Spherix
CHROMADEX CONSULTING

September 21, 2016

Jianmei (Jamie) Zhu, Ph.D.
Consumer Safety Officer
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
5001 Campus Drive
College Park, Maryland 20740

RE: Response to FDA's Questions on GRN 652

Dear Dr. Zhu:

In response to your request of September 7, 2016, following are our responses to FDA's Questions on GRN 652. FDA's questions are enumerated below in italicized text. Arcadia's responses are written in plain text. Bolded text denotes information that was included in the original version of GRN 652. All revisions have been included in the attached/revised version of GRN 652.

Critical issues that need to be addressed

1. *OFAS notes that the following advisories are provided with regard to the use of omega-6 fatty acids on the websites of the University of Maryland Med. Ctr., Penn State University Hershey Med. Ctr., WebMD (and possibly other such websites) on gamma linolenic acid (GLA):*

   #1. People taking blood thinning medications (e.g., aspirin, clopidogrel, warfarin) should not take omega-6 fatty acids without consulting the doctor because of increased risk of bleeding.
   #2. Omega-3 and Omega-6 fatty acids may increase the risk of bleeding.
   #3. Pregnant women should not take borage seed oil, and possibly other sources of GLA, because they may harm the fetus and induce early labor; talk with your physician.
   #4. DO NOT take omega-6 fatty acids if you have a seizure disorder.
   #5. Laboratory studies suggest that omega-6 fatty acids, such as the fat found in corn oil, promote the growth of prostate tumor cells. Until more research is done, health care professionals recommend not taking omega-6 fatty acids, including GLA, if you are at risk for or have prostate cancer.

   In your Notice, you estimated that the 90th percentile dietary intake is 3.9 g/d for the 13-19 age group and 3.8 g/d for the 20+ age group.

Except for those who develop epilepsy by adolescence, the populations at risk from conditions cited above primarily fall in the 20+ age group (with a 90th % EDI of ~3.8 g/d).

Given the above-mentioned warnings to consumers, which are available in the public domain from authoritative sources, please address why and how the intended use of GLA safflower oil is safe and how it is GRAS ("General Recognition" element) by addressing the following questions:

Q.1. Why would the proposed level of GLA consumption from multiple food categories not likely result in an adverse effect on people who are taking blood thinning medications?
Q.2. Why would the proposed level of GLA consumption from multiple food categories not likely result in an adverse effect in pregnant women? Please refer to the advisory #3. The GLA content of SONOVA is twice as high as that of borage oil.
Q.3. Why would the proposed level of GLA consumption from multiple food categories not likely result in an adverse effect in the population at risk for prostate cancer?
Q.4. For those who are already consuming up to 3 g/d of EPA+DHA (omega-3), please explain why would they not be at increased risk of bleeding by the additional consumption of up to 3.8 g/d of GLA through multiple food categories proposed in the Notice? Please refer to the advisory #2.

The intended uses that result in EDIs of more than 2 g/day (Table 5) have been removed from the Notification and the subsequent Table numbers have been revised accordingly. For the remaining uses, SONOVA will be used as a substitute for the γ-linolenic-rich sources that are currently used to formulate the nutritional beverages and medical foods. Therefore, the resulting EDIs will not exceed those resulting from the intake of nutritional beverages and medical foods currently enriched with other γ-linolenic-enriched sources. We have revised the Notification with the following text:

Page 1, revised 1st sentence under Section C. Intended Use, specifying the use of SONOVA as a replacement for other γ-linolenic-enriched sources and removing the salad dressing, mayonnaise, and yogurt uses:

"GLA safflower oil will replace the γ-linolenic acid-enriched sources currently used in nutritional beverages and medical foods intended for children (>2 years old) and adults."

Page 5, removed salad dressing, mayonnaise, and yogurt from the Safety conclusion:

"There is no evidence in the available information on SONOVA that demonstrates, or suggests reasonable grounds to suspect, a hazard to the public when SONOVA is used at levels that might reasonably be expected from the proposed applications of SONOVA for use in nutritional beverages and medical foods intended for adults and children (>2 years old) as proposed by Arcadia Biosciences."

Page 24, revised 1st paragraph under Chapter 4, Section C. Intended Uses and Estimated Daily Intakes of SONOVA:

"SONOVA will be used as substitute for other γ-linolenic-enriched sources that are currently used in nutritional beverages and medical foods. Therefore, dietary exposure to γ-linolenic from the ingestion of SONOVA-containing products will not exceed those that currently result from intake of nutritional beverages and medical foods enriched with other γ-linolenic-enriched sources.

Page 25, Table 5 footer now includes:

"****SONOVA will be used as a substitute for the currently used sources of GLA in the nutritional beverages and medical foods. Therefore, GLA intake from the intended use will not exceed those that currently occur."

2. *In your NDI notification (NDI 000609), you requested a condition of GLA use of 0.8 g/d, with a maximum consumption of 1.2 g/d if consuming 3 g SONOVA. You also stated that the label will include a warning like this, "Warning: If you are pregnant, nursing or taking any medications, consult your doctor before use".*

   When the consumers choose to take dietary supplement containing GLA, they are fully aware of the warning label and the possible side effects. However, consumers may not be aware of the exposure to proposed GLA levels through conventional foods. Therefore, please address the following questions.

   Q.1. Why would the health of the subpopulations (e.g., pregnant women, people with epilepsy, people on blood thinning medications, people with longer bleeding/clotting time) not be at risk by consuming the proposed levels of GLA, particularly when they are not aware of the levels of consumption through food?
   Q.2. How would the proposed consumption of GLA through food affect those who are already consuming the GLA-enriched supplements? Please explain why the health of this subpopulation would not be at risk because of the cumulative exposure through food and GLA-enriched supplements.
   Q.3. If a warning sign is needed for consuming 0.8-1.2 g GLA/d as an NDI, how can the consumption of 3 g GLA/d or higher from food be GRAS?

   Please see response to Question #1.

Other improvements needed in the narrative

3. *In the narrative provided in your notice with regard to human studies (Pages 32 -37), you should clearly state that most of these studies were not designed to evaluate the safety of GLA, and only a few studies had some hematology, clinical chemistry data or statements about the findings.*

   We have added the following sentence to the introductory paragraph under Chapter V, Section C. Human Studies, page 29 of the revised Notification.

"Although the majority of these studies were not designed to directly evaluate the safety of γ-linolenic or dihomo-γ-linolenic acid and only a few evaluated the effects on hematology and clinical chemistry parameters, the weight of the evidence shows that prolonged intakes of high amounts of γ-linolenic and dihomo-γ-linolenic acid are well tolerated and do not result in adverse effects on measures of health status, such as hematology or clinical chemistries."

4. *Please briefly discuss the human studies that measured the clinical parameters (e.g., Covar et al. (1999)), and emphasize the one(s) in which the dosage was similar or greater than the estimated exposure in your notice. Similarly, please include a brief discussion of the other studies focusing on the clinical parameters.*

We have added the following paragraph to Chapter V, Section C. Human Studies, page 30 of the revised Notification.

"Ten studies evaluated clinical parameters, including blood biochemistries, electrolytes, liver enzymes, hematologies, and coagulation indices (Covar et al., 2010; Hederos et al., 1996; Surrette et al., 2003; Surrett et al., 2008; Rilliet et al., 1988; Keen et al., 1993; Kruger et al., 1998; Guivernau et al., 1994; Gadek et al., 1999; Jacobs et al., 2013). Three studies were conducted in children who received up to 3 g γ-linolenic acid/day for up to 16 weeks from γ-linolenic acid-enriched sources (Covar et al., 2010 and Hederos et al., 1996; Jacobs et al., 2013). One of the three studies in children was in critically-ill, mechanically-ventilated children receiving sole source enteral nutrition who were diagnosed with either acute lung injury or acute respiratory distress syndrome (Jacobs et al., 2013). The remaining eight studies were conducted in adults who received up to 5.8 g γ-linolenic acid/day for up to 18 months from γ-linolenic acid-enriched sources (Surrette et al., 2003; Surrett et al., 2008; Rilliet et al., 1988; Keen et al., 1993; Kruger et al., 1998; Guivernau et al., 1994; Gadek et a., 1999; Jacobs et al., 2013). One of the eight adult studies was conducted in adults receiving sole source enteral nutrition who were diagnosed with acute respiratory distress syndrome (Gadek et al., 2013). Although Guivernau et al., 1994 reported that the ingestion of evening primrose oil by hyperlipidemic men, which resulted in exposures of approximately 0.24 g γ-linolenic acid/day, significantly decreased serum triglycerides and cholesterol, and increased HDL and bleeding time, the weight of the evidence from the remaining studies shows that the ingestion of γ-linolenic acid at levels equal to or greater than the estimated exposure from SONOVA does not significantly or reproducibly affect blood biochemistries, electrolytes, liver enzymes, hematologies, or coagulation indices."

5. *The study by Tso et al. (2012) is more relevant to your notice in terms of the test article used (high GLA). Please model your discussion of the hematology and serum biochemistries in your notice following Tso et al. (2012), but not Li et al.*

On pages 74 and 75 of our original Notification we discussed the hematology and serum biochemistry results of Tso et al. (2012). Because the Notification has now been revised to address FDA's questions, these discussions are now located on pages 73 and 74. We also now include: 1) clarifications on pages 71 and 81 of the revised Notification to note the amount of fat present in the diets used in the animal studies; and 2) a concluding paragraph

on page 76 of the revised Notification to discuss the relevance of the results reported by Tso et al. (2012) to the overall safety of ingesting SONOVA; and The revisions are as follows:

Page 71, text added to end of 2nd paragraph:

"Importantly, γ-linolenic acid levels were similar in both diets (approximately 21% of the total fat) and except for 10 times more gadoleic and nervonic acid, and 30 times more erucic acid in the borage oil-containing diet, abundance of the remaining fatty acids in the two diets was similar."

Page 76, middle paragraph added:

"In conclusion, these data show that compared to consuming a diet containing borage oil, consuming a diet containing SONOVA does not produce adverse effects on growth, body composition, hematology, organ weight or histology. Importantly, although the increases in ALT, AST, and LDH levels were noted in both the borage oil- and HGSO-treated groups relative to normal levels of these enzymes in laboratory rats, the magnitude of these increases were similar in both groups. Typical laboratory rat diets contain 5% fat and, moreover, studies have shown that rats consuming diets containing 20% fat develop non-alcoholic fatty liver disease, which is an inflammatory condition associated with increase blood levels of AST, ALT and insulin resistance (i.e., elevated blood glucose levels) (Li et al., 2013). In this study, the diets contained 10% fat, with similar levels of γ-linolenic acid. Thus, considering the anti-inflammatory activities associated with γ-linolenic acid (see Chapter 3), it is likely that the increases in ALT, AST, and LDH reported in this study are the result of the rats consuming high fat diets rather than a specific effect of consuming high levels of a particular fatty acid such as γ-linolenic acid. In addition, these increases would not occur in humans because their recommended diet should contain approximately 20-40% fat (U.S. Department of Agriculture and U.S. Department of Health and Human Services, 2010)."

Page 81, text added to end of last paragraph:

"All diets contained 10% fat and were fed to dams prior to conception and throughout pregnancy and lactation, as well as to the pups after weaning; effects on reproduction, pup development and pup brain fatty acid composition were assessed."

Page 89, text added to 1st paragraph:

"The total lipid content of each diet was 10% by weight."

6. *Please discuss and emphasize the similarity of the fatty acid content of SONOVA (other than the high GLA) with other oils currently consumed by consumers. Then please discuss the normal fatty acid metabolism and state whether the same metabolic fate is expected for the fatty acids found in SONOVA.*

Table 1 (page 8 in our original Notification and now the revised Notification), which is discussed in Chapter II, Section B. Description of SONOVA (page 7 of the original and revised Notification) and Chapter III. Intended Effect (page 20 of the original and revised Notification), shows that the fatty acids present in SONOVA are similar to those found in a wide variety of foods and dietary supplements. To flush-out the fact that the metabolism of these fatty acids and other components when obtained from SONOVA will be the same as when obtained from other sources, we have revised the first paragraph in Chapter III. Intended Effect (page 20 of the original and revised Notification):

"The intended effect of adding SONOVA to conventional and medical foods is to provide an enriched source of γ-linolenic acid. Although it contains high levels of γ-linolenic acid, SONOVA is composed of a variety of saturated and polyunsaturated fatty acids, sterols, and tocopherols that are present at levels equal to or less than those in other edible oils and conventional foods (Tables 1 and 2). Therefore, the absorption, distribution, metabolism, and excretion of these components, including the increased levels of γ-linolenic acid, will be the same as the absorption, distribution, metabolism, and excretion of these components when obtained from other dietary sources."

7. *Because the rat studies show that GLA is rapidly absorbed and partly oxidized, and the non-oxidized GLA is rapidly elongated to DGLA, the safety information of DGLA may be relevant to discuss as part of the GLA metabolism and safety narrative. Thus, please discuss relevant references emphasizing the metabolism and safety of DGLA.*

We agree and have added two sections, one that discuss the results of two clinical studies where the tolerance of dihomo-γ-linolenic acid-enriched oil was evaluated (page 35 of revised Notification) and one that discusses the results of 90-day rat toxicology study conducted with dihomo-γ-linolenic acid-enriched oil (page 86 of revised Notification). We have also added an additional point and revised the text on page 26 and page 29, respectively, to introduce the concept that the metabolism and safety of dihomo-γ-linolenic acid is relevant to the metabolism and safety of γ-linolenic acid with the following:

Page 26, point number 5:

"5. Corroborative clinical and animal studies evaluating the safety of dihomo-γ-linolenic acid."

Page 26, last paragraph:

"Although the animal studies provide data that supports the safety of consuming diets high in γ-linolenic acid, the previous GRNs, AIs, AMDRs, and clinical studies on γ-linolenic and dihomo-γ-linolenic acid provide the most relevant and compelling data to support the safe use of SONOVA because the diets used in the animals studies where the safety of γ-linolenic was evaluated contained 10% fat (Tso et al., 2012; Liu et al., 2004; Palombo et al., 2001; Wainwright et al., 2003), which is approximately double the amount that is typically in diets fed to laboratory rats (Johnson et al., 2008)."

Page 29, 1st paragraph under Chapter V, Section C. Human studies:

"To evaluate the safety of consuming products containing high levels of γ-linolenic acid, publicly available clinical studies where γ-linolenic and dihomo-γ-linolenic acid, the direct metabolite γ-linolenic acid, were administered to health and health-compromised subjects were retrieved, critically reviewed, and evaluated against the AMDRs set by IOM in 2005. Although the majority of these studies were not designed to directly evaluate the safety of γ-linolenic or dihomo-γ-linolenic acid and only a few evaluated the effects on hematology and clinical chemistry parameters, the weight of the evidence shows that prolonged intakes of high amounts of γ-linolenic and dihomo-γ-linolenic acid are well tolerated and do not result in adverse effects on measures of health status, such as hematology or clinical chemistries."

8. *Please take ownership of the safety conclusion and the GRAS conclusion. When citing the authors' conclusions from a toxicological study in the safety narrative, please state what your conclusion is, that is, whether you have arrived at the same conclusions independent of the authors' conclusions based on the data reviewed. A notifier needs to make its own safety conclusion based on the available data in order to conclude that the intended use of the ingredient is GRAS.*

On Page 5 of the original and revised Notification, the Expert Panel and Arcadia Biosciences take ownership of the safety and GRAS conclusions with the following statements:

Expert Panel conclusion:

"There is no evidence in the available information on SONOVA that demonstrates, or suggests reasonable grounds to suspect, a hazard to the public when SONOVA is used at levels that might reasonably be expected from the proposed applications of SONOVA for use in nutritional beverages and medical foods intended for adults and children (>2 years old) as proposed by Arcadia Biosciences.

Arcadia Biosciences conclusion:

"**Therefore, SONOVA is safe and GRAS at the proposed levels of addition to the intended foods. SONOVA is, therefore, excluded from the definition of a food additive, and may be used in the U.S. without the promulgation of a food additive regulation by the FDA under 21 CFR**."

We have also revised the last paragraph in the introduction for Chapter V. Safety of SONOVA and the introductory paragraphs for Chapter V, Section C. Human studies and Chapter V, Section D. Animal studies with the following text:

Page 26, revised introduction for Chapter V. Safety of SONOVA (page 29 of the original Notification):

"Although the animal studies provide data that supports the safety of consuming diets high in γ-linolenic acid, the previous GRNs, AIs, AMDRs, and clinical studies on γ-linolenic and dihomo-γ-linolenic acid provide the most relevant and compelling safety data because the diets used in the animals studies where the safety of γ-linolenic was evaluated contained 10% fat (Tso et al., 2012; Liu et al., 2004; Palombo et al., 2001; Wainwright et al., 2003), which is approximately double the amount that is typically in diets fed to laboratory rats (Johnson et al., 2008)."

Page 29, introductory paragraph for Chapter V, Section C. Human Studies (page 32 of original Notification):

"Although the majority of these studies were not designed to directly evaluate the safety of γ-linolenic or dihomo-γ-linolenic acid and only a few evaluated the effects on hematology and clinical chemistry parameters, the weight of the evidence shows that prolonged intakes of high amounts of γ-linolenic and dihomo-γ-linolenic acid are well tolerated and do not result in adverse effects on measures of health status, such as hematology or clinical chemistries."

Page 71, introductory paragraph for Chapter V, Section D. Animal Studies (page 72 or the original Notification):

"Considering the anti-inflammatory activity of γ-linolenic acid (see Chapter 3), the increases in blood glucose, AST, ALT, and LDH levels noted in studies conducted by Tso et al. (2012) and Li et al., (2004) represent a generalized effect of consuming a high fat diet rather than a specific effect of consuming high levels of γ-linolenic acid. Importantly, these effects would not occur in humans because their recommended diet should contain approximately 20-40% fat (U.S. Department of Agriculture and U.S. Department of Health and Human Services, 2010)."

Should you have additional questions, please let us know.

Sincerely,

Claire L, Kruger, Ph.D., D.A.B.T.
President

GENERALLY RECOGNIZED AS SAFE (GRAS) DETERMINATION FOR GLA SAFFLOWER OIL (SONOVA®) IN CONVENTIONAL AND MEDICAL FOODS

We, the members of the Expert Panel, qualified by scientific training and experience to evaluate the safety of substances directly or indirectly added to food, have performed a comprehensive and critical review of available information and data on the safety and Generally Recognized As Safe (GRAS) status of the use of SONOVA for the intended uses specified above has been shown to be safe and GRAS, using scientific procedures, under the Federal Food, Drug, and Cosmetic Act (FFDCA), as described under 21 CFR §170.30(b). The safety of the intake of SONOVA has been determined to be GRAS by demonstrating that the safety of this level of intake is generally recognized by experts qualified by both scientific training and experience to evaluate the safety of substances directly added to food, and is based on generally available and accepted information.

The proposed use of SONOVA as an ingredient for the intended uses in foods has been determined to be safe through scientific procedures set forth under 21 CFR §170.30(b) based on the following:

1. SONOVA is a refined, bleached, and deodorized oil that contains 400 mg γ-linolenic acid /g of oil and a variety of other fatty acids, tocopherols, and sterols, all of which are already present in conventional foods and edible oils.

2. The unique feature of SONOVA is that it contains high levels of γ-linolenic acid, approximately 2 times higher than those found in borage oil and significantly higher than those found in other edible foods and oils.

3. γ-Linolenic acid is a fatty acid that can either be obtained from the diet or synthesized *in vivo* via the desaturation of linoleic acid by Δ6 desaturase.

4. SONOVA is produced from a genetically engineered strain of *Carthamus tinctorius* L. cv. Centennial safflower that produces high amounts of γ-linolenic acid and may be blended with oil extracted from non-genetically engineered *C. tinctorius* oleic safflower varieties.

5. The genetically engineered strain of *C. tinctorius* is grown by Arcadia Biosciences under USDA authorization (BP_Number 12-102-107n) and in accordance with clearly defined standards for containment.

6. SONOVA is manufactured in a manner similar to those used for edible oils like safflower, sunflower, canola and corn oil. All processing aids used to produce SONOVA are similar to those used in the production of other edible oils (GRAS Notification (GRN) 283 and 306) and comply with appropriate federal regulations. Food grade safflower oleic acid-containing oil is used for blending and standardizing the γ-linolenic acid level in SONOVA.

7. SONOVA was introduced into the United States Market by Arcadia Biosciences in 2009 as a New Dietary Ingredient (FDA-1995S-0039). Labeling guidelines instructed consumers to limit their intake of SONOVA capsules to 4 capsules/d (approximately 800 mg γ-linolenic acid), SONOVA liquid to ¼ tsp/d (~280 mg γ-linolenic acid), SONOVA-containing omega 3-6-9 blend capsules to 3 capsules/d (~600 mg γ-linolenic acid), and SONOVA-containing omega 3-6-9 blend liquids to 3 tbsp/d (~1200 mg γ-linolenic acid).

8. Mean and 90th percentile intakes of dietary linoleic acid and γ-linolenic acid, reported in previous GRN 283 and 306 were 14 and 25 g/d and 1.42 and 2.49 g/d, respectively, which are consistent with the Adequate Intakes for n-6 polyunsaturated acids established by the Institute of Medicine (IOM).

9. The AMDR for males and females 1-year old is 0.4 – 0.9 g/d, assuming that approximately 10% of total n-6 polyunsaturated fatty acid intake is γ-linolenic acid. For males and females older than 1-year old, the lower boundaries increase to approximately 1 g/d and the upper boundaries increase to 3 to 4 g/d.

10. Ten studies were reviewed that orally administered γ-linolenic acid to both healthy or health-compromised (atopic dermatitis, asthma, and atopic eczema) infants (< 12 months old) and children (8 to 26 months old) at levels ranging from 0.1 g – 3 g γ-linolenic acid/d for up to 6 months and found that the higher dose and prolonged intake of γ-linolenic acid were well tolerated and did not result in any adverse effects on measures of health status such as hematology or clinical chemistry or reported adverse events.

