weeks
Gaullier et al., 2007	3.4	6 mo
Lambert et al., 2007	2.6	12 weeks
Gaullier et al., 2005	3.4 g CLA-TG	CLA-TG, 2 y
Comparison of CLA-FFA and CLA-TG		
Albers et al., 2003	1.7 FFA (1:1 isomers)/ 1.6 TG (4:1 isomers)	12 weeks
Gaullier et al., 2004	3.6 CLA-FFA or 3.4 g CLA in 4.5 g CLA-TG	1 yr

IV.C.4.2. Studies published since mid-2007

To find if there are any recent reports that may not be consistent with the FDA's 2008 review, we reviewed the literature published since mid-2007. The extent of the effects of CLA on insulin resistance and on markers of cardiovascular risk appears to be species-dependent (EFSA, 2010). Therefore, the focus of this safety assessment relies primarily on the large number of available human studies. Studies published since 2007 mostly employed CLA-TG although a few studies evaluated CLA-FFA, and CLA from dairy sources. Unless noted otherwise, these studies evaluated 1:1 isomers of c9,t11:t10c12-CLA.

The doses tested in most studies were 2-6.4 g/day and the duration of the studies ranged from 1 to 7 months. These studies reported no adverse effects of CLA. A 3-week randomized clinical trial (RCT) using a daily dose of 19 g of CLA-TG found no adverse effects in healthy subjects (Wanders et al., 2010a, 2010b).

No recent human studies reported the data to be inconsistent with those found from previous studies: Consumption of CLA-TG isomers (1:1) at doses of up to 6 g/day for up to 1 year (Whigham et al., 2004; Larsen et al., 2006) or 3.4 g/day for up to 2 years (Gaullier et al., 2004, 2005) was safe and had no significant effects on cardiovascular parameters (lipid metabolism, markers of inflammation, and markers of oxidative stress), insulin sensitivity and glucose metabolism, or maternal milk fat. Repeated doses of 6 g CLA-FFA isomers (1:1) for 6 months (Tarnopolsky et al., 2007) or 3.6 g CLA-FFA for 1 year were found safe. In comparative study of Gaullier et al. (2004), administration of either CLA-FFA (3.6 g/day for 1 year) or CLA-TG (3.4 g/day for 1 year) produced similar results by showing no adverse effects on body composition, glucose and lipid metabolism, and serum biochemistry. The incidence of adverse events were not different among the control, CLA-FFA and CLA-TG groups.

IV.C.4.2.1. Glucose metabolism (Table 10)

As summarized in Table 10, recent literature showed that CLA has no adverse effects on fasting blood concentrations of glucose or insulin, or insulin sensitivity. The highest dose tested were 0.8 - 6.8 g/day, and the duration of the studies ranged from 4 weeks to 7 months.

Effects of CLA on glucose metabolism in subjects with type 2 diabetes

Four studies evaluated effects of CLA on glucose metabolism in type 2 diabetes (Table 10; CLA source not specified; Carvalho et al., 2012; CLA-FFA- Shadman et al., 2013; CLA-TG – Asp et al., 2011; Norris et al., 2009). The highest dose tested for CLA-TG was 6.4 g/day for 16 weeks (Asp et al., 2011; Norris et al., 2009; Table 10). The highest dose tested for CLA-FFA was 3.0 g/day for 8 weeks (Shadman et al., 2013). Both CLA-TG and CLA-FFA had no adverse effects of glucose metabolism in subjects with types 2 diabetes.

Effects of CLA on glucose metabolism in healthy subjects or subject with other conditions

In general, CLA supplementation, regardless of CLA forms (FFA or TG), had no effects on glucose metabolism (Table 10). Doses tested were 6.4 g/day and the duration of the studies were up to 7 months. The highest doses tested for CLA-TG and CLA-FFA were 6.4 g/day for 16 weeks (Norris et al., 2009), and 6.0 g/day for 6 months (Tarnopolsky et al., 2007), repectively.

Most studies reviewed reported no beneficial or adverse effects of CLA on fasting blood concentrations of glucose or insulin, Homeostasis Model of Assessment - Insulin Resistance (HOMA-IR), or other biomarkers in healthy subjects or subjects with mildly hyercholesterolemia, hypertension, or rheumatoid arthritis (CLA-TG form: Ahren et al., 2009; Aryaeian et al., 2008; Gaullier et al., 2007; Iwata et al., 2007; Joseph et al., 2011; López-Plaza et al., 2013; MacRedmond et al., 2010; Racine et al., 2010; Sneddon et al., 2008; Syvertsen et al., 2007; Turpeinen et al., 2008; Zhao et al., 2009; CLA-FFA: Herrmann et al. (2009), Laso et al. (2007), Tarnopolsky et al. (2007), and Thrush et al. (2007; Natural form: Brown et al., 2011; CLA form not specified: Chen et al., 2012; Raff et al., 2009).

Favorable effects of CLA on glucose metabolism were noted in 3 studies (CLA-TG: Bulut et al., 201; Lambert et al., 2007; Pfeuffer et al., 2011).

Two studies reported conflicting results (Raff et al., 2009; Thrush et al., 2007). A study of Thrush et al. (2007) showed that CLA-FFA (3.1 g CLA containing 1.55g c9,t11 and 1.52 g t10,c12 CLA in the form of 1 g gel capsules with meals) increased the area under the curve (AUC) for glucose (30%) and insulin (20%) in a 3 h oral glucose tolerance test (OGTT) in 9 overweight, nondiabetic subjects (100% completed, 31.2 $\pm$ 3.7 years). Results from OGTT suggest that CLA reduced insulin sensitivity. However, the OGTT may not be a validated analytical method for determining insulin sensitivity. In addition, CLA had no effect on fasting plasma concentrations of lipids, glucose, insulin, or adiponectin after 12 weeks of supplementation. Fasting glucose and insulin concentrations may indicate CLA's effects on glucose metabolism more accurately. It is noteworthy that CLA had no effect on peroxisome proliferators activated receptor alpha (PPARα) content in muscle. The PPARα is known to regulate lipid oxidation and glucose metabolism. The study had limitations that included no control group and a small number of subjects (n=9). Overall, it is concluded that this study shows no adverse effects of CLA on glucose metabolism.

In a 2009 study of Raff et al., 81 healthy postmenopausal women were randomized to one of 3 groups: 1) 5.5 g/day of 40/40% of c9,t11-CLA-TG and t10,c12-CLA (CLA-mix; source not specified); 2) c9,t11-CLA; or 3) control (olive oil). No differences in serum insulin and glucose concentrations and HOMA-IR values were found among the groups, although post hoc analysis in the tertile of women with the highest waist circumference (WC; 94–109 cm) showed that the fasting serum insulin concentration was greater in the CLA-mix group than in the control (34%; P =0.02) and the c9,t11-CLA (28%; P = 0.04) groups. There were no differences between the effects of the supplements in the 2 lower tertiles. A large WC is a characteristic of metabolic syndrome and a risk factor for diabetes type II. Thus, this post hoc analysis indicates that participants with a large waist circumference are may be more sensitive to a negative effect of a CLA mixture on insulin metabolism. However, others did not report such effects and, thus, it requires more investigations. Overall, it is concluded that CLA supplementation had no effects on glucose metabolism.

IV.C.4.2.2. CVD risk factors (Tables 11 -13)

As summarized in Tables 11-13, current research indicated that CLA had either no adverse effects or favorable ones on CVD markers. Supplementation with CLA had no adverse effects on CVD risk factors that include endothelial functions, inflammatory biomarkers, and body weight

measures as well as fat oxidation or oxidative stress (Close et al., 2007; Kim et al., 2012; Malpuech-Brugere et al., 2010).

Three forms of CLA were employed in these studies: CLA-TG, CLA-FFA, and CLA from natural dairy sources. The CLA-TG form was employed in the studies of Aryaeian et al. (2008), Diaz et al. (2008), Gaullier et al. (2007), Iwata et al. (2007), Joseph et al. (2011), Lambert et al. (2007), Nazare et al. (2007), Norris et al. (2009), Pfeuffer et al. (2011), Racine et al. (2010), Raff et al. (2008, 2009), Sneddon et al. (2008), Steck et al. (2007), Tholstrup et al. (2008), Turpeinen et al. (2008), Watras et al. (2007), Wanders et al. (2010a), and Zhao et al. (2009). The CLA-FFA form was evaluated in the studies of Herrmann et al. (2009), Laso et al. (2007), Macaluso et al. (2012), Thrush et al. (2007), and Venkatramanan et al. (2010). CLA from natural dietary source were studied in Brown et al. (2011), Pintus et al. (2013), Sofi et al. (2010), and Venkatramanan et al. (2010). In the studies of Chen et al. (2012), Michishita et al. (2010), and Sluijs et al. (2010), CLA form not specified.

It appears that sources of CLA (TG, FFA, or natural dietary sources) did not affect the CVD risk factors. The doses tested for CLA-TG were 2-19.1 g/day, and the durations of the studies ranged from 3 weeks to 7 months. With the exception of the study of Wanders et al. (2010a) which employed 19 g CLA-TG for 3 weeks, most studies employed daily doses of 2-6.8 g and the durations of the studies ranged from 3 weeks to 7 months. The doses tested for CLA-FFA were 3.1-3.4 g/day and the durations of the studies ranged from 4-16 weeks (Herrmann et al., 2009, Laso et al., 2007, Macaluso et al., 2012, and Thrush et al., 2007).

Blood lipid profiles

Most papers reported no effects of CLA on blood lipid profiles or blood pressure (BP; Table 11: CLA-TG form - Aryaeian et al., 2008, Diaz et al., 2008, Gaullier et al., 2007, Iwata et al., 2007, Lambert et al., 2007, Lopez-Plaza et al., 2013, Racine et al. 2010, Raff et al., 2008, Steck et al., 2007, Turpeinen et al., 2008, Zhao et a., 2009; CLA-FFA form- Herrmann et al., 2009, Laso et al., 2007, Thrush et al., 2007, Venkatramanan et al., 2010; CLA from dietary sources- Brown et al., 2011, Sofi et al., 2010, Venkatramanan et al., 2010; CLA form not specified - Chen et al. (2012), Michishita et al., 2010, Sluijs et al., 2010). One study showed favorable effects (Pintus et al. 2013; CLA from dairy sources).

Three studies in 3 papers reported mixed results, with no biologically significant adverse effects from CLA (Chen et al., 2012; Tholstrup et al., 2008; Wanders et al., 2010a). Chen et al. (2012) showed that supplementation with CLA (3.4 g/day for 12 weeks; CLA form not specified) increased fasting plasma concentrations of TC, TG, LDL-C, alanine aminotransferase (ALT), and aspartate transaminase (AST), while decreasing HDL-C. However, the changes were slight and within the normal range. The authors concluded that CLA supplementation resulted in a decrease in obesity indices, with no obvious adverse effects.

Likewise, Tholstrup et al. (2008; the same study reported in Raff et al., 2008) found that a CLA-TG mixture containing t10,c12 decreased plasma HDL-C concentration (66.1 vs.77.0 mg/dl) and the ratio of HDL-C to LDL-C (2.86 vs. 3.43) while raising plasma concentrations of TG (93.0 vs. 78.8mg/dl) and CRP (by 37%) compared to controls. However, these values were within the normal ranges (HDL-C >60 mg/dl; TG<150 mg/dl; HDL-C/LDL-C ratio<4). In this study, a

daily supplement of 5.5 g of oil rich in either a CLA mixture, an oil rich in naturally occurring c9,t11 CLA, or olive oil were administered for 16 weeks in 75 healthy postmenopausal women. In prepubertal children aged 6–10 y who were overweight or obese but otherwise healthychildren, CLA supplementation (3 g/day of 80% CLA for 7 months) did not significantly influence plasma concentrations of glucose, insulin, or LDL-C although CLA decreased plasma HDL-C significantly more (P = 0.05) in the CLAgroup (-5.1 mg/dL) than in the placebo group (-0.7 mg/dL) while decreasing body fatness.

In the study of Wanders et al. (2010a), compared with the control group, the CLA-TG group had higher concentrations of TC (by 4.5%), LDL-C (7.8%), TG (5%), and apo-lipoprotein B (apo-B by 4.5%) and a lower concentration of HDL-C (5%). However, these figures are within the normal range, and are not a safety concern.

It appears that sources of CLA (TG, FFA, or natural dairy sources) did not affect blood lipid profiles.

Body weight measure and body fat

Risk factors for CVD include obesity. As shown in Table 11, a number of human trials reported favorable effects of CLA supplementation on BW and body fat mass (BFM) as well as lean body mass (LBM), body mass index (BMI), WC, fat free mass (FFM) or lean body mass (LBM) (CLA-TG form- Joseph et al., 2011; López-Plaza et al., 2013, Norris et al., 2009; Racine et al., 2010; Raff et al., 2009; Watras et al., 2007; Zhao et al., 2009; unspecified CLA form- Chen et al., 2012. However, some studies reported no significant effects of CLA on BW measures or body fat status (CLA-TG form- Diaz et al., 2008; Gaullier et al., 2007; Joseph et al., 2011; Lambert et al., 2007; Nazare et al., 2007; Pfeuffer et al., 2011; Sneddon et al., 2008; Streck et al., 2007; CLA-FFA form- Herrmann et al., 2009; Laso et al., 2007; Macaluso et al., 2012; Thrush et al., 2007; Venkatramanan et al., 2010; CLA from natural dietary source - Pintus et al., 2013; Sofi et al., 2010; Venkatramanan et al., 2010; unspecified CLA source- Michishita et al., 2010; Sluijis et al., 2010). It appears that sources of CLA (TG, FFA, or natural dairy sources) did not influence body weight measure and body fat.

Chen et al. (2012) reported that supplementation with CLA (3.4 g/day for 12 weeks; source unspecified) decreased BW, BMI, subcutaneous fat mass, and waist-to-hip ratio (WHR) in healthy Chinese subjects, with the greatest effect in those with BMI<27. A meta-analysis by Schoeller et al. (2009) demonstrated a correlation between CLA intake and increased FFM that was independent of treatment length or dosage. This indicates that FFM has a more rapid progression and a small effect that plateaus early on. A meta-analysis by Whigham et al. (2007) confirmed a linear progression in weight loss for approximately 6 months of CLA consumption. The same study found that a median dose of 3.2 g/day of CLA significantly reduced fat mass in the CLA supplemented group compared with the controls. Overall, no adverse effects from CLA were reported on BW measures or body composition.

Immune or inflammatory responses

Studies evaluating effects of CLA on immune and inflammatory responses in humans reported inconsistent results in terms of efficacy although no studies reported adverse effects of CLA (Table 12). Beneficial effects of CLA supplementation were observed in particular immuno-

challenging experiments (Dietary sources - Sofi et al., 2010; CLA-TG form - Mullen et al., 2007; Turpeinen et al., 2008; Zhao et al., 2009). In individuals with birch pollen allergy, supplementation of 2 g/day c9,t11 CLA for 12 weeks improved feelings of well-being and reduced sneezing (Turpeinen et al., 2008). Sofi et al. (2010) reported positive effects of CLA in reducing inflammatory parameters, such as IL-6 (-43%), IL-8 (-36.5%), and TNFα (-40%). However, other studies failed to show significant or consistent effects of CLA supplementation on indices of immune status (CLA-TG form - Gaullier et al., 2007; Joseph et al., 2011; MacRedmond et al., 2010; Pfeuffer et al., 2011; Raff et al., 2008; Steck et al., 2007; Stickford et al., 2011; Tholstrup et al., 2008). For example, 5.5 g of oil rich in either CLA mixture or an oil rich in naturally occurring c9,t11 CLA did not change plasma cytokines, such as IL-6, TNFα, intercellular adhesion molecule 1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1; Tholstrup et al., 2008). Steck et al. (2007) found that CLA significantly increased blood concentrations of CRP, IL-6, and white blood cells. However, all values were within normal ranges. Taken together, outcomes indicate no adverse effects of CLA on immune or inflammatory responses.

Fat oxidation or oxidative stress

Studies evaluating effects of CLA on fat oxidation or oxidative stress in humans produced inconsistent results in terms of efficacy (Table 13). Doses tested were 2.4 - 4.6 g/day and the duration of studies were 8 weeks - 6 months (CLA-TG form - Malpuech-Brugere et al., 2010; Pfeuffer et al., 2011; Tholstrup et al., 2008; Turpeinen et al., 2008; CLA-FFA form - Kim et al., 2012; CLA form not specified- Close et al., 2007). Tholstrup et al. (2008) found that control group women who received the CLA mixture group (5.1 g/day) had 60% higher 8-iso prostaglandin F2a (8-iso PGF2α; a marker of *in vivo* oxidative stress), whereas women in the CLA milk group had 25% higher 8-iso PGF2α. As most of the risk markers are new, the clinical importance of these findings is uncertain. Close et al. (2007) reported that CLA positively altered fat oxidation and energy expenditure during sleep. Other studies reported no effects of CLA on fat oxidation (Kim et al., 2012; Malpuech-Brugere et al., 2010; Pfeuffer et al., 2011). Overall, no biologically significant adverse effects of CLA were reported.

IV. C.4.2.3. Effects of CLA on human milk production

Hasin et al. (2007) reported that dietary supplementation with either c9,t11 or t10,c12 CLA (CLA source not specified) did not reduce milk fat in healthy, breast-feeding women (Table 14). No adverse effects of CLA were reported.

IV. C.4.2.4. Studies evaluating effects of CLA on other physiological outcomes in humans

Several studies evaluated the effects of CLA on bone health (CLA from dietary source - Brown et al., 2011; CLA-TG form - Gaullier et al., 2007; Racine et al., 2010), liver and kidney functions (CLA-TG form -Wanders et al., 2010b), fecal microbiota, fecal enzyme activity, or fecal composition (dietary source of CLA and CLA-TG -Farnworth et al., 2007) (Table 15). These studies reported no adverse effects from CLA. Goedecke et al. (2009) reported no adverse effects of CLA-TG in a study evaluating incorporation of CLA into adipose tissue and skeletal muscle. Wanders et al. (2010b) reported that a daily dose of 19.3 g CLA-TG (14.6 g c9,t11 CLA and 4.7 g t10,c12 CLA isomers) for 3 weeks did not cause adverse effects on liver and kidney functions in healthy subjects.

Table 10. Effects of CLA on glucose metabolism in humans

Subject	Daily dose	Duration/ Design	Measured outcomes	Results	Reference
Pateints with type 2 diabetes					
56 patients with overweight type2 diabetes	3 g CLA-FFA + 100 IU VitE, 3 g CLA + VitE placebo, or CLA placebo + VitE placebo	8 weeks	anthropometric measurements, and body composition; Fasting blood conc. of glucose, insulin, TG, TC, LDL-C, and HDL-C, HOMA-IR, and leptin	No effects of CLA on any indicators of metabolic control in overweight type2 diabetic patients	Shadman et al., 2013
14 sedentary women with metabolic syndrome (13 completed; CLA group n=7, 40 yr; Placebo; group n=7, 42 yr)	3 g CLA isomers in a sugar reduced strawberry jam (1.16 g c9,t11, + 1.19 g t10,c12); or placebo (sugar reduced strawberry jam); microencapsulated CLA-form not specified	90 days, P	Fasting blood conc. of glucose, insulin, TG, TC, LDL-C, and HDL-C, HOMA-IR, BP, % body fat, and BW measures.	No adverse effects of CLA on measured outcomes were reported. Supplementation with CLA may have a favorable effect on glycemic control and body fat mass loss at an earlier time in sedentary women with metabolic syndrome, although CLA had no effects on lipid profile and blood pressure.	Carvalho et al., 2012
55 post-menopausal, obese women with type 2 diabetes (≤ 70 yr)	6.4 g CLA as TG (3.2 g c9,t11 + 3.2 g t10,c12 in 8 g CLA oil) or safflower oil (8 g/d);	16 weeks each, X	Blood glucose, insulin, HbA1c, lipid profile, IL-6, and CRP	No adverse effects of CLA on measured outcomes were reported. No effects on metabolic parameters.	Asp et al., 2011
55 obese postmenopausal women with type 2 diabetes (35 completed; ave. 59.7 yr)	6.4 g CLA as TG (or 8 g CLA-TG oil) or placebo (safflower oil)	16 weeks, X	Plasma conc. of glucose and insulin	No effect on plasma conc. of glucose or insulin.	Norris et al., 2009

P=parallel design; X=cross over design; BP = blood pressure; BW = body weight; FFA = free fatty acid(s); HDL-C=high density lipoprotein cholesterol; HOMA-IR= Homeostasis Model of Assessment - Insulin Resistance; LDL-C=low density lipoprotein cholesterol; TG= triglycerides.

Table 10. Effects of CLA on glucose metabolism in humans

Subject	Daily dose	Duration/ Design	Measured outcomes	Results	Reference
CLA-TG					
85 overweight men (45-68 yr)	3.4 g CLA as TG (50:50 c9,t11 and t10,c12 in 4.5 g CLA-TG oil); safflower oil; heated safflower oil (240°C for 2 hours); or olive oil	28 days, P	Blood glucose, insulin, lipid profiles, HOMA-IR	No adverse events reported. Parameters associated with metabolic syndrome were not changed or were slightly improved.	Pfeuffer et al., 2011
36 overweight, mildly hypercholesterolemic men (27 completed, 18-60 yr)	1) 2.8 g CLA as TG mixture group: 1.4 g t10,c12 + 1.4 g c9,t11 (or 3.5 gCLA-TG); 2) 2.7 g CLA-TG c9,t11 isomer group: (or 3.5 g CLA oil); and 3) placebo 3.5 g safflower oil	8 weeks, X	HOMA-IR	No adverse effects on measured outcome were reported. CLA had no effects on HOMA-IR.	Joseph et al., 2011
62 prepubertal overweight or obese, but otherwise healthy, children (53 completed, 58.1% male, 6-10 yr)	2.4 g CLA as TG (1:1 mixture of c9,t11 and t10,c12 CLA isomers in 3 g CLA-oil); or placebo (sunflower oil) in chocolate milk	7 $\pm$ 0.5 months, P	Fasting blood conc. of glucose and insulin, and HOMA-IR	No significant effect on fasting glucose and insulin or HOMA-IR.	Racine et al., 2010
80 subjects with stage 1 uncontrolled hypertension (80 completed; mean age, 59.4-62.3 yr)	4.5 g CLA as TG mixture (1:1 mixture of c9,t11 and t10,c12); or placebo	8 weeks, P	Fasting blood conc. of glucose and insulin, and HOMA-IR	No significant effect on blood conc. of glucose and insulin, and HOMA-IR.	Zhao et al., 2009

P=parallel design; X=cross over design; HOMA-IR= Homeostasis Model of Assessment - Insulin Resistance.

Table 10. Effects of CLA on glucose metabolism in humans, continued

Subject	Daily dose	Duration/ Design	Measured outcomes	Results	Reference
CLA-TG					
Young men (20-37 yr)- Lean (n=12; BMI, 20-26) and Obese (n=10; BMI 29-35); Older men (50-65 yr)- Lean (n=16) and Obese (n=11)	3 g CLA as TG (76% CLA; 1:1 mixture of c9,t11 and t10,c12) plus 3 g n-3 long chain PUFA; or control oil (6 g/d)	12 weeks, X	Fasting blood conc. of glucose, insulin secretion, insulin sensitivity, C-peptide.	No effects on fasting levels of glucose, insulin or C-peptide, β-cell dysfunction or insulin resistance. In older obese subjects, estimated insulin sensitivity was reduced in both treatment and control groups.	Ahren et al., 2009
Young men (23-38 yr)- Lean (n=13; BMI, 20-26) and Obese (n=12; BMI, 30-36); Older men (50-64 yr)- Lean (n=20; BMI, 21-26) and Obese (n=4; BMI, 30-34)	1) 2.28 g CLA as TG plus 3 g n-3 LC PUFA (3 g of CLA oil; 1.14 g c9,t11 + 1.14 g t10,c12 CLA) and 3 g of n-3 LC-PUFA (30% EPA+ 21% DHA); or 2) 6 g control fat (80% palm oil and 20% soya bean oil	12 weeks, X	Fasting insulin conc. and HOMA-IR	No adverse events reported. No effects of CLA+PUFA on leptin and adiponectin, HOMA-IR, and fasting insulin conc. Data have flaws by not comparing treatment and control groups in absolute values after 12 week supplementation. Only changes after intervention within each group were compared.	Sneddon et al., 2008
38 healthy overweigth subjects (30-55 years)	3g of CLA as TG or 3g olive oil (placebo) in skim milk	24 weeks, P	Body weight measures, blood lipid profile, CRP, ALT, AST, HOMA-IR	The consumption of skimmed milk enriched with 3g CLA for 24 weeks led to a decrease in body weight and total fat mass. No significant changes were demonstrated in blood analyses, except for significant increases in glucose ($p = 0.002$) and plasma creatinine ($p = 0.001$) in the CLA group (data not shown). Insulin levels and HOMA index have no changes at the end of the intervention No adverse effects were observed.	López-Plaza et al., 2013

P=parallel design; X=cross over design; ALT = alanine aminotransferase; AST= aspartate transaminase; CRP = C-reactive protein; HOMA-IR= Homeostasis Model of Assessment - Insulin Resistance; PUFA = polyunsaturated fatty acids.

Table 10. Effects of CLA on glucose metabolism in humans, continued

Subject	Daily dose	Duration/ Design	Measured outcomes	Results	Reference
CLA-TG					
87 patients with active rheumatoid arthritis	1) 2 g CLA as TG (1 g c9,t11 + 1 g t10c12-CLA as TG); 2) 400 mg vitamin E; 3) 2 g CLAs (1 g c9,t11 and 1 g t10,c12) + 400 mg vitamin E; 4) placebo - high oleic sunflower and corn oil	3 months, P	Serum glucose conc. and blood pressure	No adverse effects of CLA on measured outcomes were reported. No CLA effects on fasting blood levels of glucose.	Aryaeian et al., 2008
40 subjects with diagnosed birch pollen allergy (20–46 yr)	2 g c9,t11 CLA as TG; or placebo	12 weeks, P	Plasma conc. of glucose and insulin, and HOMA-IR	No significant effect on plasma conc. of glucose, insulin, or HOMA-IR.	Turpeinen et al., 2008
47 healthy men (19-35 yr)	5.5 g total CLA as TG (39.4% c9,t11 and 38.5% t10,c12); or placebo (0.3 g of c9,t11 CLA) Note: CLA group received 4.6 g/day of total CLA (2.5 g of c9,t11 and 2.1 g of t10,c12) and the control group received 0.3 g of c9,t11 CLA	5 weeks, P	BMI, blood lipid profile, CRP, PAI-1, FVII, urine 8-iso-PGF2α, insulin, glucose, HOMA-IR.	A mixture of CLA isomers increased lipid peroxidation but did not affect risk markers of CVD, inflammation, or fasting insulin and glucose concentrations.	Raff et al., 2008
28 adult subjects with mild asthma (26 completed, 19-40 yr)	3.3 g CLA as TG (1.63g c9,t11+ 1.67 g t10,c12 in 4.5 g CLA-TG oil); or placebo (4.5 g olive oil)	12 weeks, P	Fasting insulin	No significant effect on insulin.	MacRedmond et al., 2010
118 healthy subjects (18-65 yr)	3.4 CLA as TG (1.7 g c9,t11+ 1.7 g t10c12 in 4.5 g CLA-oil) or placebo (4.5 g of olive oil)	6 months, P	Fasting blood conc. of glucose, HbA1c, and insulin	No adverse events reported. CLA supplementation had no effects on diabetogenic markers.	Gaullier et al., 2007

P=parallel design; X=cross over design; CRP = C-reactive protein; CVD = cardiovascular disease; FVII=factor VII;HbA1c = glycosylated hemoglobin; HOMA-IR= Homeostasis Model of Assessment - Insulin Resistance; 8-iso-PGF2α=8-iso-prostaglandin F2α; PAI-1=plasminogen activator inhibitor-1; TG = triglycerides.