11. Thirty-five clinical studies orally administered γ-linolenic acid to both healthy and health-compromised adults at levels ranging from 0.03 – 6 g γ-linolenic acid/d for up to 18 months and found that prolonged intakes of these high amounts of γ-linolenic acid were also well tolerated and did not adversely affects hematology or clinical chemistry parameters.

12. Eleven clinical studies enterally administered γ-linolenic acid-enriched formulas to adults with acute lung injury, acute respiratory distress syndrome, early sepsis, amyotrophic lateral sclerosis, or who had undergone subtotal esophagostomy and total gastroectomy.

    a. Nine of these eleven clinical studies administered 0.1 – 4.6 g γ-linolenic acid/d for up to 7 days and found that the γ-linolenic acid-enriched enteral formulas were well tolerated, did not report adverse events, and were not associated with adverse effects on hematology and clinical chemistry. In some studies, the administration of the γ-linolenic acid-enriched diets improved the clinical endpoints.

    b. Two of these eleven studies that administered γ-linolenic acid-enriched enteral formulas resulted in intake levels higher than 4.6 g γ-linolenic acid/d (Rice et al., 2011; Gadek et al., 1999).

        i. Study results from the trial reported by Rice et al. (2011) could not be used to assess safety of γ-linolenic acid-enriched enteral formulas because the study was stopped early after a futility analysis. In addition, there were confounding issues due to the macronutrient differences between test and control products, and differences in the baseline demographics and clinical characteristics between patients in the control and treatment groups.

        ii. Gadek et al. (1999) administered either a control enteral diet or γ-linolenic acid-enriched enteral diet to adults with acute respiratory distress syndrome at a constant rate to achieve a minimum of basal energy expenditure x 1.3. The diets differed only in lipid composition and level of antioxidant vitamins. The γ-linolenic acid-enriched enteral diet delivered a mean of approximately 5.8 g γ-linolenic acid/d, was well tolerated, and was associated with significant decreases in the number of adverse health effects. Adverse events that were reported were unrelated to the amount of γ-linolenic acid administered.

13. Four toxicology studies using oils derived from transgenic plant sources and borage oil determined the safety of γ-linolenic acid intake. All studies administered diets that contained greater than 10% fat to rats, which is higher than the recommended amount of 5 % (National Research Council, 1982). Rats fed diets high in fats can

develop non-alcoholic fatty liver disease, which is a pathological condition associated with increase blood levels of AST, ALT and insulin resistance (i.e., elevated blood glucose levels). Thus, the increases in blood glucose, AST, ALT, and LDH levels in both the HSGO- and borage oil-treated groups likely represent a generalized effect of consuming a high fat diet rather than a specific effect of consuming high levels of a particular fatty acid such as γ-linolenic acid. Because of this species specific response to high fat diet, clinical studies were determined to be most relevant to assess the safety of ingesting diets high in γ-linolenic acid.

14. The Estimated Daily Intake (EDI) for γ-linolenic acid from the proposed uses have been determined safe and GRAS based studies evaluating its physiological effects, and clinical studies in infants, children, and adults, which are supported by animal toxicology studies.

Determination of the GRAS status of SONOVA under the intended conditions of use has been made through the deliberations of Roger Clemens, DrPH, CNS, CFS, FACN, FIFT, A. Wallace Hayes, PhD, DABT, FATS, ERT, CNS, FACN, and Thomas Sox PhD, JD. These individuals are qualified by scientific training and experience to evaluate the safety of food and food ingredients. These experts have carefully reviewed and evaluated the publicly available information summarized in this document, including the safety of SONOVA and the human exposure to of SONOVA resulting from its intended use as an ingredient in nutritional beverages and medical foods, and concluded:

> *There is no evidence in the available information on SONOVA that demonstrates, or suggests reasonable grounds to suspect, a hazard to the public when SONOVA is used at levels that might reasonably be expected from the proposed applications of SONOVA for use in nutritional beverages and medical foods intended for adults and children (>2 years old) as proposed by Arcadia Biosciences.*

Therefore, SONOVA is safe and GRAS at the proposed levels of addition to the intended foods. SONOVA is, therefore, excluded from the definition of a food additive, and may be used in the U.S. without the promulgation of a food additive regulation by the FDA under 21 CFR.

Roger Clemens, DrPH, CNS, FACN, FIFT
GRAS Expert Panel Member
School of Pharmacy
University of Southern California

Signature: [redacted]

Date: April 19, 2016

A. Wallace Hayes, PhD, DABT, FATS, ERT
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Claire Kruger, PhD, DABT
Scientific Advisor to the Panel
Spherix Consulting, Inc.

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Date: April 19, 2016

Generally Recognized as Safe (GRAS) Determination for GLA Safflower Oil (SONOVA®) in Conventional and Medical Foods

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April 19, 2016

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TABLE OF CONTENTS

I. GRAS EXEMPTION CLAIM 1
A. NAME AND ADDRESS OF THE SPONSOR 1
B. COMMON OR USUAL NAME 1
C. INTENDED USE 1
D. BASIS FOR GRAS DETERMINATION 2
E. AVAILABILITY OF INFORMATION 6
F. SIGNATURE 6
II. DESCRIPTION OF SUBSTANCE 7
A. COMMON OR USUAL NAME 7
B. DESCRIPTION OF SONOVA 7
C. PRODUCTION PROCESS 10
1. Generation of Carthamus tinctorius L. GLA Safflower 10
2. Production of SONOVA 12
D. FINISHED PRODUCT DESCRIPTIONS AND SPECIFICATIONS 16
1. Product Specifications 16
2. Hexanes 18
3. Tocopherols 18
4. Pesticides 18
5. Allergens 19
6. Microbiologicals 19
7. Stability 19
III. INTENDED EFFECT 20
IV. HISTORY OF USE, INTENDED USE, AND ESTIMATED DAILY INTAKE 23
A. HISTORICAL EXPOSURE TO SONOVA 23
B. DIETARY INTAKES OF γ-LINOLENIC ACID 23
C. INTENDED USES AND ESTIMATED DAILY INTAKES OF SONOVA 24
V. SAFETY OF SONOVA 26
A. ADEQUATE INTAKES AND ACCEPTABLE MACRONUTRIENT INTAKE LEVELS FOR N-6 POLYUNSATURATED FATTY ACIDS 27
B. PER USER EDIS OF LINOLENIC ACID DETERMINED IN GRN 306 29
C. HUMAN STUDIES 29
1. Orally Administered Products Containing γ-Linolenic Acid 29
2. Enterally Administered Products Containing γ-Linolenic Acid 30
3. γ-Linolenic Acid and Inflammation 32
4. Orally Administered Products Containing Dihomo-γ-Linolenic Acid 35
D. ANIMAL STUDIES 71
1. Toxicology Studies 71
2. Equivalence of Fatty Acid Metabolism 88
VI. REFERENCES 91

LIST OF TABLES

TABLE 1. FATTY ACID COMPOSITIONAL COMPARISON OF SONOVA, CONVENTIONAL FOODS, AND DIETARY SUPPLEMENTS....8

TABLE 2. TOCOPHEROL AND STEROL COMPOSITION OF SONOVA, COMMON EDIBLE OILS, BORAGE OIL, AND EVENING PRIMROSE OIL....9

TABLE 3. PRODUCT SPECIFICATIONS AND BATCH DATA FOR SONOVA....17

TABLE 4. TOCOPHEROL LEVELS IN SONOVA....18

TABLE 5. MAXIMUM DAILY INTAKE OF γ-LINOLENIC ACID (GLA) FROM THE INTENDED USES OF SONOVA IN NUTRITIONAL BEVERAGES AND MEDICAL FOODS25

TABLE 6. ACCEPTABLE MACRONUTRIENT DISTRIBUTION RANGE (AMDR) FOR LINOLENIC ACIDS IN CHILDREN AND ADULTS....28

TABLE 7. CLINICAL TRIALS OF γ-LINOLENIC ACID (GLA)-ENRICHED OILS IN INFANTS AND CHILDREN....37

TABLE 8. CLINICAL TRIALS OF γ-LINOLENIC (GLA)-ENRICHED OILS IN ADULTS....43

TABLE 9. CLINICAL TRIALS OF γ-LINOLENIC (GLA)-ENRICHED OILS IN ADULTS AND CHILDREN RECEIVING SOLE-SOURCE ENTERAL NUTRITION....60

TABLE 10. ABSOLUTE (ABS) AND RELATIVE (REL) MAJOR ORGANS WEIGHTS OF MALE AND FEMALE SPRAGUE-DAWLEY RATS FED A DIET CONTAINING EITHER A BORAGE OIL BLEND OR A HIGH γ-LINOLENIC SAFFLOWER OIL (HGSO) BLEND FOR 12 WEEKS....73

TABLE 11. HEMATOLOGICAL PARAMETERS OF MALE AND FEMALE SPRAGUE-DAWLEY RATS FED A DIET CONTAINING A BORAGE OIL BLEND OR A HIGH γ-LINOLENIC SAFFLOWER OIL (HGSO) BLEND FOR 12 WEEKS....74

TABLE 12. SERUM BIOCHEMISTRY PROFILES OF MALE AND FEMALE SPRAGUE-DAWLEY RATS FED ON A DIET CONTAINING EITHER THE BORAGE OIL BLEND OR THE HIGH γ-LINOLENIC SAFFLOWER OIL (HSGO) BLEND AT THE END OF 12-WEEK FEEDING....75

TABLE 13. ABSOLUTE (ABS) AND RELATIVE (REL) LIVER, HEART, SPLEEN AND KIDNEY WEIGHTS IN MALE SPRAGUE-DAWLEY RATS FED A DIET CONTAINING 5, 10, OR 15% (W/W) OF HIGH-γ-LINOLENIC ACID CANOLA OIL (HGCO) OR 15% (W/W) OF BORAGE OIL FOR 12 WEEKS....78

TABLE 14. HEMATOLOGICAL PARAMETERS OF MALE SPRAGUE-DAWLEY RATS FED A DIET CONTAINING 5, 10, OR 15% (W/W) OF HIGH-γ-LINOLENIC ACID CANOLA OIL (HGCO) OR 15% (W/W) OF BORAGE OIL (BO) FOR 12 WEEKS....79

TABLE 15. SERUM BIOCHEMISTRY OF MALE SPRAGUE-DAWLEY RATS FED A DIET CONTAINING 5, 10, OR 15% (W/W) OF HIGH-γ-LINOLENIC ACID CANOLA OIL (HGCO) OR 15% (W/W) OF BORAGE OIL FOR 12-WEEKS....80

TABLE 16. LITTER SIZE AND PUP WEIGHT....83

TABLE 17. SELECTED FATTY ACID COMPOSITION OF BRAIN ETHANOLAMINE PHOSPHOGLYCERIDES 84

TABLE 18. SELECTED FATTY ACID COMPOSITION OF BRAIN CHOLINE PHOSPHOGLYCERIDES 84

TABLE 19. SELECTED FATTY ACID COMPOSITION OF BRAIN PS/PI (%WT FA).....85

LIST OF FIGURES

FIGURE 1. PLASMID MAP OF PSBS4119 11

FIGURE 2. PRODUCTION PROCESS FOR SONOVA.....15

FIGURE 3. METABOLISM TO γ-LINOLENIC ACID (GLA) TO THE EICOSANOIDS (PROSTAGLANDINS, THROMBOXANES), 15-(S)-HYDROXY-8, 11, 13-EICOSATRIENOIC ACID (15HETRE) AND PLATELET ACTIVATING FACTOR (PAF) 22

LIST OF ABBREVIATIONS

AA	Arachidonic Acid
AI	Adequate Intake
Alb/Glob	Albumin/Globulin Ratio
ALI	Acute Lung Injury
ALS	Amyotrophic Lateral Sclerosis
ALT	Alanine Aminotransferase
AMDR	Acceptable Macronutrient Distribution Range
AP	Alkaline Phosphatase
APHIS	Animal and Plant Health Inspection Service
araP	Arabidopsis Thaliana Oleosin Promoter
araT	Arabidopsis Thaliana Oleosin Terminator
ARDS	Acute Respiratory Distress Syndrome
AST	Aspartate Aminotransferase
BEE	Basal Energy Expenditure
BMI	Body Mass Index
BQMS	Biotechnology Quality Management System
BUN	Blood Urea Nitrogen
CFU	Colony Forming Unit
DGLA	Dihomo-γ-Linolenic Acid
EDI	Estimated Daily Intake
EER	Estimated Energy Requirement Range

ELISA	Enzyme-Linked Immunosorbant Assay
EPA	Environmental Protection Agency
FDA	Food and Drug Administration
FFDCA	Federal Food, Drug, and Cosmetic Act
GLA	γ-Linolenic Acid
GRN	GRAS Notification
HACCP	Hazard Analysis and Critical Control Point
HGCO	High- γ-Linolenic Acid Canola Oil
HGSO	SONOVA Test Diet (High- γ-Linolenic Acid Safflower Oil)
HHS	Health and Human Services
IOM	Institute of Medicine
KCS	Keratoconjunctivitis Sicca
LA	Linoleic Acid
LDH	Lactate Dehydrogenase
MCH	Mean Corpuscler Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
MCV	Mean Corpuscular Volume
NAFLD	Non-Alcoholic Fatty Liver Disease
NHANES	National Health and Nutrition Examination Survey
PAL	Physical Activity Level
PAT	Phosphinothricin-N-Acetyltransferase
PMN	Polymorphonuclear Granulocyte
PT	Prothrombin Time
PUFA	Polyunsaturated Fatty Acids
RBC	Red Blood Cell
SIRS	Systemic Inflammatory Response Syndrome
SNK	Student-Newman-Keuls
T-DNA	Transfer DNA
TG	Triacylglycerol
ubiP	Ubiquitin Promoter
ubiT	Ubiquitin Terminator
UL	Tolerable Upper Intake Level
USDA	United States Department of Agriculture
USP	United States Pharmacopeial Convention
WBC	White Blood Cell

I. GRAS EXEMPTION CLAIM

A. NAME AND ADDRESS OF THE SPONSOR

Arcadia Biosciences, Inc.
200 Cousteau Place
Suite 200
Davis, CA 95618

B. COMMON OR USUAL NAME

GLA (γ-linolenic acid) safflower oil, also known as SONOVA

C. INTENDED USE

GLA safflower oil will replace the γ-linolenic acid-enriched sources currently used in nutritional beverages and medical foods intended for children (>2 years old) and adults.

The intended uses in:

- Nutritional beverages and medical foods for children (>2 years old) and adults will result in the following intakes of γ-linolenic acid from the recommended servings per day:
    - 0.3 g GLA/day from pediatric supplemental or interim sole-source nutritional beverages;
    - 0.5 g GLA /day from adult supplemental or interim sole-source nutritional beverages;
    - 2.0 g GLA /day from adult performance nutrition products;
    - 1.0 g GLA /day in adult blood glucose management products.
- Sole-source therapeutic nutrition products containing 4 g γ-linolenic acid/L and an equivalent macronutrient and micronutrient content to the product were used in the study by Gadeck et al. (1999). γ-Linolenic acid intake will be based on the caloric need for each subject, which can be calculated using the Harris-Benedict equation to account for gender, weight, height, and age. The resulting basal

metabolic rate is multiplied by a factor to account for the energy requirement of the medical condition.

D. BASIS FOR GRAS DETERMINATION

This GRAS determination for the use of SONOVA for the intended uses specified above has been shown to be safe and GRAS, using scientific procedures, under the Federal Food, Drug, and Cosmetic Act (FFDCA), as described under 21 CFR §170.30(b). The safety of the intake of SONOVA has been determined to be GRAS by demonstrating that the safety of this level of intake is generally recognized by experts qualified by both scientific training and experience to evaluate the safety of substances directly added to food, and is based on generally available and accepted information.

The proposed use of SONOVA as an ingredient for the intended uses in foods has been determined to be safe through scientific procedures set forth under 21 CFR §170.30(b) based on the following:

1. SONOVA is a refined, bleached, and deodorized oil that contains 400 mg γ-linolenic acid /g of oil and a variety of other fatty acids, tocopherols, and sterols, all of which are already present in conventional foods and edible oils.

2. The unique feature of SONOVA is that it contains high levels of γ-linolenic acid, approximately 2 times higher than those found in borage oil and significantly higher than those found in other edible foods and oils.

3. γ-Linolenic acid is a fatty acid that can either be obtained from the diet or synthesized *in vivo* via the desaturation of linoleic acid by Δ6 desaturase.

4. SONOVA is produced from a genetically engineered strain of *Carthamus tinctorius* L. cv. Centennial safflower that produces high amounts of γ-linolenic acid and may be blended with oil extracted from non-genetically engineered *C. tinctorius* oleic safflower varieties.

5. The genetically engineered strain of *C. tinctorius* is grown by Arcadia Biosciences under USDA authorization (BP_Number 12-102-107n) and in accordance with clearly defined standards for containment.

6. SONOVA is manufactured in a manner similar to those used for edible oils like safflower, sunflower, canola and corn oil. All processing aids used to produce

SONOVA are similar to those used in the production of other edible oils (GRAS Notification (GRN) 283 and 306) and comply with appropriate federal regulations. Food grade safflower oleic acid-containing oil is used for blending and standardizing the γ-linolenic acid level in SONOVA.

7. SONOVA was introduced into the United States Market by Arcadia Biosciences in 2009 as a New Dietary Ingredient (FDA-1995S-0039). Labeling guidelines instructed consumers to limit their intake of SONOVA capsules to 4 capsules/d (approximately 800 mg γ-linolenic acid), SONOVA liquid to ¼ tsp/d (~280 mg γ-linolenic acid), SONOVA-containing omega 3-6-9 blend capsules to 3 capsules/d (~600 mg γ-linolenic acid), and SONOVA-containing omega 3-6-9 blend liquids to 3 tbsp/d (~1200 mg γ-linolenic acid).

8. Mean and 90th percentile intakes of dietary linoleic acid and γ-linolenic acid, reported in previous GRN 283 and 306 were 14 and 25 g/d and 1.42 and 2.49 g/d, respectively, which are consistent with the Adequate Intakes for n-6 polyunsaturated acids established by the Institute of Medicine (IOM).

9. The AMDR for males and females 1-year old is 0.4 – 0.9 g/d, assuming that approximately 10% of total n-6 polyunsaturated fatty acid intake is γ-linolenic acid. For males and females older than 1-year old, the lower boundaries increase to approximately 1 g/d and the upper boundaries increase to 3 to 4 g/d.

10. Ten studies were reviewed that orally administered γ-linolenic acid to both healthy or health-compromised (atopic dermatitis, asthma, and atopic eczema) infants (< 12 months old) and children (8 to 26 months old) at levels ranging from 0.1 g – 3 g γ-linolenic acid/d for up to 6 months and found that the higher dose and prolonged intake of γ-linolenic acid were well tolerated and did not result in any adverse effects on measures of health status such as hematology or clinical chemistry or reported adverse events.

11. Thirty-five clinical studies orally administered γ-linolenic acid to both healthy and health-compromised adults at levels ranging from 0.03 – 6 g γ-linolenic acid/d for up to 18 months and found that prolonged intakes of these high amounts of γ-linolenic acid were also well tolerated and did not adversely affects hematology or clinical chemistry parameters.

12. Eleven clinical studies enterally administered γ-linolenic acid-enriched formulas to adults with acute lung injury, acute respiratory distress syndrome, early sepsis, amyotrophic lateral sclerosis, or who had undergone subtotal esophagostomy and total gastroectomy.

    a. Nine of these eleven clinical studies administered 0.1 – 4.6 g γ-linolenic acid/d for up to 7 days and found that the γ-linolenic acid-enriched enteral formulas were well tolerated, did not report adverse events, and were not associated with adverse effects on hematology and clinical chemistry. In some studies, the administration of the γ-linolenic acid-enriched diets improved the clinical endpoints.

    b. Two of these eleven studies that administered γ-linolenic acid-enriched enteral formulas resulted in intake levels higher than 4.6 g γ-linolenic acid/d (Rice et al., 2011; Gadek et al., 1999).

        i. Study results from the trial reported by Rice et al. (2011) could not be used to assess safety of γ-linolenic acid-enriched enteral formulas because the study was stopped early after a futility analysis. In addition, there were confounding issues due to the macronutrient differences between test and control products, and differences in the baseline demographics and clinical characteristics between patients in the control and treatment groups.

        ii. Gadek et al. (1999) administered either a control enteral diet or γ-linolenic acid-enriched enteral diet to adults with acute respiratory distress syndrome at a constant rate to achieve a minimum of basal energy expenditure x 1.3. The diets differed only in lipid composition and level of antioxidant vitamins. The γ-linolenic acid-enriched enteral diet delivered a mean of approximately 5.8 g γ-linolenic acid/d, was well tolerated, and was associated with significant decreases in the number of adverse health effects. Adverse events that were reported were unrelated to the amount of γ-linolenic acid administered.

13. Four toxicology studies using oils derived from transgenic plant sources and borage oil determined the safety of γ-linolenic acid intake. All studies administered diets that contained greater than 10% fat to rats, which is higher than the recommended

amount of 5 % (National Research Council, 1982). Rats fed diets high in fats can develop non-alcoholic fatty liver disease, which is a pathological condition associated with increase blood levels of AST, ALT and insulin resistance (i.e., elevated blood glucose levels). Thus, the increases in blood glucose, AST, ALT, and LDH levels in both the On HSGO- and borage oil-treated groups likely represent a generalized effect of consuming a high fat diet rather than a specific effect of consuming high levels of a particular fatty acid such as γ-linolenic acid. Because of this species specific response to high fat diet, clinical studies were determined to be most relevant to assess the safety of ingesting diets high in γ-linolenic acid.