Table 10. Effects of CLA on glucose metabolism in humans, continued

Subject	Daily dose	Duration/ Design	Measured outcomes	Results	Reference
CLA-TG					
60 overweight Japanese male volunteers (25-60 yr; BMI 25-35 kg/m2)	1) 5.4 g CLA as TG (3.4 g CLA isomers); 2) 6.8 g CLA isomers (10.8 g CLA-TG -1:1 mixture of c9,t11 and t10,c12-; 3); or placebo 10.8 g high-linoleic safflower oil	12 weeks, P	Serum biochemistry (fasting serum glucose conc.)	Transient adverse events (mostly mild or moderate diarrhea, abdominal distension, and/or fever) were reported by 30% of the placebo group, 60% of the low CLA group, and 70% of the high CLA group. No differences in fasting blood glucose and insulin conc.	Iwata et al., 2007
64 regularly exercising subjects (62 completed; 21-45 yr)	2.57 g CLA (1.16 g c9,t11 + 1.20 g t10,c12 + 0.11 g other CLA isomers; 3.9 g CLA TG-oil) or placebo (3.9 g high-oleic acid sunflower oil)	12 weeks, P	Glucose homeostasis and insulin sensitivity	Adverse events did not differ between the groups. During the OGTT, CLA women group had lower mean plasma insulin conc. at 30, 120 min compared to control (20.4-24.3vs. 23.7-26.0 mU/ml). Serum NEFA levels in response to oral glucose were attenuated in both men and women in the CLA compared to control group.	Lambert et al., 2007
118 healthy overweight and obese individuals (83 completed, 22% male, mean 48 yr)	3.4 g/day CLA (4.5 g CLA TG oil - containing 37.5% c9,t11 and 38% t10,c12); or placebo (4.5 g olive oil)	6 months, P	Change in glucose uptake, insulin resistance, changes in body composition. or blood chemistry	No change in glucose uptake, insulin resistance, body composition, or blood chemistry parameters.	Syvertsen et al., 2007
18 sedentary, healthy, slightly overweight adult males (19-31 yr)	0.8-0.92 g c9,t11 + 0.8-0.92 g t10,c12 in 3 g CLA-TG; or placebo (3 g inulin)	30 days, P	Plasma lipoprotein lipase, butyryl-cholinesterase activities, and leptin, insulin, and lipid level, and HOMA-IR.	No adverse effects of CLA on measured outcomes were reported. CLA supplementation decreased butyrylcholinesterase activites (P<0.03), leptin (P=0.002), insulin (P=0.02), and HOMA-IR (P=0.02) levels.	Bulut et al., 2013

P=parallel design; X=cross over design; NEFA = non-esterified fatty acids.

Table 10. Effects of CLA on glucose metabolism in humans, continued

Subject	Daily dose	Duration/ Design	Measured outcomes	Results	Reference
CLA-FFA					
38 subjects (15 male PPARγ2 Ala12Ala homozygous and 23 BMI-matched homozygous control subjects, PPARγ2 Pro12Pro; 37 completed; 45-68 yr)	4.25 g CLA-FFA oil containing 3.4 g CLA/d; 1:1 mixture of c9,t11 and t10,c12 CLA; or control oil	4 weeks, X	Plasma glucose HOMA-IR, and Pro12Ala of PPARγ2 as an insulin resistant biomarker	No significant changes in plasma glucose conc. or HOMA-IR. The isomer specific influence of CLA on glucose and lipid metabolism was genotype dependent and partly mediated by PPARγ2.	Herrmann et al., 2009
9 overweight, non-diabetic subjects (100% completed, 31.2 ± 3.7 yr)	3.1 g CLA-FFA (1.55g c9,t11+ 1.52 g t10,c12 CLA) in the form of 1 g gel capsules with meals. No placebo group;	12 weeks	Fasting plasma conc. of glucose, insulin, and adiponectin, OGTT, and PPARα in muscle	CLA had no effects on fasting plasma conc. of lipids, glucose, insulin, and adiponectin after 12 weeks supplementation. No effect on PPARα content in muscle. CLA increased AUC for glucose (30%) and insulin (20%) in a 3 h OGTT.	Thrush et al., 2007
39 older adults (29 completed; >65 yr)	6 g CLA-FFA mixture (CLA (CLA-ONE) plus 5 g creatine; or placebo plus creatine;	6 months, P	OGTT, with and without resistance training	No effects on glucose or insulin.	Tarnopolsky et al., 2007
60 healthy men and women (43 completed, 35-65 yr)	3 g CLA-FFA (1.5 g c9t11 and 1.5 g t10c12) or placebo (500 mL of skimmed milk)	12 weeks, P	Fasting plasma insulin and HOMA-IR	No adverse events or biological changes reported. CLA had no effect on the parameters of the metabolic syndrome.	Laso et al., 2007

P=parallel design; X=cross over design; AUC = area under the curve; HOMA-IR= Homeostasis Model of Assessment - Insulin Resistance; OGTT=oral glucose tolerance test; PPARα = peroxisome proliferators activated receptor alpha.

Table 10. Effects of CLA on glucose metabolism in humans, continued

Subject	Daily dose	Duration/ Design	Measured outcomes	Results	Reference
CLA form not specified					
80 Chinese participants (63 completed; 33.3% Male; 32.8±0.8 yr)	3.4 g CLA (mixture of 1.7g c9,t11 + 1.7 g t10c12) in 400 mL of sterilized milk; or placebo (400ml of sterilized milk with salad oil); CLA form not specified	12 weeks, P	Fasting blood conc. of glucose	Supplementation with CLA had no effects on fasting blood glucose conc.	Chen et al., 2012
81 healthy postmenopausal women	1)5.5 g/day of 40/40% of 9,t11-CLA and t10,c 12-CLA; 2) c9,t11-CL A; or 3) control (olive o il)	16 weeks, P	Serum insulin and glucose concentrations, and HOMA-IR	The 1:1 mixture of c9,t11- and t10,c12-CLA isomers resulted in greater serum insulin concentrations in the highest waist circumference tertile only. Overall, Serum insulin and glucose concentrations, and HOMA-IR did not differ among the 3 groups.	Raff et al., 2009
From dietary sources					
18 women (20-40 yr)	1.17 g CLA from beef and dairy products rich in CLA from dairy cattle	56 days, P	Blood glucose and insulin, and glucagon	No significant changes in blood conc. of glucose, insulin or glucagon.	Brown et al., 2011

P=parallel design; X=cross over design; ALT = alanine aminotransferase; AST= aspartate transaminase; CRP = C-reactive protein; HOMA-IR= Homeostasis Model of Assessment - Insulin Resistance.

Table 11. Effects of CLA on CVD risk factors and body weight measures in humans

Subject	Daily dose	Duration / Design	Measured outcomes	Results	Reference
85 overweight men (100% male, 45-68 yr)	4.5 CLA-TG oil mixture containing 3.4 g of 1:1 c9,t11 and t10,c12, CLA), safflower oil, or heated safflower oil (240 °C for 2 hours); or olive oil	28 + 2 days, P	BW, PAT index as a measure of endothelial function, and blood biochemistry (lipid profiles, sVCAM, sICAM, and sE-selectin)	No adverse events reported. CLA did not impair endothelial function. Other parameters associated with metabolic syndrome and oxidative stress were not changed or were slightly improved.	Pfeuffer et al., 2011
36 overweight, mildly hypercholesterolemic men (27 completed, 18-60 yr)	1) 2.8 g CLA mixture group: 1.4 g t10,c12 + 1.4 g c9,t11 (or 3.5 g CLA-TG); 2) 2.7 g CLA c9,t11 isomer group: (or 3.5 g CLA oil); or 3) placebo 3.5 g safflower oil	8 weeks, X	BW, BMI, body fat profile, blood lipid profile, HOMA-IR, and safety outcomes (plasma CRP, adiponectin and oxidized LDL-C, serum TNFα and IL-6)	No adverse effects on measured outcomes were reported. CLA had no effects on BW, body composition and blood lipids.	Joseph et al., 2011
63 healthy subjects (61 completed, 30.9 ± 13.7 yr)	1) 19.1 g CLA isomers (16.4 g c9,t11 + 3.7 g t10,c12 CLA in 28.9 g CLA-TG oil); 2) 27.6 g oleic acid; or 3) 21.8 g industrial trans C18:1 fat diet (positive control). Note: supplements were provided in oil, margarine, and/or yogurt form	3 weeks each, X (no washout period)	Serum lipid profile and apoprotein conc.	No statistically significant differences in serum conc. of TC, HDL-C, LDL-C, and TG at the end of each treatment period among the 3 groups although, compared to the control group, CLA group tended to have higher conc. of TC (4.5%), LDL-C (8.9%), TG (6.4%), and apo-B (4.7%) and a lower conc. of HDL-C (5%). High intakes of CLA isomers also raised the ratio of TC to HDL-C (10%) and LDL-C to HDL-C (14%). However, all the values were within the normal range and were of no toxicological concern.	Wanders et al., 2010a

ALT=alanine transaminase; LT=alanine aminotransferase; AST=asparate aminotransferase; FFA=free fatty acid(s); BMI=body mass index; BW=body weight; CRP= C-reactive protein; HDL-C=high density lipoprotein cholesterol; HOMA-IR= Homeostasis Model of Assessment - Insulin Resistance; sICAM-1=soluble Intercellular adhesion molecule 1; IL=interleukin; LDL-C=low density lipoprotein cholesterol; PAT=peripheral arterial tonometry; sVCAM-1=soluble vascular cell adhesion molecule-1; TG=triglycerides; TNFα = tumor necrosis factor alpha.

Table 11. Effects of CLA on CVD risk factors and body weight measures in humans, continued

Subject	Daily dose	Duration/ Design	Measured outcomes	Results	Reference
62 prepubertal overweight or obese, but otherwise healthy, children (53 completed, 58.1% male, 6-10 yr)	2.4 g CLA (1:1 mixture of c9,t11 and t10,c12 CLA isomers in 3 g CLA-TG oil); or placebo (sunflower oil) in chocolate milk	7 + 0.5 months, RCT	BMI, body composition (body fat, bone mineral content), and blood conc. of glucose, insulin, LDL-C, HDL-C, TC, TG, AST, ALT, and γ-GTP	CLA supplementation decreased body fatness in 6-10 year old children who were overweight or obese. No differences in blood lipid profiles except HDL-C which was decreased from baseline in both groups, but CLA group had more profound effects. Reported gastrointestinal symptoms did not differ between groups. No adverse effects on measured outcomes were reported.	Racine et al. 2010
35 obese postmenopausal women with type 2 diabetes (60.1±7.9 yr)	6.4 g CLA isomers (or 8 g CLA-TG oil); or placebo-8 g safflower oil)	16 weeks, X	BW, body composition, adipose distribution, fasting blood conc. of adipokines adiponectin or leptin, ALT, and AST	Various adverse events occurred, but there were no differences between the treatments. CLA reduced BMI and total adipose mass without altering lean mass or WC. No differences in dietary energy intake and fasting blood conc. of adiponectin, leptin, ALT, or AST.	Norris et al., 2009
80 subjects taking blood pressure medication	4.5 g CLA mixture (1:1 ratio of c9,t11 and t10,c12 as TG); or placebo	8 weeks, P	BW measures and composition, blood lipid profile, and BP	CLA group had lower % body fat (14.8%) and HC (6.5%). No significant change in BW, BMI, BP, WC, WHR, TC, LDL-C, TG or VLDL-C. Increased HDL-C by 11%. Decreased SBP (30%) and DBP (22%).	Zhao et al., 2009

ALT=alanine transaminase; AST=asparate aminotransferase; FFA=free fatty acid(s); BMI=body mass index; BW=body weight; CRP= C-reactive protein; DBP = diastolic blood pressure; γ-GTP=γ-glytamyltranspeptidase; HDL-C=high density lipoprotein cholesterol; LDL-C=low density lipoprotein cholesterol; SBP = systolic blood pressure; TC=total cholesterol; TG=triglycerides; TNFα = tumor necrosis factor alpha; VLDL-C=very low density lipoprotein cholesterol; WC=waist circumference;WHR= waist-to-hip ratio.

Table 11. Effects of CLA on CVD risk factors and body weight measures in humans, continued

Subject	Daily dose	Duration/ Design	Measured outcomes	Results	Reference
CLA-TG					
47 healthy men (38 completed, 100% male, 19-35 yr)	4.6 g CLA (5.5 g CLA-TG oil, 2.5 g c9,t11 and 2.1 g t10,c12); or placebo (0.3 g/day of c9,t11 CLA)	5 weeks, P	BMI and blood lipid profile	A mixture of CLA isomers did not affect risk markers of CVD. No significant effect on blood lipid profile.	Raff et al., 2008
40 subjects with diagnosed birch pollen allergy (20–46 yr)	2.25 g CLA (2 g c9,t11- and 0.25 g t10,c12-CLA as TG); or placebo	12 weeks, P	Plasma lipids and glucose, and HOMA-IR	No significant changes in measured outcomes.	Turpeinen et al., 2008
87 patients with active rheumatoid arthritis	1) 2 g CLAs (1 g c9,t11 + 1 g t10,c12; CLA-TG) 2) 400 mg vitamin E; or 3) 2 g CLAs (1 g c9,t11 and 1 g t10,c12) + 400 mg vitamin E; or 4) placebo - high oleic sunflower and corn oil	3 months, P	Serum lipid profile, CRP, and blood pressure	No adverse effects of CLA on measured outcomes were reported. Supplementation of CLAs decreased blood pressure by 8-8.5%. No CLA effects on BMI and the fasting blood levels of prostaglandin E2, TG, TC, LDL-C, HDL-C, LDL-C/HDL-C, CRP, arylestrase activity, and platelet count.	Aryaeian et al., 2008
Young men (23-38 yr)-Lean (n=13; BMI 20-26) Obese (n=12; BMI 30-36) Older men (50-64 yr)-Lean (n=20; BMI, 21-26) Obese (n=4; BMI, 30-34)	1) 2.28 g CLA (3 g of CLA-TG oil containing 1.14 g c9,t11 + 1.14 g t10,c12 CLA) and 3 g of n-3 LC-PUFA (containing 300 mg EPA+ 210 mg DHA/g); or 2) 6 g control fat (including 800 mg palm oil/g and 200 mg soya bean oil/g)	12 weeks, X	Body composition, regional adiposity, and adiposity-related circulating hormone levels (leptin and adiponectin)	No adverse effects on measured outcomes were reported. CLA plus n-3 LC-PUFA prevented increased abdominal fat mass and fat-free mass in younger obese individuals, whereas these parameters in young and older lean and older obese individuals were unaffected. No effects of CLA+PUFA on leptin and adiponectin. Data have flaws by not comparing treatment and control groups in absolute values. Only changes after intervention were compared.	Sneddon et al., 2008

P=parallel design; X=cross over design; ALT=alanine aminotransferase; AST=asparate aminotransferase; BMI=body mass index; BUN=blood urea nitrogen; CRP=C-reactive protein; CVD = cardiovascular disease; DBP=diastolic blood pressure; DHA=docpsahexaenoic acid; EPA=eicosapentaenoic acid; FFA=free fatty acid(s); γ-GTP=γ-glytamyltranspeptidase; HR=heart rate; HDL-C=high density lipoprotein cholesterol; LDL-C=low density lipoprotein cholesterol; PUFA=polyunsaturated fatty acids; TC=total cholesterol; TG=triglycerides.

Table 11. Effects of CLA on CVD risk factors and body weight measures in humans, continued

Subject	Daily dose	Duration/ Design	Measured outcomes	Results	Reference
75 post-menopausal women with BMI<35 (CLA milk group, 58.3±3.5 yr; CLA mixture group-62.3±5.0 yr)	1) CLA mixture (4.6 g CLA-TG isomers); 2) CLA milk (5.1 g c9,t11 - CLA); or 3) olive oil (control) 5.5 g/	16 weeks, P	CVD risk markers (Plasma lipid profile)	Compared to control, a CLA mixture containing t10,c12 decreased HDL-C (by 14%) and raised plasma TG (by 15%), and CRP (by 37%), but all the values were within normal ranges. No adverse effects of CLA on measured outcomes were reported.	Tholstrup et al., 2008
44 healthy subjects (40 completed; mean age 28.9 yr)	3.8 g CLA as TG (1:1 ratio of c9,t11 and t10,c12 as TG) added to yogurt; or placebo-yogurt	14 weeks, P	BW measures including BFM	No significant change in BW or BFM.	Nazare et al., 2007
118 healthy subjects (93 completed; 18-65 yr)	3.4 CLA (4.5 g CLA-TG oil containing 1.7 g c9,t11 + 1.7 g t10c12 CLA); or placebo (4.5 g of olive oil)	6 months, P	BW measures, diet, exercise, BFM, bone mineral content, and fasting blood conc. of glucose, HbA1c, insulin, C-peptide, leptin, adiponectin, Lp(a),blood lipids , IL-6, IL-8, TNFα, CRP, and leucocytes	CLA supplementation had no effects on LBM, WHR, LBM, bone mineral content, blood lipids, and inflammatory and diabetogenic markers. However, when 6 mo changes from the baseline were compared, CLA group's changes were lower in BFM (-3·4%, P<0·043) and WHR (-0.02, P<0.05) and higher in LBM (+0.5 kg, P<0.05) then the control's. Adverse events did not differ between the groups.	Gaullier et al., 2007
55 healthy obese adults (48 completed; 18-50 yr)	1)3.2 g CLA as TG (1:1 ratio of c9,t11 and t10,c12); 2) 6.4 g CLA as TG (1:1 ratio of c9,t11 and t10,c12); or 3) placebo-sawflower oil	12 weeks, P	BW measures, body composition, blood lipids, hematology, and serum liver enzymes	CLA increased LBM by 0.64 kg, decreased serum HDL-C and sodium, hemoglobin, and hematocrit, and increased serum ALP, although all values remained within normal limits. No significant differences in BW measures and serum conc. of TC, LDL-C, TG, ALT, and AST among groups.	Steck et al., 2007

P=parallel design; X=cross over design; ALT=alanine aminotransferase; AST=aspartate transaminase; BFM=body fat mass; BMI=body mass index; BP=blood pressure; CRP=C-reactive protein; CVD = cardiovascular disease; HDL-C = high density lipoprotein cholesterol; LBM = lean body mass; LDL-C = low density lipoprotein cholesterol; TC=total cholesterol; TG=triglycerides; WC=waist circumference;WHR= waist-to-hip ratio.

Table 11. Effects of CLA on CVD risk factors and body weight measures in humans, continued

Subject	Daily dose	Duration/ Design	Measured outcomes	Results	Reference
60 overweight Japanese male volunteers (25-60 yr; BMI 25-35)	1) 5.4 g CLA-TG (3.4 g CLA isomers); 2) 10.8 g CLA-TG (6.8 g 1:1 mixture of c9,t11- and t10,c12 CLA isomers); or 3) placebo 10.8 g/day high-linoleic safflower oil	12 weeks, P	Hematology, serum biochemistry (total protein, total bilirubin, AST, ALT, γ-GTP, lipid profiles, glucose, uric acid, BUN, creatinine, PAI-1, leptin, insulin), SBP, DBP, heart rate, hepatorenal contrast, vital signs, diet, and walking	Transient adverse events (mostly mild or moderate diarrhea, abdominal distension, and/or fever which disappeared in a few days) were reported by 30% of the placebo group, 60% of the low CLA group, and 70% of the high CLA group. No differences in MCV, MCHC, AST, creatinine, LDL-C, HDL-C, FFA, hepatorenal contrast, calorie intake, or daily walking among the groups. High CLA group had lower DBP and higher ALT and γ-GTP values, but the changes were small and the values were within the normal range. Daily dose of 6.8 g CLA was found to be safe.	Iwata et al., 2007
64 regularly exercising subjects (62 completed; 21-45 yrs)	2.57 g CLA-TG (1.16 g c9,t11 + 1.20 g t10,c12 + 0.11 g other CLA isomers; 3.9 g CLA-oil); or placebo (3.9 g high-oleic acid sunflower oil)	12 weeks, P	Primary: body composition, and regional adipose tissue distribution, whole body substrate oxidation at rest and during exercise, and resting energy expenditure. Secondary: blood lipid profiles, glucose homeostasis and insulin sensitivity.	Adverse events did not differ between the groups. No CLA-specific effects were observed on body composition, energy expenditure, or appetite. No significant differences in blood lipid profiles between the 2 groups.	Lambert et al., 2007
40 healthy, overweight subjects (18–44 yr; BMI, 25–30).	3.2 g CLA-TG mixture	6 months, P	Body composition, RMR, dietary intake, and blood chemistry	Significant decrease in BFM (4%) and BW (1%). No effect on RMR, physical activity, or dietary intake. No adverse effects on measured outcomes were reported.	Watras et al., 2007

P=parallel design; X=cross over design; ALT=alanine aminotransferase; AST=aspartate transaminase; BP=blood pressure; BFM=body fat mass; DBP=diastolic blood pressure; FA=free fatty acid(s); HDL-C=high density lipoprotein cholesterol; LDL-C=low density lipoprotein cholesterol; MCHC = mean corpuscular hemoglobin concentration; MCV = mean corpuscular volume RMR =resting metabolic rate.

Table 11. Effects of CLA on CVD risk factors and body weight measures in humans, continued

Subject	Daily dose	Duration/ Design	Measured outcomes	Results	Reference
CLA-TG					
38 healthy overweigth subjects (30-55 years)	3g of CLA as TG or 3g olive oil (placebo) in skim milk	24 weeks, P	Body weight measures, blood lipid profile, CRP, ALT, AST,	The consumption of skimmed milk enriched with 3g CLA for 24 weeks led to a decrease in body weight and total fat mass. No adverse effects were observed.	López-Plaza et al., 2013
35 overweight, postmenopausal women (36±1 yr)	1.8 g CLA as TG + 400 ug chromium picolinate-CLA or placebo (<0.3 ug Cr as chromium picolinate and 2.4 g canola oil):	12 weeks, P	BW, body composition, VO_2 max, serum lipid profile, and BP	No adverse effects on measured outcomes were reported. The use of a combined chromium picolinate and CLA did not affect BW, body composition, plasma glucose and insulin conc., serum lipid profiles, or BP.	Diaz et al., 2008
18 moderately overweight and hyperlipidemic subjects (15 completed, 30-60 yr)	1) milk naturally enriched in CLA (4.2%, containing c9,t11 only providing 1.3 g/day CLA); 2) milk enriched with 1.3 g/day synthetic CLA-TG -0.65 g t10,c12 + 0.65 g c9,t11; or 3) control-untreated milk (0.2 g/day CLA)	8 weeks, X	Blood lipid profile, indices of liver function (plasma ALT, total bilirubin), CRP, TNFα, BW, and composition	No adverse effects of CLA on measured outcomes were reported. Both types of CLA had no effects on plasma concentrations of TC, LDL-C, HDL-C, or TG, BW, body fat composition, indices of liver function, CRP, or TNFα.	Venkatramanan et al., 2010

P=parallel design; RCT=randomized controlled trial; X=cross over design; ALT=alanine aminotransferase; AST=aspartate transaminase; BP=blood pressure; BW= body weight; CRP = C-reactive protein; HDL-C=high density lipoprotein cholesterol; IDL= Intermediate-density lipoproteins; LDL-C=low density lipoprotein cholesterol; TC=total cholesterol; TG=triglycerides; VLDL= very low density lipoprotein cholesterol; VO_2 max = maximal oxygen consumption.

Table 11. Effects of CLA on CVD risk factors and body weight measures in humans, continued

Subject	Daily dose	Duration/ Design	Measured outcomes	Results	Reference
CLA-FFA					
10 physically active males (27.4±3.7 yr)	4.8 g CLA-FFA (2.4 g c9,t11 + 2.4 g t10,c12, with 1.2 g other fatty acids mixture); or 6 g placebo (sunflower oil)	3 weeks each, X	Body composition (body mass, BFM, LBM), repetition maximum strength test, resistance exercise bout, total conc. of testosterone, estradiol, cortisol, and SHBG	CLA supplementation had no effects on measured outcomes including body composition.	Macaluso et al., 2012
38 subjects (15 male PPARγ2 Ala12Ala homozygous and 23 BMI-matched homozygous control subjects, PPARγ2 Pro12Pro; 45-68 yr)	1) 3.4 g c9,t11 CLA FFA; 2) 3.4 g t10,c12 CLA FFA; 3) 3.4 g CLA FFA mixture (1.7 g c9,t11 + 1.7 g t10,c12), or 4) placebo (3.23 g linoleic acid in a total of 4.25 g FA)	4 weeks, X	BW measures, plasma glucose, blood lipid profile, BP, and genes (including: PPARγ, FA transport and metabolism, adipokines, insulin signaling, etc)	No effects of CLA on BW measures, blood lipid profile, and BP. No adverse effects on measured outcomes were reported.	Herrmann et al., 2009
60 healthy men and women (43 completed, 35-65 yrs)	3 g CLA-FFA (1.5 g c9t11 and 1.5 g t10,c12 in 500 mL skimmed milk); or placebo (500 mL of skimmed milk)	12 weeks, P	BFM, LBM, BMI, WC, BP, and lipid profiles, and security parameters (hematological profile, renal and hepatic function)	No adverse events were reported. CLA had no effect on WC, BP, calorie intake, CVD risk factors, hematological parameters, or renal function. CLA reduced fat mass (–pre vs.post CLA: 29.1 vs. 28.5 kg, changes -0.06; P<0.05) in overweight but not in obese subjects.	Laso et al., 2007
9 overweight, non-diabetic subjects (100% completed, 44.4% male, 31.2 ± 3.7 yrs)	3.1 g CLA-FFA (1.55g c9,t11+ 1.52 g t10,c12 CLA) in the form of 1 g gel capsules with meals. No placebo group	12 weeks, X	Skeletal muscle lipid composition (muscle biopsy), body composition, and fasting plasma conc. of lipids, adiponectin, TNFα, and PPARα.	CLA had no effects on body composition, fasting plasma conc. of lipids, adiponectin and TNFα after 12 weeks supplementation.	Thrush et al., 2007

P=parallel design; X=cross over design; BFM=body fat mass; BMI = body mass index; BP=blood presseure; LBM=lean body mass; Lp(a)=lipoprotein a; PPARα = peroxisome proliferators activated receptor alpha; TNFα=Tumor necrosis factor alpha; WC = waist circumference.

Table 11. Effects of CLA on CVD risk factors and body weight measures in humans, continued

Subject	Daily dose	Duration/ Design	Measured outcomes	Results	Reference
CLA from dietary sources					
10 subjects (30-65 yr)	1) Pecorino cheese, ~ 200 g/wk (containing 1.56% lipids c9,t11); or 2) Placebo cheese, ~200 g/wk (containing 0.19% lipids c9,t11)	10 weeks, X	Blood lipid profile, inflammatory biomarkers, and platelet function	No adverse effects of CLA on measured outcomes were reported. No effects on blood lipid profile. CLA reduced in inflammatory parameters such as IL-6 (-43%), IL-8 (-36.5%), and TNF-α (-40%).	Sofi et al., 2010
18 moderately overweight and hyperlipidemic subjects (15 completed, 30-60 yr)	1) milk naturally enriched in CLA (4.2%, containing c9,t11 only providing 1.3 g/day CLA); 2) milk enriched with 1.3 g/day synthetic CLA-TG -0.65 g t10,c12 + 0.65 g c9,t11; or 3) control-untreated milk (0.2 g/day CLA)	8 weeks, X	Blood lipid profile, indices of liver function (plasma ALT, total bilirubin), CRP, TNFα, BW, and composition	No adverse effects of CLA on measured outcomes were reported. Both types of CLA had no effects on plasma concentrations of TC, LDL-C, HDL-C, or TG, BW, body fat composition, indices of liver function, CRP, or TNFα.	Venkatramanan et al., 2010
42 hypercholesterolemic adults (~45% male, 30-60 yr)	CLA (0.6 g/day total CLA with 0.5 g/day c9,t11 in 90 g/day enriched sheep cheese); or placebo (0.2 g/day total CLA with 0.2g/d c9,t11 CLA in 90 g/day control cheese). 45 g/day supplement contained half the amount of CLA as the 90 g/day serving.	3 weeks, X	Plasma lipid profile and endocannabinoids, adipokines, inflammatory markers, height, weight, waist circumference, BMI, plasma FA profile, creatinine, and uricaemia.	Consumption of CLA enriched cheese significantly increased plasma concentrations of CLA, VA, the n-3 FAs (including EPA), and decreased the endocannabinoid anandamide and LDL-C. No changes were detected in the levels of inflammatory markers and body weight, and blood lipid profiles.	Pintus et al. 2013
18 women (20-40 yr)	1.17 g CLA from beef and dairy products rich in CLA from dairy cattle	56 days, P	Blood lipid profile	No significant changes in TC, TG, HDL, LDL, VLDL or IDL	Brown et al., 2011

ALT=alanine transaminase; BMI=body mass index; BW=body weight; CRP= C-reactive protein; DBP = diastolic blood pressure; EPA = eicosapentaenoic acid; FA=fatty acid; HDL-C=high density lipoprotein cholesterol; LDL-C=low density lipoprotein cholesterol; SBP = systolic blood pressure; TC=total cholesterol; TG=triglycerides; TNFα = tumor necrosis factor alpha.

Table 11. Effects of CLA on CVD risk factors and body weight measures in humans, continued

Subject	Daily dose	Duration/ Design	Measured outcomes	Results	Reference
CLA form unspecified					
80 Chinese participants (63 completed; 33.3% Male; 32.8±0.8 yr)	3.4 g CLA in 400 mL of sterilized milk -1:1 mixture of c9,t11 and t10,c12 isomers); or placebo (400ml of sterilized milk with salad oil); CLA form not specified	12 weeks, P	BW, BMI, LBM, BFM, WHR, internal organ fat mass, subcutaneous fat mass, DBP, SBP, ALT, AST, and lipid profiles	Supplementation with CLA decreased the BW, BMI, subcutaneous fat mass, and waist-to-hip ratio and subjects with a BMI < 27 had more profound effects. CLA also increased fasting plasma TC, TG, LDL-C, ALT and AST while decreasing HDL-C. However, the changes were slight and the values were within the normal range. Authors concluded that CLA supplementation resulted in a decrease in obesity indices with no obvious adverse effects.	Chen et al., 2012
59 healthy subjects (41 completed, 20-60 yr)	1) 0.76 g amino acid mixture (lysine, proline, alanine, and arginine); 2) 1.52 g amino acid mixture; 3) 1.6 g CLA+ 1.52 g amino acid mixture; or 4) a placebo-corn starch +soybean oil; CLA form not specified	12 weeks, P	BW measures, hematological tests, blood biochemical tests, and urinalyses	No adverse effects on measured outcomes were reported. CLA+amino acid mixture decreased waist (-3.6%) and hip (-2.4%) circumferences without changing body fat, BW, and BMI. No clinically significant changes in blood biochemistry.	Michishita et al., 2010
401 overweight or obese, otherwise healthy subjects (346 completed, 58.4±0.5 yr; BMI>25)	3.1 g CLA (2.5 g c9,t11 + 0.6 g t10,c12 in 4 g CLA oil); or placebo (a blend of 80% palm oil and 20% soybean oil); CLA form not specified	6 months, P	Aortic pulse wave velocity, blood pressure, blood lipids, body composition, and CRP	No significant effect on blood conc. of HDL-C, LDL-C, TG, TC, or CRP, BW, BMI, WC, WHR, SBP, and DBP.	Sluijs et al., 2010

P=parallel design; RCT=randomized controlled trial; X=cross over design; ALT=alanine aminotransferase; AST= aspartate transaminase; BFM= body fat mass; BMI = body mass index; BW=body weight; γ-GTP=γ-glytamyltranspeptidase; DBP=diastolic blood pressure; HC= hip circumference; HDL-C=high density lipoprotein cholesterol; LDL-C=low density lipoprotein cholesterol; TC=total cholesterol; TG=triglycerides; WHR=waist-to-hip ratio.

Table 12. Effects of CLA on inflammatory biomarkers in humans

Subject	Daily dose	Duration/ Design	Measured outcomes	Results	Reference
28 adult subjects with mild asthma (26 completed, 19-40 yr)	3.3 g CLA as TG (1.63g c9,t11+ 1.67 g t10,c12 or 4.5 g CLA oil); or placebo (4.5 g olive oil)	12 weeks, P	Primary: tolerability and ARH; Secondary: AQLQ, BMI, systemic cytokine and serum conc. of inflammatory mediators (IL-5, IL-6, IL-8, PAI-1, TNF-a, MCP-1, HGF, ECP and IFNγ), metabolic hormones (leptin, adiponectin and insulin), and adverse events	No significant effects of CLA on adiponectin, leptin, IL-6, IL-8, IL-5, IFN-γ, TNFα, PAI-1, MCP-1, HGF, and ECP. No adverse effects on measured outcomes were reported.	MacRedmond et al., 2010
36 overweight, mildly hypercholesterolemic men (27 completed, 18-60 yr)	1) 2.8 g CLA (3.5 g CLA-TG oil containing 1.4 g t10,c12 + 1.4 g c9,t11 CLA); 2) 2.7 g CLA- c9,t11 isomers in; or 3) placebo 3.5 g/day safflower oil	8 weeks, X	Safety outcomes (plasma conc. of CRP, adiponectin and oxidized LDL-C, serum conc. of TNFα and IL-6)	No significant effect on CRP, IL-6, TNFα, or adiponectin. No adverse effects on measured outcomes were reported.	Joseph et al., 2011
6 subjects with physician-diagnosed asthma (6 completed)	4.8 g CLA -TG; or placebo	8 weeks, X	Urinary markers of airway inflammation (LT C_4-E_4 and 9_a,11beta-PGF_2)	No significant effect on urinary markers of airway inflammation (LT C_4-E_4 and 9_a,11beta-PGF_2)	Stickford et al., 2011

P = parallel design; X = cross over design; ARH=airway hyper-responsiveness; AQLQ=asthma quality of life questionnaire; BMI = body mass index; CRP=C-reactive protein; ECP = enterocutaneous fistulas; HGF=hepatocyte growth factor; IFN-γ=interferon gamma; IL= interleukin; LDL-C = low density lipoprotein cholesterol; LT= leukotrienes; MCP-1= monocyte chemotactic protein-1; PAI-1=plasminogen activator inhibitor-1; PG= prostaglandin; TNFα =tumor necrosis factor alpha.

Table 12. Effects of CLA on inflammatory biomarkers in humans

Subject	Daily dose	Duration/ Design	Measured outcomes	Results	Reference
80 subjects taking BP medication	4.5 g CLA as TG (a 1:1 isomer blend of c9,t11 and t10,c12 CLA); or placebo	8 weeks, P	Plasma conc. of adiponectin, leptin, and angiotensinogen and ACE activity	CLA increased plasma adiponectin concentration (37%, $P < 0.05$) and decreased plasma concentrations of leptin (22%) and angiotensinogen (8.9%). No significant effect on ACE activity. No adverse effects on measured outcomes were reported.	Zhao et al., 2009
40 subjects with diagnosed birch pollen allergy (38 completed; 20–46 yr)	2 g c9,t11 CLA as TG; or placebo	12 weeks, P	Inflammatory markers	No adverse effects on measured outcomes were reported. The CLA group reported less sneezing ($P<0.05$) during the pollen season. CLA supplementation decreased the in vitro production of TNFα ($P<0.01$), interferon-gamma ($P<0.05$) and IL-5 ($P<0.05$). Total plasma IgE and birch-specific IgE concentrations did not differ between groups, whereas plasma IgA ($P<0.05$), granulocyte macrophage colony-stimulating factor ($P<0.05$) and eosinophil-derived neurotoxin ($P<0.05$) concentrations were lower after CLA supplementation. No effects on CRP. The results suggest that c9,t11 CLA has modest anti-inflammatory effects in allergic subjects.	Turpeinen et al., 2008
75 post-menopausal women with BMI<35 (CLA milk group, 58.3±3.5 yr; CLA mixture group-62.3±5.0 yr)	1) 4.6 g CLA mixture (5.5 g CLA-TG isomers); 2) CLA milk (5.1 g c9,t11 CLA); or 3) olive oil (control) 5.5 g	16 weeks, P	CVD risk markers (Plasma lipid profile, inflammatory risk markers, and urine F2-isoprostane (free radical-mediated lipid peroxidation).	No differences in Il-6, TNFα, ICAM-1, and VCAM-1 among the groups. Compared to control, the CLA mixture group had 60% higher 8-iso PGF 2α, whereas women in the CLA milk group had 25% higher 8-iso PGF2α.	Tholstrup et al., 2008

P=parallel design; X=cross over design; ACE = angiotensin-converting enzyme; BP=blood pressure; CRP = C-reactive protein; ICAM-1 = Intercellular adhesion molecule 1; Ig=immunoglobulin; IL=interleukin; 8-iso PGF2α =8-iso-prostaglandin F2α; TNFα=Tumor necrosis factor alpha; VCAM-1 = vascular cell adhesion molecule-1.

Table 12. Effects of CLA on inflammatory biomarkers in humans, continued

Subject	Daily dose	Duration/ Design	Measured outcomes	Results	Reference
47 healthy men (38 completed, 100% male, 19-35 yr)	4.6 g CLA as TG (5.5 g CLA-TG oil containing 2.5 g of c9,t11 and 2.1 g of t10,c12); control (0.3 g of c9,t11); or placebo	5 weeks, P	CRP, PAI-1, FVIIc, and urine 8-iso-PGF2α	No significant effect on inflammatory markers (CRP, PAI-1, FVII-C). No adverse effects on measured outcomes were reported.	Raff et al., 2008
118 healthy subjects (93 completed; 18-65 yr)	3.4 g CLA as TG (4.5 g CLA-TG oil containing 1.7 g c9,t11 + 1.7 g t10c12 CLA); or placebo (4.5 g of olive oil)	6 months, P	IL-6, IL-8, TNFα, CRP, and leukocytes	CLA supplementation had no effects on inflammatory markers. Adverse events did not differ between the groups.	Gaullier et al., 2007
55 healthy obese adults (48 completed; 18-50 yr)	1)3.2 CLA as TG (1:1 ratio of c9,t11 and t10,c12), 2) 6.4 g CLA as TG (1:1 ratio of c9,t11 and t10,c12); or 3) placebo	12 weeks, P	CRP, IL-6, and white blood cells	CLA significantly increased CRP, IL-6, and white blood cells although all the values were within normal ranges.	Steck et al., 2007
30 healthy males (40-60 yr)	2.2 g CLA as TG (1:1 ratio of c9,t11 and t10,c12); or placebo	8 weeks, P	Serum IL-6, conc. of IL-2, IL-10, TNFα of PMBC supernatant, and plasma fibrinogen and CRP	CLA supplementation reduced PBMC IL-2 secretion (37%) from Con A-stimulated PBMC but lacked effect on other markers of the human inflammatory response	Mullen et al., 2007
Natural dietary sources					
10 healthy subjects (mean age: 45.6yr)	0.25 g c9,t11CLA from sheep's cheese naturally rich in c9,t11-CLA; or placebo	10 weeks, X	Cytokines and platelet aggregation	Significant decrease in platelet aggregation. CLA had positive effects on reducing inflammatory parameters such as IL-6 (-43%), IL-8 (-36.5%), and TNFα (-40%). No adverse effects on measured outcomes were reported.	Sofi et al., 2010

P=parallel design; X=cross over design; IL = interleukin; CRP= C-reactive protein; FVII-C= Factor VII coagulation activity; PBMC=peripheral blood mononuclear cells; ICAM-1=Intercellular adhesion molecule 1; IL=interleukin; 8-iso PGF2α =8-iso-prostaglandin F2α; PAI-1=plasminogen activator inhibitor-1; PG= prostaglandin; TNFα = tumor necrosis factor alpha.

Table 13. Effects of CLA on fat oxidation or oxidative stress in humans

Subject	Daily dose	Duration/ Design	Measured outcomes	Results	Reference
30 healthy overweight/obese participants (29 completed; 19-65 yr)	CLA –FFA (2.4 g CLA/4 capsules); or placebo (2.4 g oilve oil/4 capsules)	8 weeks, P	Lipid peroxidation and antioxidant metabolism: plasma TRAP, conjugated dienes in LDL, plasma concentrations of retinol, carotenoids, lycophene, tocopherol, and coenzyme Q10, GSH-Px, CAT, SOD, DNA damage.	No adverse effects on measured outcomes were reported. The data suggest that short-term supplementation with CLA (2.4 g/day) might have no significant effects on lipid peroxidation and antioxidant metabolism.	Kim et al., 2012
48 subjects (40 completed; CLA group: 33±7 yr, placebo group: 33±6 yr)	3.2 g CLA (1.57 g c9,t11 and 1.54 g t10,c12 in 4 g CLA oil); or placebo (4 g safflower oil); CLA source not specified	6 months, P	24-hr energy expenditure and substrate utilization, and dietary fat oxidation	No adverse effects on measured outcomes were reported. CLA supplementation, but not placebo, positively altered fat oxidation and energy expenditure during sleep.	Close et al., 2007
24 healthy moderately overweight subjects (22 completed, ~49.8±8.2 yr)	CLA as TG (3g of c9,t11 or t10,c12; TG); or placebo (3g 9c-18:1)	24 weeks, P	Excretion of CO_2 in the breath, and FA oxidation	No adverse effects on measured outcomes were reported. No adverse events reported. c9,t11 is more rapidly oxidized compared than t10,c12 CLA.	Malpuech-Brugere et al., 2010
75 post-menopausal women with BMI<35 (CLA milk group, 58.3±3.5 yr; CLA mixture group-62.3±5.0 yr)	1) 4.6 CLA as TG (5.5 g CLA-TG isomers); 2) CLA milk (5.1 g c9,t11 - CLA); or 3) olive oil (control) 5.5 g	16 weeks, P	Urinary free 8-iso-PGF2α (a marker of in vivo oxidative stress)	Compared to control, the CLA mixture group had 60% higher 8-iso PGF 2α, whereas women in the CLA milk group had 25% higher 8-iso PGF2α. No adverse effects on measured outcomes were reported.	Tholstrup et al., 2008; (also reported in Raff et al. 2008)

P=parallel design; X=cross over design; CAT=Catalas activity; GSH-Px=glutathione peroxidase activity; 8-iso PGF2α =8-iso-prostaglandin F2α; SOD=superoxide dismutase; TRAP=total radical-trapping antioxidant potential.

Table 13. Effects of CLA on fat oxidation or oxidative stress in humans, continued

Subject	Daily dose	Duration/ Design	Measured outcomes	Results	Reference
40 subjects with diagnosed birch pollen allergy (38 completed; 20–46 yr)	2 g c9,t11-CLA as TG; or placebo	12 weeks, P	Free 8-iso-PGF2α, an indicator of free radical-mediated lipid peroxidation and 15-keto-dihydro-PGF2α, an indicator of cyclo-oxygenase-catalysed lipid peroxidation.	Urinary excretion of 8-iso-PGF2α, a major F2-isoprostane (P<0.01), and 15-keto-dihydro-PGF2α, a primary PGF2α metabolite (P<0.05), increased (43-59%) in the CLA group. However, other inflammatory markers measured in the present study did not suggest a pro-inflammatory effect for CLA.	Turpeinen et al., 2008
85 overweight men (100% male, 45-68 yr)	3.4 g CLA (4.5 g CLA-TG oil -1:1 mixture of c9,t11 and t10,c12 CLA isomers in); safflower oil; heated safflower oil (240 °C for 2 h); or olive oil	28 + 2 days, P	PAT index as a measure of endothelial function: oxidized LDL-C, PGF2α, paraoxonase arylesterase activity, and activity of paraoxonase toward paraoxon	CLA did not impair endothelial function. Other parameters associated with metabolic syndrome and oxidative stress were not changed or were slightly improved. Increased F2-isoprostane concentrations in this context may not indicate increased oxidative stress.	Pfeuffer et al., 2011

P=parallel design; PGF2α =F2-isoprostane 8-iso-prostaglandin F; 8-iso PGF2a =8-iso-prostaglandin F2a; LDL-C = low density lipoprotein cholesterol; PAT=peripheral arterial tonometry.

Table 14. Effects of CLA on Milk fat production in humans

Subject	Daily dose	Duration/ Design	Measured outcomes	Results	Reference
12 breast-feeding women (100% female; 29.4±1.6 yr)	CLA (750 mg/d; c9,t11 or t10,c12 CLA); or placebo (750 mg/d; olive oil); CLA source not specified	5 days, X	Milk fat content, maternal body fat, infant milk consumption, milk lipids, and milk FA profile	No adverse events reported. Dietary supplementation with either c9,t11 CLA or t10,c12 CLA did not reduce milk fat in healthy, breast-feeding women.	Hasin et al., 2007

X=cross over design.

Table 15. Effects of CLA on other physiological outcomes in humans

Subject	Daily dose	Duration/ Design	Measured outcomes	Results	Reference
20 healthy subjects (19 completed; 18-65 yr)	19.3 g CLA-TG (14.6 g c9,t11 CLA and 4.7 g t10,c12 CLA isomers); or placebo	3 weeks, X	Liver and kidney functions	Mean values of all tests remained within normal limits. Lactate dehydrogenase increased from 290.9±43.6 to 322.5±60.7 U/L (p = 0.04) on day 21. One subject exceeded the upper limit of normal of 450 U/L on day 21, to 472 U/L and another showed an isolated elevation to 555 U/L on day 7. c-Lutamyltranspeptidase increased from 12.1 ±5.9 to 13.5±6.2 U/L (p = 0.002). No one exceeded the upper limit of 50 U/L for men and 40 U/L for women. Overall, CLA did not produce clinically relevant effects on markers of liver and kidney function.	Wanders et al., 2010b
25 non-obese, regularly exercising, healthy white adults (25 completed; 21-45 yr)	3.9 g CLA-TG (29.7% c9,t11, 30.9% t10,c12 isomers, 2.9% other CLA isomers, and 24.7% oleic acid, 3.5% palmitic acid, 1.9% linoleic acid, and 1.3% stearic acid); or placebo (3.9 g of high oleic acid sunflower oil)	12 weeks, P	Incorporation into adipose tissue and skeletal muscle	No adverse events reported. Following supplementation, the t10,c12 CLA isomer was incorporated into adipose tissue TG, and c9,t11 CLA isomer tended to increase in skeletal muscle phospholipids.	Goedecke et al., 2009
21 subjects (15 completed; 30-60 yrs)	3 Types of milk: 1) 4% fat homogenized cows' milk, containing 5 mg/g c9,t11CLA; 2) 4% fat homogenized cows' milk naturally enriched in c9,t11 CLA (32 mg/g fat); or 3) 4% fat homogenized cows' milk that was enriched with t10, c12 CLA and c9,t11 CLA-TG (each CLA-32 mg/g fat) in the form of TG; Dairy source of CLA	8 weeks, X	Fecal microbiota composition, fecal enzyme activity, or fecal composition.	No adverse events reported. Inconclusive evidence but reductions in fecal enzyme activity evident, but not attributable to changes in the population of one or more faecal bacteria – long-term effects of reducing these enzymes may be desirable.	Farnworth et al., 2007

P=parallel design; X=cross over design; TG = triglycerides.

Table 15. Effects of CLA on other physiological outcomes in humans, continued

Subject	Daily dose	Duration/ Design	Measured outcomes	Results	Reference
18 women (20-40 yr)	1.17 g CLA from beef and dairy products rich in CLA from pasture-fed dairy cattle; or placebo	56 days, P	Bone health parameters	No significant changes in BMC or bone area (cm^2).	Brown et al., 2011
62 prepubertal overweight or obese, but otherwise healthy, children (53 completed, 6-10 yr)	2.4 g CLA as TG (3 g CLA-TG oil -1:1 mixture of c9,t11 and t10,c12 CLA isomers); or placebo (sunflower oil in chocolate milk)	7 $\pm$ 0.5 months, P	BMC	BMC accrual in CLA group was significantly less than control group.	Racine et al., 2010
118 healthy subjects (93 completed; 18-65 yr)	3.4 g CLA as TG (1:1 mixture of c9,t11 + t10c12 CLA isomers in 4.5 g CLA-TG oil); or placebo (4.5 g of olive oil)	6 months, P	BW measures, body fat mass, BMC, fasting blood conc. of glucose, HbA1c, insulin, C-peptide, leptin, adiponectin, Lp(a),blood lipids , IL-6, IL-8, TNFα, CRP, and leucocytes	CLA supplementation had no effects on BMC. Adverse events did not differ between the groups.	Gaullier et al., 2007

P=parallel design; BMC=bone mineral content; CRP = C-reactive protein; IL=interleukin; Lp(a)=lipoprotein a; TNFα =Tumor necrosis factor alpha.

Table 16 summarizes the highest dose levels of CLA-FFA and CLA-TG showing no adverse effects in humans. Longer term studies show that a daily dose of 6 g CLA-TG and CLA-FFA were not associated with adverse effects (Larsen et al., 2006; Tarnopolsky et al., 2007; Whigham et al., 2004) . Short term studies showed that daily doses of 6.8 g CLA-FFA and 19 g CLA-TG were well tolerated with no serious side effects (Blankson et al., 2000; Wanders 2010a,2010b).

Table 16. Summary of highest dose levels of CLA showing no adverse effects in humans

	GRN 232		Since 2007 (after GRN 232)	
	CLA-FFA	CLA-TG	CLA-FFA	CLA-TG
Longer term studies	3.6 g CLA/day for 1 year (Gaullier et al, 2004, 2005)	6 g CLA/day for 1 year (Larsen et al., 2006; Whigham et al., 2004); 3.4 g CLA/day (4.5 g CLA-TG oil for 2 years (Gaullier et al, 2004, 2005)	6 g CLA/day for 6 months (Tarnopolsky et al., 2007)	
Short term studies	6.8 g CLA/day for 8 weeks (Blankson et al., 2000)			19 g CLA for 3 weeks (Wanders 2010a,2010b)
Single dose study	11.2 g CLA (Hoye et al., 2004)	8.9 g CLA (Hoye et al., 2004)		

IV.C.5. Potential adverse effects (updated from GRN 232)
Of the adverse events reported in clinical trials, the most frequent complaints were gastrointestinal (Blankson et al., 2000; Smedman and Vessby, 2001). These are common complaints with high fat diets and dietary supplement capsules. Several studies reported rapid adaptation to the addition of dietary CLA. Some investigators suggested that gastrointestinal upset could be due to either the CLA oil itself or the large number of gelatin capsules administered in most of the trials (up to 12 in the case of Blankson et al., 2000). Other studies show no such effects, including Whigham et al. (2004), which administered 6 gelatin capsules containing CLA TG.

Gastrointestinal complaints were evenly distributed between treatment and placebo groups in many studies. This suggests that complaints were not treatment-related. For example, in the 3-arm study by Gaullier et al. (2004), adverse events did not differ significantly among the groups. In this study, 3.4-3.6 g CLA isomers in 2 different forms (FFA and TG) in 4.5 g CLA-oil, or placebo (olive oil) were administered to 180 healthy overweight adults consuming an ad libitum diet for a year. Adverse events were reported by 68% of all randomly assigned subjects and with similar frequency in all 3 study groups (P = 0.68). Of 264 single events, the investigators considered 30 to be treatment-related. The treatment-related adverse events were evenly

distributed among the 3 study arms. All adverse events were rated as either “mild” or “moderate,” and the symptoms were transient. Abdominal discomfort or pain, loose stools, and dyspepsia were the most frequently reported treatment-related adverse events.

Adverse events in Iwata et al. (2007; mostly mild or moderate diarrhea, abdominal distension, and/or fever) disappeared in a few days. When subjects received daily doses of 3.4 g or 6.8 g CLA for 12 weeks, these effects were reported by 30% of the placebo group, 60% of the low CLA group, and 70% of the high CLA group. But most symptoms disappeared spontaneously or went into remission after a few days, indicating that the human gastrointestinal tract rapidly adapts to the addition of dietary CLA.

A daily dose of 1-19.3 g CLA for 3 weeks-7 months did not produce adverse effects on liver and kidney functions, CVD risk factors, or other outcomes in healthy subjects (Wanders et al., 2010a, 2010b). Overall evidence indicates that daily doses of 1-19 g for 3 weeks -7 months were well-tolerated.

V. SAFETY DETERMINTATION

Numerous human and animal studies have examined the health benefits of CLA with no major adverse effects reported. INNOBIO® uses a HACCP-controlled manufacturing process and rigorously tests its final production batches to verify adherence to quality control specifications. There is broad-based and widely disseminated knowledge concerning the chemistry of CLA. This GRAS determination is based on the data and information generally available and consented opinion about the safety of CLA. The literature indicates that CLA offers consumers health benefits without serious adverse effects.

The following safety evaluation fully considers the composition, intake, nutritional, microbiological, and toxicological properties of CLA as well as appropriate corroborative data.