14. The Estimated Daily Intake (EDI) for γ-linolenic acid from the proposed uses have been determined safe and GRAS based studies evaluating its physiological effects, and clinical studies in infants, children, and adults, which are supported by animal toxicology studies.

Determination of the GRAS status of SONOVA under the intended conditions of use has been made through the deliberations of Roger Clemens, DrPH, CNS, CFS, FACN, FIFT, A. Wallace Hayes, PhD, DABT, FATS, ERT, CNS, FACN, and Thomas Sox PhD, JD. These individuals are qualified by scientific training and experience to evaluate the safety of food and food ingredients. These experts have carefully reviewed and evaluated the publicly available information summarized in this document, including the safety of SONOVA and the human exposure to of SONOVA resulting from its intended use as an ingredient in nutritional beverages and medical foods, and concluded:

There is no evidence in the available information on SONOVA that demonstrates, or suggests reasonable grounds to suspect, a hazard to the public when SONOVA is used at levels that might reasonably be expected from the proposed applications of SONOVA for use in nutritional beverages and medical foods intended for adults and children (>2 years old) as proposed by Arcadia Biosciences.

Therefore, SONOVA is safe and GRAS at the proposed levels of addition to the intended foods. SONOVA is, therefore, excluded from the definition of a food additive, and may be used in the U.S. without the promulgation of a food additive regulation by the FDA under 21 CFR.

E. AVAILABILITY OF INFORMATION

The data and information that serve as the basis for this GRAS determination will be available for review and copying at reasonable times at the office of Claire L. Kruger, Ph.D., D.A.B.T., President, Spherix Consulting, A Division of ChromaDex, Inc., at 11821 Parklawn Drive, Suite 310, Rockville, MD 20852. Telephone: 301-897-0613; Facsimile: 240- 621-7549; Email: clairek@chromadex.com, or be sent to FDA upon request.

F. SIGNATURE

Pursuant to the criteria provided in proposed 21 CFR 170.36, Arcadia Biosciences hereby notifies the United States Food and Drug Administration (FDA) that the use of SONOVA in foods under the intended conditions of use is exempt from the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act, because Arcadia Biosciences has determined that such use is Generally Recognized As Safe through scientific procedures.

Signature
Keith Redenbaugh, Ph.D.
Authorized Representative of Arcadia Biosciences, Inc.

May 26, 2016
Date

II. DESCRIPTION OF SUBSTANCE

A. COMMON OR USUAL NAME

GLA (γ-linolenic acid) safflower oil

B. DESCRIPTION OF SONOVA

GLA safflower oil, also referred to as SONOVA® and SONOVA 400, is a refined, bleached, and deodorized oil that contains 400 mg γ-linolenic acid (18:3Δ 6, 9, 12)/g oil and a variety of other fatty acids, tocopherols, and sterols, all of which are present in other edible oils and conventional foods (Tables 1 and 2). SONOVA 400 was the subject of the New Dietary Ingredient Notification (FDA-19955-0039) filed with the FDA by Arcadia Biosciences on August 27, 2009 without comment.

Table 1. Fatty Acid Compositional Comparison of SONOVA, Conventional Foods, and Dietary Supplements

Fatty Acids		SONOVA 400[1] (average % of oil)	Foods (%)									Dietary Supplements (%)			
			Oleic Safflower Oil[2]	Linoleic Safflower Oil[2]	Chicken Thigh Meat[2]	Chicken Wing Meat[2]	Atlantic Salmon Meat[4]	Sunflower Oil[3]	Peanut Oil[3]	Corn Oil[3]	Soybean Oil[3]	Evening Primrose Oil[2]	Black Currant Oil[2]	Borage Oil[2]	Hemp Seed Oil[2]
14:0	Myristic	0.1	0.1	0.1	0.8	0.5	4.6	0.1	0.1	0.0	0.1	0.1	0.0	0.0	0.0
16:0	Palmitic	7.1	6.2	7.2	21.8	23.5	14.8	6.4	10.4	12.3	10.8	6.5	9.7	9.7	6.3
18:0	Stearic	2.1	2.0	2.1	7.1	8.9	2.8	4.5	3.0	1.9	3.9	1.9	2.3	3.9	2.8
18.1	Oleic	35.0	80.9	13.1	38.9	35.8	15.2	22.1	47.9	27.7	23.9	7.9	12.9	18.2	12.1
18:2	Linoleic	12.2	11.1	73.6	20.2	19.4	4.4	65.6	30.3	56.1	52.1	73.7	51.1	40.4	55.9
18:2	18:2Δ6, 9	0.1	0.0	0.0	0.1	0.1	-	-	-	-	-	0.0	0.0	0.0	0.1
γ18:3	γ-linolenic	41.7	0.0	0.0	0.3	0.3	-	-	-	-	-	9.1	10.5	18.4	2.8
α18:3	α-linolenic	0.2	0.0	0.1	1.0	0.8	1.4	0.5	0.4	1.0	7.8	0.2	12.9	0.3	19.7
18:4	Stearidonic	0.1	0.0	0.0	0.1	0.0	1.7	-	-	-	-	0.0	1.9	0.1	0.0
20:0	Arachidic	0.6	0.5	0.4	0.1	0.1	-	0.3	1.2	0.3	0.3	0.3	0.3	0.3	0.7
20:2	Gadoleic	0.3	0.5	0.2	0.6	0.6	8.1	0.2	1.3	0.2	0.1	0.2	1.4	5.3	0.0
22:2	Behenic	0.4	0.4	0.3	0.1	0.1	-	0.8	2.3	0.8	0.2	0.1	0.1	0.2	0.3
24:2	Lignoceric	0.2	0.0	0.2	0.0	0.2	-	0.2	1.4	0.2	-	0.0	0.0	0.0	0.0

"-" denotes "not reported"; "0.0" indicates that amount was below the detection limit.

[1]Fatty acid composition for SONOVA was determined by gas chromatography and mass spectrometry. Values represent averages of 2 pilot and 2 laboratory scale lots.

[2]Data obtained from Arcadia Biosciences analysis of chicken, commercial refined, bleached, and deodorized oil (evening primrose, black currant, borage, and safflower oils), hempseed oil from crude oil, chicken meat purchase in June 2009.

[3]Reported in Dubois et al. (2007).

[4]Reported in Bell et al. (2003).

Table 2. Tocopherol and Sterol Composition of SONOVA, Common Edible Oils, Borage Oil, and Evening Primrose Oil

Parameter (mg/g)	Edible Oils							Dietary Supplements	
	Sonova 400[1]	Oleic Safflower Oil[2]	Linoleic Safflower Oil[3]	Sunflower Oil[3, 6]	Peanut Oil[4]	Corn Oil[3,7]	Soybean Oil[3]	Evening Primrose Oil[5]	Borage Oil[5]
Total Tocopherols (α, β, δ, and γ)	0.301	0.179	0.237	0.671	0.408	1.087	1.046	0.543	3.168
Total Sterols (Stigmasterol, Campesterol, and β-sitosterol)	2.338	1.227	6.242	3.915	1.876	9.520	3.500	15.957	6.460

[1]Tocopherol and sterol concentrations in SONOVA before the addition of antioxidants as determined by the modified version of AOAC method 992.04 (Holen, 1985). Values represent averages of 2 pilot and 2 laboratory scale lots.

[2]Tocopherol levels obtained from Ortega-García et al. (2006).

[3]Tocopherol levels obtained from Zilch (2000).

[4]Tocopherol levels obtained from Padley (1994).

[5]Tocopherol levels obtained from Clough (2001).

[6]Sterol levels obtained from Weber and Mukherjee (2006).

[7]Sterol levels obtained from Kritchevsky and Bonfield (1997).

C. PRODUCTION PROCESS

SONOVA is produced by extracting oil from the seeds of *Carthamus tinctorius* L. GLA safflower, a genetically engineered strain of *C. tinctorius* L. cv. Centennial safflower that ectopically expresses *Saprolegnia diclina* Δ6 desaturase and produces high amounts of γ-linolenic acid. To achieve γ-linolenic acid levels within the product specification of 380-475 mg/g, *Carthamus tinctorius* L. GLA safflower raw seed and/or crude degummed oil may be mixed with *C. tinctorius* oleic safflower seed or refined and bleached oleic safflower oil.

1. Generation of *Carthamus tinctorius* L. GLA Safflower

The *C. tinctorius* L. GLA safflower was generated by genetically engineering *Carthamus tinctorius* L. cv. Centennial to ectopically express *Saprolegnia diclina* Δ6 desaturase and produce high amounts of γ-linolenic acid using *Agrobacterium tumefaciens*-mediated transformation of two day-old cotyledons and pSBS4119, a plasmid containing cDNA encoding *Saprolegnia diclina* Humphrey Δ6 desaturase controlled by the *Arabidopsis thaliana* oleosin promoter (araP; GeneBank Accession X62353) and terminator (araT; GenBank Accession NM_118647). pSBS4119 also contains cDNA encoding *Streptomyces viridochromogenes* phosphinothricin-N-acetyltransferase (PAT; GenBank Accession M22827) controlled by the *Petroselinum crispum* ubiquitin promoter (ubiT; GenBank Accession X64345) and the *P. crispum* ubiquitin terminator (ubiT) located between the right border and left border regions of the transfer DNA (T-DNA) region obtained from the *A. tumefaciens* plasmid pTiC58 T-DNA (GeneBank Accession AJ237588) (Figure 1).

S. diclina Δ6 desaturase converts linoleic acid to γ-linolenic acid. *S. viridochromogenes* phosphinothricin-N-acetyltransferase is a selectable marker used for the glufosinate-mediated selection of the genetically modified two day-old cotyledons that had undergone genetic recombination following the transformation of *C. tinctorius* with pSBS4119. The right and left border regions are recognized by endonucleases that mediate the transfer of DNA from pSBS4119 to the genome of *C. tinctorius* during *Agrobacterium tumefaciens*-mediated transformation. Notably, Δ6 desaturases also desaturate oleic acid (18:1Δ 9 cis) and α-linolenic acid (18:3Δ 9, 12, 15) to octadecadienoic acid (18:2Δ 6, 9) and steridonic acid (18:4Δ 6, 9, 12, 15), although not as efficiently as γ-linolenic acid (Brenner, 1974).

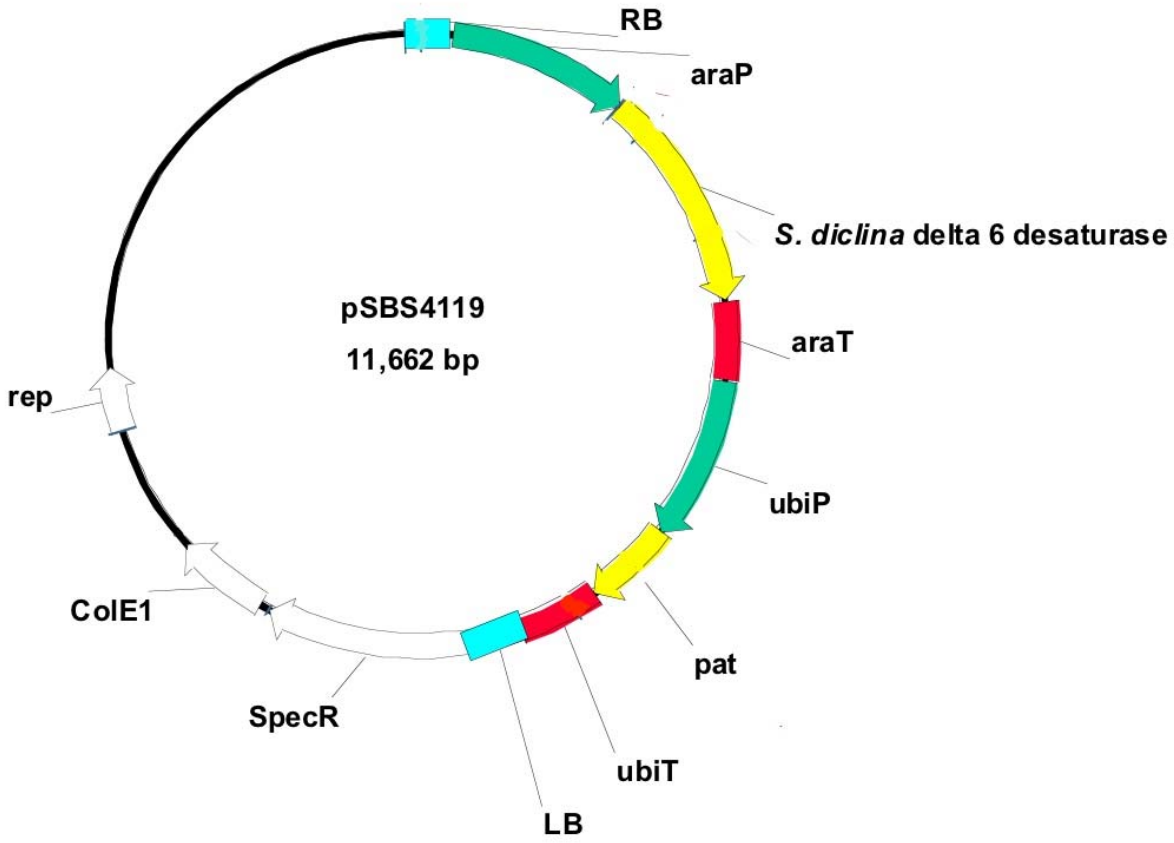

Figure 1. Plasmid Map of pSBS4119

pSBS4119 contains DNA sequences that define the extent of the T-DNA that are normally transferred into the plant genome. These are termed the right border (RB) and left border (LB) regions, and they contain sequences necessary for efficient transfer of the T-DNA into the plant cell. In pSBS4119, the RB region is located 5' to the delta 6 desaturase cassette (nucleotides 5 to 183) and the LB region is located adjacent the 3' end of the selectable marker cassette (nucleotides 5,193 to 5,503). ColE1 is the origin of replication, SpecR is the spectinomycin resistance marker, and rep is the origin of replication.

Southern blot analyses and subsequent direct sequencing confirmed that the genome of the genetically engineered *C. tinctorius* contained a single copy of the genetic material between the right and left border regions of pSBS4119 (Appendices 3 and 4). The single copy contained 41 base pairs of the right border region, intact araP, *S. declina* Δ6 desaturase, araT, ubiP and PAT cDNAs, and 423 base pairs of ubiT. There was no integration of either the left border region or the vector backbone. Subsequent functional analyses showed that the genetically engineered safflower produced high amounts of γ-linolenic acid and low amounts of linoleic acid (Nykiforuk et al., 2012).

2. Production of SONOVA

a. Cultivation of C. tinctorius GLA Safflower

C. tinctorius GLA safflower seed used for GLA safflower oil production and subsequent planting is grown on an annual basis in exactly the same manner (farming practices, equipment, and applied chemicals), in the same locations, and during the same growing season as conventional safflower grown for edible oil production. Conventional safflower requires 300 – 500 mm of moisture during the growing season and no moisture when seed is present because germination can occur. Moreover, the crop is ready for harvest approximately 45 days after full flower and requires 120 frost-free days (Smith, 1996).

The amount of *C. tinctorius* GLA safflower seed grown is based on the demands of oil production, future planting, unanticipated sales increases, crop failures, and inclement weather. To safeguard against catastrophic failure, a sufficient volume of planting seed is reserved to produce adequate quantities of oil and planting seed.

Importantly, Arcadia Biosciences grows *C. tinctorius* GLA safflower seed under strict Identity Preservation conditions and USDA authorization (BP_Number 12-102-107n). Arcadia Biosciences is also a participating member of the Animal and Plant Health Inspection Service (APHIS) Biotechnology Quality Management System (BQMS), which is an auditable quality system that provides external verification of compliance with 7 CFR 340, the *Introduction of Organisms and Products Altered or Produced Through Genetic Engineering Which Are Plant Pests or Which There is Reason to Believe Are Plant Pests*. The scope of BQMS audit includes the field release, importation, and movement of plant materials regulated by the USDA through procedures and records. Following harvest each truckload of seed is delivered to the seed handling facility at Arcadia Biosciences and sampled. All seed from a particular field or farm is pooled for analysis. Composite samples are tested for debris, moisture, oil content, and γ-linolenic acid concentration. Prior to storage, the seed is cleaned by screening out extraneous plant debris, such as stones, dirt, and sticks, and if necessary, seed moisture levels are adjusted to 8 to 10% by drying the seed. Seed earmarked for seed production is stored in nitrogen blanketed sealed containers and maintained in a warehouse under ambient storage conditions. Seed earmarked for SONOVA production is delivered to the vegetable oil processing facility.

b. Processing of C. tinctorius GLA Safflower Seed and Production of SONOVA

At the vegetable oil processing facility SONOVA is manufactured in a continuous process that occurs over days and in a manner that is similar to the production processes used for manufacturing other edible oils, such as safflower, sunflower, canola and corn oil. The different manufacturing stages such as crushing, refining, bleaching and deodorization are outlined in Figure 2.

C. tinctorius GLA safflower seed harvested from all the production fields are mixed and analyzed for γ-linolenic acid composition. If γ-linolenic acid levels are greater than 70%, *C. tinctorius* oleic safflower seed may be mixed with *C. tinctorius* GLA safflower seed to reduce γ-linolenic acid levels to less than 70%. The seeds are then cleaned with a mechanical sieve or by wind sifting, cracked with a roller mill, and crushed with an expeller press, producing "expeller oil" and an "expeller cake". The residual oil present in the expeller cake is extracted using recycled hexanes, which is removed via distillation. The extracted oil is mixed with the expeller oil, producing crude γ-linolenic acid safflower oil. Depending on the γ-linolenic acid concentration in the crude oil, the γ-linolenic acid level may be adjusted to approximately 50% with the addition of oleic safflower oil prior to refining. The crude γ-linolenic acid safflower oil is stored in a single tank (Figure 2).

During refining, crude γ-linolenic acid safflower oil is mixed with a 50% citric acid solution, heated at 60 – 65°C, agitated for 20 – 30 minutes in a process known as "degumming". The resulting water phase, which contains the phospholipids, free fatty acids, monoglycerides, and diglycerides from the oil, is decanted. The degummed oil is then neutralized with 50% sodium hydroxide, agitated and heated at 60 – 65°C until the alkaline fraction or soapstock, separates from the oil. The oil/soapstock mixture is heated to 85°C, mixed with water and centrifuged. The soapstock is then removed and the resulting oil is mixed with acid-activated clays, such as bentonite and possibly silica, heated to 100 - 120°C under a vacuum, and agitated for 20 to 30 minutes for bleaching. The oil/silica/clay mixture is vacuum-dried to remove residual moisture, filtered using silicone dioxide-imbedded filters to remove the acid activated clays and dispensed into a storage tank. The level of γ-linolenic acid is then standardized to 400 to 450 mg/g oil with the addition of food grade oleic oil extracted from *C. tinctorius* oleic safflower, which is also produced by the vegetable oil processing facility. The product is de-aerated at 85 - 90°C with a vacuum, heated to 240°C, and steam-stripped for approximately 20 minutes yielding the deodorized oil. The oil is then cooled to ambient temperature and mixed with citric acid to chelate any remaining trace metals and a mixture of antioxidants, to prevent oxidation. For storage, SONOVA is placed in air-tight polyethylene food-grade containers, which are sparged, blanketed with nitrogen, sealed, and placed at 4°C.

The vegetable oil processing facility meets the requirements of Level 2 Hazard Analysis and Critical Control Point (HACCP)-based food safety plan and utilizes segregation procedures to prevent cross contamination with other raw materials. Prior to delivery of *C. tinctorius* GLA safflower seeds to the vegetable oil processing facility, all grain-handling areas (silos, conveyors, etc.) are dry-cleaned and swept to remove all non-*C. tinctorius* materials. Ideally, SONOVA is produced immediately after the production of oleic safflower oil. If an oil or seed other than oleic safflower seed or oil was processed immediately prior to SONOVA production, the crush plant (elevators, augers, etc.), storage tanks, and refinery are cleaned and flushed with oleic safflower,

the production lines and hoses are flushed with nitrogen, and separate lines and hoses are used for crude and refined oil transfers.

Spherix Consulting reviewed the Certificates of Analysis and conditions of use for all the processing aids currently used in the production of SONOVA. Except for hexanes, all of the processing aids used in the production of SONOVA and the oleic acid extracted from *C. tinctorius* oleic safflower comply with 21 CFR. For hexanes, although there are no specific federal regulations stating that it can be used as a processing aid in the extraction of vegetable oils, GRN 306 determined it to be safe and similar to paraffinic hydrocarbons that have a long history of safe use in the production of food oils (pre-1958), GRN 94 and 326 determined it to be safe for use as an extraction solvent for edible oils used in infant formulas, and Directive 2009/32/EC establishes a maximum residue limit for hexanes in the production of fats and oils of 1 mg/kg (1 ppm) fat or oil. Importantly, vegetable oil processing facility occasionally changes their suppliers of the processing aids, and all future processing aids and their uses shall be either GRAS or comply with 21 CFR. In addition, all other manufacturers used to produce SONOVA will comply with the processing conditions described in this GRAS determination.

c. Quality Control

Quality control measures span both the agricultural and production processes. Prior to being unloaded, all oilseeds are tested for oil content, moisture, and fatty acid profile. During expelling, the oil is tested for free fatty acid content, peroxide value, fatty acid composition, refractive index, color (Gardner) and general appearance. The crude oil is analyzed for color, moisture, free fatty acids and peroxide value. Before the refining process is started, the crude *C. tinctorius* GLA safflower oil is blended down to 50% γ-linolenic acid using oleic safflower oil; γ-linolenic acid levels are confirmed by fatty acid composition analysis. During the alkali refining process, the oil is tested for free fatty acids and soaps. During the bleaching process, the oil is tested for color and fatty acid salts. During the deodorization process, the oil is tested for free fatty acids, peroxide value, color, clarity and flavor. After deodorization, the oil is blended with oleic safflower oil to meet the γ-linolenic acid specification of SONOVA and analyzed for fatty acid composition, free fatty acid content, peroxide value, moisture, color, appearance, odor, flavor, and iodine value. During the drumming process, a composite sample is taken and the same analyses performed.