1. Numerous reviews conducted by experts in the field unanimously support the safety of CLA. Numerous clinical trials have evaluated the effects of a 1:1 CLA mixture (c9,t11 plus t10,c12) and a number of other isomers on similar parameters. Human studies show that administration of daily dose of 3.4-3.6 g CLA (either FFA or TG form) for 1-2 years resulted in no serious adverse effects. Also, a daily dose 6 g CLA (either FFA or TG form) for 6 months-1 year was not associated with adverse effects (Larsen et al., 2006; Tarnopolsky et al., 2007; Whigham et al., 2004). Short term studies showed that daily doses of 6.8 g CLA-FFA and 19 g CLA-TG for 3-8 weeks were well tolerated with no serious side effects (Blankson et al., 2000; Wanders 2010a, 2010b). In addition, Both CLA-TG and CLA-FFA had no adverse effects of glucose metabolism in subjects with types 2 diabetes (Asp et al., 2011; Norris et al., 2009; Shadman et al., 2013). For these “pivotal” studies, the levels of consumption represent the maximum dose consumed, rather than absolute safety endpoints.

2. Conjugated linoleic acids are intended to be used in the same categories of foods as those described in GRN 232 (FDA, 2008) at 1.5 g CLA per serving for specific food categories. The maximum proposed uses of CLA by weight percent in each food category are as follows: 0.625% in specific soy milk beverages, meal replacement beverages, milk (filled), flavored milk products, and fruit juice products; 3.75% in meal replacement bars; and 0.667% in specific yogurt products. Using the NHANES (2009-2010) dataset, mean intake of users is estimated to be 0.84 g/person/day (16.5 mg/kg BW/day) and the 90th percentile intake of users is estimated to be 2.64 g/person/day (47.8 mg/kg BW/day) for Americans aged 2 years and older. These EDIs are well within safe intake levels: consumption up to 6-6.4 g/day is known to be safe for both CLA-TG and CLA-FFA in humans.

3. The compositional data and product properties are consistent with carefully controlled cGMP production and purification. INNOBIO® CLA contains no impurities or contaminants of concern for human health.

4. The US FDA (GRN 232 [FDA, 2008]) has not raised questions regarding the safety of CLA-TG as currently used in food. Since the FDA's last review of CLA in 2007-2008 (GRN 232, [FDA, 2008]), no literature contradicting the agency's prior reviews/decisions has been published.

5. Several reviews by experts in the field also have documented the safety of CLA.

VI. CONCLUSIONS AND GENERAL RECOGNITION OF THE SAFETY OF CLA

The intended uses of CLA (both TG and FFA) have been determined to be safe though scientific procedures as set forth in 21 CFR 170.3(b), thus satisfying the so-called "technical" element of the GRAS determination. In addition, because this safety evaluation was based on generally available and widely accepted data and information, it also satisfies the so-called "common knowledge" element of a GRAS determination.

On behalf of INNOBIO® Limited (INNOBIO®), the expert panel members, Susan Cho, Ph.D., Steven Heymsfield, M.D., Donald Schmitt, M.P.H., and Yeonhwa Park, Ph.D., have independently evaluated the materials summarized in the CLA Generally Recognized as Safe (GRAS) report. These individuals are qualified by scientific training and experience to evaluate the safety of substances intended to be added to foods. They have critically reviewed and evaluated the publicly available information summarized in this document and have individually and collectively concluded that CLA, produced consistent with cGMP and meeting the specifications described herein, is safe under its intended conditions of use. The Panel further unanimously concludes that these uses of CLA are GRAS based on scientific procedures, and other experts qualified to assess the safety of food and food ingredients would concur with these conclusions.

It is also INNOBIO® opinion that the proposed use of CLA (TG and FFA) is not only safe within the terms of the Federal Food, Drug, and Cosmetic Act (meeting the standard of reasonable certainty of no harm), but it is also GRAS according to 21 CFR according to consensus among experts.

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APPENDIX A.
EXPERT PANEL OPINION
THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF CONJUGATED LINOLEIC ACIDS (CLA) FOR USE AS A FOOD INGREDIENT

INTRODUCTION

The undersigned, an independent panel of experts, qualified by their scientific training and national and international experience to evaluate the safety of food ingredients (the "Expert Panel") was specially convened by INNOBIO® Limited (INNOBIO®) and asked to evaluate the safety and Generally Recognized as Safe (GRAS) status of the proposed use of conjugated linoleic acid (CLA) as a food ingredient.

A comprehensive search of the literature bearing on the safety of CLA was performed by INNOBIO®. The results of the literature search were summarized in the safety dossier "GRAS Determination of CLA for Use as a Food ingredient" for consideration by the Expert Panel.

The Expert Panel reviewed the safety documentation, as well as other available data and information that the Panel members believed to be pertinent to the safety of CLA under the conditions of intended use as a food ingredient. In addition, the Expert Panel reviewed the methods of manufacture and specifications for CLA, analytical data confirming compliance with specifications, and the proposed use and use levels of the proposed food ingredient. The Expert Panel independently and unanimously concluded that CLA, produced consistent with current good manufacturing practice (cGMP) and meeting appropriate specifications, is safe for its intended use. The Expert Panel further concluded that this intended use is GRAS based on scientific procedures. It is also the opinion of the Expert Panel that other qualified experts would concur with our conclusions. Summarized below is the Panel's scientific analysis supporting our conclusions.

DESCRIPTION

Common or trade name

CLAs mostly consist of two isomers: cis-9, trans 11 (c9,t11) and trans-10, cis-12 (t10,c12) CLA. They are available in two forms: free fatty acids (FFA) and triglycerides (TG).
INNOBIO® CLA 80 TG (oil form),
INNOBIO® CLA TG 40 Powder CWD,
INNOBIO® CLA TG 40 Powder WDP,
INNOBIO® CLA TG 60 Powder WDP,
INNOBIO® CLA 80 FFA (oil form),
INNOBIO® CLA 95 FFA (oil form),
INNOBIO® CLA FFA 40 Powder CWD, and
INNOBIO® CLA FFA 40 Powder WDP.

Background

CLAs refer to a mixture of positional and geometric isomers of octadecadienoic acid (linoleic acid, LA; c9,c12-C18:2) characterized by conjugated double bonds (Danicke et al., 2012). The conjugation of the double bond results in conformational properties different from the unconjugated linoleic acid forms. It is a natural compound mainly found in ruminant products, such as meat, milk, and other dairy foods. These represent the main source of CLA for humans. Of the two biologically important isomers, c9,t11 (rumenic acid, RA) is the most prevalent one in foods. It comprises about 80 to 90% of total CLA in ruminant products. In processed vegetable oils, the t10,c12-isomer is found in approximately equal amounts with the c9,t11-isomer. In addition, heated frying fats and partially hydrogenated vegetable oils contain a mixture of several CLA isomers.

Since RA (c9,t11-CLA isomer) is bioactive when supplied as a natural food component in the form of CLA-enriched milk fat (Anadon et al., 2011), it confers a number of beneficial biological effects that include anti-carcinogenesis, immunomodulation, and anti-atherosclerosis. It is formed as an intermediate product of the biohydrogenation process that occurs in rumen; a multistep mechanism carried out by different microorganisms on unsaturated fatty acids to produce stearic acid (C18:0). It can also be produced by desaturation of trans vaccenic acid (t11-C18:1, TVA).

From a nutritional and labeling perspective, CLA is not a trans fatty acid. The United States Food and Drug Administration (FDA) has stated, "Under FDA's definition, conjugated linoleic acid would be excluded from the definition of trans fat" (68 Fed. Reg. 41433, 41462 [July 11, 2003; FDA, 2003]). The FDA has also concluded that synthetically produced CLA is a component of certain foods, such as processed vegetable oils (FDA letter to I.S. Bass, Mar. 12, 2003).

CHEMISTRY AND COMPOSITION

The molecular weight of CLA is 280.4, and its molecular formula is $C_{18}H_{32}O_2$. The CAS numbers of c9,t11 and t10,c12 CLA isomers are 872-23-1 and 2420-44-2, respectively.

INNOBIO® CLAs are available in 2 chemical forms—free fatty acids (FFA) and triglycerides (TG). Conjugated linoleic acid oils (in a chemical form of either FFA or TG) are food-grade preparations derived from processed safflower oil. They consist of approximately 1:1 mixture of c9, t11 and t10, c12 CLA isomers ("1:1 mixture"). INNOBIO® CLAs are available in different physical forms as either powder or liquid and these are available in different concentrations. INNOBIO® CLA liquid oils are CLA 80 TG, CLA 95 FFA, and CLA 80 FFA. INNOBIO® CLA powdered products are CLA TG 40 Powder CWD, CLA TG 40 Powder WDP, CLA TG 60 Powder WDP, CLA FFA 40 Powder CWD, and CLA FFA 40 Powder WDP.

INNOBIO® CLA 80 TG has a 1:1 ratio of the c9, t11 and t10, c12 isomers in TG form with a concentration of approximately 80% (78-84%) CLA, derived from 100% pure, natural safflower seed oil. The remaining oil consists of other fatty acids, such as linoleic, oleic, palmitic, and stearic.

INNOBIO® CLA 80 FFA has a 1:1 ratio of the c9, t11 and t10, c12 isomers in free FA form with approximately 80% (78-84%) CLA, derived from 100% pure, natural safflower seed oil with molecular distillation process.

INNOBIO® CLA 95 FFA is the highest concentration INNOBIO® CLA with approximately 95% (93-97%) CLA in FFA form, derived from 100% pure, natural safflower seed oil with molecular distillation process. It has at least 88% of a 1:1 mixture of the c9, t11 and t10, c12 isomers. The remaining oil consists of other fatty acids, such as linoleic, oleic, palmitic, and stearic.

INNOBIO® CLA powder products include CLA FFA and TG chemical forms. These powders are available with two different shell compositions for the WDP (water dispersible powder) and CWD (cold water dispersible). The WDP is protein based. The CWD is starch based.

MANUFACTURING PROCESS OF INNOBIO® CLA

Current Good Manufacturing Practice (cGMP), which includes Hazard Analysis and Critical Control Point (HACCP) risk analysis as well as hygiene and transport standards, is applied throughout the manufacturing process; continuous monitoring is used for control. Sampling schemes and analysis schedules are applied to raw materials, products in all stages of processing, and end products. The finished product is routinely tested to ensure that it meets specifications. Highly modern production facilities and electronic monitoring systems enable precise process control at all stages. Every step in the production of CLA is carried out under nitrogen, eliminating contact with air. Parameters are set to ensure a high degree of purity that is consistent with its use as a food ingredient. Fatty acids and oils are processed by following standard procedures common to the current fat and oil industry.

CLA 80 FFA

CLA 80 FFA is prepared using commercially available food grade safflower oil derived from safflower seeds rich with linoleic acid. Safflower oil is trans-esterified with methanol to generate fatty acid (FA) methyl esters (ME) that are rich in linoleic acid (LA) ME using sodium hydroxide as a catalyst. Then the sodium hydroxide is neutralized by adding sulfuric acid. The FA ME become CLA isomers by an isomerization process using sodium hydroxide and methanol under high pH conditions and controlled temperature. The product of above step, CLA sodium salt and excess sodium hydroxide, are neutralized by adding sulfuric acid. Solvent is removed by exsolution. After acidification, the CLA isomers are decolorized, distilled and purified. Activated carbon, a processing aid, is used as an adsorbent to remove natural pigments and trace impurities from CLA during the decolorization process. The used activated carbon is effectively removed by filtration and thus is not present in the finished product. Food grade mixed tocopherols are added to the product as an antioxidant before packaging. Main difference between manufacturing process of CLA FFA and CLA-TG is that CLA-FFA does not employ trans-esterification of CLA ME/EE isomers with triacetate to form CLA-TG.

CLA 95 FFA

CLA 95 FFA is prepared using commercially available food grade safflower oil derived from safflower seeds rich with linoleic acid. Safflower oil is trans-esterified with methanol to generate

FA ME that are rich in LA ME by using sodium hydroxide as a catalyst. The sodium hydroxide is neutralized by adding sulfuric acid. The FA ME become CLA isomers by an isomerization process using sodium hydroxide and methanol under high pH conditions and controlled temperature. The products of above step, CLA sodium salt and excess sodium hydroxide, are neutralized by adding sulfuric acid. Solvent is removed by exsolution. After acidification, the CLA isomers go through decolorization, vacuum rectification and molecular distillation stages.One additional vacuum rectification step is incorporated into the process to remove the saturated or monounsaturated fatty acid (SFA; MUFA) for concentration of the CLA FFA from 80% up to 95-97%. Activated carbon, a processing aid, is used as an adsorbent to remove natural pigments and trace impurities from CLA during the decolorization process. The used activated carbon is effectively removed by filtration and thus is not present in the finished product. Food grade mixed tocopherols are added to the product as an antioxidant before packaging.

CLA 80 TG

CLA 80 TG is prepared using commercially available food grade safflower oil derived from safflower seeds rich with LA. Safflower oil trans-esterifies with methanol/ethanol to generate FA ME or ethyl ester (EE) that are rich in LA ME/EE, using sodium hydroxide as a catalyst. Methanol or ethanol is removed by exsolution and then washed from FA ME or EE using water. The FA ME/EE become CLA ME/EE isomers by an isomerization process using potassium methylate methanol / potassium ethylate ethanol under high pH conditions and controlled temperature. The CLA ME/EE isomers are washed with methanol / ethanol aqueous solution to remove the potassium methylate/potassium ethylate and some small amount of fatty acid salt. The CLA ME/EE isomers will trans-esterify with triacetate using sodium methylate methanol or sodium ethylate ethanol to generate CLA TG. The CLA TG are then decolorized, distilled, and purified. Activated carbon is used in the manufacturing process as an adsorbent to remove natural pigments and trace impurities from CLA TG. Diatomite is used as a processing aid for removing impurities from CLA TG. The activated carbon and diatomite are effectively removed by filtration and are not present in the finished product. Mixed tocopherol is added to the product as an antioxidant before packaging.

CLA FFA 40 Powder CWD, CLA FFA 40 Powder WDP, CLA TG 40 Powder CWD, CLA TG 40 Powder WDP, and CLA TG 60 Powder WDP

The CWD (cold water dispersible) and the WDP (water dispersible powder) powders have different shell material only and the manufacturing process is identical. Food grade CLA FFA or CLA TG is combined with a food grade emulsifier and an antioxidant (food grade mixed tocopherols) then mixed at a set temperature until all ingredients combine to form an oil phase. The CWD powders are starch based and the WDP powders are protein based. Water, base ingredients (either protein or starch), and food grade antioxidants (sodium ascorbate and/or mixed tocopherols) are combined and mixed together to form an aqueous phase. Both the oil phase and the water phase mixtures are combined and mixed to form an initial emulsion. This initial emulsion becomes a homogenous emulsion under a high pressure homogenization process. The resulting homogenous emulsion is dried to powder as a fine grain by spraying. Food grade SiO_2 is added at less than 1.5% to the final powder. The final steps include blending, sieving, and packaging.

Stability of CLA preparations
Various CLA preparations are stabilized with approximately 250-300 ppm of mixed tocopherol and are stable at ambient temperature for up to 2 years.

INTENDED USES AND EXPOSURE ESTIMATES

Food sources of CLA
Conjugated linoleic acids naturally occur in ruminant products, such as beef, lamb, and dairy goods. This is due to the bacterial biohydrogenation of LA in the rumen, with the c9,t11 isomer accounting for more than 90% of CLA intake in the diet (Bhattacharya et al., 2006). Production of CLA in ruminants is subject to seasonal variation and depends on animal feed type. Total CLA content in milk or dairy products ranges from 0.34 to 1.07% of total fat (Dhiman et al., 2005). Total CLA content in raw or processed beef ranges from 0.12 to 0.68% of total fat. It is currently estimated that the average adult consumes only one third to one half of the amount of CLA that has been shown to reduce cancer in animal studies (Dhiman et al., 2005).

Intended use
Conjugated linoleic acids are intended to be used in the same categories of foods as those described in GRN 232 (FDA, 2008) at 1.5 g CLA per serving for specific food categories. The maximum proposed uses of CLA by weight percent in each food category are as follows: 0.625% in specific soy milk beverages, meal replacement beverages, milk (filled), flavored milk products, and fruit juice products; 3.75% in meal replacement bars; and 0.667% in specific yogurt products. To deliver 1.5 g CLA, it requires 1.58 g of CLA 95 FFA, 1.875 g CLA 80 FFA, 1.875 g CLA 80 TG, 3.75 g CLA FFA 40 Powder, 3.75 g CLA TG 40 Powder, or 2.5 g CLA TG 60 Powder.

Exposure estimates
Estimated Daily Intakes (EDI) from before the intended use
The mean daily dietary CLA intake from the background diet is an estimated 212 mg/day for men and 151 mg/day for women in the general U.S. population, with subgroups consuming up to 300 mg/day (Ritzenthaler et al., 2001). The Ritzenthaler et al. (2001) study, with 51 men and 51 women between the ages of 18 and 60 years, compared 3-day food records and food frequency questionnaires. It was well-designed, fully reported, and representative of the general population—a very relevant report for this GRAS determination.

EDI under the intended use
Using the National Health and Nutrition Examination Survey (NHANES) 2009-2010 dataset, mean intake of users is estimated to be 0.84 g/person/day (16.5 mg/kg body weight[BW]/day) and the 90th percentile intake of users is 2.64 g/person/day (48.4 mg/kg BW/day) for Americans aged 2 years and older. Among specific groups, children aged 2-12 years and male teens aged 13-18 years consumed the highest EDIs with a 90th percentile value of 3.12 g/person/day which corresponds to 134.0 and 48.2 mg/kg body weight/day for children and males teens, respectively. These EDIs are well within safe intake levels: consumption up to 6-6.4 g/day is known to be safe for both CLA-TG and CLA-FFA in humans.

SAFETY DATA

The metabolism of CLA (in a form of either TG or FFA) has been widely studied and reported, and follows the standard pathway of dietary TG or FFA (GRN 232 [FDA, 2008]), thus so systematic toxicity is expected after ingestion and metabolism of 1.5 g/serving in selected foods. Regulation of lipid metabolism is controlled by the liver and involves catabolism of TG for use as energy, desaturation, and elongation of FA, and synthesis of TG and phospholipids (Krummel, 1996). The metabolism of absorbed FA depends, in part, upon their chemical structure. Medium-chain length FA are used mainly as energy sources (β-oxidation), longer-chain saturated and monounsaturated FA (SFA; MUFA) are generally stored (i.e., in adipose tissue as TG), and polyunsaturated fatty acids (PUFAs) are typically taken up by cells for incorporation into membrane phospholipids (Linder, 1991).

Hoye et al. (2004) evaluated the relative bioavailability of CLA from CLA-TG or in FFA form in healthy male volunteers (n=12). The bioavailability of CLA was measured in the serum as area under the curve (AUC). The authors reported that CLA in the form of FFA was 15-19% greater than CLA in the TG form. However, they noted that this might be due to the high load of the FFA form (11.4 g vs 8.9 g), changing the rate of absorption and disposition. The CLA reacheda peak concentration at 2 to 8 hours (depending on the isomer and form) and after 24 hours there was only a small fraction left. Half-lives of the isomers in the two forms ranged between 16 and 29 hours. No adverse events were noted during the trial period of 10 to 15 days.

Park and Pariza (2007) evaluated effects of CLA-FFA, CLA-diacylglyceride (DG-CLA), and CLA-TG on body composition. Four-week old ICR male mice were fed semipurified diet containing 0.7% of CLA (w/w, equivalent to 0.5% CLA at final) with different preparations for 4 weeks. All CLA preparations contained about 73% CLA (cis-9,trans-11, 33.4–33.9; trans-10,cis-12, 34.4–35.5; cis, cis, 2.3-2.4; trans, trans, 1.6–2.0). Comapred to the control group, body protein and water composition were significantly higher in CLA groups, but no differernces were noted among the 3 CLA groups. Body fat composition was significantly lower in CLA groups, but the 3 CLA groups had comparable body fat content. The data suggest that CLA-FFA and CLA-TG have comparable metabolic effects.

Since the FDA's last review of CLA in 2007-2008 (GRN232 [FDA, 2008]), no literature contradicting the agency's prior decision has been published. To find out if there are any recent reports that are inconsistent with FDA's 2008 review, we reviewed literature published since 2007 on the safety of CLA from 3 perspectives: 1) glucose metabolism, 2) cardiovascular (CVD) risk factors, including body weight measures, inflammatory biomarkers, and fat oxidation, and 3) human milk fat production and milk yield.

<u>Mutagenicity and genotoxicity studies</u>

Preclinical data demonstrate an absence of significant toxicological, mutagenic or reproductive and developmental effects of CLA-TG (O'Hagan and Menzel, 2003). Similarly, there was no mutagenic potential of CLA-TG in two *in vitro* assays (reverse mutation and chromosomal aberration in human lymphocytes; O'Hagan and Menzel, 2003).

<u>Animal toxicity studies published in or before 2007</u>

Preclinical data demonstrate an absence of significant toxicological, mutagenic, or reproductive

and developmental effects. Based on a 13-week feeding study, the reported no-observed-adverse-effect level (NOAEL) for the 1:1 CLA isomer mixture in rats was 5% in the diet, the highest level fed. This is equivalent to 2,433 and 2,728 mg/kg body weight (BW)/day for males and females, respectively (O'Hagan and Menzel, 2003).

Such observations are further supported by studies conducted by Park et al. (2005). They examined the effects of long-term feeding of male Fischer 344 rats with a diet containing 1% CLA (41.9% c9,t11 and 43.5% t10,c12; 1,000 mg/kg BW/day) for a period of 18 months. On molecular structure and metabolic levels, it was clear that CLA did not represent a carcinogenic risk above that of normal dietary TG.

Reproductive and developmental toxicity studies in rats and pigs have also shown a lack of adverse effects on maternal food consumption and BW, litter size, and offspring growth and development following exposure to CLA (0.25 to 2% in the diet) throughout gestation, lactation, and/or during a post-weaning period (Chin et al., 1994; Bee, 2000a, 2000b; Poulos et al., 2001).

It is noteworthy that these animal toxicity studies were done both CLA-TG and CLA-FFA. Since CLA-TG was thought to have a similar metabolic pathway to that of CLA-FFA, GRN 232 included the animal toxicity data from CLA-FFA studies to justify the safety of CLA-TG. The subchronic toxicity study of O'Hagan and Menzel (2003) is based on CLA-TG, but the reproductive and developmental toxicity studies of Bee (2000a, 2000b), Chin et al. (1994), and Poulos et al. (2001) as well as a chronic toxicity study of Park et al. (2005) employed CLA-FFA.

Animal toxicity studies published since 2007

Animal toxicity studies published since 2007 indicate that conclusions from a previous review reporting the NOAEL for the 1:1 CLA isomer mixture as 5% in the diet of rats are still valid. This was the highest level fed, which is equivalent to 2,433 and 2,728 mg/kg BW/day for males and females, respectively (O'Hagan and Menzel, 2003; GRN 232).

Animal studies evaluating reproductive performance of CLA

No recent literature contradicted the 2007 review of reproductive and developmental toxicity studies, which reported on the NOAEL of 0.25 to 2% CLA in the diets of rats and pigs (Chin et al., 1994; Bee, 2000a, 2000b; Poulos et al., 2001; GRN 232 [FDA, 2008]).

Recent studies (Danicke et al., 2012; de Veth et al., 2009; Peng et al., 2010; Veaute et al., 2007) also showed that CLA supplementation did not adversely impact reproductive performance or fetus weight in mice and dairy cows. In these recent studies, a CLA form was not specified. Danicke et al. (2012) tested supplementation of 8 g CLA/day (1:1 mixture of c9, t11 and t10, c12 CLA) from day 190 to 262 of pregnancy in cows, or fetal exposure times of day 19 to 102. De Veth et al. (2009) administered 18.3 g of t10,c12 CLA/day during early lactation (from day 37 to day 154) in dairy cows to show that reproductive performance of dairy cows may be improved by feeding of CLA supplements during early lactation. In the study of Peng et al. (2010), CLA was administered in pigs at 1% of diet (containing 28.02% c9,t11 CLA and 30.07% t10,c12 CLA) during the last 50 day of gestation and throughout a 26 day lactation to demonstrate no adverse effects of CLA on reproductive performance and piglet growth.

In a two-generation mouse study, Veaute et al. (2007) found no differences in BW gain, fetus number, and estrus cycle among groups of animals when fed one of 4 diets; control (7% total fat) or one of 3 experimental high fat (20% total fat) diets (cis FA, trans FA, or CLA). The CLA diet contained 3% CLA-rich oil (purity not specified). In both first and second generations, females receiving high levels of dietary fat tended to have higher rates of resorptions irrespective of the type of dietary fat. No CLA-specific effects were noted. In the first and second generation, CLA had lower values of placental interleukin (IL)-4 (~80-85%) and transforming growth factor-β (TGF-β) expressions (by 60-80%). Second generation males fed trans FA and CLA had lower rates of induced sperm acrosome reaction (~15% at 90 min and 40% at 30 min). Whether the specific changes are of clinical importance is uncertain; sperm acrosome reaction is not an established biomarker of reproductive performance. It is noteworthy that tumor necrosis factor alpha (TNF-α), sperm number, and motility were not different among groups.

In a study by Hayashi et al. (2007), 1.35% CLA in diets (containing 24% c9,t11; 35% t10,c12; 15% c8,t10; 17% c11,t13; and 9% of others) was administered to Wistar rats from day 1 of parturition (considered day 1 of lactation) to day 15 of lactation. On day 15 post-partum, CLA supplementation reduced pup growth by 21%. Conjugated linoleic acids treatment in the mammary glands and liver, reduced FA synthase, glucose 6-phosphate dehydrogenase, and 6-phosphogluconate dehydrogenase. The activity of nicotinamide adenine dinucleotide phosphate (NADP)-malate dehydrogenase was unchanged. CLA supplementation during lactation did not affect the dam's food intake or BW. Overall, CLA supplementation, regardless of CLA form (TG or FFA), did not adversely impact reproductive performance in various animals.

Animal studies evaluating metabolic effects of CLA

Supplementation with CLA did not induce major metabolic changes, including risk factors of CVD, diabetes, and obesity and immune responses, in dairy cows, rats, and pigs (Akter et al., 2011; Corino et al., 2009; Fernandez-Figares et al., 2012; Soares et al., 2012). Two studies specified CLA source in FFA form (Atker et al., 2011; Corino et al., 2009). Other studies did not specify the chemical form of CLA.

Atker et al. (2011) reported that CLA-FFA induced decreases in adipocyte size indicated lipolytic or anti-lipogenic effects of CLA on adipose tissue in primiparous dairy cows. Corino et al. (2009) reported that CLA-FFA supplementation improved BW at weaning, and immune responses in piglets, with no changes in TG content in the liver, serum low density lipoprotein cholesterol (LDL-C), non-esterified fatty acids (NEFAs), and histology.

Soares et al. (2012) found no intergroup differences in body and brain weights, body length, body mass index (BMI), abdominal and thoracic circumferences, and the abdominal to thoracic circumference ratio in progeny rats at 35-45 days of life. A pig study of Fernandez-Figares et al. (2012) determined possible mechanisms of action that might explain the nutrient partitioning effect of betaine and CLA in pigs. Twenty gilts were restrictively fed from 20 to 50 kg BW control, 0.5% betaine, 1% CLA or 0.5% betaine plus 1% CLA diets. Conjugated linoleic acids and CLA plus betaine did not influence serum concentrations of glucose, LDL-C, and NEFA, indices of insulin resistance, liver TG conc. or histopathology. However, increases in serum insulin (28% and 83%, respectively; $P=0.0001$) and serum TG (58% and 28%, respectively; $P<0.01$) were noted in 1% CLA and in 0.5% betaine + 1% CLA pigs. The increase in serum TG

indicated that CLA and betaine + CLA could have reduced adipose tissue TG synthesis from preformed FA.

Overall, none of these studies report data that contradict the prior review, which concluded that supplementation with CLA-FFA or CLA-TG did not impair glucose metabolism and did not increase CVD factors.

Animal studies evaluating milk yield or milk fat production

Most studies evaluating milk yield or milk fat production employed encapsulated CLA-FFA (Castaneda-Gutierrez et al., 2007; Hutchinson et al., 2012; Medeiros et al., 2010) or calcium salt of CLA-FFA (Medeiros et al., 2010). Castaneda-Gutierrez et al. (2007) reported that in Holstein cows, supplementation with 9.5-14.2 g/day of CLA-FFA isomer mixtures had no significant effect on milk fat yield or content over the entire supplementation period. Milk fat content, however, decreased by week 5 by 14.1 and 6.1% for 14.2 g CLA (c9,t11:t10,c12=:1:1) and 9.5 g CLA (c9,t11:t10,c12=75:25), respectively (P =0.005) compared with the controls. After termination of supplementation, fat content became similar to that of controls. No difference was observed in milk protein or milk lactose content or yield. Hayashi et al. (2007) administered 1.35% CLA (CLA form, not specified) in the diet of Wistar rats (containing 24% c9,t11; 35% t10,c12; 15% c8,t10; 17% c11,t13; and 9% others) from day 1 of parturition (considered day 1 of lactation) to day 15 of lactation. On day 15 post-partum, CLA supplementation during lactation reduced milk fat content by 33% without affecting the dam's food intake or BW. Total milk production was not measured in this study.

Energy spared from milk fat depression is repartitioned for milk production to increase yield from dairy cows (Hutchinson et al., 2012; Medeiros et al., 2010). Milk energy output and energy balances were similar for CLA and controls in dairy cows (Medeiros et al., 2010). Also, Sigl et al. (2010) reported no significant effects of CLA-FFA on milk composition and yield in brown Swiss cows. Overall, due to increased milk yield, there were no adverse effects of CLA on milk energy output despite depressed milk fat production which were found in some studies.

Animal studies evaluating anti-carcinogenicity of CLA

Several studies reported anticarcinogenic activity of CLA in rats and mice (Kim et al., 2010; Shiraishi et al., 2010). In a study by Shiraishi et al. (2010), both CLA-TG and CLA-FFA attenuated the increase in aberrant crypt foci (ACFs) induced by 10% beef tallow and azoxymethane (AOM) pretreatment at week 12 in Sprague-Dawley rats. At 44 weeks, both forms of CLA attenuated multiple colon cancers, although CLA-FFA reduced the incidence of colon cancer to 50% of that seen with CLA-TG. These results suggested that both CLA-TG and CLA-FFA suppressed colon carcinogenesis in rats with long-term feeding of a 10% beef tallow diet through several mechanisms. The authors concluded that results of this rat study might be applicable to humans.

The study of Kim et al. (2010) employed CLA-FFA to report anticarcinogenic potential of CLA in a mouse forestomach carcinogenesis regimen induced by benzo(a)pyrene.

In summary, a subchronic toxicity study reporting the NOAEL for the 1:1 CLA-TG isomer mixture as 5% in the diet of rats is still valid. This was the highest level fed, which is equivalent

to 2,433 and 2,728 mg/kg BW/day for males and females, respectively (O'Hagan and Menzel, 2003; GRN 232). The GRN 232 included the NOAEL of CLA-FFA found from developmental and reproductive toxicity studies to justify the safety of CLA-TG: the NOAELs of 0.25 to 2% CLA-FFA in the diets of rats and pigs (Chin et al., 1994; Bee, 2000a, 2000b; Poulos et al., 2001; GRN 232) are still valid.

Human clinical studies

Human clinical data presented in GRN 232

Human clinical studies on CLA-TG first appeared in the literature in 2003 (Albers et al., 2003) since CLA-TG has been available since early 2000. In GRN 232, CLA-FFA studies were included to justify the safety of CLA-TG since CLA-TG and CLA-FFA follow similar metabolic fates in human bodies, Comparative studies of Gaullier et al. (2004, 2005) and a single dose metabolic study of Hoye et al. (2004) confirmed that CLA-TG and CLA-FFA have similar metabolic effects.

Repeated dose human clinical studies presented in Tables 1-2. The studies on CLA-FFA only are found from Atkinson et al., 1999; Basu et al., 2000a (Riserus et al., 2001); Belury et al., 2003; Berven et al., 2000; Blankson et al., 2000; Eyjolfson et al., 2004; Kamphuis et al., 2003a,b ; Masters et al., 2002; Mougios et al., 2001; Noone et al., 2002 (Nugent et al., 2005); Smedman and Vessby, 2001(Basu et al., 2000b); Smedman et al., 2004 (Basu et al., 2000a), 2005; Taylor et al., 2006; Thom et al., 2001. Data for CLA-TG only are available from the studies of Doyle et al., 2005; Gaullier et al., 2005, 2007; Lambert et al**.,** 2007; Larsen et al., 2006; Moloney et al., 2004; Nazare et al., 2007; Pinkoski et al., 2006; Raff et al., 2006; Song et al., 2005; Steck et al**.,** 2007; Syvertsen et al., 2007; Watras et al., 2007; Whigham et al., 2004. A comparative study results for CLA-FFA and CLA-TG were reported in Albers et al. (2003) and Gaullier et al., (2004).

The highest dose of CLA-FFA was 6.1 g/day for 8 weeks (Belury et al., 2003). The highest chronic dose tested with CLA-TG was 6 g/day for 1 year, which did not produce adverse effects (Larsen et al., 2006; Whigham et al., 2004). In a comparative study of Gaullier et al. (2004), administration of both CLA-FFA (3.6 g/day for 1 year) and CLA-TG (3.4 g/day for 1 year) produced no adverse effects when body composition, glucose and lipid metabolism, serum biochemistry, and adverse events were compared. A single dose study demonstrated that administration of CLA-FFA at 11.2 g and CLA-TG at 8.9 g was not associated with adverse effects (Hoye et al., 2004).

Table 1. Studies on CLA-FFA presented in GRN 232

Studies on CLA-FFA	Daily dose, g/day	Duration
Atkinson et al., 1999	2.7	26 weeks
Thom et al., 2001	1.8	12 weeks
Mougios et al., 2001	0.7, and1.4	4 weeks
Blankson et al., 2000	1.7, 3.4, 5.1, and 6.8	12 weeks
Berven et al., 2000	3.4	12 weeks
Basu et al., 2000a: Riserus et al., 2001	4.2	4 weeks
Smedman and Vessby, 2001, Basu et al., 2000b	3.2	12 weeks
Noone et al., 2002: Nugent et al., 2005	2.0 mix/1.8 (1.3 as c9,t11)	8 weeks
Kamphuis et al., 2003a,b	1.8, and 3.6	13 weeks
Eyjolfson et al., 2004	3.0	8 weeks
Smedman et al., 2004: Basu et al., 2000a	3.5 as mix/4.0 as t10,c12	6 weeks
Masters et al., 2002	1.2	5 days
Belury et al., 2003	6.1	8 weeks
Smedman et al., 2005	3.2	12 weeks
Taylor et al., 2006	4.5	12 weeks

Table 2. Studies on CLA-TG presented in GRN 232

	Daily dose, g/day	Duration
CLA-TG alone		
Whigham et al., 2004	6.0	1 y
Moloney et al., 2004	2.2	8 weeks
Song et al., 2005	3.0	12 weeks
Doyle et al., 2005	2.2	8 weeks
Raff et al., 2006	4.4 g CLA/3.6 g Vaccenic acid	5 weeks
Pinkoski et al., 2006	5.1	7 weeks
Syvertsen et al., 2007	3.4	6 mo
Nazare et al., 2007	2.8	14 weeks
Larsen et al., 2006	3.4	1 y
Watras et al., 2007	3.2	6 mo
Steck et al., 2007	3.2 and 6.4	12 weeks
Gaullier et al., 2007	3.4	6 mo
Lambert et al., 2007	2.6	12 weeks
Gaullier et al., 2005	3.4 g CLA-TG	CLA-TG, 2 y
Comparison of CLA-FFA and CLA-TG		
Albers et al., 2003	1.7 FFA (1:1 isomers)/ 1.6 TG (4:1 isomers)	12 weeks
Gaullier et al., 2004	3.6 CLA-FFA or 3.4 g CLA in 4.5 g CLA-TG	1 yr

Studies published since mid-2007

To find if there are any recent reports that may not be consistent with the FDA's 2008 review, we reviewed the literature published since mid-2007. The extent of the effects of CLA on insulin resistance and on markers of cardiovascular risk appears to be species-dependent (EFSA, 2010). Therefore, the focus of this safety assessment relies primarily on the large number of available human studies. Studies published since 2007 mostly employed CLA-TG although a few studies evaluated CLA-FFA, and CLA from dairy sources. Unless noted otherwise, these studies evaluated 1:1 isomers (c9,t11: t10,c12) of CLA.

The doses tested in most studies were 2-6.4 g/day and the duration of the studies ranged from 1 to 7 months. These studies reported no adverse effects of CLA. A 3-week randomized clinical trial (RCT) using a daily dose of 19 g of CLA-TG found no adverse effects in healthy subjects (Wanders et al., 2010a, 2010b).

No recent human studies reported the data to be inconsistent with those found from previous studies: Consumption of CLA-TG isomers (1:1) at doses of up to 6 g/day for up to 1 year (Whigham et al., 2004; Larsen et al., 2006) and 3.4 g/day for up to 2 years (Gaullier et al., 2004, 2005) was safe and had no significant effects on cardiovascular parameters (lipid metabolism, markers of inflammation, and markers of oxidative stress), insulin sensitivity and glucose metabolism, or maternal milk fat. Repeated doses of 6 g CLA-FFA isomers (1:1) for 6 months (Tarnopolsky et al., 2007) and 3.6 g CLA-FFA for 1 year were found safe. In comparative study of Gaullier et al. (2004), administration of both CLA-FFA (3.6 g/day for 1 year) and CLA-TG (3.4 g/day for 1 year) produced similar results by showing no adverse effects on body composition, glucose and lipid metabolism, and serum biochemistry. The incidence of adverse events was not different among control, CLA-FFA and CLA-TG groups.

Glucose metabolism

Recent literature showed that CLA has no adverse effects on fasting blood concentrations of glucose or insulin, or insulin sensitivity. The dose tested were 0.8 - 6.8 g/day, and the duration of the studies ranged from 4 weeks to 7 months.

Effects of CLA on glucose metabolism in subjects with type 2 diabetes

Four studies evaluated effects of CLA on glucose metabolism in type 2 diabetes (CLA source not specified; Carvalho et al., 2012; CLA-FFA- Shadman et al., 2013; CLA-TG – Asp et al., 2011; Norris et al., 2009). The highest dose tested for CLA-TG was 6.4 g/day for 16 weeks (Asp et al., 2011; Norris et al., 2009; Table 10). The highest dose tested for CLA-FFA was 3.0 g/day for 8 weeks (Shadman et al., 2013). Both CLA-TG and CLA-FFA had no adverse effects of glucose metabolism in subjects with types 2 diabetes.

Effects of CLA on glucose metabolism in healthy subjects or subject with other conditions

In general, CLA supplementation, regardless of CLA forms (FFA or TG), had no effects on glucose metabolism. Doses tested were 6.4 g/day and the duration of the studies were up to 7 months. The highest doses tested for CLA-TG and CLA-FFA were 6.4 g/day for 16 weeks (Norris et al., 2009), and 6.0 g/day for 6 months (Tarnopolsky et al., 2007), repectively. Most studies reviewed reported no beneficial or adverse effects of CLA on fasting blood concentrations of glucose or insulin, Homeostasis Model of Assessment - Insulin Resistance

(HOMA-IR), or other biomarkers in healthy subjects or subjects with mildly hyercholesterolemia, hypertension, or rheumatoid arthritis (CLA-TG form: Ahren et al., 2009; Aryaeian et al., 2008; Gaullier et al., 2007; Iwata et al., 2007; Joseph et al., 2011; López-Plaza et al., 2013; MacRedmond et al., 2010; Racine et al., 2010; Sneddon et al., 2008; Syvertsen et al., 2007; Turpeinen et al., 2008; Zhao et al., 2009; CLA-FFA: Herrmann et al. (2009), Laso et al. (2007), Tarnopolsky et al. (2007), and Thrush et al. (2007; Natural form: Brown et al., 2011; CLA form not specified: Chen et al., 2012; Raff et al., 2009).

Favorable effects of CLA on glucose metabolism were noted in 3 studies (CLA-TG: Bulut et al., 201; Lambert et al., 2007; Pfeuffer et al., 2011).

Two studies reported conflicting results (Raff et al., 2009; Thrush et al., 2007). A study of Thrush et al. (2007) showed that CLA-FFA (3.1 g CLA containing 1.55g c9,t11 and 1.52 g t10,c12 CLA in the form of 1 g gel capsules with meals) increased the area under the curve (AUC) for glucose (30%) and insulin (20%) in a 3 h oral glucose tolerance test (OGTT) in 9 overweight, nondiabetic subjects (100% completed, 31.2 $\pm$ 3.7 years). Results from OGTT suggest that CLA reduced insulin sensitivity. However, the OGTT may not be a validated analytical method for determining insulin sensitivity. In addition, CLA had no effect on fasting plasma concentrations of lipids, glucose, insulin, or adiponectin after 12 weeks of supplementation. Fasting glucose and insulin concentrations may indicate CLA's effects on glucose metabolism more accurately. It is noteworthy that CLA had no effect on peroxisome proliferators activated receptor alpha (PPARα) content in muscle. The PPARα is known to regulate lipid oxidation and glucose metabolism. The study had limitations that included no control group and a small number of subjects (n=9). Overall, it is concluded that this study shows no adverse effects of CLA on glucose metabolism.

In a 2009 study of Raff et al., 81 healthy postmenopausal women were randomized to one of 3 groups: 1) 5.5 g/day of 40/40% of c9,t11-CLA-TG and t10,c12-CLA (CLA-mix; source not specified); 2) c9,t11-CLA; or 3) control (olive oil). No differences in serum insulin and glucose concentrations and HOMA-IR values were found among the groups, although post hoc analysis in the tertile of women with the highest waist circumference (WC; 94–109 cm) showed that the fasting serum insulin concentration was greater in the CLA-mix group than in the control (34%; P =0.02) and the c9,t11-CLA (28%; P = 0.04) groups. There were no differences between the effects of the supplements in the 2 lower tertiles. A large WC is a characteristic of metabolic syndrome and a risk factor for diabetes type II. Thus, this post hoc analysis indicates that participants with a large waist circumference are may be more sensitive to a negative effect of a CLA mixture on insulin metabolism. However, others did not report such effects and, thus, it requires more investigations. Overall, it is concluded that CLA supplementation had no effects on glucose metabolism.

CVD risk factors

As summarized in Tables 11-13, current research indicated that CLA had either no adverse effects or favorable ones on CVD markers. Supplementation with CLA had no adverse effects on CVD risk factors that include endothelial functions, inflammatory biomarkers, and body weight measures as well as fat oxidation or oxidative stress (Close et al., 2007; Kim et al., 2012; Malpuech-Brugere et al., 2010).

Three forms of CLA were employed in these studies: CLA-TG, CLA-FFA, and CLA from natural dairy sources. The CLA-TG form was employed in the studies of Aryaeian et al. (2008), Diaz et al. (2008), Gaullier et al. (2007), Iwata et al. (2007), Joseph et al. (2011), Lambert et al. (2007), Nazare et al. (2007), Norris et al. (2009), Pfeuffer et al. (2011), Racine et al. (2010), Raff et al. (2008, 2009), Sneddon et al. (2008), Steck et al. (2007), Tholstrup et al. (2008), Turpeinen et al. (2008), Watras et al. (2007), Wanders et al. (2010a), and Zhao et al. (2009). The CLA-FFA form was evaluated in the studies of Herrmann et al. (2009), Laso et al. (2007), Macaluso et al. (2012), Thrush et al. (2007), and Venkatramanan et al. (2010). CLA from natural dietary source were studied in Brown et al. (2011), Pintus et al. (2013), Sofi et al. (2010), and Venkatramanan et al. (2010). In the studies of Chen et al. (2012), Michishita et al. (2010), and Sluijs et al. (2010), CLA form not specified.

It appears that sources of CLA (TG, FFA, or natural dietary sources) did not affect the CVD risk factors. The doses tested for CLA-TG were 2-19.1 g/day, and the durations of the studies ranged from 3 weeks to 7 months. With the exception of the study of Wanders et al. (2010a) which employed 19 g CLA-TG for 3 weeks, most studies employed daily doses of 2-6.8 g and the durations of the studies ranged from 3 weeks to 7 months. The doses tested for CLA-FFA were 3.1-3.4 g/day and the durations of the studies ranged from 4-16 weeks (Herrmann et al., 2009, Laso et al., 2007, Macaluso et al., 2012, and Thrush et al., 2007).

Blood lipid profiles

Most papers reported no effects of CLA on blood lipid profiles or blood pressure (BP; Table 11: CLA-TG form - Aryaeian et al., 2008, Diaz et al., 2008, Gaullier et al., 2007, Iwata et al., 2007, Lambert et al., 2007, Lopez-Plaza et al., 2013, Racine et al. 2010, Raff et al., 2008, Steck et al., 2007, Turpeinen et al., 2008, Zhao et a., 2009; CLA-FFA form- Herrmann et al., 2009, Laso et al., 2007, Thrush et al., 2007, Venkatramanan et al., 2010; CLA from dietary sources- Brown et al., 2011, Sofi et al., 2010, Venkatramanan et al., 2010; CLA form not specified - Chen et al. (2012), Michishita et al., 2010, Sluijs et al., 2010). One study showed favorable effects (Pintus et al. 2013; CLA from dairy sources).

Three studies in 3 papers reported mixed results, with no biologically significant adverse effects from CLA (Chen et al., 2012; Tholstrup et al., 2008; Wanders et al., 2010a). Chen et al. (2012) showed that supplementation with CLA (3.4 g/day for 12 weeks; CLA form not specified) increased fasting plasma concentrations of TC, TG, LDL-C, alanine aminotransferase (ALT), and aspartate transaminase (AST), while decreasing HDL-C. However, the changes were slight and within the normal range. The authors concluded that CLA supplementation resulted in a decrease in obesity indices, with no obvious adverse effects.

Likewise, Tholstrup et al. (2008; the same study reported in Raff et al., 2008) found that a CLA-TG mixture containing t10,c12 decreased plasma HDL-C concentration (66.1 vs.77.0 mg/dl) and the ratio of HDL-C to LDL-C (2.86 vs. 3.43) while raising plasma concentrations of TG (93.0 vs. 78.8mg/dl) and CRP (by 37%) compared to controls. However, these values were within the normal ranges (HDL-C >60 mg/dl; TG<150 mg/dl; HDL-C/LDL-C ratio<4). In this study, a daily supplement of 5.5 g of oil rich in either a CLA mixture, an oil rich in naturally occurring c9,t11 CLA, or olive oil were administered for 16 weeks in 75 healthy postmenopausal women. In prepubertal children aged 6–10 y who were overweight or obese but otherwise healthychildren,

CLA supplementation (3 g/day of 80% CLA for 7 months) did not significantly influence plasma concentrations of glucose, insulin, or LDL-C although CLA decreased plasma HDL-C significantly more (P = 0.05) in the CLAgroup (-5.1 mg/dL) than in the placebo group (-0.7 mg/dL) while decreasing body fatness.

In the study of Wanders et al. (2010a), compared with the control group, the CLA-TG group had higher concentrations of TC (by 4.5%), LDL-C (7.8%), TG (5%), and apo-lipoprotein B (apo-B by 4.5%) and a lower concentration of HDL-C (5%). However, these figures are within the normal range, and are not a safety concern.

It appears that sources of CLA (TG, FFA, or natural dairy sources) did not affect blood lipid profiles.

Body weight measure and body fat

Risk factors for CVD include obesity. A number of human trials reported favorable effects of CLA supplementation on BW and body fat mass (BFM) as well as lean body mass (LBM), body mass index (BMI), WC, fat free mass (FFM) or lean body mass (LBM) (CLA-TG form- Joseph et al., 2011; López-Plaza et al., 2013, Norris et al., 2009; Racine et al., 2010; Raff et al., 2009; Watras et al., 2007; Zhao et al., 2009; unspecified CLA form- Chen et al., 2012.

However, some studies reported no significant effects of CLA on BW measures or body fat status (CLA-TG form- Diaz et al., 2008; Gaullier et al., 2007; Joseph et al., 2011; Lambert et al., 2007; Nazare et al., 2007; Pfeuffer et al., 2011; Sneddon et al., 2008; Streck et al., 2007; CLA-FFA form- Herrmann et al., 2009; Laso et al., 2007; Macaluso et al., 2012; Thrush et al., 2007; Venkatramanan et al., 2010; CLA from natural dietary source - Pintus et al., 2013; Sofi et al., 2010; Venkatramanan et al., 2010; unspecified CLA source- Michishita et al., 2010; Sluijis et al., 2010). It appears that sources of CLA (TG, FFA, or natural dairy sources) did not influence body weight measure and body fat.

Chen et al. (2012) reported that supplementation with CLA (3.4 g/day for 12 weeks; source unspecified) decreased BW, BMI, subcutaneous fat mass, and waist-to-hip ratio (WHR) in healthy Chinese subjects, with the greatest effect in those with BMI<27. A meta-analysis by Schoeller et al. (2009) demonstrated a correlation between CLA intake and increased FFM that was independent of treatment length or dosage. This indicates that FFM has a more rapid progression and a small effect that plateaus early on. A meta-analysis by Whigham et al. (2007) confirmed a linear progression in weight loss for approximately 6 months of CLA consumption. The same study found that a median dose of 3.2 g/day of CLA significantly reduced fat mass in the CLA supplemented group compared with the controls. Overall, no adverse effects from CLA were reported on BW measures or body composition.

Immune or inflammatory responses

Studies evaluating effects of CLA on immune and inflammatory responses in humans reported inconsistent results in terms of efficacy although no studies reported adverse effects of CLA. Beneficial effects of CLA supplementation were observed in particular immuno-challenging experiments (Dietary sources - Sofi et al., 2010; CLA-TG form - Mullen et al., 2007; Turpeinen et al., 2008; Zhao et al., 2009). In individuals with birch pollen allergy, supplementation of 2

g/day c9,t11 CLA for 12 weeks improved feelings of well-being and reduced sneezing (Turpeinen et al., 2008). Sofi et al. (2010) reported positive effects of CLA in reducing inflammatory parameters, such as IL-6 (-43%), IL-8 (-36.5%), and TNFα (-40%). However, other studies failed to show significant or consistent effects of CLA supplementation on indices of immune status (CLA-TG form - Gaullier et al., 2007; Joseph et al., 2011; MacRedmond et al., 2010; Pfeuffer et al., 2011; Raff et al., 2008; Steck et al., 2007; Stickford et al., 2011; Tholstrup et al., 2008). For example, 5.5 g of oil rich in either CLA mixture or an oil rich in naturally occurring c9,t11 CLA did not change plasma cytokines, such as IL-6, TNFα, intercellular adhesion molecule 1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1; Tholstrup et al., 2008). Steck et al. (2007) found that CLA significantly increased blood concentrations of CRP, IL-6, and white blood cells. However, all values were within normal ranges. Taken together, outcomes indicate no adverse effects of CLA on immune or inflammatory responses.

Fat oxidation or oxidative stress

Studies evaluating effects of CLA on fat oxidation or oxidative stress in humans produced inconsistent results in terms of efficacy. Doses tested were 2.4 - 4.6 g/day and the duration of studies were 8 weeks - 6 months (CLA-TG form - Malpuech-Brugere et al., 2010; Pfeuffer et al., 2011; Tholstrup et al., 2008; Turpeinen et al., 2008; CLA-FFA form - Kim et al., 2012; CLA form not specified- Close et al., 2007). Tholstrup et al. (2008) found that control group women who received the CLA mixture group (5.1 g/day) had 60% higher 8-iso prostaglandin F2a (8-iso PGF2α; a marker of *in vivo* oxidative stress), whereas women in the CLA milk group had 25% higher 8-iso PGF2α. As most of the risk markers are new, the clinical importance of these findings is uncertain. Close et al. (2007) reported that CLA positively altered fat oxidation and energy expenditure during sleep. Other studies reported no effects of CLA on fat oxidation (Kim et al., 2012; Malpuech-Brugere et al., 2010; Pfeuffer et al., 2011). Overall, no biologically significant adverse effects of CLA were reported.

Effects of CLA on Human milk production

Hasin et al. (2007) reported that dietary supplementation with either c9,t11 or t10,c12 CLA (CLA source not specified) did not reduce milk fat in healthy, breast-feeding women. No adverse effects of CLA were reported.

Studies evaluating effects of CLA on other physiological outcomes in humans

Several studies evaluated the effects of CLA on bone health (CLA from dietary source - Brown et al., 2011; CLA-TG form - Gaullier et al., 2007; Racine et al., 2010), liver and kidney functions (CLA-TG form -Wanders et al., 2010b), fecal microbiota, fecal enzyme activity, or fecal composition (dietary source of CLA and CLA-TG -Farnworth et al., 2007). These studies reported no adverse effects from CLA. Goedecke et al. (2009) reported no adverse effects of CLA-TG in a study evaluating incorporation of CLA into adipose tissue and skeletal muscle. Wanders et al. (2010b) reported that a daily dose of 19.3 g CLA-TG (14.6 g c9,t11 CLA and 4.7 g t10,c12 CLA isomers) for 3 weeks did not cause adverse effects on liver and kidney functions in healthy subjects.

Table 3 summarizes the highest dose levels of CLA-FFA and CLA-TG showing no adverse effects in humans. Longer term studies show that a daily dose of 6 g CLA-TG and CLA-FFA were not associated with adverse effects (Larsen et al., 2006; Tarnopolsky et al., 2007; Whigham et al., 2004). Short term studies showed that daily doses of 6.8 g CLA-FFA and 19 g CLA-TG were well tolerated with no serious side effects (Blankson et al., 2000; Wanders 2010a,2010b).

Table 3. Summary of highest dose levels of CLA showing no adverse effects in humans

	GRN 232		Since 2007 (after GRN 232)	
	CLA-FFA	CLA-TG	CLA-FFA	CLA-TG
Longer term studies	3.6 g/day for 1 year (Gaullier et al, 2004, 2005)	6 g/day for 1 years (Larsen et al., 2006; Whigham et al., 2004); 3.4 g/day for 2 years Gaullier et al, 2004, 2005)	6 g/day for 6 months (Tarnopolsky et al., 2007)	
Short term studies	6.8 g/day for 8 weeks (Blankson et al., 2000)			19 g for 3 weeks (Wanders 2010a,2010b)
Single dose study	11.2 g (Hoye et al., 2004)	8.9 g (Hoye et al., 2004)		

POTENTIAL ADVERSE EFFECTS (updated from GRN 232 [FDA, 2008])
Of the adverse events reported in clinical trials, the most frequent complaints were gastrointestinal (Blankson et al., 2000; Smedman and Vessby, 2001). These are common complaints with high fat diets and dietary supplement capsules. Several studies also reported rapid adaptation to the addition of dietary CLA. Some investigators suggested that gastrointestinal upset could be due to either the CLA oil itself or the large number of gelatin capsules administered in most of the trials (up to 12 in the case of Blankson et al., 2000). Other studies show no such effects, including Whigham et al. (2004), which administered 6 gelatin capsules containing CLA TG.