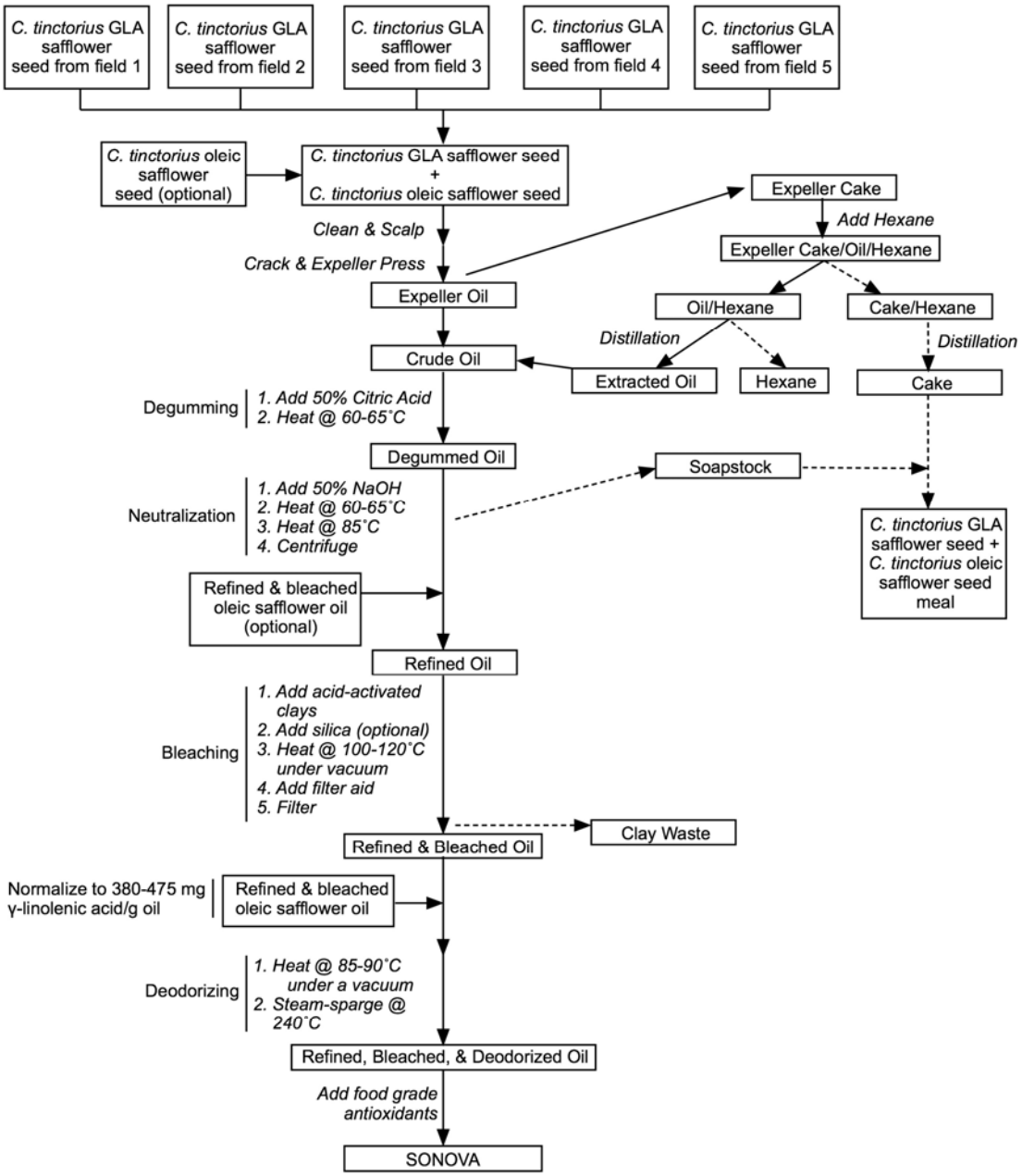

Figure 2. Production Process for SONOVA

SONOVA is produced from C. tinctorius GLA safflower seed obtained from multiple fields. To obtain a γ-linolenic acid levels within the product specification, raw seed and/or crude degummed oil may be mixed with C. tinctorius oleic safflower seed or refined and bleached oleic safflower oil. Mixing with C. tinctorius oleic safflower seed or refined and bleached oleic safflower oil does not occur when starting γ-linolenic acid levels in the γ-linolenic acid safflower seed are less than 70% or the amount of γ-linolenic acid levels in the crude degummed oil are less than 50%. Waste streams are highlighted with dashed lines.

D. FINISHED PRODUCT DESCRIPTIONS AND SPECIFICATIONS

1. Product Specifications

From crushing the seeds to packaging the oil, the production of SONOVA is a continuous process that occurs over days. All seed delivered to vegetable oil processing facility is mixed and expeller pressed. The resulting expeller oil and solvent-extracted oil are combined and stored in a single tank, resulting in a lot of crude *C. tinctorius* GLA safflower oil, which is then refined, bleached, and deodorized to produce SONOVA (also referred to as SONOVA 400). During normalization to 400 mg γ-linolenic acid/g oil with oleic safflower oil, one lot of SONOVA may be subdivided. If normalization occurs in a single tank, one third of the volume constitutes a lot of finished product. If more than one tank is required for normalization, each tank is treated as a single lot of finished product and, if necessary, subdivided to produce at least three lots of finished product. During drumming, samples are taken and analyzed to produce the certificate of analysis for each lot of finished product. Each drum is clearly labeled with the lot number. To date, three crushes have been completed yielding a total of 14 lots (Table 3). In 2012, Arcadia Biosciences separated the expeller oil from the solvent oil. Batch analyses for the 14 lots produced either from the expeller oil, solvent oil, or combination of the two demonstrates that Arcadia Biosciences and the vegetable oil processing facility have tight control over the manufacturing processes.

Table 3. Product Specifications and Batch Data for SONOVA

Parameter	Method	Spec.	Year:	2011					2012						2014			Mean +/- St. Dev.
			Lot:	1	2	3	4	5	6	7	8	9	10	11	12	13	14	
			Crude Source:	Exp. +Sol.[1]	Exp. +Sol.	Exp. +Sol.	Exp. +Sol.	Exp. +Sol.	Exp.	Sol.	Exp.	Exp.	Exp.	Exp.	Exp. +Sol.	Exp. +Sol.	Exp. +Sol.	
Fatty Acids																		
Palmitic Acid (C16:0)	AOCS Ce 1e-91	50-200 mg/g		66.34	69.33	67.33	66.88	65.54	69.70	69.80	65.80	67.90	67.30	67.30	73.90	74.00	73.70	68.92 +/- 3.01
Stearic Acid (C18:0)	AOCS Ce 1e-91	10-100 mg/g		20.56	21.06	20.46	20.41	20.36	19.30	19.80	19.30	19.70	19.70	19.40	20.90	20.90	20.90	20.20 +/- 0.66
Oleic Acid (C18:1n-9)	AOCS Ce 1e-91	100-500 mg/g		297.36	285.45	285.80	290.17	288.86	292.00	288.00	331.00	309.00	309.00	310.00	240.00	240.00	240.00	286.19 +/- 28.96
Linoleic Acid (C18:2n-6)	AOCS Ce 1e-91	100-300 mg/g		146.26	146.63	147.45	147.28	146.74	132.00	136.00	140.00	137.00	138.00	138.00	180.00	180.00	180.00	149.67 +/- 17.83
γ-Linolenic Acid (C18:3n-6)	AOCS Ce 1e-91	380-475 mg/g		435.21	442.96	445.60	438.71	441.00	443.00	451.00	383.00	422.00	421.00	432.00	464.00	464.00	465.00	439.17 +/- 22.26
Heavy Metals																		
Lead	ICP-AOCS Ca 17-01[3]	<0.2 ppm		ND[2]	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Arsenic	ICP-AOCS Ca 17-01[3]	<0.2 ppm		ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Cadmium	ICP-AOCS Ca 17-01[3]	<0.2 ppm		ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Mercury	ICP-AOCS Ca 17-01[3]	<0.2 ppm		ND	ND	ND		ND	ND		ND	ND	ND	ND	ND	ND	ND	ND
Other																		
Color	5 ¼" Lovibond	≤ 20 Red		6.1	6.1	ND 6.8	7.2	3.4	ND 10.8	20.0	7.8	8.1	7.6	8.3	8.0	7.9	7.7	8.27 +/- 3.84
Peroxide Value	AOCS Cd 8-53	≤ 10 meq/kg		1.2	1.0	1.1	1.0	1.2	1.1	2.2	1.1	1.2	1.3	1.7	1.3	1.6	1.9	1.35 +/- 0.38
Acid Value	AOCS Cd 3a-63	< 4.0 mg KOH/g		0.12	0.08	0.10	0.10	0.10	0.03	0.10	0.05	0.10	0.10	0.10	0.04	0.02	0.04	0.08 +/- 0.03
Moisture	AOCS Ca 2c-25	≤ 0.2%		0.06	0.07	0.06	0.06	0.08	0.06	0.05	0.05	0.04	0.05	0.04	0.04	0.04	0.02	0.05 +/- 0.02

[1]Exp = Expeller extracted oil; Solvent extracted oil.
[2]ND=None detected. Limit of detection = 0.1 ppm.

2. Hexanes

Hexane is the most commonly used solvent in edible oil processing (Williams, 2010). During the production of SONOVA, the hexane used to extract the residual amount of oil in the expeller cake is removed by distillation. To confirm that SONOVA does not contain hexanes or other solvents, Arcadia Biosciences analyzed four lots of SONOVA using the United States Pharmacopeial (USP) Convention method for detecting residual solvents in pharmaceutical products (USP <476>). Hexanes were undetectable at a limit of quantitation of 1 ppm, which is the maximum residue limit for hexane in fats and oils in the European Union (Directive 2009/32/EC).

3. Tocopherols

Tocopherols are fat soluble compounds produced by plants that are commonly found in vegetable-derived edible oils, and play an important role in minimizing fatty acid oxidation (Papas, 2006; Weber and Mukherjee, 2006). Because a majority of tocopherols are removed during the production process (O'Brien, 2000; Ortega-García et al., 2006), the vegetable oil processing facility supplements SONOVA with a mixture of α- and γ-tocopherols, citric acid, ascorbyl palmitate, and rosemary extract, to reduce the rate of oxidation. All the components in the mixture comply with 21 CFR 182 and 184. Although the analysis of tocopherol levels in SONOVA is not routine, an analysis of α- and γ-tocopherol levels in five samples were taken during the production of a pilot scale lot of SONOVA revealed that the average amount of α- and γ-tocopherol was 294 mg and 307 mg/kg SONOVA, respectively (Table 4).

Table 4. Tocopherol Levels in SONOVA

Tocopherol[1]	Sample Number					Average
	1	2	3	4	5	
α (mg/kg)	328	294	298	279	272	294
γ (mg/kg)	256	266	265	247	505	307

[1]Determined according to Total Tocopherol (TTLC_S:8) at Covance Laboratories.

4. Pesticides

Only safflower-specific pesticides registered by the Environmental Protection Agency (EPA), such as Dow Sonalan® HFP, Gowan Eptam® 7E, DuPont™ Harmony® SG, Syngenta Quadris® and Monsanto Roundup®, are used according to label guidance during the farming of *C. tinctorius* GLA safflower seed. Although the analysis of pesticide residues is not routine, Arcadia Biosciences has also analyzed SONOVA for the presence of pesticides not approved for use on safflower for due diligence purposes. All pesticides that were analyzed were undetectable.

5. Allergens

There are no reports of food hypersensitivity responses to safflower, safflower oils, or any of the genetic elements used to generate the genetically engineered *C. tinctorius* GLA safflower. However, SONOVA and the oleic safflower oil used to standardize γ-linolenic acid content are produced in a facility and on a manufacturing line that is used for the production of peanuts and peanut products, soybean and soybean products, sesame seed and sesame seed products, tree and tree nut products and other vegetable oils. As described in Section II.C.2.b appropriate containment and washing procedures are in place at the vegetable oil processing facility to prevent cross-contamination. Furthermore, Arcadia Biosciences determined that the protein concentration in the finished products is approximately 30 µg/kg (0.03 ppm). In addition, *S. diclina* Δ6 desaturase and *S. viridochromogenes* PAT gene products were undetectable by mass spectrometry and an enzyme-linked immunosorbant assay (ELISA; detection limit 0.5 ng), respectively. Assuming that the maximum intake of γ-linolenic acid would be approximately 2 g through the ingestion of performance nutrition products (Table 5), the resulting protein intake from SONOVA would be 0.15 µg, which is approximately 1000-fold lower that the lowest reported eliciting dose (0.1 mg) to peanut protein, one of the most potent allergens (Wensing et al., 2002).

6. Microbiologicals

Although microbiological contamination is not routinely monitored, *Escherichia coli*, *Salmonella*, total aerobes, yeast and mold colony forming units (CFUs) were determined in 5 samples harvested during a five day production run of a pilot-scale lot of SONOVA. No *E. coli*, *Salmonella*, yeast, mold, or other aerobic bacteria CFUs were detected. It is noteworthy that refining edible oils involves incubations at extreme temperatures for extended periods of time (i.e., 240°C for 20 min), thus greatly reducing the potential for microbial contamination.

7. Stability

SONOVA stability was determined when the material was stored frozen or at 23°C for 13 months. All drums were nitrogen purged, samples were taken monthly from previously unopened drums, and oil quality was evaluated using the peroxide value. When stored at both temperatures, the peroxide values were always less than the product specification limit of 10 meq/kg. Furthermore, SONOVA met all product specifications when drums were maintained at 23°C for 15 months.

III. INTENDED EFFECT

The intended effect of adding SONOVA to conventional and medical foods is to provide an enriched source of γ-linolenic acid. Although it contains high levels of γ-linolenic acid, SONOVA is composed of a variety of saturated and polyunsaturated fatty acids, sterols, and tocopherols that are present at levels equal to or less than those in other edible oils and conventional foods (Tables 1 and 2). Therefore, the absorption, distribution, metabolism, and excretion of these components, including the increased levels of γ-linolenic acid, will be the same as the absorption, distribution, metabolism, and excretion of these components when obtained from other dietary sources.

Fatty acids are a major source of fuel for the body and play integral roles in cell signaling, lipid and carbohydrate metabolism, inflammation, neurological function, and cell membrane structural integrity and dynamics (reviewed in IOM (US) Panel on Macronutrients and IOM (US) Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, 2005). Fatty acids can be either obtained from the diet or synthesized *in vivo*, and can be classified into 5 major categories: saturated fatty acids; cis-monounsaturated fatty acids; *n-6* polyunsaturated fatty acids; n-3 polyunsaturated fatty acids; and trans-fatty acids. Saturated fatty acids [caprylic acid (8:0), caproic acid (10:0), lauric acid (12:0), myristic acid (14:0), palmitic acid (16:0), stearic acid (18:0)] contain no double bonds. Cis-monounsaturated fatty acids [oleic acid (18:1), myristoleic acid (14:1), palmitoleic acid (16:1), vaccenic acid (18:1), eicosenoic acid (20:1), erucic acid (22:1)] have one double bond located 7 (*n*-7) or 9 (*n*-9) carbon atoms from the methyl tail of the fatty acid and the hydrogen atoms in double bond are present on the same side. *n-6* Polyunsaturated fatty acids [linoleic acid, γ-linolenic acid, dihomo γ-linolenic acid (C20:3Δ 8, 11, 14), arachidonic acid (C20:4Δ 4, 8, 11, 14), adrenic acid (22:4), docosapentaenoic acid (22:5)] contain more than one double bond with the most terminal bond located 6 (*n*-6) carbons atoms from the methyl end. *n*-3 Polyunsaturated fatty acids [α–linolenic acid (18:3), eicoapentanoic acid (20:5), docosapentaenoic acid (22:5), and docosahexaenoic acid (22:6)] also contain more than one double, but the most terminal double bond is located 3 carbon atoms from the methyl end. Trans unsaturated fatty acids contain at least one double bond with the hydrogen atoms present on opposite sides of the double bond.

Functionally, saturated fatty acids are used for fuel and are components of cell membranes. Monounsaturated fatty acids are components of cell membranes. Polyunsaturated fatty acids are components of cell membranes, activate signal transduction pathways, and are precursors for the production of eicosanoids and platelet activating factor (PAF), which are key modulators of inflammation, muscle tone, hemostasis, thrombosis, partuition, and gastrointestinal secretions. Trans fatty acids, in contrast, are not essential and do not provide health benefits.

Mammalian cells do not have the ability to insert a cis double bond at the *n*-6 position of a fatty acid chain. As a result, *n*-6 polyunsaturated fatty acids, specifically linoleic acid, can only be obtained from the diet. *In vivo,* linoleic acid is desaturated by Δ6 desaturase in a rate-limiting reaction to generate γ-linolenic acid (Figure 3). γ-Linolenic acid is then elongated to dihomo γ-linolenic acid (C20:3Δ 8, 11, 14) and further desaturated by Δ5 desaturase in another rate-limiting reaction to form arachidonic acid (C20:4Δ 4, 8, 11, 14), which is the primary precursor for eicosanoids and PAF (for review Smyth et al., 2009). Importantly, a diet deficient in linoleic acid results in adverse clinical symptoms such as scaly rashes and reduced growth rate (IOM (US) Panel on Macronutrients and IOM (US) Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, 2005). Moreover, reduced rates of γ-linolenic acid synthesis have been associated with aging, diabetes, alcoholism, rheumatoid arthritis, viral infections, hypercholesterolemia, and stress (reviewed in Horrobin, 1992). In contrast, diets containing high amounts of γ-linolenic acid (0.8 to 3 g/d) have been reported to improve symptoms associated with several inflammatory disorders (see Chapter 5, Section C.3; Biagi et al., 1994; Kawamura et al., 2011; Stewart et al., 1991; Schalin-Karrila et al., 1987; Wright and Burton, 1982; Belch et al., 1988; Brzeski et al., 1991; Leventhal et al., 1993; Leventhal et al., 1994; Zurier et al., 1996; Barabino et al., 2003; Ranieri et al., 2009; Jamal and Carmichael, 1990; Keen et al., 1993; Pacht et al., 2003; Gadek et al., 1999; Surette et al., 2003). Although the mechanism(s) by which this occurs is unclear, evidence in human and animal studies suggest that the ingestion of γ-linolenic acid can result in an accumulation of dihomo-γ-linolenic acid, the subsequent production of series-1 prostaglandins and 15-(S)-hydroxy-8,11,13-eicosatrienoic aid (15-HETrE), and inhibition of the inflammatory process (reviewed in Fan and Chapkin, 1998). Thus, in addition to provide an additional source of essential *n*-6 polyunsaturated fatty acids, the ingestion of products containing high amounts of γ-linolenic acid may increase endogenous dihomo γ-linolenic acid levels, reduce anti-inflammatory eicosanoid production, and provide some benefit to consumers with inflammatory conditions.

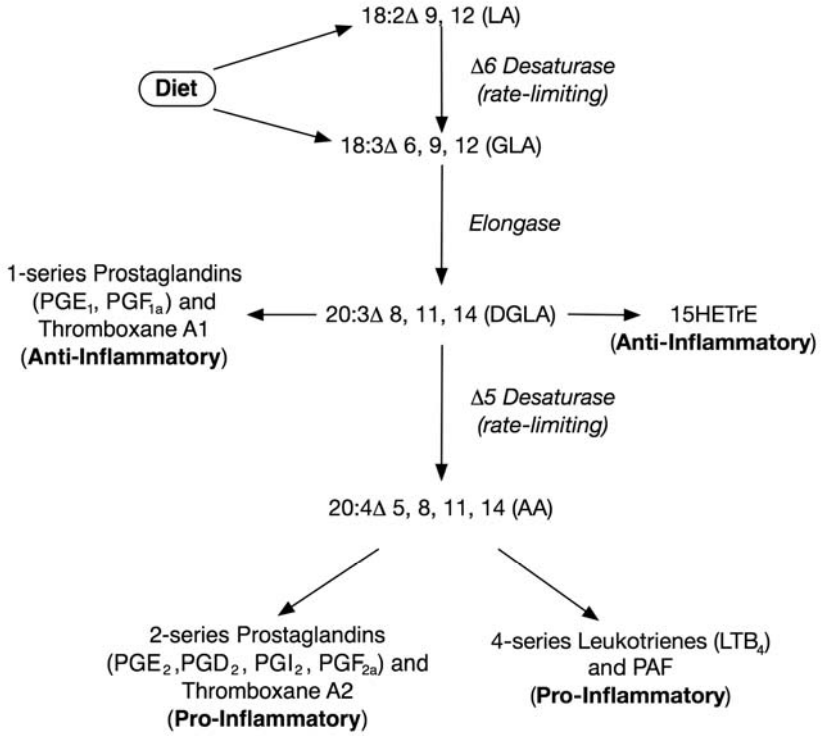

Figure 3. Metabolism to γ-linolenic Acid (GLA) to the Eicosanoids (Prostaglandins, Thromboxanes), 15-(S)-hydroxy-8, 11, 13-eicosatrienoic Acid (15HETrE) and Platelet Activating Factor (PAF)

Linoleic (LA) and GLA are obtained from the diet, but GLA can also be synthesized from LA via a rate-limiting reaction involving Δ6 desaturase. GLA is then elongated to dihomo-γ-linolenic acid (DGLA), which is desaturated to arachidonic acid (AA) via another rate limiting reaction involving Δ5 desaturase. DGLA is used to produce 1-series prostaglandins, thromboxane A1 and 15-(S)-hydroxy-8, 11, 13-eicosatrienoic acid (15HETrE), which are predominantly anti-inflammatory. Arachidonic acid is used to produce 2-series prostaglandins, thromboxane A2, 4-series leukotrienes, and platelet activating factor, which are predominantly pro-inflammatory.