A daily dose of 1-19.3 g CLA for 3 weeks-7 months did not produce adverse effects on liver and kidney function tests or other outcomes in healthy subjects in the Netherlands (Wanders et al., 2010b).

SAFETY DETERMINTATION
Numerous human and animal studies have examined the health benefits of CLA with no major adverse effects reported. INNOBIO® uses a HACCP-controlled manufacturing process and rigorously tests its final production batches to verify adherence to quality control specifications. There is broad-based and widely disseminated knowledge concerning the chemistry of CLA. This GRAS determination is based on the data and information generally available and

consented opinion about the safety of CLA. The literature indicates that CLA offers consumers health benefits without serious adverse effects.

The following safety evaluation fully considers the composition, intake, nutritional, microbiological, and toxicological properties of CLA as well as appropriate corroborative data.

1. Numerous reviews conducted by experts in the field unanimously support the safety of CLA. Numerous clinical trials have evaluated the effects of a 1:1 CLA mixture and a number of other isomers on similar parameters. Human clinical studies show that administration of daily dose of 3.4-3.6 g CLA (either FFA or TG form) for 1-2 years resulted in no serious adverse effects. Also, a daily dose 6 g CLA (either FFA or TG form) for 6 months-1 year was not associated with no adverse effects (Larsen et al., 2006; Tarnopolsky et al., 2007; Whigham et al., 2004). Short-term studies (3-8 weeks) showed that daily doses of 6.4-6.8 g CLA-FFA and 19 g CLA-TG were well tolerated with no serious side effects (Blankson et al., 2000; Wanders 2010a, 2010b). In addition, In addition, Both CLA-TG and CLA-FFA had no adverse effects of glucose metabolism in subjects with types 2 diabetes (Asp et al., 2011; Norris et al., 2009; Shadman et al., 2013). For these “pivotal” studies, the levels of consumption represent the maximum dose consumed, rather than absolute safety endpoints.

2. The proposed food uses result in EDIs for users (mean of 0.84 g/person/day or 16.5 mg/kg BW/day and 90th percentile intake of 2.64 g/person/day or 47.8 mg/kg BW/day) at levels significantly below those associated with any side effects. These EDIs are well within safe intake levels: consumption up to 6-6.4 g/day is known to be safe for both CLA-TG and CLA-FFA in humans.

3. The compositional data and product properties are consistent with carefully controlled cGMP production and purification. CLA contains no impurities or contaminants of concern for human health.

4. The US FDA (GRN 232 [FDA, 2008]) has not raised questions regarding the safety of CLA-TG as currently used in food. Since the FDA’s last review of CLA in 2007-2008 (GRN 232), no literature contradicting the agency’s prior reviews/decisions has been published.

5. Several reviews by experts in the field also have documented the safety of CLA.

CONCLUSIONS AND GENERAL RECOGNITION OF THE SAFETY OF CLA

The intended uses of CLA (TG and FFA forms) have been determined to be safe though scientific procedures as set forth in 21 CFR 170.3(b), thus satisfying the so-called "technical" element of the GRAS determination. In addition, because this safety evaluation was based on generally available and widely accepted data and information, it also satisfies the so-called "common knowledge" element of a GRAS determination.

On behalf of INNOBIO® Limited (INNOBIO®), we, the undersigned expert panel members, Susan Cho, Ph.D., Steven Heymsfield, M.D., Donald Schmitt, M.P.H., and Yeonhwa Park, Ph.D., have independently evaluated the materials summarized in the CLA Generally Recognized as Safe (GRAS) report. These individuals are qualified by scientific training and experience to evaluate the safety of substances intended to be added to foods. They have critically reviewed and evaluated the publicly available information summarized in this document and have individually and collectively concluded that CLA, produced consistent with Good Manufacturing Practice and meeting the specifications described herein, is safe under its intended conditions of use. The Panel further unanimously concludes that these uses of CLA are GRAS based on scientific procedures, and other experts qualified to assess the safety of food and food ingredients would concur with these conclusions.

It is also INNOBIO's opinion that the proposed use of CLA (TG and FFA) is not only safe within the terms of the Federal Food, Drug, and Cosmetic Act (meeting the standard of reasonable certainty of no harm), but it is also *Generally Recognized as Safe* (GRAS) according to Title 21 Code of Federal Regulations (21 CFR) according to consensus among experts.

_____ _____

Susan Cho, Ph.D. Date
NutraSource, Inc.

_____ _____

Steven Heymsfield, M.D. Date
Pennington Biomedical Research Center

_____ _____

Donald Schmitt, M.P.H. Date
ToxStrategies, Inc.

_____ _____

Yeonhwa Park, Ph.D. Date
University of Massachusettes

CONCLUSIONS AND GENERAL RECOGNITION OF THE SAFETY OF CLA

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(b) (6)

5/10/2014

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Date

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APPENDIX C. CERTIFICATE OF ANALYSIS

INNOBIO® Conjugated Linoleic Acid 80 FFA

Code: 10101-80
Lot No.: (b) (6) Date of Manufacture: 02/25/2013

Description: INNOBIO® Conjugated Linoleic Acid 80 FFA is a 1:1 ratio of the cis9, trans11 (c9, t11) and trans10, cis12 (t10, c12) isomers in free fatty acid form, derived from 100% pure, natural safflower seed oil using molecular distillation.

Tests	Limits	Methods	Results
Appearance	Clear colorless or light yellow oil	Visual	Conforms
Conjugated Linoleic Acid (%)	78.0-84.0	ISO 15304	81.1
-c9, t11 + t10, c12 CLA (%)	75.0 Min.	ISO 15304	79.1
-c9, t11 CLA (%)	37.5 Min.	ISO 15304	40.0
-t10, c12 CLA (%)	37.5 Min.	ISO 15304	39.1
-t, t CLA (%)	3.0 Max.	ISO 15304	0.8
Palmitic Acid (%)	6.0 Max.	ISO 15304	3.9
Stearic Acid (%)	4.0 Max.	ISO 15304	0.5
Linoleic Acid (%)	2.5 Max.	ISO 15304	1.6
Oleic Acid (%)	15.0 Max.	ISO 15304	12.3
Color (LOVIBOND 5 1/4")	Y5.0, R0.5 Max.	AOCS Cc 13e-92	Y2.9,R0.1
Acid Value (mg KOH/g)	195.0-204.0	AOCS Cd 3d-63	196.1
Peroxide Value (meq/kg)	5.0 Max.	AOCS Cd 8-53	0.2
Moisture (%)	0.1 Max.	AOCS Td 2-64	0.02
Saponification Value (mg KOH/g)	195.0-204.0	AOCS Cd 3-25	196.4
Unsaponifiable Matter (%)	1.0 Max.	AOCS Ca 6a-40	0.2
Methanol (ppm)	10 Max	GC-FID	3.9
Lead (ppm)	0.2 Max.	EN ISO 17294	< 0.05
Arsenic (ppm)	0.2 Max.	EN ISO 17294	< 0.05
Cadmium (ppm)	0.2 Max.	EN ISO 17294	< 0.01
Mercury (ppm)	0.1 Max.	BS EN 13806	0.02

Packaging: Packaged in HDPE drums under nitrogen blanket, 190 kg net.
Storage and Handling: Store in a cool dry place, protect from light, heat and oxygen. The preferred storage temperature is below 30°C. If precipitation occurs before using, please warm the product to 30-40°C under nitrogen to achieve complete dissolution.
Shelf Life: Three years in the original package. Once opened, the remaining oil should be blanketed under nitrogen and used as quickly as possible.

(b) (6)

Approval:_____ Date: 02/27/2013

INNOBIO® Conjugated Linoleic Acid 80 FFA

Code: 10101-80
Lot No.: (b) (6) Date of Manufacture: 09/02/2013

Description: INNOBIO® Conjugated Linoleic Acid 80 FFA is a 1:1 ratio of the cis9, trans11 (c9, t11) and trans10, cis12 (t10, c12) isomers in free fatty acid form, derived from 100% pure, natural safflower seed oil using molecular distillation.

Tests	Limits	Methods	Results
Appearance	Clear colorless or light yellow oil	Visual	Conforms
Conjugated Linoleic Acid (%)	78.0-84.0	ISO 15304	80.5
-c9, t11 + t10, c12 (%)	75.0 Min.	ISO 15304	76.8
-c9, t11 CLA (%)	37.5Min.	ISO 15304	38.4
-t10, c12 CLA (%)	37.5 Min.	ISO 15304	38.4
-t, t CLA (%)	3.0 Max.	ISO 15304	0.8
Palmitic Acid (%)	6.0 Max.	ISO 15304	5.4
Stearic Acid (%)	4.0 Max.	ISO 15304	1.7
Linoleic Acid (%)	2.5 Max.	ISO 15304	1.4
Oleic Acid (%)	15.0 Max.	ISO 15304	10.4
Color (LOVIBOND 5 1/4")	Y5.0, R0.5 Max.	AOCS Cc 13e-92	Y2.5,R0.3
Acid Value (mg KOH/g)	195.0-204.0	AOCS Cd 3d-63	195.6
Peroxide Value (meq/kg)	5.0 Max.	AOCS Cd 8-53	0.7
Moisture (%)	0.1 Max.	AOCS Td 2-64	0.02
Saponification Value (mg KOH/g)	195.0-204.0	AOCS Cd 3-25	201.2
Unsaponifiable Matter (%)	1.0 Max.	AOCS Ca 6a-40	0.3
Methanol (ppm)	10 Max	GC-FID	3.3
Lead (ppm)	0.2 Max.	EN ISO 17294	< 0.05
Arsenic (ppm)	0.2 Max.	EN ISO 17294	< 0.05
Cadmium (ppm)	0.2 Max.	EN ISO 17294	< 0.01
Mercury (ppm)	0.1 Max.	BS EN 13806	< 0.005

Packaging: Packaged in HDPE drums under nitrogen blanket, 190 kg net.
Storage and Handling: Store in a cool dry place, protect from light, heat and oxygen. The preferred storage temperature is below 30°C. If precipitation occurs before using, please warm the product to 30-40°C under nitrogen to achieve complete dissolution.
Shelf Life: Three years in the original package. Once opened, the remaining oil should be blanketed under nitrogen and used as quickly as possible.

Approval:_____ (b) (6) Date: 09/03/2013

INNOBIO® Conjugated Linoleic Acid 80 FFA

Code: 10101-80
Lot No.: (b) (6) Date of Manufacture: 07/31/2013

Description: INNOBIO® Conjugated Linoleic Acid 80 FFA is a 1:1 ratio of the cis9, trans11 (c9, t11) and trans10, cis12 (t10, c12) isomers in free fatty acid form, derived from 100% pure, natural safflower seed oil using molecular distillation.

Tests	Limits	Methods	Results
Appearance	Clear colorless or light yellow oil	Visual	Conforms
Conjugated Linoleic Acid (%)	78.0-84.0	ISO 15304	80.4
-c9, t11 + t10, c12 (%)	75.0 Min.	ISO 15304	76.7
-c9, t11 CLA (%)	37.5Min.	ISO 15304	38.4
-t10, c12 CLA (%)	37.5 Min.	ISO 15304	38.3
-t, t CLA (%)	3.0 Max.	ISO 15304	0.9
Palmitic Acid (%)	6.0 Max.	ISO 15304	5.4
Stearic Acid (%)	4.0 Max.	ISO 15304	1.7
Linoleic Acid (%)	2.5 Max.	ISO 15304	1.4
Oleic Acid (%)	15.0 Max.	ISO 15304	10.5
Color (LOVIBOND 5 1/4")	Y5.0, R0.5 Max.	AOCS Cc 13e-92	Y2.6,R0.2
Acid Value (mg KOH/g)	195.0-204.0	AOCS Cd 3d-63	198.1
Peroxide Value (meq/kg)	5.0 Max.	AOCS Cd 8-53	0.5
Moisture (%)	0.1 Max.	AOCS Td 2-64	0.06
Saponification Value (mg KOH/g)	195.0-204.0	AOCS Cd 3-25	197.2
Unsaponifiable Matter (%)	1.0 Max.	AOCS Ca 6a-40	0.5
Methanol (ppm)	10 Max	GC-FID	4.3
Lead (ppm)	0.2 Max.	EN ISO 17294	< 0.05
Arsenic (ppm)	0.2 Max.	EN ISO 17294	< 0.05
Cadmium (ppm)	0.2 Max.	EN ISO 17294	< 0.01
Mercury (ppm)	0.1 Max.	BS EN 13806	< 0.005

Packaging: Packaged in HDPE drums under nitrogen blanket, 190 kg net.
Storage and Handling: Store in a cool dry place, protect from light, heat and oxygen. The preferred storage temperature is below 30°C. If precipitation occurs before using, please warm the product to 30-40°C under nitrogen to achieve complete dissolution.
Shelf Life: Three years in the original package. Once opened, the remaining oil should be blanketed under nitrogen and used as quickly as possible.

Approval:_____ (b) (6) Date: 08/02/2013

INNOBIO® Conjugated Linoleic Acid 95 FFA

Code: 10101-95
Lot No.: (b) (6) Date of Manufacture: 01/14/2013

Description: INNOBIO® Conjugated Linoleic Acid 95 FFA is a 1:1 ratio of the cis9, trans11 (c9, t11) and trans10, cis12 (t10, c12) isomers, derived from 100% pure, natural safflower seed oil using molecular distillation.

Tests	Limits	Methods	Results
Appearance	Clear colorless or light yellow oil	Visual	Conforms
Conjugated Linoleic Acid (%)	93.0 Min.	ISO 15304	94.1
-c9, t11 + t10, c12 (%)	88.0 Min.	ISO 15304	89.1
-c9, t11 CLA (%)	44.0 Min.	ISO 15304	44.1
-t10, c12 CLA (%)	44.0 Min.	ISO 15304	45.0
-t, t CLA (%)	3.0 Max.	ISO 15304	0.9
Palmitic Acid (%)	1.0 Max.	ISO 15304	0.1
Stearic Acid (%)	1.0 Max.	ISO 15304	0.04
Linoleic Acid (%)	2.5 Max.	ISO 15304	1.1
Oleic Acid (%)	5.0 Max.	ISO 15304	4.5
Color (LOVIBOND 5 1/4")	Y10.0, R1.0 Max.	AOCS Cc 13e-92	Y2.0,R0.1
Acid Value (mg KOH/g)	195.0-204.0	AOCS Cd 3d-63	200.2
Peroxide Value (meq/kg)	5.0 Max.	AOCS Cd 8-53	0.2
Moisture (%)	0.1 Max.	AOCS Td 2-64	0.04
Saponification Value (mg KOH/g)	195.0-204.0	AOCS Cd 3-25	200.0
Unsaponifiable Matter (%)	1.0 Max.	AOCS Ca 6a-40	0.2
Methanol (ppm)	10 Max	GC-FID	2.1
Lead (ppm)	0.2 Max.	EN ISO 17294	< 0.05
Arsenic (ppm)	0.2 Max.	EN ISO 17294	< 0.05
Cadmium (ppm)	0.2 Max.	EN ISO 17294	< 0.01
Mercury (ppm)	0.1 Max.	BS EN 13806	< 0.005

P Packaging: Packaged in HDPE drums under nitrogen blanket, 190 kg net.
Storage and Handling: Store in a cool dry place, protect from light, heat and oxygen. The preferred storage temperature is below 30°C.
Shelf Life: Three years in the original package. Once opened, the remaining oil should be blanketed under nitrogen and used as quickly as possible.

Approval:_____ (b) (6) Date: 01/16/2013

INNOBIO® Conjugated Linoleic Acid 95 FFA

Code: 10101-95
Lot No.: (b) (6) Date of Manufacture: 01/09/2013

Description: INNOBIO® Conjugated Linoleic Acid 95 FFA is a 1:1 ratio of the cis9, trans11 (c9, t11) and trans10, cis12 (t10, c12) isomers, derived from 100% pure, natural safflower seed oil using molecular distillation.

Tests	Limits	Methods	Results
Appearance	Clear colorless or light yellow oil	Visual	Conforms
Conjugated Linoleic Acid (%)	93.0 Min.	ISO 15304	95.2
-c9, t11 + t10, c12 (%)	88.0 Min.	ISO 15304	90.4
-c9, t11 CLA (%)	44.0 Min.	ISO 15304	45.1
-t10, c12 CLA (%)	44.0 Min.	ISO 15304	45.3
-t, t CLA (%)	3.0 Max.	ISO 15304	0.8
Palmitic Acid (%)	1.0 Max.	ISO 15304	0.2
Stearic Acid (%)	1.0 Max.	ISO 15304	0.05
Linoleic Acid (%)	2.5 Max.	ISO 15304	1.2
Oleic Acid (%)	5.0 Max.	ISO 15304	2.9
Color (LOVIBOND 5 1/4")	Y10.0, R1.0 Max.	AOCS Cc 13e-92	Y1.2,R0.1
Acid Value (mg KOH/g)	195.0-204.0	AOCS Cd 3d-63	197.0
Peroxide Value (meq/kg)	5.0 Max.	AOCS Cd 8-53	0.5
Moisture (%)	0.1 Max.	AOCS Td 2-64	0.05
Saponification Value (mg KOH/g)	195.0-204.0	AOCS Cd 3-25	198.3
Unsaponifiable Matter (%)	1.0 Max.	AOCS Ca 6a-40	0.5
Methanol (ppm)	10 Max	GC-FID	3.2
Lead (ppm)	0.2 Max.	EN ISO 17294	< 0.05
Arsenic (ppm)	0.2 Max.	EN ISO 17294	< 0.05
Cadmium (ppm)	0.2 Max.	EN ISO 17294	< 0.01
Mercury (ppm)	0.1 Max.	BS EN 13806	< 0.005

Packaging: Packaged in HDPE drums under nitrogen blanket, 190 kg net.
Storage and Handling: Store in a cool dry place, protect from light, heat and oxygen. The preferred storage temperature is below 30°C.
Shelf Life: Three years in the original package. Once opened, the remaining oil should be blanketed under nitrogen and used as quickly as possible.

Approval:_____ (b) (6) Date: 01/30/2013

INNOBIO® Conjugated Linoleic Acid 95 FFA

Code: 10101-95
Lot No.: (b) (6) Date of Manufacture: 06/17/2013

Description: INNOBIO® Conjugated Linoleic Acid 95 FFA is a 1:1 ratio of the cis9, trans11 (c9, t11) and trans10, cis12 (t10, c12) isomers, derived from 100% pure, natural safflower seed oil using molecular distillation.

Tests	Limits	Methods	Results
Appearance	Clear colorless or light yellow oil	Visual	Conforms
Conjugated Linoleic Acid (%)	93.0 Min.	ISO 15304	95.3
-c9, t11 + t10, c12 (%)	88.0 Min.	ISO 15304	90.9
-c9, t11 CLA (%)	44.0 Min.	ISO 15304	45.3
-t10, c12 CLA (%)	44.0 Min.	ISO 15304	45.6
-t, t CLA (%)	3.0 Max.	ISO 15304	0.9
Palmitic Acid (%)	1.0 Max.	ISO 15304	0.2
Stearic Acid (%)	1.0 Max.	ISO 15304	0.04
Linoleic Acid (%)	2.5 Max.	ISO 15304	1.1
Oleic Acid (%)	5.0 Max.	ISO 15304	3.2
Color (LOVIBOND 5 1/4")	Y10.0, R1.0 Max.	AOCS Cc 13e-92	Y2.1,R0.1
Acid Value (mg KOH/g)	195.0-204.0	AOCS Cd 3d-63	196.7
Peroxide Value (meq/kg)	5.0 Max.	AOCS Cd 8-53	0.7
Moisture (%)	0.1 Max.	AOCS Td 2-64	0.05
Saponification Value (mg KOH/g)	195.0-204.0	AOCS Cd 3-25	189.4
Unsaponifiable Matter (%)	1.0 Max.	AOCS Ca 6a-40	0.3
Methanol (ppm)	10 Max	GC-FID	2.4
Lead (ppm)	0.2 Max.	EN ISO 17294	< 0.05
Arsenic (ppm)	0.2 Max.	EN ISO 17294	< 0.05
Cadmium (ppm)	0.2 Max.	EN ISO 17294	< 0.01
Mercury (ppm)	0.1 Max.	BS EN 13806	< 0.005

Packaging: Packaged in HDPE drums under nitrogen blanket, 190 kg net.
Storage and Handling: Store in a cool dry place, protect from light, heat and oxygen. The preferred storage temperature is below 30°C.
Shelf Life: Three years in the original package. Once opened, the remaining oil should be blanketed under nitrogen and used as quickly as possible.

Approval:_____ (b) (6) Date: 07/31/2013

INNOBIO® Conjugated Linoleic Acid 80 TG

Code: 10102-80
Lot No.: (b) (6) Date of Manufacture: 04/08/2013

Description: INNOBIO® Conjugated Linoleic Acid 80 TG is a 1:1 ratio of the cis 9, trans 11 (c9, t11) and trans10, cis12 (t10, c12) isomers in triglyceride form, derived from 100% pure, natural safflower seed oil using molecular distillation.

Tests	Limits	Methods	Results
Appearance	Clear colorless or light yellow oil	Visual	Conforms
Conjugated Linoleic Acid (%)	78.0-84.0	ISO 15304	79.6
-c9, t11 + t10, c12 (%)	75.0 Min.	ISO 15304	77.2
-c9, t11 CLA (%)	37.5 Min.	ISO 15304	38.6
-t10, c12 CLA (%)	37.5 Min.	ISO 15304	38.6
-t, t CLA (%)	3.0 Max.	ISO 15304	0.8
Palmitic Acid (%)	6.0 Max.	ISO 15304	5.3
Stearic Acid (%)	4.0 Max.	ISO 15304	2.3
Linoleic Acid (%)	2.5 Max.	ISO 15304	1.3
Oleic Acid (%)	15.0 Max.	ISO 15304	10.9
Color (GARDNER)	4.0 Max.	AOCS Cc 13e-92	4.0
Acid Value (mg KOH/g)	2.0 Max.	AOCS Cd 3d-63	0.1
Peroxide Value (meq/kg)	5.0 Max.	AOCS Cd 8-53	4.5
Moisture (%)	0.1 Max.	AOCS Td 2-64	0.01
Saponification Value (mg KOH/g)	185.0-195.0	AOCS Cd 3-25	189.7
Unsaponifiable Matter (%)	1.0 Max.	AOCS Ca 6a-40	0.3
Methanol / Ethanol (ppm)	10 Max	GC-FID	3.2
Lead (ppm)	0.2 Max.	EN ISO 17294	< 0.05
Arsenic (ppm)	0.2 Max.	EN ISO 17294	< 0.05
Cadmium (ppm)	0.2 Max.	EN ISO 17294	< 0.01
Mercury (ppm)	0.1 Max.	BS EN 13806	< 0.005

Packaging: Packaged in HDPE drums under nitrogen blanket, 190 kg net.
Storage and Handling: Store in a cool dry place, protect from light, heat and oxygen. The preferred storage temperature is below 30°C.
Shelf Life: Three years in the original package. Once opened, the remaining oil should be blanketed under nitrogen and used as quickly as possible.

Approval:_____ (b) (6) Date: 04/13/2013

INNOBIO® Conjugated Linoleic Acid 80 TG

Code: 10102-80
Lot No.:(b) (6) Date of Manufacture: 09/17/2012

Description: INNOBIO® Conjugated Linoleic Acid 80 TG is a 1:1 ratio of the cis 9, trans 11 (c9, t11) and trans10, cis12 (t10, c12) isomers in triglyceride form, derived from 100% pure, natural safflower seed oil using molecular distillation.

Tests	Limits	Methods	Results
Appearance	Clear colorless or light yellow oil	Visual	Conforms
Conjugated Linoleic Acid (%)	78.0-84.0	ISO 15304	80.4
-c9, t11 + t10, c12 (%)	75.0 Min.	ISO 15304	77.7
-c9, t11 CLA (%)	37.5 Min.	ISO 15304	38.7
-t10, c12 CLA (%)	37.5 Min.	ISO 15304	39.0
-t, t CLA (%)	3.0 Max.	ISO 15304	0.8
Palmitic Acid (%)	6.0 Max.	ISO 15304	5.2
Stearic Acid (%)	4.0 Max.	ISO 15304	2.3
Linoleic Acid (%)	2.5 Max.	ISO 15304	0.8
Oleic Acid (%)	15.0 Max.	ISO 15304	11.0
Color (GARDNER)	4.0 Max.	AOCS Cc 13e-92	3.0
Acid Value (mg KOH/g)	2.0 Max.	AOCS Cd 3d-63	0.2
Peroxide Value (meq/kg)	5.0 Max.	AOCS Cd 8-53	2.9
Moisture (%)	0.1 Max.	AOCS Td 2-64	0.07
Saponification Value (mg KOH/g)	185.0-195.0	AOCS Cd 3-25	188
Unsaponifiable Matter (%)	1.0 Max.	AOCS Ca 6a-40	0.2
Methanol / Ethanol (ppm)	10 Max	GC-FID	2.8
Lead (ppm)	0.2 Max.	EN ISO 17294	< 0.05
Arsenic (ppm)	0.2 Max.	EN ISO 17294	< 0.05
Cadmium (ppm)	0.2 Max.	EN ISO 17294	< 0.01
Mercury (ppm)	0.1 Max.	BS EN 13806	< 0.005

Packaging: Packaged in HDPE drums under nitrogen blanket, 190 kg net.
Storage and Handling: Store in a cool dry place, protect from light, heat and oxygen. The preferred storage temperature is below 30°C.
Shelf Life: Three years in the original package. Once opened, the remaining oil should be blanketed under nitrogen and used as quickly as possible.

Approval:_____ (b) (6) Date: 09/19/2012

INNOBIO® Conjugated Linoleic Acid 80 TG

Code: 10102-80
Lot No.: (b) (6) Date of Manufacture: 09/18/2012

Description: INNOBIO® Conjugated Linoleic Acid 80 TG is a 1:1 ratio of the cis 9, trans 11 (c9, t11) and trans10, cis12 (t10, c12) isomers in triglyceride form, derived from 100% pure, natural safflower seed oil using molecular distillation.

Tests	Limits	Methods	Results
Appearance	Clear colorless or light yellow oil	Visual	Conforms
Conjugated Linoleic Acid (%)	78.0-84.0	ISO 15304	80.1
-c9, t11 + t10, c12 (%)	75.0 Min.	ISO 15304	79.3
-c9, t11 CLA (%)	37.5 Min.	ISO 15304	39.2
-t10, c12 CLA (%)	37.5 Min.	ISO 15304	40.1
-t, t CLA (%)	3.0 Max.	ISO 15304	0.8
Palmitic Acid (%)	6.0 Max.	ISO 15304	5.5
Stearic Acid (%)	4.0 Max.	ISO 15304	2.1
Linoleic Acid (%)	2.5 Max.	ISO 15304	0.8
Oleic Acid (%)	15.0 Max.	ISO 15304	10.5
Color (GARDNER)	4.0 Max.	AOCS Cc 13e-92	3.0
Acid Value (mg KOH/g)	2.0 Max.	AOCS Cd 3d-63	0.1
Peroxide Value (meq/kg)	5.0 Max.	AOCS Cd 8-53	1.7
Moisture (%)	0.1 Max.	AOCS Td 2-64	0.03
Saponification Value (mg KOH/g)	185.0-195.0	AOCS Cd 3-25	194.6
Unsaponifiable Matter (%)	1.0 Max.	AOCS Ca 6a-40	0.2
Methanol / Ethanol (ppm)	10 Max	GC-FID	2.6
Lead (ppm)	0.2 Max.	EN ISO 17294	< 0.05
Arsenic (ppm)	0.2 Max.	EN ISO 17294	< 0.05
Cadmium (ppm)	0.2 Max.	EN ISO 17294	< 0.01
Mercury (ppm)	0.1 Max.	BS EN 13806	0.024

Packaging: Packaged in HDPE drums under nitrogen blanket, 190 kg net.
Storage and Handling: Store in a cool dry place, protect from light, heat and oxygen. The preferred storage temperature is below 30°C.
Shelf Life: Three years in the original package. Once opened, the remaining oil should be blanketed under nitrogen and used as quickly as possible.

Approval:____ (b) (6) Date: 09/19/2012

INNOBIO® Conjugated Linoleic Acid FFA 40 Powder CWD

Code: 40102-40
Lot No.: (b) (6) Date of Manufacture: 08/09/2012

Description: INNOBIO® Conjugated Linoleic Acid FFA 40 Powder CWD is a free flowing and water dispersible powder. It contains approximately 40% (38-44%) of conjugated linoleic acid in free fatty acid form derived from 100% natural safflower seed oil and is spray dried with modified starch, maltodextrin, sodium ascorbate, silicon dioxide, mono- and di-glycerides, natural mixed tocopherols and sunflower seed oil derived lecithin.

Tests	Limits	Methods	Results
Appearance	White or off white, free-flowing powder	Visual	Conforms
Pure CLA Content (%)	38.0-44.0	AOAC 996.06	43.8
Peroxide Value (meq/kg)	10.0 Max.	AOCS Cd 8-53	0.1
Loss on Drying (%)	4.0 Max.	USP 731	0.8
Bulk Density (g/mL)	0.40-0.60	USP 616	0.46
Particle Size	95% through 40 mesh	USP 786	99.9
Lead (ppm)	1.0 Max.	EN ISO 17294	< 0.05
Arsenic (ppm)	1.0 Max.	EN ISO 17294	< 0.05
Cadmium (ppm)	1.0 Max.	EN ISO 17294	< 0.01
Mercury (ppm)	0.1 Max.	BS EN 13806	< 0.005
Total Plate Count (CFU/g)	3,000 Max.	ISO 4833	< 10
Yeasts and Molds (CFU/g)	300 Max.	ISO 7954	< 10
Coliform (CFU/g)	10 Max.	ISO 4832	< 10
E. Coli	Negative	ISO 16649	Negative
Salmonella	Negative/25g	ISO 6579	Negative
Staphyloccous aureus	Negative/g	ISO 6888	Negative

Packaging: Packaged in heat sealed aluminum foil bags under nitrogen blanket, 10 kg net. Two bags packed per corrugated paper carton.
Storage and Handling: Store in a cool dry place, protect from light, heat and oxygen. The preferred storage temperature is below 30°C.
Shelf Life: Two years in the original package. It is recommended to use the entire contents after opening.

Approval:_____ (b) (6) Date: 08/27/2012

INNOBIO® Conjugated Linoleic Acid FFA 40 Powder CWD

Code: 40102-40
Lot No.: (b) (6) Date of Manufacture: 08/21/2012

Description: INNOBIO® Conjugated Linoleic Acid FFA 40 Powder CWD is a free flowing and water dispersible powder. It contains approximately 40% (38-44%) of conjugated linoleic acid in free fatty acid form derived from 100% natural safflower seed oil and is spray dried with modified starch, maltodextrin, sodium ascorbate, silicon dioxide, mono- and di-glycerides, natural mixed tocopherols and sunflower seed oil derived lecithin.

Tests	Limits	Methods	Results
Appearance	White or off white, free-flowing powder	Visual	Conforms
Pure CLA Content (%)	38.0-44.0	AOAC 996.06	41.8
Peroxide Value (meq/kg)	10.0 Max.	AOCS Cd 8-53	0.1
Loss on Drying (%)	4.0 Max.	USP 731	0.6
Bulk Density (g/mL)	0.40-0.60	USP 616	0.46
Particle Size	95% through 40 mesh	USP 786	98.8
Lead (ppm)	1.0 Max.	EN ISO 17294	< 0.05
Arsenic (ppm)	1.0 Max.	EN ISO 17294	< 0.05
Cadmium (ppm)	1.0 Max.	EN ISO 17294	< 0.01
Mercury (ppm)	0.1 Max.	BS EN 13806	< 0.005
Total Plate Count (CFU/g)	3,000 Max.	ISO 4833	< 10
Yeasts and Molds (CFU/g)	300 Max.	ISO 7954	< 10
Coliform (CFU/g)	10 Max.	ISO 4832	< 10
E. Coli	Negative	ISO 16649	Negative
Salmonella	Negative/25g	ISO 6579	Negative
Staphyloccous aureus	Negative/g	ISO 6888	Negative

Packaging: Packaged in heat sealed aluminum foil bags under nitrogen blanket, 10 kg net. Two bags packed per corrugated paper carton.
Storage and Handling: Store in a cool dry place, protect from light, heat and oxygen. The preferred storage temperature is below 30°C.
Shelf Life: Two years in the original package. It is recommended to use the entire contents after opening.

Approval: (b) (6) Date: 08/30/2012

INNOBIO® Conjugated Linoleic Acid FFA 40 Powder CWD

Code: 40102-40
Lot No.: (b) (6) Date of Manufacture: 08/24/2012

Description: INNOBIO® Conjugated Linoleic Acid FFA 40 Powder CWD is a free flowing and water dispersible powder. It contains approximately 40% (38-44%) of conjugated linoleic acid in free fatty acid form derived from 100% natural safflower seed oil and is spray dried with modified starch, maltodextrin, sodium ascorbate, silicon dioxide, mono- and di-glycerides, natural mixed tocopherols and sunflower seed oil derived lecithin.

Tests	Limits	Methods	Results
Appearance	White or off white, free-flowing powder	Visual	Conforms
Pure CLA Content (%)	38.0-44.0	AOAC 996.06	43.0
Peroxide Value (meq/kg)	10.0 Max.	AOCS Cd 8-53	0.1
Loss on Drying (%)	4.0 Max.	USP 731	0.5
Bulk Density (g/mL)	0.40-0.60	USP 616	0.47
Particle Size	95% through 40 mesh	USP 786	98.7
Lead (ppm)	1.0 Max.	EN ISO 17294	< 0.05
Arsenic (ppm)	1.0 Max.	EN ISO 17294	< 0.05
Cadmium (ppm)	1.0 Max.	EN ISO 17294	< 0.01
Mercury (ppm)	0.1 Max.	BS EN 13806	< 0.005
Total Plate Count (CFU/g)	3,000 Max.	ISO 4833	70
Yeasts and Molds (CFU/g)	300 Max.	ISO 7954	< 10
Coliform (CFU/g)	10 Max.	ISO 4832	< 10
E. Coli	Negative	ISO 16649	Negative
Salmonella	Negative/25g	ISO 6579	Negative
Staphyloccous aureus	Negative/g	ISO 6888	Negative

Packaging: Packaged in heat sealed aluminum foil bags under nitrogen blanket, 10 kg net. Two bags packed per corrugated paper carton.
Storage and Handling: Store in a cool dry place, protect from light, heat and oxygen. The preferred storage temperature is below 30°C.
Shelf Life: Two years in the original package. It is recommended to use the entire contents after opening.

Approval: (b) (6) Date: 08/30/2012

INNOBIO® Conjugated Linoleic Acid FFA 40 Powder WDP

Code: 40112-40
Lot No.: (b) (6) Date of Manufacture: 10/10/2012

Description: INNOBIO® Conjugated Linoleic Acid FFA 40 Powder WDP is a free flowing and water dispersible powder. It contains approximately 40% (38-44%) of conjugated linoleic acid in free fatty acid form derived from 100% natural safflower seed oil and is spray dried with sodium caseinate, maltodextrin, silicon dioxide, mono- and di-glycerides, sodium tripolyphosphate, natural mixed tocopherols and sunflower seed oil derived lecithin.

Tests	Limits	Methods	Results
Appearance	White or off white, free-flowing powder	Visual	Conforms
Pure CLA Content (%)	38.0-44.0	AOAC 996.06	42.7
Peroxide Value (meq/kg)	10.0 Max.	AOCS Cd 8-53	0.1
Loss on Drying (%)	4.0 Max.	USP 731	1.0
Bulk Density (g/mL)	0.20-0.50	USP 616	0.47
Particle Size	95% through 40 mesh	USP 786	99.8
Lead (ppm)	1.0 Max.	EN ISO 17294	< 0.05
Arsenic (ppm)	1.0 Max.	EN ISO 17294	< 0.05
Cadmium (ppm)	1.0 Max.	EN ISO 17294	< 0.01
Mercury (ppm)	0.1 Max.	BS EN 13806	< 0.005
Total Plate Count (CFU/g)	3,000 Max.	ISO 4833	<10
Yeasts and Molds (CFU/g)	300 Max.	ISO 7954	<10
Coliform (CFU/g)	10 Max.	ISO 4832	< 10
E. Coli	Negative	ISO 16649	Negative
Salmonella	Negative/25g	ISO 6579	Negative
Staphyloccous aureus	Negative/g	ISO 6888	Negative

Packaging: Packaged in heat sealed aluminum foil bags under nitrogen blanket, 10 kg net. Two bags packed per corrugated paper carton.
Storage and Handling: Store in a cool dry place, protect from light, heat and oxygen. The preferred storage temperature is below 30°C.
Shelf Life: Two years in the original package. It is recommended to use the entire contents after opening.

Approval:_____ (b) (6) Date: 10/24/2012

INNOBIO® Conjugated Linoleic Acid FFA 40 Powder WDP

Code: 40112-40
Lot No.: (b) (6) Date of Manufacture: 09/17/2013

Description: INNOBIO® Conjugated Linoleic Acid FFA 40 Powder WDP is a free flowing and water dispersible powder. It contains approximately 40% (38-44%) of conjugated linoleic acid in free fatty acid form derived from 100% natural safflower seed oil and is spray dried with sodium caseinate, maltodextrin, silicon dioxide, mono- and di-glycerides, sodium tripolyphosphate, natural mixed tocopherols and sunflower seed oil derived lecithin.

Tests	Limits	Methods	Results
Appearance	White or off white, free-flowing powder	Visual	Conforms
Pure CLA Content (%)	38.0-44.0	AOAC 996.06	40.9
Peroxide Value (meq/kg)	10.0 Max.	AOCS Cd 8-53	0.08
Loss on Drying (%)	4.0 Max.	USP 731	0.5
Bulk Density (g/mL)	0.20-0.50	USP 616	0.42
Particle Size	95% through 40 mesh	USP 786	98.9
Lead (ppm)	1.0 Max.	EN ISO 17294	< 0.05
Arsenic (ppm)	1.0 Max.	EN ISO 17294	< 0.05
Cadmium (ppm)	1.0 Max.	EN ISO 17294	< 0.01
Mercury (ppm)	0.1 Max.	BS EN 13806	< 0.005
Total Plate Count (CFU/g)	3,000 Max.	ISO 4833	<10
Yeasts and Molds (CFU/g)	300 Max.	ISO 7954	<10
Coliform (CFU/g)	10 Max.	ISO 4832	< 10
E. Coli	Negative	ISO 16649	Negative
Salmonella	Negative/25g	ISO 6579	Negative
Staphyloccous aureus	Negative/g	ISO 6888	Negative

Packaging: Packaged in heat sealed aluminum foil bags under nitrogen blanket, 10 kg net. Two bags packed per corrugated paper carton.
Storage and Handling: Store in a cool dry place, protect from light, heat and oxygen. The preferred storage temperature is below 30°C.
Shelf Life: Two years in the original package. It is recommended to use the entire contents after opening.

Approval:_____ (b) (6) Date: 09/30/2013

INNOBIO® Conjugated Linoleic Acid FFA 40 Powder WDP

Code: 40112-40
Lot No.:(b) (6) Date of Manufacture: 07/06/2011

Description: INNOBIO® Conjugated Linoleic Acid FFA 40 Powder WDP is a free flowing and water dispersible powder. It contains approximately 40% (38-44%) of conjugated linoleic acid in free fatty acid form derived from 100% natural safflower seed oil and is spray dried with sodium caseinate, maltodextrin, silicon dioxide, mono- and di-glycerides, sodium tripolyphosphate, natural mixed tocopherols and sunflower seed oil derived lecithin.

Tests	Limits	Methods	Results
Appearance	White or off white, free-flowing powder	Visual	Conforms
Pure CLA Content (%)	38.0-44.0	AOAC 996.06	43.1
Peroxide Value (meq/kg)	10.0 Max.	AOCS Cd 8-53	0.1
Loss on Drying (%)	4.0 Max.	USP 731	0.6
Bulk Density (g/mL)	0.20-0.50	USP 616	0.44
Particle Size	95% through 40 mesh	USP 786	99.9
Lead (ppm)	1.0 Max.	EN ISO 17294	< 0.05
Arsenic (ppm)	1.0 Max.	EN ISO 17294	< 0.05
Cadmium (ppm)	1.0 Max.	EN ISO 17294	< 0.01
Mercury (ppm)	0.1 Max.	BS EN 13806	< 0.005
Total Plate Count (CFU/g)	3,000 Max.	ISO 4833	<10
Yeasts and Molds (CFU/g)	300 Max.	ISO 7954	<10
Coliform (CFU/g)	10 Max.	ISO 4832	< 10
E. Coli	Negative	ISO 16649	Negative
Salmonella	Negative/25g	ISO 6579	Negative
Staphyloccous aureus	Negative/g	ISO 6888	Negative

Packaging: Packaging: Packaged in heat sealed aluminum foil bags under nitrogen blanket, 10 kg net. Two bags packed per corrugated paper carton.
Storage and Handling: Store in a cool dry place, protect from light, heat and oxygen. The preferred storage temperature is below 30°C.
Shelf Life: Two years in the original package. It is recommended to use the entire contents after opening.

Approval:_____(b) (6) Date: 07/13/2011

INNOBIO® Conjugated Linoleic Acid TG 40 Powder CWD

Code: 40101-40
Lot No.: (b) (6) Date of Manufacture: 11/09/2011

Description: INNOBIO® Conjugated Linoleic Acid TG 40 Powder CWD is a free flowing and water dispersible powder. It contains approximately 40% (38-43%) of conjugated linoleic acid in triglyceride form derived from 100% natural safflower seed oil and is spray dried with gum arabic, maltodextrin, silicon dioxide, mono- and di-glycerides, natural mixed tocopherols and sunflower seed oil derived lecithin.

Tests	Limits	Methods	Results
Appearance	White or off white, free-flowing powder	Visual	Conforms
Pure CLA Content (%)	38.0-44.0	AOAC 996.06	40.8
Peroxide Value (meq/kg)	10.0 Max.	AOCS Cd 8-53	0.2
Loss on Drying (%)	4.0 Max.	USP 731	0.02
Bulk Density (g/mL)	0.40-0.60	USP 616	0.44
Particle Size	95% through 40 mesh	USP 786	99.1
Lead (ppm)	1.0 Max.	EN ISO 17294	< 0.05
Arsenic (ppm)	1.0 Max.	EN ISO 17294	< 0.05
Cadmium (ppm)	1.0 Max.	EN ISO 17294	< 0.01
Mercury (ppm)	0.1 Max.	BS EN 13806	< 0.005
Total Plate Count (CFU/g)	3,000 Max.	ISO 4833	<10
Yeasts and Molds (CFU/g)	300 Max.	ISO 7954	<10
Coliform (CFU/g)	10 Max.	ISO 4832	<10
E. Coli	Negative	ISO 16649	Negative
Salmonella	Negative/25g	ISO 6579	Negative
Staphyloccous aureus	Negative/g	ISO 6888	Negative

Packaging: Packaged in heat sealed aluminum foil bags under nitrogen blanket, 10 kg net. Two bags packed per corrugated paper carton.
Storage and Handling: Store in a cool dry place, protect from light, heat and oxygen. The preferred storage temperature is below 30°C.
Shelf Life: Two years in the original package. It is recommended to use the entire contents after opening.

Approval:_____ (b) (6) Date:_11/25/2011_

INNOBIO® Conjugated Linoleic Acid TG 40 Powder CWD

Code: 40101-40
Lot No.: (b) (6) Date of Manufacture: 12/05/2011

Description: INNOBIO® Conjugated Linoleic Acid TG 40 Powder CWD is a free flowing and water dispersible powder. It contains approximately 40% (38-43%) of conjugated linoleic acid in triglyceride form derived from 100% natural safflower seed oil and is spray dried with gum arabic, maltodextrin, silicon dioxide, mono- and di-glycerides, natural mixed tocopherols and sunflower seed oil derived lecithin.

Tests	Limits	Methods	Results
Appearance	White or off white, free-flowing powder	Visual	Conforms
Pure CLA Content (%)	38.0-44.0	AOAC 996.06	39.0
Peroxide Value (meq/kg)	10.0 Max.	AOCS Cd 8-53	0.08
Loss on Drying (%)	4.0 Max.	USP 731	1.0
Bulk Density (g/mL)	0.40-0.60	USP 616	0.45
Particle Size	95% through 40 mesh	USP 786	98.9
Lead (ppm)	1.0 Max.	EN ISO 17294	< 0.05
Arsenic (ppm)	1.0 Max.	EN ISO 17294	< 0.05
Cadmium (ppm)	1.0 Max.	EN ISO 17294	< 0.01
Mercury (ppm)	0.1 Max.	BS EN 13806	< 0.005
Total Plate Count (CFU/g)	3,000 Max.	ISO 4833	<10
Yeasts and Molds (CFU/g)	300 Max.	ISO 7954	<10
Coliform (CFU/g)	10 Max.	ISO 4832	< 0.3
E. Coli	Negative	ISO 16649	Negative
Salmonella	Negative/25g	ISO 6579	Negative
Staphyloccous aureus	Negative/g	ISO 6888	Negative

Packaging: Packaged in heat sealed aluminum foil bags under nitrogen blanket, 10 kg net. Two bags packed per corrugated paper carton.
Storage and Handling: Store in a cool dry place, protect from light, heat and oxygen. The preferred storage temperature is below 30°C.
Shelf Life: Two years in the original package. It is recommended to use the entire contents after opening.

Approval:_____ (b) (6) Date: 12/23/2011

INNOBIO® Conjugated Linoleic Acid TG 40 Powder CWD

Code: 40101-40
Lot No.: (b) (6) Date of Manufacture: 11/23/2011

Description: INNOBIO® Conjugated Linoleic Acid TG 40 Powder CWD is a free flowing and water dispersible powder. It contains approximately 40% (38-43%) of conjugated linoleic acid in triglyceride form derived from 100% natural safflower seed oil and is spray dried with gum arabic, maltodextrin, silicon dioxide, mono- and di-glycerides, natural mixed tocopherols and sunflower seed oil derived lecithin.

Tests	Limits	Methods	Results
Appearance	White or off white, free-flowing powder	Visual	Conforms
Pure CLA Content (%)	38.0-44.0	AOAC 996.06	40.5
Peroxide Value (meq/kg)	10.0 Max.	AOCS Cd 8-53	0.04
Loss on Drying (%)	4.0 Max.	USP 731	1.6
Bulk Density (g/mL)	0.40-0.60	USP 616	0.44
Particle Size	95% through 40 mesh	USP 786	99.9
Lead (ppm)	1.0 Max.	EN ISO 17294	< 0.05
Arsenic (ppm)	1.0 Max.	EN ISO 17294	< 0.05
Cadmium (ppm)	1.0 Max.	EN ISO 17294	< 0.01
Mercury (ppm)	0.1 Max.	BS EN 13806	< 0.005
Total Plate Count (CFU/g)	3,000 Max.	ISO 4833	<10
Yeasts and Molds (CFU/g)	300 Max.	ISO 7954	<10
Coliform (CFU/g)	10 Max.	ISO 4832	<10
E. Coli	Negative	ISO 16649	Negative
Salmonella	Negative/25g	ISO 6579	Negative
Staphyloccous aureus	Negative/g	ISO 6888	Negative

Packaging: Packaged in heat sealed aluminum foil bags under nitrogen blanket, 10 kg net. Two bags packed per corrugated paper carton.
Storage and Handling: Store in a cool dry place, protect from light, heat and oxygen. The preferred storage temperature is below 30°C.
Shelf Life: Two years in the original package. It is recommended to use the entire contents after opening.

Approval:_____ (b) (6) Date:_12/04/2011_

INNOBIO® Conjugated Linoleic Acid TG 40 Powder WDP

Code: 40111-40
Lot No.: (b) (6) Date of Manufacture: 07/17/2011

Description: INNOBIO® Conjugated Linoleic Acid TG 40 Powder WDP is a free flowing and water dispersible powder. It contains approximately 40% (38-43%) of conjugated linoleic acid in triglyceride form derived from 100% natural safflower seed oil and is spray dried with sodium caseinate, maltodextrin, silicon dioxide, mono- and di-glycerides, sodium tripolyphosphate, natural mixed tocopherols and sunflower seed oil derived lecithin.

Tests	Limits	Methods	Results
Appearance	White or off white, free-flowing powder	Visual	Conforms
Pure CLA Content (%)	38.0-44.0	AOAC 996.06	41.0
Peroxide Value (meq/kg)	10.0 Max.	AOCS Cd 8-53	0.3
Loss on Drying (%)	4.0 Max.	USP 731	0.04
Bulk Density (g/mL)	0.20-0.50	USP 616	0.41
Particle Size	95% through 40 mesh	USP 786	99.2
Lead (ppm)	1.0 Max.	EN ISO 17294	< 0.05
Arsenic (ppm)	1.0 Max.	EN ISO 17294	< 0.05
Cadmium (ppm)	1.0 Max.	EN ISO 17294	< 0.01
Mercury (ppm)	0.1 Max.	BS EN 13806	< 0.005
Total Plate Count (CFU/g)	3,000 Max.	ISO 4833	<10
Yeasts and Molds (CFU/g)	300 Max.	ISO 7954	<10
Coliform (CFU/g)	10 Max.	ISO 4832	<10
E. Coli	Negative	ISO 16649	Negative
Salmonella	Negative/25g	ISO 6579	Negative
Staphyloccous aureus	Negative/g	ISO 6888	Negative

Packaging: Packaged in heat sealed aluminum foil bags under nitrogen blanket, 10 kg net. Two bags packed per corrugated paper carton.
Storage and Handling: Store in a cool dry place, protect from light, heat and oxygen. The preferred storage temperature is below 30°C.
Shelf Life: Two years in the original package. It is recommended to use the entire contents after opening.

Approval:_____ (b) (6) Date:_07/25/2011_

INNOBIO® Conjugated Linoleic Acid TG 40 Powder WDP

Code: 40111-40
Lot No.: (b) (6) Date of Manufacture: 09/05/2011

Description: INNOBIO® Conjugated Linoleic Acid TG 40 Powder WDP is a free flowing and water dispersible powder. It contains approximately 40% (38-43%) of conjugated linoleic acid in triglyceride form derived from 100% natural safflower seed oil and is spray dried with sodium caseinate, maltodextrin, silicon dioxide, mono- and di-glycerides, sodium tripolyphosphate, natural mixed tocopherols and sunflower seed oil derived lecithin.

Tests	Limits	Methods	Results
Appearance	White or off white, free-flowing powder	Visual	Conforms
Pure CLA Content (%)	38.0-44.0	AOAC 996.06	38.7
Peroxide Value (meq/kg)	10.0 Max.	AOCS Cd 8-53	0.1
Loss on Drying (%)	4.0 Max.	USP 731	1.0
Bulk Density (g/mL)	0.20-0.50	USP 616	0.42
Particle Size	95% through 40 mesh	USP 786	99.7
Lead (ppm)	1.0 Max.	EN ISO 17294	< 0.05
Arsenic (ppm)	1.0 Max.	EN ISO 17294	< 0.05
Cadmium (ppm)	1.0 Max.	EN ISO 17294	< 0.01
Mercury (ppm)	0.1 Max.	BS EN 13806	< 0.005
Total Plate Count (CFU/g)	3,000 Max.	ISO 4833	<10
Yeasts and Molds (CFU/g)	300 Max.	ISO 7954	<10
Coliform (CFU/g)	10 Max.	ISO 4832	<10
E. Coli	Negative	ISO 16649	Negative
Salmonella	Negative/25g	ISO 6579	Negative
Staphyloccous aureus	Negative/g	ISO 6888	Negative

Packaging: Packaged in heat sealed aluminum foil bags under nitrogen blanket, 10 kg net. Two bags packed per corrugated paper carton.
Storage and Handling: Store in a cool dry place, protect from light, heat and oxygen. The preferred storage temperature is below 30°C.
Shelf Life: Two years in the original package. It is recommended to use the entire contents after opening.

Approval:_____ (b) (6) Date:_09/13/2011_

INNOBIO® Conjugated Linoleic Acid TG 40 Powder WDP

Code: 40111-40
Lot No.: (b) (6) Date of Manufacture: 03/28/2012

Description: INNOBIO® Conjugated Linoleic Acid TG 40 Powder WDP is a free flowing and water dispersible powder. It contains approximately 40% (38-43%) of conjugated linoleic acid in triglyceride form derived from 100% natural safflower seed oil and is spray dried with sodium caseinate, maltodextrin, silicon dioxide, mono- and di-glycerides, sodium tripolyphosphate, natural mixed tocopherols and sunflower seed oil derived lecithin.

Tests	Limits	Methods	Results
Appearance	White or off white, free-flowing powder	Visual	Conforms
Pure CLA Content (%)	38.0-44.0	AOAC 996.06	40.9
Peroxide Value (meq/kg)	10.0 Max.	AOCS Cd 8-53	0.07
Loss on Drying (%)	4.0 Max.	USP 731	1.2
Bulk Density (g/mL)	0.20-0.50	USP 616	0.44
Particle Size	95% through 40 mesh	USP 786	99.1
Lead (ppm)	1.0 Max.	EN ISO 17294	< 0.05
Arsenic (ppm)	1.0 Max.	EN ISO 17294	< 0.05
Cadmium (ppm)	1.0 Max.	EN ISO 17294	< 0.01
Mercury (ppm)	0.1 Max.	BS EN 13806	< 0.005
Total Plate Count (CFU/g)	3,000 Max.	ISO 4833	<10
Yeasts and Molds (CFU/g)	300 Max.	ISO 7954	<10
Coliform (CFU/g)	10 Max.	ISO 4832	<10
E. Coli	Negative	ISO 16649	Negative
Salmonella	Negative/25g	ISO 6579	Negative
Staphyloccous aureus	Negative/g	ISO 6888	Negative

Packaging: Packaged in heat sealed aluminum foil bags under nitrogen blanket, 10 kg net. Two bags packed per corrugated paper carton.
Storage and Handling: Store in a cool dry place, protect from light, heat and oxygen. The preferred storage temperature is below 30°C.
Shelf Life: Two years in the original package. It is recommended to use the entire contents after opening.