Adopted from Fan et al., 1998.

IV. HISTORY OF USE, INTENDED USE, AND ESTIMATED DAILY INTAKE

A. HISTORICAL EXPOSURE TO SONOVA

SONOVA was introduced into the United States market in 2009 as a dietary supplement (FDA-1995S-0039). Labeling guidelines instructed consumers to limit their intake of SONOVA capsules to 4 capsules/d (approximately 800 mg γ-linolenic acid), SONOVA liquid to ¼ tsp/d (~280 mg γ-linolenic acid), SONOVA-containing omega 3-6-9 blend capsules to 3 capsules/d (~600 mg γ-linolenic acid), and SONOVA-containing Omega 3-6-9 blend liquids to 3 tbsp/d (~1200 mg γ-linolenic acid).

Borage seed oil contains approximately half the amount of γ-linolenic acid as SONOVA and has been commercially available as a dietary supplement since the early- to mid-1980s (Clough, 2001).

B. DIETARY INTAKES OF γ-LINOLENIC ACID

γ-Linolenic acid is an n-6 polyunsaturated fatty acid that can be either obtained from the diet or synthesized *in vivo* from linoleic acid, which cannot be made by humans and must therefore be obtained from the diet. γ-Linolenic acid is present at low levels in a wide variety of foods and edible oils (Table 1). Sources of n-6 polyunsaturated fatty acids include nuts, seeds, certain vegetables, and vegetable oils such as soybean, safflower oil, and corn oil.

According to GRN 306, the per capita mean and 90th percentile total dietary intakes for linoleic and γ-linolenic acid in the United States population were approximately 15 and 26 g linoleic acid/d, respectively, and 1 and 2 g γ-linolenic acid/d, respectively. These results were calculated using the National Health and Nutrition Examination Survey (NHANES) surveys conducted between 2003 and 2007. According to GRN 283, which calculated per capita mean and 90th percentile total dietary intakes of linoleic acid and γ-linolenic acid using the NHANES 1999 to 2002 surveys, mean and 90th percentile total dietary intakes of linoleic acid were 10 and 19.5 g/d, respectively, and mean and 90th percentile EDIs of γ-linolenic acid were 0.03 and 0.1 g/d, respectively.

C. INTENDED USES AND ESTIMATED DAILY INTAKES OF SONOVA

SONOVA will be used as substitute for other γ-linolenic-enriched sources that are currently used in nutritional beverages and medical foods. Therefore, dietary exposure to γ-linolenic from the ingestion of SONOVA-containing products will not exceed those currently resulting from the intake of nutritional beverages and medical foods enriched with other γ-linolenic-enriched sources.

Nutritional beverage and medical foods include meal replacement drinks, pediatric supplemental beverages, adult nutritional beverages, blood glucose management products, performance nutrition products, and tube-feeding or sole-source nutrition products (Table 5). The levels of addition range from 0.05 to 4 g γ-linolenic acid/serving (Table 5). Importantly, products that will be formulated with SONOVA as a source of γ-linolenic acid for use in these foods are not widely consumed by the U.S. population, have recommended directions for consumer use and some may be used as a sole source of nutrition. Assuming that these products are used as a sole source of nutrition, the resulting maximum γ-linolenic acid estimated daily intakes (EDIs) will range from 0.3 to 2 g γ-linolenic acid/d from ingested products and up to approximately 6 g/d from adult enterally-fed nutrition products.

The pediatric supplemental beverage products are intended for children 1 to 13 years of age, although they are primarily consumed by children of 3-6 years old at 1-2 servings/d (8 fl oz per serving). They may be used as a sole source of nutrition to provide approximately 946 ml/d for children of 1-8 years old and approximately 1420 ml/d for children of 9-13 years old.

The adult nutritional beverage products provide a source of nutrients to help fill nutritional gaps. It is used mostly as supplemental nutrition (1-2 servings/d), but can also be used as interim sole source nutrition, at 4-6 servings/d (providing approximately 1000-2000 kcal/d), for patients who have special nutritional needs.

Blood glucose management products are designed specifically to help adults with diabetes manage their blood glucose levels as part of an overall diabetes management plan. They are mainly used as a supplemental source of nutrition, up to 3 servings/d.

Performance nutrition products are used by fitness enthusiasts and athletes at up to 2 servings/d.

Tube-feeding or sole-source nutrition products may be high caloric (1.5 Kcal/ml) products intended for critically ill, mechanically ventilated patients, especially those with SIRS (systemic inflammatory response syndrome, e.g., sepsis, trauma, burns), ALI (acute lung injury), or ARDS (acute respiratory distress syndrome). Importantly, intake is dependent upon caloric need.

Table 5. Maximum Daily Intake of γ-Linolenic Acid (GLA) from the Intended Uses of SONOVA in Nutritional Beverages and Medical Foods****

Intended population	Product type	Intended use	Maximum amount of GLA	Recommended servings/d	GLA intake from product (g/d)
Pediatric	Supplemental or interim sole-source nutritional beverage	Nutritional beverage use	0.15 g/serving	2	0.3
		Medical food use (1 – 8 years old)	0.075 g/serving	4	0.3
		Medical food use (9 – 13 years old)	0.05 g/serving	6	0.3
Adult	Supplemental or interim sole-source nutritional beverage	Nutritional beverage use	0.25 g/serving	2	0.5
		Medical food use	0.075 g/serving	6	0. 5
	Performance nutrition product	Nutritional beverage use	1.0 g/serving	2	2.0
	Blood glucose management product	Medical food use	0.33 g/serving	3	1.0
	Tube-feeding or sole-source nutrition product for critically-ill, mechanically ventilated patients, especially those with SIRS (systemic inflammatory response syndrome, e.g., sepsis, trauma, burns), ALI (acute lung injury), or ARDS (acute respiratory distress syndrome)**	Medical food use	4 g/L	NA*	Based on caloric goal***

*NA=not applicable.
**These products contain approximately 1.5 Kcal/L and are administered according to the caloric need of the patient.
***Caloric goal for each subject can be calculated using the Harris-Benedict equation, which accounts for the gender, weight, height, and age. The resulting basal metabolic rate is multiplied by a factor that accounts for the energy requirement of the medical condition.
****SONOVA will be used as a substitute for the currently used sources of GLA in the nutritional beverages and medical foods. Therefore, GLA intake from the intended use will not exceed those that currently occur.

V. SAFETY OF SONOVA

The safe ingestion of γ-linolenic acid from the intended uses is supported by:

1. Adequate Intakes (AI; the recommended average daily intake levels of n-6 polyunsaturated fatty acids based on observed or experimentally determined approximations or estimates of nutrient intake by a group (or groups) of apparently, healthy people that are assumed to be adequate) and the Acceptable Macronutrient Distribution Range (AMDR; the range of intakes that are associated with reduced risk of chronic diseases while providing an adequate level of intake) for n-6 polyunsaturated fatty acids established by IOM (Table 6);

2. Per user EDIs of linolenic acid determined in GRN 306;

3. A wide variety of clinical studies involving healthy and health-compromised adults and children (i.e. subjects suffering for atopic dermatitis, acute lung injury, acute respiratory distress syndrome, etc.) (Tables 8, 9, and 10);

4. Four animal studies that administered diets containing up to 15% of γ-linolenic acid (Tso et al., 2012; Liu et al., 2004; Wainwright et al., 2003; Palombo et al., 2001).

5. Corroborative clinical and animal studies evaluating the safety of dihomo-γ-linolenic acid.

Although the animal studies provide data that supports the safety of consuming diets high in γ-linolenic acid, the previous GRNs, AIs, AMDRs, and clinical studies on γ-linolenic and dihomo-γ-linolenic acid provide the most relevant and compelling safety data because the diets used in the animals studies where the safety of γ-linolenic was evaluated contained 10% fat (Tso et al., 2012; Liu et al., 2004; Palombo et al., 2001; Wainwright et al., 2003), which is approximately double the amount that is typically in diets fed to laboratory rats (Johnson et al., 2008).

A. ADEQUATE INTAKES AND ACCEPTABLE MACRONUTRIENT INTAKE LEVELS FOR N-6 POLYUNSATURATED FATTY ACIDS

A Tolerable Upper Intake Level (UL), or the intake level at which adverse effects occur, for n-6 polyunsaturated fatty acids could not be set by the IOM because a defined intake level at which an adverse effect occurs has not been determined (IOM (US) Panel on Macronutrients and IOM (US) Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, 2005). However, IOM did establish an AMDR for n-6 polyunsaturated fatty acids based on the adverse effects of consuming a diet low or high in n-6 polyunsaturated fatty acids. According to IOM, the AMDR for n-6 polyunsaturated fatty acids is estimated to be 5 to 10% of total energy intake (Table 6). The minimum intake of 5 % of energy through the ingestion of linoleic acid would be needed to meet the AI for n-6 polyunsaturated fatty acids whereas the upper boundary of 10% is based on three factors: 1) individual dietary intakes in the North American population rarely exceeding 10% of energy; 2) epidemiological evidence for the safety of intakes greater than 10% of energy is generally lacking; and 3) high intakes of linoleic acid create a pro-oxidant state that may predispose consumers to severe chronic diseases such as coronary heart disease and cancer. Assuming that approximately 10% of total n-6 polyunsaturated fatty acid intake is γ-linolenic acid, the AMDR for males and females 1-year old is 0.4 – 0.9 g/d. For males and females older than 1-year old, the lower boundaries increase to approximately 1 g/d and the upper boundaries increase to 3 to 4 g/d. In addition, the AMDRs for males are generally higher than those for females after 8 years-of-age. Importantly, the EDIs from the intended uses (Table 5) fall below the upper boundaries for the AMDRs based on linoleic acid intake and the calculated AMDRs based on the estimated intake of linolenic acid (Table 6).

Table 6. Acceptable Macronutrient Distribution Range (AMDR) for Linolenic Acids in Children and Adults

Population	Age (years)	EER Range[a] (kcal/d)	AMDR for Linoleic Acids (g/d)	Approximate AMDR for Linolenic Acids (g/d)[e]
Females	1	768	4.3 – 8.5 [b]	0.4 – 0.9
	8	1360 - 2173	7.5 - 24.1 [c]	0.8 – 2.4
	14	1718 - 2831	9.5 – 31.5[c]	1 – 3.2
	Adults (BMI[a]=18.5 kg/m^2; height =67 inches)	1881 - 2662	10.5 – 29.6[d]	1.0 – 3.0
	Adults (BMI of 24.99 kg/m^2; height =67 inches)	2057 - 2916	11.4 – 32.4[d]	1 – 3.0
Males	1	844	4.7 – 9.4 [b]	0.4 - 0.9
	8	1453 - 2225	8.0 – 24.7 [c]	0.8 – 2.4
	14	2090 - 3283	11.6 – 36.5[c]	1.2 – 3.7
	Adults (BMI[a]=18.5 kg/m^2; height =71 inches)	2301 - 3225	12.8 - 35.8[d]	1.3 – 3.6
	Adults (BMI of 24.99 kg/m^2; height =71 inches)	2636 - 3720	14.6 – 41.3[d]	1.5 – 4.1

All data except AMDRs were obtained from IOM (US) Panel on Macronutrients and IOM (US) Standing Committee on the Scientific Evaluation of Dietary Reference Intakes (2005).

[a] EER = Estimated Energy Requirement Range for the indicated sex and age group; BMI = Body Mass Index.

[b]The lower boundary was calculated by multiplying the EER of girls and boys 1 year old by the minimum percentage of dietary fat recommended by IOM (5%) and the caloric density of fat (9 kcal/g). The upper boundary was calculated by multiplying the EER of girls and boys 1 year old by the maximum percentage of dietary fat recommended by IOM (10%) and the caloric density of fat (9 kcal/g).

[c] The lower boundary was calculated by multiplying the EER of sedentary (Physical Activity Level (PAL) ≥ 1.0 < 1.4) girls and boys 8 and 14 years old by the minimum percentage of dietary fat recommended by IOM (5%) and the caloric density of fat (9 kcal/g). The upper boundary was calculated by multiplying the EER of very active (PAL ≥ 1.9 < 2.5) girls and boys 8 and 14 years old by the maximum percentage of dietary fat recommended by IOM (10%) and the caloric density of fat (9 kcal/g).

[d] The lower boundary was calculated by multiplying the EER of sedentary (Physical Activity Level (PAL) ≥ 1.0 < 1.4) women and men, at the indicated BMI and height by the minimum percentage of dietary fat recommended by IOM (5%) and the caloric density of fat (9 kcal/g). The upper boundary was calculated by multiplying the EER of very active (PAL ≥ 1.9 < 2.5) women and men, at the indicated BMI and height by the maximum percentage of dietary fat recommended by IOM (10%) and the caloric density of fat (9 kcal/g). AMDRs for women and men with heights and BMIs greater or smaller than those shown will be larger or smaller, respectively.

[e] The AMDR for linolenic acids was calculated by dividing the AMDR for n-6 polyunsaturated fatty acids by a factor of 10, based on the results of GRN 306.

B. PER USER EDIS OF LINOLENIC ACID DETERMINED IN GRN 306

GRN 306, which received a "no questions" letter from the FDA on April 10, 2010, and calculated per user 90th percentile EDIs for linolenic acid and linoleic acid using the 2003-2006 National Health and Nutrition Examination Surveys (NHANES). The resulting 90th percentile EDIs for linolenic acids and linoleic acid were 2.49 g/d and 25 g/d, respectively. Thus, although other n-6 polyunsaturated fatty acids exist, the EDI of linolenic acid represents approximately 10% of total n-6 polyunsaturated fatty acid intake.

C. HUMAN STUDIES

To evaluate the safety of consuming products containing high levels of γ-linolenic acid, publicly available clinical studies where γ-linolenic and dihomo-γ-linolenic acid, the direct metabolite γ-linolenic acid, were administered to health and health-compromised subjects were retrieved, critically reviewed, and evaluated against the AMDRs set by IOM in 2005. Although the majority of these studies were not designed to directly evaluate the safety of γ-linolenic or dihomo-γ-linolenic acid and only a few evaluated the effects on hematology and clinical chemistry parameters, the weight of the evidence shows that prolonged intakes of high amounts of γ-linolenic and dihomo-γ-linolenic acid are well tolerated and do not result in adverse effects on measures of health status, such as hematology or clinical chemistries.

1. Orally Administered Products Containing γ-Linolenic Acid

Ten studies orally administered products containing γ-linolenic acid to healthy or health-compromised [atopic dermatitis (n=7), asthma (n=1), and atopic eczema (n=2)] infants and children at levels ranging from 0.1 g – 3 g γ-linolenic acid/d for up to 6 months (Table 7). The highest dose of 3 g γ-linolenic acid/d was administered for 4 weeks to children with atopic dermatitis who were 8 – 26 months old. In general, prolonged intakes of high amounts of γ-linolenic acid were well tolerated.

Thirty-five studies orally administered γ-linolenic acid to either healthy (n=6) and health-compromised adults such as those with hyperlipidemia (n=1), eczema (n=5), atopic dermatitis (n=5), transitional cell carcinoma (n=1), rheumatoid arthritis (n=7), diabetic neuropathy (n=2), osteoporosis (n=1), periodontitis (n=1), compressive root syndrome (n=1), ulcerative colitis (n=1), metastatic breast cancer (n=1), asthma (n=2) and aqueous-deficient keratoconjunctivitis (n=1) at levels ranging from 0.03 – 6 g γ-linolenic acid/day for up to 18 months (Table 8). The highest dose of 6 g/day was administered to healthy adults for 21 days.

Ten studies evaluated clinical parameters, including blood biochemistries, electrolytes, liver enzymes, hematologies, and coagulation indices (Covar et al., 2010; Hederos et al., 1996; Surrette et al., 2003; Surrett et al., 2008; Rilliet et al., 1988; Keen et al., 1993; Kruger et al., 1998; Guivernau et al., 1994; Gadek et a., 1999; Jacobs et al., 2013). Three studies were conducted in children who received up to 3 g γ-linolenic acid/day for up to 16 weeks from γ-linolenic acid-enriched sources (Covar et al., 2010 and Hederos et al., 1996; Jacobs et al., 2013). One of the three studies in children was in critically-ill, mechanically-ventilated children receiving sole source enteral nutrition that were diagnosed with either acute lung injury or acute respiratory distress syndrome (Jacobs et al., 2013). The remaining eight studies were conducted in adults who received up to 5.8 g γ-linolenic acid/day for up to 18 months from γ-linolenic acid-enriched sources (Surrette et al., 2003; Surrett et al., 2008; Rilliet et al., 1988; Keen et al., 1993; Kruger et al., 1998; Guivernau et al., 1994; Gadek et a., 1999; Jacobs et al., 2013). One of the eight adult studies was conducted in adults receiving sole source enteral nutrition that were diagnosed with acute respiratory distress syndrome (Gadek et al., 2013). Although Guivernau et al. (1994) reported that the ingestion of evening primrose oil by hyperlipidemic men, which resulted in approximately 0.24 g γ-linolenic acid/day, significantly decreased serum triglycerides and cholesterol, and increased HDL and bleeding time, the weight of the evidence from the remaining studies shows that the ingestion of γ-linolenic acid at levels equal to or greater than the estimated exposure for SONOVA does not significantly or reproducibly affect blood biochemistries, electrolytes, liver enzymes, hematologies, or coagulation indices.

2. Enterally Administered Products Containing γ-Linolenic Acid

Eleven studies administered γ-linolenic acid-enriched enteral diets to subjects with acute lung injury, acute respiratory distress syndrome, early sepsis, amyotrophic lateral sclerosis (ALS) or who had undergone subtotal oesophagectomy and total gastroectomy (Table 9).

The level of γ-linolenic acid administered and duration of administration in nine of these studies ranged from 0.1 – 4.6 g /day and for up to 7 days (Pontes-Arruda et al., 2006; Pontes-Arruda et al., 2011; Singer et al., 2006; Jacobs et al., 2013; Grau-Carmona et al., 2011; Sultan et al., 2012, Wills et al., 2014, Kushta et al., 2011; Pacht et al., 2003). Compared to the control formulas, the γ-linolenic acid-enriched formulas were well-tolerated, did not produce adverse events, and were not associated with adverse effects on hematology or clinical chemistries. In some studies, the administration of the γ-linolenic acid-enriched diets improved the clinical endpoints, such as survival rate, gas exchange, ventilator-free days, and organ dysfunction.

The remaining two studies administered γ-linolenic acid-enriched enteral diets at levels higher than 4.6 g γ-linolenic acid/day (Gadek et al., 1999; Rice et al., 2011). Gadek et al. (1999) administered enteral diet to adults with acute respiratory distress at a constant rate to achieve a minimum of 50% of basal energy expenditure (BEE), calculated using the Harris-Benedict equation, x 1.3 within the first 24 hr. Continuous enteral nutrition was then advanced as tolerated with the goal of achieving a minimum of 75% of BEE x 1.3 within 72 hours of initiation of enteral feeding. Enteral nutrition was delivered continuously for a minimum of four study days ± 1 day at a rate not to exceed BEE x 1.3. Daily enteral intake was recorded for total volume and calories delivered to the patient. The average intake of γ-linolenic acid was 5.8 +/- 0.3 g/day. Consistent with the results of the other studies (Pontes-Arruda et al., 2006; Singer et al., 2006), γ-linolenic acid-enriched enteral diets were well tolerated and associated with statistically significant decreases in the number of adverse health effects. Adverse events that were reported were unrelated to the amount of GLA administered. In contrast, Rice et al. (2011) administered an enteral diet containing high amounts of γ-linolenic acid to adults with developing acute lung injury in twice daily bolus doses for 3 weeks resulting in a γ-linolenic acid intake of 5.9 g/day. Compared to the control group, which received a high carbohydrate/high protein enteral diet, administration of the γ-linolenic acid-enriched diet was associated with significant increases in mortality and diarrhea, and the study was terminated after the first interim analysis because the primary end point (ventilator free days) and the major secondary end point (mortality) crossed the predefined futility boundaries. Notably, the statistically significant effect on mortality, which was identified in the futility analysis, could not be confidently attributed to the γ-linolenic acid - enriched formula.

Other differences in the Gadek et al. (1999) and Rice et al. (2011) studies may have also accounted for the disparity in the results such as the types of formula used and the age of the subjects. Although the formulas used by Rice et al. were isocaloric, the γ-linolenic acid-enriched formula contained 5-times less protein, 12-times less carbohydrate, and higher amounts of vitamin C, vitamin E, zinc, selenium, L-carnitine, and taurine than the control formula. In a study conducted by Gadek et al., the test and control formulas were similar in protein, carbohydrate, and lipid content and the only differences were in the amount of γ-linolenic acid, vitamin C, vitamin E, β-carotine, L-carnitine, and taurine. In addition, there was a slight imbalance in age, APACHE (Acute Physiology, Age, Chronic Health Evaluation) III score, Pa_{O2}:FI_{O2} ratio, and minute ventilation in the study conducted by Rice et al., which may have favored the control group. Thus, due to confounding factors in the design of the Rice et al. study, it is not possible to determine the safety of administration of γ-linolenic acid enriched enteral diets to critically-ill patients when given a bolus administration of the enteral formula containing GLA used in that

study. However, continuous administration of enteral formula containing 4 g/liter of GLA in critically-ill patients demonstrated safety and tolerance (Gadek et al., 1999, Wills et al., 2014; Sultan et al., 2012; Kushta et al., 2011).

It is also noteworthy that seven of the 11 studies that administered γ-linolenic acid-enriched enteral diets to humans, used a γ-linolenic acid-containing enteral diet by Abbott Laboratories (Pontes-Arruda et al., 2011; Singer et al., 2006; Jacobs et al., 2013; Grau-Carmona et al., 2011; Gadek et al., 1999, Sultan et al., 2012, Wills et al., 2014, Kushta et al., 2011). This diet has been distributed in the United States for more than 10 years and approximately 511,000 L were distributed from April 1, 2012 to March 31, 2014. During this time the adverse event reports were primarily related to gastrointestinal symptoms that are within the expected safety profile of this product when fed to the intended population according to directions provided on the label or as instructed by a health care professional. Further analysis of these and all other adverse events do not suggest atypical trends in the frequency and/or severity of the adverse events reported. Based on the adverse event reports received, when aligned with patient exposure, the likelihood of a patient experiencing any specific adverse event coincident with the use of γ-linolenic acid-enriched enteral diets is unlikely (i.e. <1 report/100,000 L distributed).

3. γ-Linolenic Acid and Inflammation

The effects of diets containing γ-linolenic acid on inflammation have been investigated in a wide variety of clinical studies involving healthy and health-compromised subjects.

In healthy subjects, three studies have shown that the ingestion of γ-linolenic acid at levels ranging from 0.8 g/day to 3.0 g/d for periods ranging from 3 to 4 weeks (Table 7; Johnson et al., 1997; Chilton et al., 2008; Weaver et al., 2009) can attenuate inflammatory metabolites. Johnson et al. (1997) orally administered 3.0 g γ-linolenic acid/day for 3 weeks to healthy adults and evaluated its effect on serum and neutrophil membrane γ-linolenic acid, dihomo-γ-linolenic acid, and arachidonic acid levels. Circulating neutrophils were also harvested from the subjects before and after the dihomo-γ-linolenic acid treatment. The production of the proinflammatory leukotriene, LTB_4, and PAF by the cells was determined following their stimulation with the calcium ionophore A23187. Although γ-linolenic acid administration led to significant, dose-dependent increases in arachidonic, dihomo-γ-linolenic, and γ-linolenic acid serum levels relative to baseline, it significantly and dose-dependently increased only dihomo-γ-linolenic acid levels in neutrophil membranes. *Ex vivo* stimulation of the neutrophils showed that the γ-linolenic acid administration reduced LTB_4 and PAF production. Chilton et al. (2008) administered 1.5 g γ-linolenic acid/day in diets supplemented with borage oil for 3 weeks. Plasma fatty acids and *ex*

vivo stimulated whole-blood LTB_4 production were measured in whole blood samples collected at baseline, weekly for 3 weeks and after a 2-week washout period. γ-Linolenic acid supplementation resulted in increased circulating plasma arachidonic acid concentrations, but because dihomo-γ-linolenic acid is efficiently incorporated into the polymorphonuclear granulocyte (PMN) lipids and PMNs lack Δ5 desaturase activity, the PMN content of arachidonic acid did not increase. Furthermore, *ex vivo* stimulation of whole blood leukocytes with the calcium ionophore, A23187, revealed that 2 weeks of γ-linolenic acid supplementation significantly decreased LTB_4 production, which returned to baseline levels after the 2-week washout period. Weaver et al. (2009) obtained similar results administering 0.8 g γ-linolenic acid/day for 4 weeks.

To evaluate the effect of ingesting γ-linolenic acid on inflammation, formulations containing γ-linolenic acid alone or in combination with other polyunsaturated fatty acids, such as eicosapentanoic acid, have been administered to subjects with inflammatory conditions such as atopic dermatitis and eczema, lung injury, respiratory distress syndrome, rheumatoid arthritis, aqueous-deficient keratoconjunctivitis sicca (KCS; dry eye syndrome), and cancer (Tables 8 and 9). Although the effects are not entirely reproducible, the evidence suggests that consuming a diet containing γ-linolenic acid may be beneficial.

Atopic dermatitis is associated with a number of abnormalities of non-immunological nature, including changes in the fatty acid composition of blood lipids, particularly an increase in linoleic acid and a decrease in arachidonic acid and other linolenic metabolites (Manku et al., 1984; Biagi et al., 1994; van Gool et al., 2003). The increase in linolenic acid and decrease in arachidonic acid suggest that the Δ6 desaturase may be defective in subjects with atopic dermatitis, and result in a decrease in the amount γ-linolenic acid and an imbalance in prostaglandin production. Other groups have speculated that administering high amounts of γ-linolenic acid may improve inflammatory skin disease by increasing the relative amount of non-inflammatory prostaglandins and leukotrienes (Berth-Jones and Graham-Brown, 1993; Hederos and Berg, 1996). Five studies have reported that direct administration of γ-linolenic acid (from evening primrose oil or γ-linolenic acid-enriched oil extracted from the fungus *Mucor circinelloides*) led to an improvement in the symptoms associated with atopic dermatitis (Table 7 and 9; Biagi et al., 1994; Kawamura et al., 2011; Stewart et al., 1991; Schalin-Karrila et al., 1987; Wright and Burton, 1982). In contrast, a meta-analysis of nineteen placebo-controlled trials of γ-linolenic acid from borage oil, evening primrose oil and blackcurrant seed oil and five trials of fish oil did not confirm that supplementation with γ-linolenic acid exerts a large effect

on reducing severity of atopic dermatitis (van Gool et al., 2004), although the study had a low power to detect a moderate effect of supplementation with γ-linolenic acid on atopic dermatitis.

γ-Linolenic acid administration has also been evaluated in subjects with rheumatoid arthritis (Table 8; Belch et al., 1988; Brzeski et al., 1991; Leventhal et al., 1993; Leventhal et al., 1994; Zurier et al., 1996). In five studies, the administration of formulations containing γ-linolenic acid ranging from 0.54 g to 2.8 g/d resulted in significantly and clinically relevant reductions in the signs and symptoms of disease activity in patients with rheumatoid arthritis were noted (Belch et al., 1988; Brzeski et al., 1991; Leventhal et al., 1993; Leventhal et al., 1994; Zurier et al., 1996). In contrast, two studies that administered 0.36 g and 1 g γ-linolenic acid/d reported no improvements in the signs and symptoms of disease (Hansen et al., 1983; Pullman-Mooar et al., 1990).

Barabino et al. (2003) administered capsules delivering approximately 30 mg γ-linolenic acid/d to subjects with aqueous-deficient keratoconjunctivitis sicca (dry eye syndrome) and reported that the γ-linolenic acid-containing formulation reduced ocular surface inflammation and improved the associated symptoms (Table 9).

Ranieri et al., 2009 administered a combination of 0.36 g γ-linolenic acid and 0.6 g α-lipoic acid/d to patients with compressive radiculopathy syndrome undergoing rehabilitation for 6 weeks. Compared to a group that received the rehabilitation program alone, the linolenic acid α-lipoic acid combination improved the associated neuropathic symptoms of paresthesia, stabbing and back pain, possibly through the modulation of prostaglandins (Table 8).

Two studies conducted in patients with diabetic neuropathy showed that the administration of formulation containing 0.3 and 0.48 g γ-linolenic acid/d significantly improved a variety of symptoms, such as neuropathy symptom scores, median nerve motor conduction velocity and compound muscle action potential amplitude, peroneal nerve motor conduction velocity and compound muscle action potential amplitude, median and sural sensory nerve action potential amplitude and ankle heat and cold threshold values (Table 8; Jamal and Carmichael, 1990; Keen et al., 1993).

One study found that a borage seed oil delivering approximately 1.4 g γ-linolenic acid/d to patients with ulcerative colitis provided no benefit (Table 8; Middleton et al., 2002).

Three studies evaluated the effect of γ-linolenic and eicosapentanoic acid-enriched formulations in modulating the inflammatory responses in subjects with acute lung injury (Table 9; Rice et al., 2011; Pacht et al., 2003; Gadek et al., 1999). As discussed above, Rice et al. was

prematurely terminated because the primary and major secondary endpoints crossed predefined futility boundaries. In the remaining two studies, Pacht et al. (2003) and Gadek et al. (1999) both found that the ingestion of the γ-linolenic and eicosapentanoic acid-containing formulations significantly increased arterial oxygenation and reduced the number of neutrophils in the bronchoalveolar lavage fluid.

Two studies evaluated the effects of a γ-linolenic acid-containing formulation in the dietary management of asthmatic subjects (Table 8). One study found that the administration of 0.75 and 1.13 g γ-linolenic acid/d to asthmatic patients reduced zymosan-induced LTB_4 production from ex vivo neutrophils (Surette et al., 2003). The other found that formulation delivering 0.75 g γ-linolenic acid/d improved quality of life measurements, determined using validated asthma questionnaires, and reduced bronchodilator use (Surette et al., 2008).

4. Orally Administered Products Containing Dihomo-γ-Linolenic Acid

Two studies orally administered products containing dihomo-γ-linolenic acid to healthy adults (Teraoka et al., 2009; Tanaka et al., 2012).

In a randomized, double-blind, placebo-controlled study, Tanaka et al. (2012) evaluated the safety and tolerability of ingesting dihomo-γ-linolenic acid by administering either a dihomo-γ-linolenic acid-enriched oil, delivering approximately 450 mg dihomo-γ-linolenic acid, or placebo (olive oil) to healthy males and females for 4 weeks, followed by a 4-week washout period. Eleven subjects received dihomo-γ-linolenic acid and ten subjects received placebo. Only one subject in the dihomo-γ-linolenic acid-treated group withdrew due to having a cold. There were no side effects and all adverse events observed were considered unrelated to supplementation. After 4 weeks of supplementation, significant increases in dihomo-γ-linolenic and arachidonic acid levels serum phospholipids were observed in the dihomo-γ-linolenic acid-treated group, both of which returned to baseline levels after the 4-week washout. Importantly, although significant differences in the levels of lactate dehydrogenase, albumin, total cholesterol, the number of red blood cells, hematocrits relative to their respective levels at baseline occurred in both dihomo-γ-linolenic acid- and placebo-treated groups, all values were with the normal ranges for the enrolled subjects. Moreover, diastolic and systolic blood pressures, coagulation (prothrombin time, activated partial thromboplastin time and antithrombin III) and urinalysis parameters were all within the normal ranges throughout the study and did not significantly different between the two groups.

To evaluate the effects of dietary dihomo-γ-linolenic acid on physical characteristics, blood and urine chemistries, platelet aggregation, and serum phospholipids, Teraoka et al. (2009)

administered either placebo (olive oil; n=10) or a dihomo-γ-linolenic acid-enriched oil, delivering 50 (n=10) and 150 mg dihomoγ-linolenic acid/day (n=10) to healthy men for 4 weeks followed by a 4 week washout period in a randomized, double-blind, placebo-controlled study. One subject in the 50 mg-treated group withdrew due to personal reasons. No side effects were reported and all adverse events that occurred were considered unrelated to ingestion of the dihomo-γ-linolenic acid-enriched oil. After 4 weeks, dihomo-γ-linolenic acid levels increased in a dose-dependent fashion in the serum phospholipids in the dihomo-γ-linolenic acid-treated subjects, and the increase was significant ($p<0.05$) in 150 mg/day-treated group compared to the placebo-treated group. After the 4-week washout period, the significant increase in serum dihomo-γ-linolenic acid levels in 150 mg/day-treated group returned to baseline. There were no significant effects of dihomo-γ-linolenic acid supplementation on platelet aggregation and, although significant differences in serum albumin in the low-dose group compared to the placebo group, creatinine in the high dose group relative to baseline, and prothrombin time in the high-dose group compared to the placebo group were reported, all effects on blood and urine chemistries were not dose-dependent and within the normal range for the males enrolled in the study.

Table 7. Clinical Trials of γ-linolenic Acid (GLA)-enriched Oils in Infants and Children

Reference	Study Design and Population	Treatments and Number of Subjects	Duration	Safety Outcomes
Fiocchi et al., 1994	Open-label, uncontrolled Children between 8-26 mo with atopic dermatitis	3 g GLA/d; n=10 (5M/5F)	4 wk	Relative to baseline, GLA supplementation significantly increased percent of CD8+ lymphocytes at 4 wk. Relative to baseline, GLA supplementation had no effect on IgE levels. Relative to baseline, GLA supplementation significantly reduced the presence, intensity and site of itching at 4 wk.
Covar et al., 2010	Randomized, double-blind, placebo-controlled Children, aged 6-14 years old, with mild to moderate persistent asthma. Of 43 randomized, 19 receiving test formula (NNF) and 18 receiving control formula completed the study.	Group 1: Control, given complete ready-to-drink nutritional formula with an oil blend (100% high-oleic safflower oil); n=18 Group 2: NNF, complete ready-to-drink nutritional formula with a blend of oils (fish oil and borage oil), and a mix of antioxidant vitamins and minerals. Equivalent to 3 g GLA/d; n=19 Of those who did not complete: in the NNF group, four withdrew, one was due to hospitalization for asthma immediately after randomization, one disliked the taste of formula, one due to physician discretion and one withdrew consent. In the control group, two withdrew, one due to formula taste intolerance and one due to elevated serum potassium.	12 wk	At baseline and at 4 and 12 weeks of study, the control and NNF groups had similar mean values for serum chemistries, electrolytes, liver function, hematology and blood coagulation indices, with all variable remaining within the normal range. No significant differences in body weight, height, or body mass index changes between study groups. Adverse events were reported in 16 NNF and 10 control patients; of the 42 adverse events reported, 26 were in the NNF group and 16 in the control group. Only three of these events were considered possible related to study formula: one in the NNF group (mild elevation of liver enzymes) and two in the control group (one mild elevated potassium and one moderate episode of vomiting). The only severe event, an upper respiratory infection, probably not related to the study formula, occurred in the control group. No difference in asthma free days between active treatment and control.

Table 7. Clinical Trials of γ-linolenic Acid (GLA)-enriched Oils in Infants and Children

Reference	Study Design and Population	Treatments and Number of Subjects	Duration	Safety Outcomes
Biagi et al., 1994	Double-blind, placebo-controlled Children aged 2.2-8.5 yr with atopic dermatitis and abnormalities of IgE-mediated immune responses	Subjects were sorted into allergic and non-allergic groups and then randomized to three Different groups Group 1: Olive oil + 10 mg Vitamin E (Placebo); n=16 Group 2: Olive oil mixed with evening primrose oil (Low GLA; equivalent to 0.02 g GLA/kg•d); n=16 Group 3: Evening primrose oil (High GLA; equivalent to 0.4 g GLA/kg•d); n=16	8 wk	Three children did not attend for follow-up (reasons not given). Overall severity of atopic dermatitis was improved at 8 wk vs. baseline. Individual fatty acid content of erythrocyte membranes was altered more after treatment in children without concomitant allergies, in comparison with those having allergies. GLA was only increased in erythrocyte membranes of non-allergic children receiving the higher dose of oil. Overall *n*-6/*n*-3 ratio was significantly increased from baseline to study's end in the allergic children but not in non-allergic children. GLA supplementation had no effect on erythrocyte membrane fluidity. The clinical significance of these findings was unclear.

Table 7. Clinical Trials of γ-linolenic Acid (GLA)-enriched Oils in Infants and Children

Reference	Study Design and Population	Treatments and Number of Subjects	Duration	Safety Outcomes
Berth-Jones and Graham-Brown, 1993	Double-blind, placebo-controlled, parallel-group Children and adults with atopic dermatitis	Group 1: Paraffin and olive oil (Placebo); n=41 (25M/16F; 20 subjects were 12-yr old or younger) Group 2: Capsules containing evening primrose oil (0.48 g GLA/d); n=41 (22M/19F; 21 subjects were 12-yr old or less) Group 3: Capsules containing evening primrose oil and fish oil (0.4 g GLA/d); n=20 (22M/19F; 20 subjects were 12-yr or less)	16 wk	At 4 weeks, 9 subjects had defaulted or been withdrawn. By 18 weeks, 14 had defaulted or been withdrawn. By 16 weeks, 21 had defaulted or been withdrawn. In the placebo group, four subjects experienced deterioration of eczema, two were non-compliant, and 1 defaulted. In the evening primrose oil -treated group, one subject experienced deterioration of eczema, two were excluded due to non-compliance, three withdrew consent, and two experienced nausea. In the evening primrose oil + fish oil group, three subjects experienced deterioration of eczema, two defaulted, and one had diarrhea. Baseline symptoms were similar across all groups. With the exception of patients withdrawn due to deterioration, there were no apparent differences in response to treatment.

Table 7. Clinical Trials of γ-linolenic Acid (GLA)-enriched Oils in Infants and Children

Reference	Study Design and Population	Treatments and Number of Subjects	Duration	Safety Outcomes
Hederos and Berg, 1996	Double-blind, randomized, placebo-controlled, parallel Children (1-16 yr old) with atopic dermatitis	Dosage was according to age Group 1: Sunflower oil + vitamin E (Placebo); n = 30 (13M/17F). Group 2: 1-12 yr received 4 capsules containing evening primrose oil (equiv. to 0.3 g GLA/d); > 12 yr received 6 capsules containing evening primrose oil (equiv. to 0.4 g GLA/d)	16 wk	Routine hematological and biochemical analyses showed one significant difference between treatments and that was for urate, but all serum urate concentrations remained within the normal range. Five patients in the evening primrose oil group and six patients in the placebo group reported seven adverse events. None were considered serious and only one in each group was considered a possible effect of treatment.
Guenther and Wexler, 1987	Double-blind, crossover Children with atopic dermatitis	Treatment 1: Placebo Treatment 2: evening primrose oil Dosage was according to age. < 7 yr received 4 capsules of evening primrose oil/d 7-14 yr received 6 capsules of evening primrose oil /d > 14 yr received 8 capsules of evening primrose oil /d GLA content of Efamol is not reported; calculated estimated doses at 0.18, 0.27, and 0.36 g GLA/d, based on estimated 9% GLA per 0.5 g capsule.	12 wk	Fourteen patients completed the study and three withdrew after the first visit. Both groups showed improvement, but no statistically significant differences in the severity of eczema between the two groups. No other safety parameters reported; fidgeting was reduced versus placebo and versus baseline.

Table 7. Clinical Trials of γ-linolenic Acid (GLA)-enriched Oils in Infants and Children

Reference	Study Design and Population	Treatments and Number of Subjects	Duration	Safety Outcomes
Bordoni et al., 1988 [Note: authors mention that data suggest that in atopic eczema there is a reduced ability to convert linoleic acid to GLA and DGLA]	Randomized Children 2-4 yr old with atopic eczema	Group 1: Olive oil (placebo); n =12 Group 2: Evening primrose oil (equiv. to 0.3 g GLA/d); n=12	4 wk	Relative to baseline, the percent of DGLA in plasma increased significantly in the evening primrose oil -treated group. Adrenic acid (22:4 *n*-6) levels also increased significantly in neutrophils relative to baseline levels in the EPO-treated group. Authors reported that no side effects were apparent in either group.
Shimasaki, 1995	Intervention-only Japanese children (3-10 yr; 30M/21F) with and without atopic dermatitis	Group 1: Japanese children without atopic dermatitis; n =30 Group 2: Japanese children with atopic dermatitis that received candy-type jellies containing GLA (equiv. to 0.18 g GLA/d); n=51	8 wk	GLA as plasma levels increased in Group 2 relative to Group 1 Other fatty acids, which were increased in the plasma of treated subjects, included 15:0, DGLA (20:3 *n*-6), ARA (20:4 *n*-6), DPA (22:5 *n*-3) and α-LA (18:3 *n*-3). Adverse effects were not mentioned by the authors.

Table 7. Clinical Trials of γ-linolenic Acid (GLA)-enriched Oils in Infants and Children

Reference	Study Design and Population	Treatments and Number of Subjects	Duration	Safety Outcomes
Lovell et al., 1981	Randomized, double-blind, cross-over Children and adults with atopic eczema	Treatment 1: Liquid paraffin Treatment 2: Evening primrose oil (equiv. 0.2 g GLA/d) n=32	3 wk	Efamol caused no adverse reactions in either adults or children. Subjects treated with evening primrose oil exhibited a modest, but statistically significant, improvement on both the doctor's and self-assessments.
van Gool et al., 2003	Randomized, double-blind, placebo-controlled Formula-fed infants at high risk for atopic dermatitis 121 infants were included, 118 completed follow-up; gestational age of ≥ 38 wk, birth weight > 2500 g, uncomplicated perinatal period, exclusive formula-feeding from 2 wk of age	Group 1: Sunflower oil (Placebo); n=60 Group 2: Borage oil (equiv. to 0.1 g GLA/d); n=61	6 mo	Two infants in Group 1 were lost to follow-up due to lack of time to parent and due to abdominal cramps). One infant in Group 2 was lost to follow-up due to lack of time of parent. Adverse events were similar across two groups and among the subjects that completed the study, there were 4 reports of milk reflux and 1 report of lactose intolerance in Group 1, and 1 report of food aversion, 2 reports of abdominal cramps, 1 report of milk reflux, 1 report of reflux oesophagitis, and 1 report of obstipation in Group 2. At 1-yr follow up, there was a non-significant reduction in the severity of atopic dermatitis.