Approval:_____ (b) (6) Date:_04/10/2012_

INNOBIO® Conjugated Linoleic Acid TG 60 Powder WDP

Code: 40111-60
Lot No.: (b) (6) Date of Manufacture: 04/26/2013

Description: INNOBIO® Conjugated Linoleic Acid TG 60 Powder WDP is a free flowing and water dispersible powder. It contains approximately 60% (57-64%) of conjugated linoleic acid in triglyceride form derived from 100% natural safflower seed oil and is spray dried with sodium caseinate, maltodextrin, silicon dioxide, mono- and di-glycerides, sodium tripolyphosphate, natural mixed tocopherols and sunflower seed oil derived lecithin.

Tests	Limits	Methods	Results
Appearance	White or off white, free-flowing powder	Visual	Conforms
Pure CLA Content (%)	57.0-64.0	AOAC 996.06	58.0
Peroxide Value (meq/kg)	10.0 Max.	AOCS Cd 8-53	0.1
Loss on Drying (%)	4.0 Max.	USP 731	0.1
Bulk Density (g/mL)	0.20-0.50	USP 616	0.41
Particle Size	95% through 40 mesh	USP 786	99.5
Lead (ppm)	1.0 Max.	EN ISO 17294	< 0.05
Arsenic (ppm)	1.0 Max.	EN ISO 17294	< 0.05
Cadmium (ppm)	1.0 Max.	EN ISO 17294	< 0.01
Mercury (ppm)	0.1 Max.	BS EN 13806	< 0.005
Total Plate Count (CFU/g)	3,000 Max.	ISO 4833	<10
Yeasts and Molds (CFU/g)	300 Max.	ISO 7954	<10
Coliform (CFU/g)	10 Max.	ISO 4832	<10
E. Coli	Negative	ISO 16649	Negative
Salmonella	Negative/25g	ISO 6579	Negative
Staphyloccous aureus	Negative/g	ISO 6888	Negative

Packaging: Packaged in heat sealed aluminum foil bags under nitrogen blanket, 10 kg net. Two bags packed per corrugated paper carton.
Storage and Handling: Store in a cool dry place, protect from light, heat and oxygen. The preferred storage temperature is below 30°C.
Shelf Life: Two years in the original package. It is recommended to use the entire contents after opening.

Approval: (b) (6) Date: 05/03/2013

INNOBIO® Conjugated Linoleic Acid TG 60 Powder WDP

Code: 40111-60
Lot No.: (b) (6) Date of Manufacture: 05/12/2013

Description: INNOBIO® Conjugated Linoleic Acid TG 60 Powder WDP is a free flowing and water dispersible powder. It contains approximately 60% (57-64%) of conjugated linoleic acid in triglyceride form derived from 100% natural safflower seed oil and is spray dried with sodium caseinate, maltodextrin, silicon dioxide, mono- and di-glycerides, sodium tripolyphosphate, natural mixed tocopherols and sunflower seed oil derived lecithin.

Tests	Limits	Methods	Results
Appearance	White or off white,free-flowing powder	Visual	Conforms
Pure CLA Content (%)	57.0-64.0	AOAC 996.06	61.9
Peroxide Value (meq/kg)	10.0 Max.	AOCS Cd 8-53	0.6
Loss on Drying (%)	4.0 Max.	USP 731	1.0
Bulk Density (g/mL)	0.20-0.50	USP 616	0.42
Particle Size	95% through 40 mesh	USP 786	98.8
Lead (ppm)	1.0 Max.	EN ISO 17294	< 0.05
Arsenic (ppm)	1.0 Max.	EN ISO 17294	< 0.05
Cadmium (ppm)	1.0 Max.	EN ISO 17294	< 0.01
Mercury (ppm)	0.1 Max.	BS EN 13806	< 0.005
Total Plate Count (CFU/g)	3,000 Max.	ISO 4833	<10
Yeasts and Molds (CFU/g)	300 Max.	ISO 7954	<10
Coliform (CFU/g)	10 Max.	ISO 4832	<10
E. Coli	Negative	ISO 16649	Negative
Salmonella	Negative/25g	ISO 6579	Negative
Staphyloccous aureus	Negative/g	ISO 6888	Negative

Packaging: Packaged in heat sealed aluminum foil bags under nitrogen blanket, 10 kg net. Two bags packed per corrugated paper carton.
Storage and Handling: Store in a cool dry place, protect from light, heat and oxygen. The preferred storage temperature is below 30°C.
Shelf Life: Two years in the original package. It is recommended to use the entire contents after opening.

Approval: (b) (6) Date: 05/24/2013

INNOBIO® Conjugated Linoleic Acid TG 60 Powder WDP

Code: 40111-60
Lot N (b) (6) Date of Manufacture: 05/21/2012

Description: INNOBIO® Conjugated Linoleic Acid TG 60 Powder WDP is a free flowing and water dispersible powder. It contains approximately 60% (57-64%) of conjugated linoleic acid in triglyceride form derived from 100% natural safflower seed oil and is spray dried with sodium caseinate, maltodextrin, silicon dioxide, mono- and di-glycerides, sodium tripolyphosphate, natural mixed tocopherols and sunflower seed oil derived lecithin.

Tests	Limits	Methods	Results
Appearance	White or off white,free-flowing powder	Visual	Conforms
Pure CLA Content (%)	57.0-64.0	AOAC 996.06	60.4
Peroxide Value (meq/kg)	10.0 Max.	AOCS Cd 8-53	0.8
Loss on Drying (%)	4.0 Max.	USP 731	1.0
Bulk Density (g/mL)	0.20-0.50	USP 616	0.44
Particle Size	95% through 40 mesh	USP 786	99.8
Lead (ppm)	1.0 Max.	EN ISO 17294	< 0.05
Arsenic (ppm)	1.0 Max.	EN ISO 17294	< 0.05
Cadmium (ppm)	1.0 Max.	EN ISO 17294	< 0.01
Mercury (ppm)	0.1 Max.	BS EN 13806	< 0.005
Total Plate Count (CFU/g)	3,000 Max.	ISO 4833	<10
Yeasts and Molds (CFU/g)	300 Max.	ISO 7954	<10
Coliform (CFU/g)	10 Max.	ISO 4832	<10
E. Coli	Negative	ISO 16649	Negative
Salmonella	Negative/25g	ISO 6579	Negative
Staphyloccous aureus	Negative/g	ISO 6888	Negative

Packaging: Packaged in heat sealed aluminum foil bags under nitrogen blanket, 10 kg net. Two bags packed per corrugated paper carton.
Storage and Handling: Store in a cool dry place, protect from light, heat and oxygen. The preferred storage temperature is below 30°C.
Shelf Life: Two years in the original package. It is recommended to use the entire contents after opening.

Approval: (b) (6) Date: 05/31/2012

APPENDIX D. NHANES FOOD CODES

Group No.	Group description	Intended use level, %	NHANES food codes	Food description
1	Soymilk	0.625	11320000	Milk, soy, ready-to-drink, not baby's
1	Soymilk	0.625	11320100	Milk, soy, light, ready-to-drink, not baby's
1	Soymilk	0.625	11320200	Milk, soy, nonfat, ready-to-drink, not baby's
1	Soymilk	0.625	11321000	Milk, soy, ready-to-drink, not baby's, chocolate
1	Soymilk	0.625	11321100	Milk, soy, light, ready-to-drink, not baby's, chocolate
1	Soymilk	0.625	11321200	Milk, soy, nonfat, ready-to-drink, not baby's, chocolate
2	Meal Replacement Beverages	0.625	11611000	Instant breakfast, fluid, canned
2	Meal Replacement Beverages	0.625	11612000	Instant breakfast, powder, milk added
2	Meal Replacement Beverages	0.625	11613000	Instant breakfast, powder, sweetened with low calorie sweetener, milk added
2	Meal Replacement Beverages	0.625	11622010	Diet beverage, powder, reconstituted with skim milk
2	Meal Replacement Beverages	0.625	11623000	Meal supplement or replacement, commercially prepared, ready-to-drink
2	Meal Replacement Beverages	0.625	11631000	High calorie beverage, canned or powdered, reconstituted
2	Meal Replacement Beverages	0.625	11641000	Meal supplement or replacement, milk-based, high protein, liquid
2	Meal Replacement Beverages	0.625	11641020	Meal replacement or supplement, milk based, ready-to-drink
2	Meal Replacement Beverages	0.625	11830940	Meal replacement, high protein, milk based, fruit juice mixable formula, powdered, not reconstituted
4	Meal Replacement Bars	3.75	41435000	Fiber One Fulfill Bar
4	Meal Replacement Bars	3.75	41435110	High protein bar, candy-like, soy and milk base
4	Meal Replacement Bars	3.75	41435120	Zone Perfect Classic Crunch nutrition bar
4	Meal Replacement Bars	3.75	41435300	Balance Original Bar
4	Meal Replacement Bars	3.75	41435500	Clif Bar
4	Meal Replacement Bars	3.75	41435700	South Beach Living High Protein Cereal Bar
4	Meal Replacement Bars	3.75	41435710	South Beach Living Meal Replacement Bar
4	Meal Replacement Bars	3.75	53540000	Breakfast bar, NFS
4	Meal Replacement Bars	3.75	53540200	Breakfast bar, cereal crust with fruit filling, lowfat
4	Meal Replacement Bars	3.75	53540300	Fiber One Chewy Bar
4	Meal Replacement Bars	3.75	53540400	Kellogg's Nutri-Grain Cereal Bar
4	Meal Replacement Bars	3.75	53540402	Kellogg's Nutri-Grain Yogurt Bar
4	Meal Replacement Bars	3.75	53540404	Kellogg's Nutri-Grain Fruit and Nut Bar

4	Meal Replacement Bars	3.75	53540500	Breakfast bar, date, with yogurt coating
4	Meal Replacement Bars	3.75	53540600	Milk 'n Cereal bar
4	Meal Replacement Bars	3.75	53540700	Kellogg's Special K bar
4	Meal Replacement Bars	3.75	53540800	Kashi GOLEAN Chewy Bars
4	Meal Replacement Bars	3.75	53540802	Kashi TLC Chewy Granola Bar
4	Meal Replacement Bars	3.75	53540804	Kashi GOLEAN Crunchy Bars
4	Meal Replacement Bars	3.75	53540806	Kashi TLC Crunchy Granola Bar
4	Meal Replacement Bars	3.75	53540900	Nature Valley Chewy Trail Mix Granola Bar
4	Meal Replacement Bars	3.75	53540902	Nature Valley Chewy Granola Bar with Yogurt Coating
4	Meal Replacement Bars	3.75	53540904	Nature Valley Sweet and Salty Nut Granola Bar
4	Meal Replacement Bars	3.75	53540906	Nature Valley Crunchy Granola Bar
4	Meal Replacement Bars	3.75	53541000	Quaker Chewy Granola Bar
4	Meal Replacement Bars	3.75	53541002	Quaker Chewy 90 Calorie Granola Bar
4	Meal Replacement Bars	3.75	53541004	Quaker Chewy 25% Less Sugar Granola Bar
4	Meal Replacement Bars	3.75	41435000	Fiber One Fulfill Bar
4	Meal Replacement Bars	3.75	41435110	High protein bar, candy-like, soy and milk base
4	Meal Replacement Bars	3.75	41435120	Zone Perfect Classic Crunch nutrition bar
4	Meal Replacement Bars	3.75	41435300	Balance Original Bar
4	Meal Replacement Bars	3.75	41435500	Clif Bar
4	Meal Replacement Bars	3.75	41435700	South Beach Living High Protein Cereal Bar
4	Meal Replacement Bars	3.75	41435710	South Beach Living Meal Replacement Bar
4	Meal Replacement Bars	3.75	53540000	Breakfast bar, NFS
4	Meal Replacement Bars	3.75	53540200	Breakfast bar, cereal crust with fruit filling, lowfat
4	Meal Replacement Bars	3.75	53540300	Fiber One Chewy Bar
4	Meal Replacement Bars	3.75	53540400	Kellogg's Nutri-Grain Cereal Bar
4	Meal Replacement Bars	3.75	53540402	Kellogg's Nutri-Grain Yogurt Bar
4	Meal Replacement Bars	3.75	53540404	Kellogg's Nutri-Grain Fruit and Nut Bar
4	Meal Replacement Bars	3.75	53540500	Breakfast bar, date, with yogurt coating
4	Meal Replacement Bars	3.75	53540600	Milk 'n Cereal bar
4	Meal Replacement Bars	3.75	53540700	Kellogg's Special K bar
4	Meal Replacement Bars	3.75	53540800	Kashi GOLEAN Chewy Bars
4	Meal Replacement Bars	3.75	53540802	Kashi TLC Chewy Granola Bar
4	Meal Replacement Bars	3.75	53540804	Kashi GOLEAN Crunchy Bars
4	Meal Replacement Bars	3.75	53540806	Kashi TLC Crunchy Granola Bar
4	Meal Replacement Bars	3.75	53540900	Nature Valley Chewy Trail Mix Granola Bar
4	Meal Replacement Bars	3.75	53540902	Nature Valley Chewy Granola Bar with Yogurt Coating

4	Meal Replacement Bars	3.75	53540904	Nature Valley Sweet and Salty Nut Granola Bar
4	Meal Replacement Bars	3.75	53540906	Nature Valley Crunchy Granola Bar
4	Meal Replacement Bars	3.75	53541000	Quaker Chewy Granola Bar
4	Meal Replacement Bars	3.75	53541002	Quaker Chewy 90 Calorie Granola Bar
4	Meal Replacement Bars	3.75	53541004	Quaker Chewy 25% Less Sugar Granola Bar
4	Meal Replacement Bars	3.75	53541006	Quaker Chewy Dipps Granola Bar
4	Meal Replacement Bars	3.75	53541200	Meal replacement bar
4	Meal Replacement Bars	3.75	53541300	Slim Fast Original Meal Bar
4	Meal Replacement Bars	3.75	53542000	Snack bar, oatmeal
4	Meal Replacement Bars	3.75	53542100	Granola bar, NFS
4	Meal Replacement Bars	3.75	53542200	Granola bar, lowfat, NFS
4	Meal Replacement Bars	3.75	53542210	Granola bar, nonfat
4	Meal Replacement Bars	3.75	53543000	Granola bar, reduced sugar, NFS
4	Meal Replacement Bars	3.75	53543100	Granola bar, peanuts, oats, sugar, wheat germ
4	Meal Replacement Bars	3.75	53544200	Granola bar, chocolate-coated, NFS
4	Meal Replacement Bars	3.75	53544210	Granola bar, with coconut, chocolate-coated
4	Meal Replacement Bars	3.75	53544220	Granola bar with nuts, chocolate-coated
4	Meal Replacement Bars	3.75	53544230	Granola bar, oats, nuts, coated with non-chocolate coating
4	Meal Replacement Bars	3.75	53544250	Granola bar, coated with non-chocolate coating
4	Meal Replacement Bars	3.75	53544300	Granola bar, high fiber, coated with non-chocolate yogurt coating
4	Meal Replacement Bars	3.75	53544400	Granola bar, with rice cereal
4	Meal Replacement Bars	3.75	53544410	Quaker Granola Bites
4	Meal Replacement Bars	3.75	53544450	PowerBar (fortified high energy bar)
5	Flavored Milk	0.625	11511000	Milk, chocolate, NFS
5	Flavored Milk	0.625	11511100	Milk, chocolate, whole milk-based
5	Flavored Milk	0.625	11511200	Milk, chocolate, reduced fat milk-based, 2% (formerly "lowfat")
5	Flavored Milk	0.625	11511300	Milk, chocolate, skim milk-based
5	Flavored Milk	0.625	11511400	Milk, chocolate, lowfat milk-based
5	Flavored Milk	0.625	11513000	Cocoa and sugar mixture, milk added, NS as to type of milk
5	Flavored Milk	0.625	11513100	Cocoa and sugar mixture, whole milk added
5	Flavored Milk	0.625	11513150	Cocoa and sugar mixture, reduced fat milk added
5	Flavored Milk	0.625	11513200	Cocoa and sugar mixture, lowfat milk added
5	Flavored Milk	0.625	11513300	Cocoa and sugar mixture, skim milk added
5	Flavored Milk	0.625	11513400	Chocolate syrup, milk added, NS as to type of milk
5	Flavored Milk	0.625	11513500	Chocolate syrup, whole milk added
5	Flavored Milk	0.625	11513550	Chocolate syrup, reduced fat milk added

5	Flavored Milk	0.625	11513600	Chocolate syrup, lowfat milk added
5	Flavored Milk	0.625	11513700	Chocolate syrup, skim milk added
5	Flavored Milk	0.625	11514100	Cocoa, sugar, and dry milk mixture, water added
5	Flavored Milk	0.625	11514300	Cocoa with nonfat dry milk and low calorie sweetener, mixture, water added
5	Flavored Milk	0.625	11514500	Cocoa, whey, and low calorie sweetener, mixture, fortified, water added
5	Flavored Milk	0.625	11515100	Cocoa and sugar mixture fortified with vitamins and minerals, milk added, NS as to type of milk, Puerto Rican style
5	Flavored Milk	0.625	11515400	Cocoa with nonfat dry milk and low calorie sweetener, high calcium, water added
5	Flavored Milk	0.625	11516000	Cocoa, whey, and low-calorie sweetener mixture, lowfat milk added
5	Flavored Milk	0.625	11518000	Milk beverage with nonfat dry milk and low calorie sweetener, water added, chocolate
5	Flavored Milk	0.625	11518050	Milk beverage with nonfat dry milk and low calorie sweetener, water added, flavors other than chocolate
5	Flavored Milk	0.625	11518100	Milk beverage with nonfat dry milk and low calorie sweetener, high calcium, water added, chocolate
5	Flavored Milk	0.625	11519000	Milk beverage, made with whole milk, flavors other than chocolate
5	Flavored Milk	0.625	11519040	Milk, flavors other than chocolate, NFS
5	Flavored Milk	0.625	11519050	Milk, flavors other than chocolate, whole milk-based
5	Flavored Milk	0.625	11519105	Milk, flavors other than chocolate, reduced fat milk-based
5	Flavored Milk	0.625	11519200	Milk, flavors other than chocolate, lowfat milk-based
5	Flavored Milk	0.625	11519205	Milk, flavors other than chocolate, skim-milk based
5	Flavored Milk	0.625	11520000	Milk, malted, unfortified, NS as to flavor, made with milk
5	Flavored Milk	0.625	11521000	Milk, malted, unfortified, chocolate, made with milk
5	Flavored Milk	0.625	11522000	Milk, malted, unfortified, natural flavor, made with milk
5	Flavored Milk	0.625	11525000	Milk, malted, fortified, natural flavor, made with milk
5	Flavored Milk	0.625	11526000	Milk, malted, fortified, chocolate, made with milk
5	Flavored Milk	0.625	11527000	Milk, malted, fortified, NS as to flavor, made with milk
5	Flavored Milk	0.625	11541000	Milk shake, NS as to flavor or type
5	Flavored Milk	0.625	11541400	Milk shake with malt
5	Flavored Milk	0.625	11541500	Milk shake, made with skim milk, chocolate
5	Flavored Milk	0.625	11541510	Milk shake, made with skim milk, flavors other than chocolate
5	Flavored Milk	0.625	11551050	Milk fruit drink
5	Flavored Milk	0.625	11553000	Fruit smoothie drink, made with fruit or fruit juice and dairy products
5	Flavored Milk	0.625	11553100	Fruit smoothie drink, NFS

5	Flavored Milk	0.625	11560000	Chocolate-flavored drink, whey- and milk-based
5	Flavored Milk	0.625	11560020	Flavored milk drink, whey- and milk-based, flavors other than chocolate
5	Flavored Milk	0.625	11561000	Cafe con leche
5	Flavored Milk	0.625	11561010	Cafe con leche prepared with sugar
6	Milk	0.625	11114000	Milk, cow's, fluid, filled with vegetable oil, NS as to percent fat
6	Milk	0.625	11114100	Milk, cow's, fluid, filled with vegetable oil, whole
6	Milk	0.625	11114200	Milk, cow's, fluid, filled with vegetable oil, lowfat
7	Yogurt	0.667	11410000	Yogurt, NS as to type of milk or flavor
7	Yogurt	0.667	11411010	Yogurt, plain, NS as to type of milk
7	Yogurt	0.667	11411100	Yogurt, plain, whole milk
7	Yogurt	0.667	11411200	Yogurt, plain, lowfat milk
7	Yogurt	0.667	11411300	Yogurt, plain, nonfat milk
7	Yogurt	0.667	11420000	Yogurt, vanilla, lemon, or coffee flavor, NS as to type of milk
7	Yogurt	0.667	11421000	Yogurt, vanilla, lemon, or coffee flavor, whole milk
7	Yogurt	0.667	11422000	Yogurt, vanilla, lemon, maple, or coffee flavor, lowfat milk
7	Yogurt	0.667	11422100	Yogurt, vanilla, lemon, maple, or coffee flavor, lowfat milk, sweetened with low calorie sweetener
7	Yogurt	0.667	11423000	Yogurt, vanilla, lemon, maple, or coffee flavor, nonfat milk
7	Yogurt	0.667	11424000	Yogurt, vanilla, lemon, maple, or coffee flavor, nonfat milk, sweetened with low calorie sweetener
7	Yogurt	0.667	11425000	Yogurt, chocolate, NS as to type of milk
7	Yogurt	0.667	11426000	Yogurt, chocolate, whole milk
7	Yogurt	0.667	11427000	Yogurt, chocolate, nonfat milk
7	Yogurt	0.667	11430000	Yogurt, fruit variety, NS as to type of milk
7	Yogurt	0.667	11431000	Yogurt, fruit variety, whole milk
7	Yogurt	0.667	11432000	Yogurt, fruit variety, lowfat milk
7	Yogurt	0.667	11432500	Yogurt, fruit variety, lowfat milk, sweetened with low-calorie sweetener
7	Yogurt	0.667	11433000	Yogurt, fruit variety, nonfat milk
7	Yogurt	0.667	11433500	Yogurt, fruit variety, nonfat milk, sweetened with low-calorie sweetener
7	Yogurt	0.667	11444000	Yogurt, fruit and nuts, NS as to type of milk
7	Yogurt	0.667	11445000	Yogurt, fruit and nuts, lowfat milk
7	Yogurt	0.667	11446000	Fruit and lowfat yogurt parfait
7	Yogurt	0.667	11459990	Yogurt, frozen, NS as to flavor, NS as to type of milk

7	Yogurt	0.667	11460000	Yogurt, frozen, flavors other than chocolate, NS as to type of milk
7	Yogurt	0.667	11460100	Yogurt, frozen, chocolate, NS as to type of milk
7	Yogurt	0.667	11460150	Yogurt, frozen, NS as to flavor, lowfat milk
7	Yogurt	0.667	11460160	Yogurt, frozen, chocolate, lowfat milk
7	Yogurt	0.667	11460170	Yogurt, frozen, flavors other than chocolate, lowfat milk
7	Yogurt	0.667	11460190	Yogurt, frozen, NS as to flavor, nonfat milk
7	Yogurt	0.667	11460200	Yogurt, frozen, chocolate, nonfat milk
7	Yogurt	0.667	11460250	Yogurt, frozen, flavors other than chocolate, with sorbet or sorbet-coated
7	Yogurt	0.667	11460300	Yogurt, frozen, flavors other than chocolate, nonfat milk
7	Yogurt	0.667	11460400	Yogurt, frozen, chocolate, nonfat milk, with low-calorie sweetener
7	Yogurt	0.667	11460410	Yogurt, frozen, flavors other than chocolate, nonfat milk, with low-calorie sweetener
7	Yogurt	0.667	11460420	Yogurt, frozen, NS as to flavor, whole milk
7	Yogurt	0.667	11460430	Yogurt, frozen, chocolate, whole milk
7	Yogurt	0.667	11460440	Yogurt, frozen, flavors other than chocolate, whole milk
7	Yogurt	0.667	11461000	Yogurt, frozen, chocolate-coated
7	Yogurt	0.667	11461100	Yogurt, frozen, carob-coated
7	Yogurt	0.667	11461200	Yogurt, frozen, sandwich
7	Yogurt	0.667	11461250	Yogurt, frozen, cone, chocolate
7	Yogurt	0.667	11461260	Yogurt, frozen, cone, flavors other than chocolate
7	Yogurt	0.667	11461270	Yogurt, frozen, cone, flavors other than chocolate, lowfat milk
7	Yogurt	0.667	11461280	Yogurt, frozen, cone, chocolate, lowfat milk
8	Fruit Juice	0.625	61201010	Grapefruit juice, freshly squeezed
8	Fruit Juice	0.625	61201020	Grapefruit juice, NS as to form
8	Fruit Juice	0.625	61201220	Grapefruit juice, canned, bottled or in a carton
8	Fruit Juice	0.625	61201620	Grapefruit juice, frozen (reconstituted with water)
8	Fruit Juice	0.625	61210000	Orange juice, NFS
8	Fruit Juice	0.625	61210010	Orange juice, freshly squeezed
8	Fruit Juice	0.625	61210220	Orange juice, canned, bottled or in a carton
8	Fruit Juice	0.625	61210250	Orange juice, with calcium added, canned, bottled or in a carton
8	Fruit Juice	0.625	61210620	Orange juice, frozen (reconstituted with water)
8	Fruit Juice	0.625	61210720	Orange juice, frozen, not reconstituted
8	Fruit Juice	0.625	61210820	Orange juice, frozen, with calcium added (reconstituted with water)
8	Fruit Juice	0.625	61213800	Fruit juice blend, including citrus, 100% juice
8	Fruit Juice	0.625	61213900	Fruit juice blend, including citrus, 100% juice, with calcium added

8	Fruit Juice	0.625	64100100	Fruit juice, NFS
8	Fruit Juice	0.625	64100110	Fruit juice blend, 100% juice
8	Fruit Juice	0.625	64100200	Fruit juice blend, with cranberry, 100% juice
8	Fruit Juice	0.625	64132010	Prune juice
8	Fruit Juice	0.625	64134000	Fruit smoothie drink, made with fruit or fruit juice only (no dairy products)
8	Fruit Juice	0.625	74301100	Tomato juice
8	Fruit Juice	0.625	74301150	Tomato juice, low sodium
8	Fruit Juice	0.625	74302000	Tomato juice cocktail
8	Fruit Juice	0.625	74303000	Tomato and vegetable juice, mostly tomato
8	Fruit Juice	0.625	74303100	Tomato and vegetable juice, mostly tomato, low sodium