Table 8. Clinical Trials of γ-linolenic (GLA)-enriched oils in Adults

Reference	Study Design and Population	Treatment and Number of Subjects	Duration	Safety Outcomes
Johnson et al., 1997	GLA supplementation (4 studies involving healthy adults).	Study 1: Capsules containing a proprietary blend of oil (Ultra GLA or BIO-EFA; equiv to 3.0 g GLA/d); n=9 Study 2: Capsules containing a proprietary blend of oil (Ultra GLA or BIO-EFA; equiv to 3.0 g GLA/d); n=5. Study 3: 4 groups Group 1: no supplementation; n =3 Group 2: Equiv. to 1.5 g GLA/d; n=3 Group 3: Equiv. to 3.0 g GLA/d; n=3 Group 4: Equiv. to 6 g GLA/d; n=3 Study 4: Equiv. to 5 g GLA/d; n=3.	Study 1, 2, and 3 - 3 wk Study 4 - 12 wk	GLA supplementation significantly increased serum concentrations of AA at all doses whereas only 3 and 6 g GLA/day significantly increased the serum concentrations of DGLA and GLA; led to significant increases in GLA levels in serum phospholipids, and cholesterol esters, and DGLA and AA levels in serum phospholipids, but had no effect on GLA, DGLA, or AA levels in diglyceride, free fatty acid, or triglyceride fractions; increased DGLA concentrations in the glycerolipid fraction of neutrophil membranes, specifically in the neutral lipid and phosphatidylethanolamine but not the phosphatidylinositol/phosphatidylserine and phosphatidylcholine fractions; and increased the amount of DGLA release from A23187-stimulated neutrophils harvested from the different groups in study 4 (increased appeared to be dose-dependent) Significant increases in serum concentrations of AA and DGLA were seen as early as 2 weeks after supplementation began and continued until supplementation was stopped. GLA levels were also significantly increased at 2, 8 and 12 weeks but were not increased at 4 and 10 week. AA, DGLA, and GLA levels returned to normal when GLA supplementation was stopped. GLA supplementation reduced LTB_4 and PAF production from A23187-stimulated neutrophils harvested from subjects consuming 3 g GLA/day for 3 weeks. The authors stated that no adverse effects were reported before or after supplementation.

Table 8. Clinical Trials of γ-linolenic (GLA)-enriched oils in Adults

Reference	Study Design and Population	Treatment and Number of Subjects	Duration	Safety Outcomes
Laidlaw and Holub, 2003	Placebo-controlled clinical trial involving healthy women	Group 1: Fish oil concentrate (no GLA); n=8 Group 2: Fish oil conc. + borage oil conc. (equiv. 1 g GLA/d); n=8 Group 3: Fish oil conc. + borage oil conc. (equiv to 2 g GLA/d); n=7 Group 4: Fish oil conc. + borage oil conc. (equiv. 4 g GLA/d); n=8	28 d	No adverse side effects were reported by the investigators. The mixture of EPA + DHA and GLA favorably altered blood lipid and fatty acid profiles.
Chilton-Lopez et al., 1996	Healthy subjects	Capsules containing borage oil (equiv. to 3.0 g GLA/d); n=12	3 wk	No significant side effects were reported by any of the subjects before or after the supplementation period. GLA supplementation significantly increased the amount of DGLA in the membranes of neutrophils and had no effect on the amount of AA.
Zurier et al., 1996	Randomized, double-blinded, placebo-controlled trial followed by single-blinded trial. Adults with active rheumatoid arthritis.	Group 1: Capsules containing sunflower seed oil (placebo); n=28 Group 2: Capsules containing modified borage oil (equiv. to 2.8 g GLA/d); n=28	6-mo randomized double-blind comparison of GLA and placebo, followed by 6-mo GLA administration for all patients. GLA stopped at 12 mo, and patients were evaluated again at 15 mo.	Twenty-three patients in the placebo group and 20 patients in the modified borage oil-treated group were being treated with NSAIDs. Thirteen women patients in the placebo group and 15 in the borage oil-treated group were taking corticosteroids (5.1 and 5.2 mean daily dose, respectively). In the placebo group, 5 patients were taking methotrexate, 5 were taking gold salts, and 5 were taking hydroxychloroquine. In the borage oil-treated group, 10 patients were taking methotrexate, 3 were taking gold salts, and 4 were taking hydroxychloroquine. During the first 6 months, 6 patients in the modified borage oil-treated group withdrew (2 due to lack of

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				efficacy, 1 due to diarrhea, 1 due to surgery, 1 due to unrelated illness, and one due to protocol violation) and 9 patients in the placebo withdrew (4 due to lack of efficacy, 1 due to constipation, 2 due to unrelated illness, 1 due to a rash, and 1 due to protocol violation). The GLA group demonstrated statistically significant and clinically relevant reductions in the signs and symptoms of disease activity (physician's global assessment of disease activity, patient's global assessment of disease activity and a pain assessment, the number of joints with tenderness and/or pain on pressure or passive motion, the number of joints with swelling, joint pain/tenderness score; joint swelling score, duration of morning stiffness, grip strength, and degree of disability). Patients taking GLA during the entire 12 mo showed progressive improvement during the second 6 mo. GLA was reported to be well-tolerated. Representative analyses on selected patients showed enrichment of plasma and platelet lipids with GLA and DGLA during borage-oil but not placebo treatment.

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Reference	**Study Design and Population**	**Treatment and Number of Subjects**	**Duration**	**Safety Outcomes**
Kenny et al., 2000	Clinical trial of GLA supplementation compared to control cases selected on the basis of matching disease stage and initial estrogen receptor expression. Adults with locally advanced or metastatic breast cancer all of whom primary endocrine therapy was indicated as appropriate initial treatment.	Group 1: 20 mg of Tamoxifan/day; n=47 Group 2: 20 g tamoxifan + capsules containing GLA (equiv. to 2.8 g GLA/d); n=38 2 patients in each group received monthly subcutaneous depot injections of 3.6 mg goserelin	6 mo	GLA was well tolerated with no major side effects reported. 16 patients reported no side effects; no adverse effects results in discontinuation of treatment. 22 patients reported minor side effects: 13 patients reported mild alteration of bowel habit with tendency towards loose stool; several patients found the mild laxative effect beneficial. 2 patients had difficulty swallowing the capsules. 2 patients reported of a bitter aftertaste. 2 patients reported sleepiness, although researchers attributed this more likely to the concurrent tamoxifen administration. Faster clinical response of GLA + tamoxifen compared to tamoxifen; apparent enhancement of tamoxifen-induced estrogen receptor down-regulation by GLA was noted.

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Reference	**Study Design and Population**	**Treatment and Number of Subjects**	**Duration**	**Safety Outcomes**
Leventhal et al., 1994	Randomized, double-blind, placebo-controlled trial. Adults with rheumatoid arthritis and active synovitis.	Group 1: Capsules containing soybean oil; n=14 Group 2: Capsules containing black currant seed oil (equiv. to 1.99 g GLA/d; n=11	6 mo	7 patients in the treatment group and 13 in the placebo group withdrew from the study No patients in the treatment group withdrew because of adverse reactions. The vast majority withdrew because of the large size of the capsules. Treated subjects showed reduction in overall pain and joint tenderness, other measures of disease activity did not change.
Middleton et al., 2002	Double-blind, randomized, placebo-controlled trial. Adults with quiescent ulcerative colitis 63 patients randomized, 31 received treatment and 32 placebo (sunflower oil).	Group 1: Capsules containing sunflower oil; n=32 Group 2: Capsules containing 1.6 g GLA/d, EPA, and DHA.	12 mo	No statistically significant difference in adverse events between the groups. Disease relapse rates were similar at 12 months as were changes in sigmoidoscopic grade from baseline in both groups.
Leventhal et al., 1993	Randomized, double-blind, placebo-controlled study. Adults with rheumatoid arthritis and active synovitis. 37 patients enrolled, 19 received treatment and 18 placebo.	Group 1: Cotton seed oil (placebo); n=18 Group 2: Capsules containing borage seed oil (equiv. to 1.4 g GLA/day); n=19	6 mo	Adverse reactions included soft stools (2 patients in the borage seed oil-treated group, 1 in the placebo-treated group), constipation (1 patient in placebo group), flatulence (1 patient in borage seed oil-treated group), belching (1 patient in borage seed oil-treated group); no patients in the borage seed oil-treated group withdrew from the study because of adverse reactions. Treatment with borage seed oil resulted in clinically relevant and statistically significant reduction in signs and symptoms of disease activity compared to placebo.

Table 8. Clinical Trials of γ-linolenic (GLA)-enriched oils in Adults

Reference	Study Design and Population	Treatment and Number of Subjects	Duration	Safety Outcomes
Surette et al., 2008	Trial 1: Randomized, prospective, double-blind, placebo-controlled, parallel group trial. Atopic adults with mild-to-moderate asthma *Follow-on study to Surette et al., 2003 Trial 2: Open-label study Adults with a history of asthma	Trial 1: Group 1: A medical food emulsion containing olive oil (placebo); n=8 Group 2: A medical food emulsion containing borage seed oil and fish oil (equiv. to 0.75 g GLA/d); n=12 Group 3: A medical food emulsion containing borage seed oil and fish oil (equiv. to 1.13 g GLA/d) delivered in a medical food emulsion; n=15 Trial 2: A medical food emulsion containing borage seed oil and fish oil (equiv. to 0.75 g GLA/d); n=65	Trial 1: 4 wk Trial 2: 4 wk	Trial 1: Although the administration of the GLA containing formulas did not improve self-reported asthma status and bronchodilator use compared to placebo, the administration of the GLA-containing product reduced zymosan-induced neutrophil leukotriene B_4 (LTB_4) biosynthesis, which was reported in Surette et al., 2003. Trial 2: Asthma management scores, as determined by the validated Mini Asthma Quality of Life Questionnaire (MiniAQLQ) and the Asthma Control Questionnaire (ACQ), improved in the GLA-treated groups. No serious adverse events occurred during either trial. As reported in Surette et al., 2003, there were no significant between-group differences in adverse events or mean clinical chemistry values in Trial 1. In trial 2, two subjects reports gastrointestinal upset.
Surette et al., 2003	Randomized, double-blind, placebo-controlled, parallel group, prospective trial. Adults with mild to moderate atopic asthma	Group 1: A medical food emulsion containing olive oil (placebo); n=8 Group 2: A medical food emulsion containing borage seed oil and fish oil (equiv. to 0.75 g GLA/d); n=10 Group 3: A medical food emulsion containing borage seed oil and fish oil (equiv. to 1.13 g GLA/d) delivered in a medical food emulsion; n=11	4 wk	No clinically significant changes in vital signs were observed and there were no significant between-group differences in treatment-emergent adverse events or mean clinical laboratory values (hematology and clinical chemistry panels). None of the patients had elevations >2 times the upper limit of normal for any clinical laboratory measurements. GLA inhibited leukotriene biosynthesis.

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Reference	**Study Design and Population**	**Treatment and Number of Subjects**	**Duration**	**Safety Outcomes**
Pullman-Mooar et al., 1990	Open, uncontrolled study involving patients with active rheumatoid arthritis	All patients received in the form of borage seed oil capsules (equiv. of 1.062 g GLA/d); n=7 Patients were also treated with nonsteroidal anti-inflammatory drugs and analgesics and may have taken up to 10 mg prednisone/day.	12 wk	There were no adverse events other than softening of stools and occasional sensation of bloatedness. In general, administration of borage oil resulted in apparent clinical improvement in 6 patients (sleep patterns, joint scores, morning stiffness and the patients overall assessment of disease activity. No changes were seen in 50-foot walk time, grip strength, complete blood count, platelet count, erythrocyte sedimentation rate, or blood chemistry. One patients joint score worsened after borage oil supplementation was discontinued and when placed back on borage oil, the joint score improved.
Weaver et al., 2009	Uncontrolled study involving healthy volunteers. Volunteers were provided a background diet for five weeks and after the first week were given dietary supplements containing fish oil and borage oil for four weeks.	Capsules containing borage oil and fish oil (equiv 0.8 g GLA/d); n=27.	4 wk	Although fish oil and borage oil were combined and adverse events were not mentioned, there appeared to be a small and significant increase in the number of lymphocytes and neutrophils in the blood. Borage/fish oil supplementation also significantly reduced A23187-induced LTB_4 production from whole blood neutrophils.
Brosche and Platt, 2000	Open uncontrolled study involving 29 elderly people (mean age of 68.6 years) showing no signs of skin disease.	Capsules containing borage oil (equiv. to 0.36 0.72 g GLA/d); n=27	8 wk	Adverse events were not mentioned. Borage oil induced a statistically significant improvement in cutaneous barrier function.

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Reference	**Study Design and Population**	**Treatment and Number of Subjects**	**Duration**	**Safety Outcomes**
Bamford et al., 1985	Randomized, double-blind, blocked, cross-over trail involving patients (children and adults) with active eczema. *During the trial patients were allowed to continue the use of emollients, topical steroids, or oral antihistamines.	Test substance was capsules containing evening primrose oil and placebo was capsules containing liquid paraffin Children: Treatment 1: Capsules containing liquid paraffin (placebo); n=49 Treatment 2: Capsules containing evening primrose oil (equiv. 0.18 g GLA/d); n=33 Treatment 3: Capsules containing evening primrose oil (equivalent of 0.36 g GLA/d); n=16 Adults: Treatment 1: Capsules containing liquid paraffin (placebo); n=74 Treatment 2: Capsules containing evening primrose oil (equiv. to 0.54 g GLA/d); n=40 Treatment 3: Capsules containing evening primrose oil (equiv. to 0.72 GLA/d); n=34	12 wk	31 subjects dropped out (14 while taking evening primrose oil and 17 while taking placebo). 29 subjects dropped out for personal reasons and the remaining 3 believed that they had a reaction to the capsules (one stopped taking the evening primrose oil because of increased dermatitis; one child discontinued placebo because of hyperactivity developed). 80 subjects achieved 50% compliance and 56 achieved 75% compliance. Side effects, which were minor and temporary, were noted by 26 of the subjects The total number of complaints was equal between the evening primrose oil and placebo groups. Nausea and bloating occurred in five of the subjects taking evening primrose oil and one taking the placebo. Hyperactivity developed in three children taking the placebo and one taking evening primrose oil. There was no difference in weight, triceps weight, skin-fold thickness, or blood pressure, or in the ratings of appetite and stress.

Table 8. Clinical Trials of γ-linolenic (GLA)-enriched oils in Adults				
Reference	**Study Design and Population**	**Treatment and Number of Subjects**	**Duration**	**Safety Outcomes**
Rilliet et al., 1988(abstract)	Randomized; double-blind; placebo-controlled trial of adults with atopic dermatitis	Group 1: Grape seed oil (placebo); n=13 Group 2: Blackcurrant seed oil (equiv. to 0.6 g GLA/d); n=11	13 wk	Adverse events were not mentioned. Clinical parameters (such as transepidermal water loss, hematology, total IgE levels) in both groups improved substantially.
Andreassi et al., 1997	Placebo-controlled study involving patients with atopic dermatitis	Group 1: Placebo; n=30 Group 2: Borage oil (equiv. to 0.5 g GLA/d); n=30	12 wk	There were no side effects associated with the borage oil treatment.
Henz et al., 1999	Randomized, double-blind, placebo-controlled multicenter study involving otherwise healthy patients with atopic eczema. *Patients were allowed to use difucortolone 21-valerate cream during the trial if necessary.	Group 1: Bland oil (placebo); n=80 Group 2: Borage oil (equivalent to 0.5 g GLA/d assuming that borage oil contains approximately 18% GLA); n=80	24 wk	Nineteen and 17 patients dropped out of the placebo and borage oil groups. No significant differences were found between the groups with regard to corticosteroid dosage until response. Borage oil decreased serum IgE levels although the difference was not significant. Two patients in the placebo group withdrew because of hyperlipidemia and one patient in the borage oil group withdrew because of headache, diarrhea, and vomiting. Adverse events were reported by 27 patients in the placebo group and 29 patients in the borage oil group, and were mostly were mostly influenza-like symptoms

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Reference	**Study Design and Population**	**Treatment and Number of Subjects**	**Duration**	**Safety Outcomes**
Rosenstein et al., 2003	Single-site, randomized, placebo-controlled, double blind study involving patients with periodontitis (mean age of 41) 30 patients	Group 1: Capsules containing a mixture of olive and corn oil (placebo); n=6 Group 2: Capsules containing fish oil; n=5 Group 3: Capsules containing borage oil (equiv. to 0.5 g GLA assuming that borage oil is 18% GLA); n=7 Group 4: Capsules containing fish oil and borage oil; n=6	12 wk	6 patients dropped out due to adverse effects (gastrointestinal distress in three subjects and rest were due to non-compliance). The treatments these patients received were not noted.
Belch et al., 1988	Double-blind, placebo-controlled trial. Adults with classical or definite rheumatoid arthritis as defined by American Rheumatism Association criteria	Group 1: Liquid paraffin (placebo); n=18 Group 2: Capsules containing evening primrose oil and fish oil (equiv. to 0.45 g GLA/d); n= 15 Group 3: Capsules containing evening primrose oil (equiv. to 0.54 mg GLA/d); n= 16	15 mo	Four patients experienced side effects; 2 in Group 3, one with nausea and one with diarrhea that required withdrawal from the study. Two patients in Group 2 experienced nausea and headache but neither required withdrawal from the study. At 12 mo, significant subjective improvement in the condition of the subjects compared to placebo was reported for both 94% and 93% of Group 3 and Group 2, respectively.
Brzeski et al., 1991	Prospective, double-blind, placebo-controlled study. Adults with classical or definite rheumatoid arthritis	Group 1: Olive oil (placebo); n=21 Group 2: evening primrose oil (equiv. to 0.54 g GLA/day); n=19	6 mo	6/19 treated patients withdrew from the study, 2 with nausea, 2 for joint surgery, 1 with deteriorating arthritis and 1 due to flu-like symptoms. 4/21 from the placebo group withdrew, 3 with nausea and 1 leaving the area. Of the 13 completing treatment, 10 showed a significant rise in plasma DGLA, suggesting good compliance. In the evening primrose oil-treated group, arthritis was reduced at 3 months with a trend to reduction at 6 months as measured by indices of pain, articular index and morning stiffness.

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Reference	**Study Design and Population**	**Treatment and Number of Subjects**	**Duration**	**Safety Outcomes**
Wright and Burton, 1982	Double-blind, placebo-controlled, cross-over study Adults and children with atopic eczema	<u>Adults</u> Treatment 1: Liquid paraffin (placebo) Treatment 2: Evening primrose oil Group 1: Evening primrose oil (equiv. to 0.18 g GLA/d); n=20 Group 2: Evening primrose oil (equiv. to 0.36 g GLA/d); n=20 Group 3: Evening primrose oil (equiv. to 0.54 g GLA/d); n=20 <u>Children</u> Treatment 1: Liquid paraffin (placebo) Treatment 2: Evening primrose oil Group 1: Evening primrose oil (equiv. to 0.09 g GLA/d); n=20 Group 2: Evening primrose oil (equiv. to 0.18 g GLA/d); n =18	12 wk treatment followed by 12 wk placebo in random order.	16/60 adults dropped out before completing the 6 months; 8 in the treated group and 8 in placebo. 11/16 were lost to follow-up or moved out of the area and 2 with previous psychiatric illness became ill. One patient felt faint; 1 patient felt his eczema was getting worse, both were on placebo. One patient became pregnant and stopped. 3/39 children dropped out. Mean symptom scores showed an improvement in symptoms that was greatest in the high dose groups; adults responded better than children.

Table 8. Clinical Trials of γ-linolenic (GLA)-enriched oils in Adults				
Reference	**Study Design and Population**	**Treatment and Number of Subjects**	**Duration**	**Safety Outcomes**
Keen et al., 1993	Double-blind, placebo-controlled, parallel study Adults with mild diabetic neuropathy	Group 1: Liquid paraffin (placebo); n=57 (12M/45F) Group 2: Capsules containing evening primrose oil (equiv. to 0.48 g GLA/d); n=54 (18M/36F)	1 yr	10 patients withdrew from the evening primrose oil -treated group; 17 withdrew from the placebo group. 4 adverse events were reported in the evening primrose oil -treated group and 6 in the placebo group; only 2 cases of nausea and vomiting in each group were thought to be treatment-related. No clinically important changes in hematological parameters, urea, electrolytes, protein, albumin liver enzymes or fructosamine were observed. Substantial rises in aspartate aminotransferase appeared in 2 patients, but in both cases, the rise was found to be related to acute ingestion of alcohol. Neurophysiological and neurological parameters showed improvement with evening primrose oil -treatment.
Kruger et al., 1998	Placebo-controlled study Women with confirmed osteoporosis or who were osteopenic	Group 1: Coconut oil (placebo); n=31 Group 2: Evening primrose oil + Fish oil (equiv. 0.48 g GLA/d); n=29	18 mo; 21 patients continued for an additional 18 mo treatment period.	6 patients (3 from the evening primrose oil +fish oil treatment group and 1 from the placebo and 2 from the second evening primrose oil + fish oil treatment period withdrew due to non-compliance). No side effects were recorded, except soft stools in a few patients. Clinical chemistry values for hematology, kidney and liver functions were normal in all patients. Beneficial effects on bone density were seen in evening primrose oil + fish oil-treated patients.

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Reference	**Study Design and Population**	**Treatment and Number of Subjects**	**Duration**	**Safety Outcomes**
Berth-Jones and Graham-Brown, 1993	Double-blind, placebo-controlled, parallel-group Children and adults with atopic dermatitis	Group 1: Paraffin and olive oil (Placebo); n=41 (25M/16F; 20 subjects were 12-yr old or younger) Group 2: Capsules containing evening primrose oil (0.48 g GLA/d); n=41 (22M/19F; 21 subjects were 12-yr old or less) Group 3: Capsules containing evening primrose oil and fish oil (0.4 g GLA/d); n=20 (22M/19F; 20 subjects were 12-yr or less)	16 wk	At 4 weeks, 9 subjects had defaulted or been withdrawn. By 18 weeks, 14 had defaulted or been withdrawn. By 16 weeks, 21 had defaulted or been withdrawn. In the placebo group, four subjects experienced deterioration of eczema, two were non-compliant, and 1 defaulted. In the evening primrose oil -treated group, one subject experienced deterioration of eczema, two were excluded due to non-compliance, three withdrew consent, and two experienced nausea. In the evening primrose oil + fish oil group, three subjects experienced deterioration of eczema, two defaulted, and one had diarrhea. Baseline symptoms were similar across all groups. With the exception of patients withdrawn due to deterioration, there were no apparent differences in response to treatment.
Jamal and Carmichael, 1990	Double-blind, placebo-controlled, randomized, parallel Patients with distal diabetic polyneuropathy (21-74 yr)	Group 1: Placebo Group 2: EPO (equiv. 0.36 g GLA/d)	6 mo	Statistically significant improvements in various compound muscle action potential measurements, sensory nerve action potentials, and heat thresholds were observed in the treatment group vs. placebo. There was no mention of any adverse effects.

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Reference	**Study Design and Population**	**Treatment and Number of Subjects**	**Duration**	**Safety Outcomes**
Schalin-Karrila et al., 1987	Double-blind, placebo-controlled, randomized Young adults (19-31 yr) with moderate to severe eczema	Group 1: Liquid paraffin (placebo); n=11 Group 2: Evening primrose oil (equiv. to 0.36 g GLA/d); n=14	12 wk	One subject in the treatment group dropped out due to a severe allergic reaction to topical dequaline chloride. No side effects due to evening primrose oil were observed. Less topical steroid use was reported in the evening primrose oil -treated group vs. placebo. There was improvement in the overall severity of symptoms in all but one subject in the treatment group. However, the mean initial status of the eczema was worse in the evening primrose oil -treated group at baseline.
Hansen et al., 1983	Open-label, uncontrolled study Patients (39-81 yr) with definite or classical rheumatoid arthritis	A proprietary evening primrose oil formulation with zinc, ascorbic acid, niacin, and pyridoxine (equiv. to 0.36 g GLA/d); n=20	12 wk	Seventeen subjects completed the study; one died suddenly (81 yr old) but the cause of death was not considered treatment-related. One patient was unable to complete the treatment without non-steroidal anti-inflammatory drugs, and one patient was started on glucocorticosteroids and cyclophosphamide after 8 wk due to vasculitis and neuropathy. Only 3 patients reported definite improvement during the study.
Ranieri et al., 2009	Observational, two-arm Patients with compressive root syndromes from conflict disk root	Group 1: No treatment other than a rehabilitation program Group 2: A rehabilitation program + 0.36 g GLA and 0.6 g ALA	6 wk	Thirteen subjects were excluded during follow-up as they had not completed the trial. Of these, seven were in the treatment group and five were in the control group. One person discontinued treatment due to an adverse reaction to an excipient (polyvinylpyrrolidone), manifesting as a skin rash in the thoracoabdominal region.

Table 8. Clinical Trials of γ-linolenic (GLA)-enriched oils in Adults

Reference	Study Design and Population	Treatment and Number of Subjects	Duration	Safety Outcomes
Lovell et al., 1981	Randomized, double-blind, cross-over Children and adults with atopic eczema	Treatment 1: Liquid paraffin Treatment 2: Evening primrose oil (equiv. 0.2 g GLA/d) n=32	3 wk	Efamol caused no adverse reactions in either adults or children. Subjects treated with evening primrose oil exhibited a modest, but statistically significant, improvement on both the doctor's and self-assessments.
Stewart et al., 1991	Open, uncontrolled Patients with moderate to severe atopic dermatitis	Capsules containing a proprietary evening primrose oil (equiv. to 0.32g GLA/d); n=179	Subjects took the formulation for 12 wk and had the option to continue afterward. Median duration of treatment among responders was 11 mo.	Relative to baseline, 111 subjects improved and 68 did not improve (improvement was primarily noted for parameters such as erythema, dryness, scaling, and itch). Two adverse events were noted: stomach pains and mild fluid retention. It is unknown whether these were related to evening primrose oil.
Guivernau et al., 1994	Placebo-controlled, double-blinded, crossover Hyperlipidemic men with known family history of premature coronary artery disease	Treatment 1: Liquid paraffin (placebo); n=12 Treatment 2: Evening primrose oil (equiv. to 240 mg GLA/d); n=12	4 mo each treatment, with a 4 wk washout in-between	Relative to baseline, evening primrose oil supplementation caused a significant decrease in serum triglyceride levels, total serum cholesterol, LDL, and a significant increase in HDL. Some *in vitro* assays of platelet aggregation exhibited decreased values in the evening primrose oil -treated group, versus control (endpoints included adenosine diphosphate- and adrenaline-aggregated platelet assays and production of platelet-derived thromboxane B_2). Bleeding time was not affected after 1 mo of treatment (versus baseline). After 3-4 mo supplementation, there was a significant mean 40% increase in bleeding time.

Table 8. Clinical Trials of γ-linolenic (GLA)-enriched oils in Adults				
Reference	**Study Design and Population**	**Treatment and Number of Subjects**	**Duration**	**Safety Outcomes**
Kawamura et al., 2011	Double-blind, placebo-controlled Adults with dry skin and mild to moderate atopic dermatitis	Group 1: A mixture of rapeseed and soybean oil (placebo; equiv. to 0.00006 g GLA/d); n= 61 Group 2: Oil extracted from the fungus *Mucor circinelloides* (equiv. to 0.2 g GLA/d); n=61 [*Note: This is the only GLA preparation which is derived from a fungus, rather than from evening primrose oil]	4 wk observation period; 12 wk test period; 4 wk follow-up period	Transepidermal water loss at the cheek and forearm was improved at 4 wk in the *M. circinelloides* oil-treated vs. placebo-treated group. Systolic and diastolic blood pressure and heart rate were not significantly different between groups. The authors mention that there were some changes in parameters measured, but they were either slight and within normal reference ranges, or temporary. The monitoring physician judged them as not being clinically important. No adverse events attributable to the test diet were noted by the monitoring physician.
Harris et al., 2002	Uncontrolled study Adults with non-muscle-invasive transitional cell carcinoma (TCC) Fifteen patients received the low dose therapy; 15 additional patients received the high dose therapy.	Group 1: 0.05 g of a novel intravesical formulation of meglumine gamma-linolenic acid (GLA content unknown) Group 2: 0.125 g of a novel intravesical formulation of meglumine gamma-linolenic acid (GLA content unknown)	A single intravesical instillation was administered and retained for 1 h	There were no significant local or systemic side effects. There were four complete responses, nine partial responses and 17 non-responders. Histology indicated no evidence of damage to the urothelium.
Schubert et al., 2007	Randomized, placebo-controlled double-blind, parallel study Healthy volunteers (20-38 yr)	Group 1: Mixture of olive oil and a fat blend rich in AA; n= 15 Group 2: Fat blend enriched with GLA (equiv. to 0.073 g GLA/d); n= 15	2 wk, plus 2 wk follow-up	Prostaglandins E_1, E_2, leukotriene B_4 production from lipopolysaccharide stimulated whole blood cells was significantly reduced in the GLA-treated group 2 wk after stopping supplementation. Other cytokines were not significantly affected. Authors did not mention adverse effects.

Table 8. Clinical Trials of γ-linolenic (GLA)-enriched oils in Adults				
Reference	**Study Design and Population**	**Treatment and Number of Subjects**	**Duration**	**Safety Outcomes**
Barabino et al., 2003	Randomized 26 patients with aqueous-deficient keratoconjunctivitis sicca	Group 1: Preservative-free substitute tears and the oral administration of a capsule containing a low amount of sugar; n=13 Group 2: Preservative-free substitute tears and the oral administration capsules containing GLA (Medilar tablets, Fidia Oftal-Bausch & Lomb Pharmaceuticals; equiv. to 30 mg GLA/d); n=13	45 d	There was a significant reduction in the percentage of HLA-DR conjunctival epithelial cells, symptoms score, and lissamine green staining score in the GLA-treated group by the end of the study. The authors did not mention adverse effects.

Table 9. Clinical Trials of γ-linolenic (GLA)-enriched oils in Adults and Children Receiving Sole-Source Enteral Nutrition

Reference	Study Design and Population	Treatment and Number of Subjects	Duration	Safety Outcomes
Rice et al., 2011	Randomized, double-blind, placebo-controlled, multicenter trial. Adults within 48 hours of developing acute lung injury resulting from pneumonia, sepsis, aspiration, or trauma.	Group 1: An isocaloric-isovolemic carbohydrate-rich control (control); n=129 Group 2: Enteral supplementation that contained EPA, DHA, GLA, and antioxidants (equiv. to 5.92 g GLA/d); n=143 *Both study diets were administered enterally as twice-daily boluses of 120 ml beginning within 6 hr of randomization.	3 wk	Study was stopped early as a result of the futility analysis. Enteral supplementation with n-3 fatty acids, GLA and antioxidants did not improve lung physiology or clinical outcomes and did not protect from nosocomial infections or improve nonpulmonary organ function. Increased incidence of diarrhea was noted and may have been caused by the bolus administration. The enteral-supplementation-treated group had fewer ventilator free days ($p= 0.02$) and higher mortality ($p=0.054$), however this may have been a chance observation as a result of the futility analysis. If stopping boundaries for efficacy and futility had been the same, $p < 0.001$ would have been required to establish statistical significance. More patients in Group 2 were receiving vasorepressors at enrollment and through day 7.

Table 9. Clinical Trials of γ-linolenic (GLA)-enriched oils in Adults and Children Receiving Sole-Source Enteral Nutrition

Reference	Study Design and Population	Treatment and Number of Subjects	Duration	Safety Outcomes
Gadek et al., 1999	Prospective, multicentered, double-blind, randomized controlled trial. Adults with acute respiratory distress syndrome (ARDS, as defined by the American-European Consensus Conference) caused by sepsis/pneumonia, trauma or aspiration injury.	Group 1: Isonitrogenous, isocaloric standard diet without these fatty acids; n=47 Group 2: Mixture of fish and borage oil (equiv. to 5.8 ± 0.3 g GLA/d); n=51. *Both diets were delivered at a constant rate to achieve a minimum of 50% basal energy expenditure (BEE) x 1.3 within the first 24 hrs. Continuous enteral nutrition was advanced as tolerated with the goal of achieving a minimum of 75% of BEE x 1.3 within 72 hrs of initiation. Enteral nutrition was delivered continuously for a minimum of 4+/- 1 study days at a rate not to exceed BEE x 1.3.	At least 4-7 d	No statistically significant changes in the treatment group compared to control were noted in values for serum chemistries, electrolytes, liver function, hematology, and blood coagulation. Modulation of plasma phospholipid fatty acids indicated increases in DGLA and EPA and increases in the ratio of EPA to ARA in Group 2 compared to Group 1. Formula tolerance compared to control was demonstrated by similar time to caloric goal, decrease in serum triglycerides indicating better ability to clear circulating lipids in the treatment group and fewer adverse events, particularly gastrointestinal complications in the treatment group. Ingestion of the GLA-containing diet was associated with a significant decrease in the number of total cells and neutrophils in the alveolar fluid, an increase in arterial oxygenation, and significant decreases in ventilator, intensive care unit, and supplemental oxygen days.

Table 9. Clinical Trials of γ-linolenic (GLA)-enriched oils in Adults and Children Receiving Sole-Source Enteral Nutrition

Reference	Study Design and Population	Treatment and Number of Subjects	Duration	Safety Outcomes
Pontes-Arruda et al., 2006	Prospective, single-center, randomized, double-blinded, placebo controlled trial. Adults requiring mechanical ventilation, having enteral access, and having a clinical diagnosis of either sepsis or septic shock	Group 1 (Placebo): High fat, low carbohydrate enteral formulation for patients with pulmonary diseases; n=48. Group 2 (EPA + GLA enriched diet): High fat, low carbohydrate enteral formulation for patients with pulmonary diseases enriched with 4.3 g GLA/L, 4.5 g EPA/L, and 2.0 g DHA/L; n=55. *Both diets were delivered at a constant rate to achieve a minimum of 50% basal energy expenditure (BEE) x 1.3 within the first 24 hrs. Continuous enteral nutrition was advanced as tolerated with the goal of achieving a minimum of 75% of BEE x 1.3 within 72 hrs of initiation. Enteral nutrition was delivered continuously for a minimum of 7 days or until they were interrupted at the physician's discretion or because of the development of any adverse event that could be related to the enteral feeding.	Diets were delivered until patients were extubated or until interrupted at physician's discretion or due to the development of any adverse event that could be related to the enteral feeding (4 – 7 days)	Sixty-two patients were excluded from the final analysis before unblinding because of protocol violations such as unable to meet caloric goal; extubation; pulling out the feeding tube; withdrawal by the attending physician; and death. Total number and type of adverse events was similar in both groups (Group 1: n=48; Group 2: n=55). Mean dietary intake was similar between groups for total calories. Group 2 received an additional 4.6 g +/- 0.13 g GLA/day, 4.9 +/- 0.14 g EPA/day, and 2.2 +/- 0.06 g DHA/day and higher amounts of vitamin C and E. Group 2 had a higher survival rate ($p=0.037$); statistically significant increase in Pao_2/FIo_2 ratios on study days 4 and 7; more ventilator-free days (13.4 +/- 1.2 vs 5.8 +/- 1.0; $p<0.001$; developed less new organ dysfunction.

Table 9. Clinical Trials of γ-linolenic (GLA)-enriched oils in Adults and Children Receiving Sole-Source Enteral Nutrition

Reference	Study Design and Population	Treatment and Number of Subjects	Duration	Safety Outcomes
Pontes-Arruda et al., 2011	Randomized, multicenter, double-blinded, placebo-controlled trial Adults with clinically diagnoses early sepsis that required enteral nutrition	Group 1 (Control): Ensure Plus HN (higher carbohydrates than the study formula); n=53 Group 2 (Study Formula): Enteral nutrition enriched with EPA, GLA and elevated levels of antioxidants (Oxepa, Abbott Nutrition); n=53 *Both diets were delivered at a constant rate to achieve a minimum of 50% basal energy expenditure (BEE) x 1.3 within the first 24 hrs. Continuous enteral nutrition was advanced as tolerated with the goal of achieving a minimum of 75% of BEE x 1.3 within 72 hrs of initiation. Enteral nutrition was delivered continuously for a minimum of 7 days or until they were interrupted at the physician's discretion or because of the development of any adverse event that could be related to the enteral feeding. Resulting intake of GLA was equiv. to 4.4 g/day	7 days	No serious adverse events were recorded over the course of the study. Although some patients experienced diarrhea and vomiting, there were no statistically significant differences between the two groups. Group 2 developed less severe sepsis and septic shock than Group 1. Less patients in Group 2 developed respiratory failure (14 vs. 23) and cardiovascular failure (12 vs. 21). No differences between the groups in the development of coagulation failure, renal failure, metabolic failure, or hepatic failure.

Table 9. Clinical Trials of γ-linolenic (GLA)-enriched oils in Adults and Children Receiving Sole-Source Enteral Nutrition

Reference	Study Design and Population	Treatment and Number of Subjects	Duration	Safety Outcomes
Pacht et al., 2003	Prospective, randomized, double-blind, controlled trial. Adults with illnesses known to be associated with acute lung injury, pulmonary inflammation and ARDS.	Group 1: An isonitrogenous, isocaloric standard diet without fatty acids; n=22 Group 2: Mixture of fish and borage oil (equiv. to 4.4 g GLA/d); n= 21 *Both diets were delivered at a constant rate to achieve a minimum of 50% basal energy expenditure (BEE) x 1.3 within the first 24 hrs. Continuous enteral nutrition was advanced as tolerated with the goal of achieving a minimum of 75% of BEE x 1.3 within 72 hrs of initiation. Enteral nutrition was delivered continuously for a minimum of 4+/- 1 study days at a rate not to exceed BEE x 1.3.	At least 4-7 d	43 patients were deemed evaluable. A total of 17 patients did not complete from study day 4 through study day 7 (seven patients in Group 1 and 10 patients in Group 2). 60% of the patients in Group 2 were extubated whereas only 14 % of patients in Group 1 were weaned from the ventilator. Although ingestion of the mixture of fish and borage oil had no affect on total protein, neutrophil count, and leukotriene B_4 levels in the bronchoalveolar fluid, the mixture significantly reduced brochoalveolar lavage fluid ceruloplasmin and IL-8 levels, reduced pulmonary inflammation, increased oxygenation, and improved clinical outcomes.

Table 9. Clinical Trials of γ-linolenic (GLA)-enriched oils in Adults and Children Receiving Sole-Source Enteral Nutrition				
Reference	**Study Design and Population**	**Treatment and Number of Subjects**	**Duration**	**Safety Outcomes**
Sultan et al., 2012	Prospective, double-blind randomized in patients undergoing subtotal oesphagectomy and total gastrectomy	Group 1 (control): Osmolite (1 kcal/ml); n=66 Group 2 (Standard enteral nutrition (SEN)): Ensure (1.5 kcal/ml); n=66 Group 3 (Immuno-enhancing diet (IED)): Oxepa, Abbott (1.5 kcal/ml; 4 g GLA/L); n=63 Supplementation was performed 7 days before surgery in the SEN and IED groups at a fixed rate of 1000 kcal/day, resulting in a daily desired volume of 675 mg/day. On the second day after surgery, feeding was commenced at 25 ml/hr and was increased to 50 ml/hr on the third day, reaching the maximum at some point during that day. Feeding was continued at the maximum rate between 4 and 7 days after surgery. The maximum rate of administration for all diets and for all patients was calculated according to the predicted proteins and calories that the individual thought to require based on Schofield equations for estimating basal metabolic rate, adjusting for stress or weight loss, and adding a combined factor for activity and diet-induced thermogenesis.	14 days (7 days before and after surgery)	21 patients were eligible and 195 patients were randomized and were used in the analysis. There was no difference between the three groups in the infective complications, proportion of patients who develop an infective complication, those requiring therapeutic antibiotics, other complications, critical care stay, hospital stay or mortality rate. There was no difference in the volume of feed received ether before or after surgery. Only 55 and 63% of the patients in the IED and SEN groups, respectively, achieved the desired maximum hourly feed. Only 22% of the patients in the control group achieved the maximum feed rate. GLA intake was not specifically noted, although based on overall pre- and post-operative feed volumes, and durations of feeding, the pre- and post-operative GLA intakes are estimated to be 2.4 +/- 0.5 g/day and 3.2 +/- 1.4 g/day, respectively.

Table 9. Clinical Trials of γ-linolenic (GLA)-enriched oils in Adults and Children Receiving Sole-Source Enteral Nutrition

Reference	Study Design and Population	Treatment and Number of Subjects	Duration	Safety Outcomes
Singer et al., 2006	Open, prospective, randomized, unblinded, and placebo-controlled Patients with acute lung injury (ALI)/acute respiratory distress syndrome (ARDS)	Group 1 (Control): ready-to-feed, high-fat, low-carbohydrate, enteral formula; n=49. Group 2 (EPA + GLA diet): Oxepa (differed from the control formula only in lipid composition) and in the level of antioxidants; n=46. Diet feeding in both groups was adjusted to achieve greater or equal to 50% of the resting energy expenditure (REE) x 1.2 on the first day and 70% REE x 1.2 on the second day.	Enteral feeding was administered continuously' patients received the same enteral formula throughout their stay in the ICU (approx. 7 days)	All patients included in the study were fed successfully for greater than or equal to 14 days through the gastric, duodenal, or jejunal route at a rate that did not exceed REE X 1.25. No patients were dropped because of formula-related safety concerns. Nutritional intake (kcal/day) as similar between the two groups on day 1 (1055 +/- 378 vs. 1053 +/- 351) and day 7 (1420 +/- 437 vs 1624 +/- 512). Borage oil accounted for 20% of the fat fraction EPA + GLA diet. Assuming that 18% of borage oil is GLA, the resulting intake of GLA at day 1 was 2.4 vs 2.4 g/day and at day 7, 3.05 vs 3.6 g/day. There were no significant differences in nutritional variables (weight variations, plasma albumin, and prealbumin).

Table 9. Clinical Trials of γ-linolenic (GLA)-enriched oils in Adults and Children Receiving Sole-Source Enteral Nutrition

Reference	Study Design and Population	Treatment and Number of Subjects	Duration	Safety Outcomes
Jacobs et al., 2013	Prospective, blinded, randomized, controlled, multicenter trial. Critically-ill mechanically ventilated children with a diagnosis of acute lung injury or acute respiratory distress syndrome	Group 1 (PEF): Ready-to-feed, concentrated version of a pediatric enteral nutrition formula (Pediasure, Abbott Nutrition); n=12. Group 2 (Experimental diet): A commercially available adult formula (Oxepa, Abbott Nutrition) that was isocaloric to the PEF diet, differing only in terms of its lipid composition and level of antioxidants; n=14. Diet feeding in both groups was adjusted to achieve greater or equal to 50% of the estimated energy expenditure (EEE) x 1.3 on the first day and advanced to 75% REE x 1.3 within 48 hr of initiating the enteral feeding. Enteral feeding was delivered continuously for a minimum of 4 +/- 1 study days at a rate not to exceed EEE x 1.3.	4 days	Thirty-seven patients were enrolled and 11 dropped out due to non-compliance such as unable to meet caloric goal; extubation; pulling out the feeding tube; withdrawal by the attending physician; and death. There were no significant differences in the daily intakes of calories or total lipids. Group 2 consumed approximately 2.6 g GLA/day. Except for statistically significant increases in serum blood urea nitrogen (BUN) and urine osmolality, which were within their normal ranges, there were no statistically significant differences noted in serum biochemistries, renal and hepatic function, blood coagulation parameters, and creatine clearance. Although there was a greater number of adverse events in Group 2 vs. Group 1 (n=14 vs. n=12), none was severe or directly related to the study formula, and there were no significant differences between the two groups with regard to specific categories (e.g., gastrointestinal, cardiac, hematological, respiratory, skin, and infections). One patient died in groups 1 and 2.

Table 9. Clinical Trials of γ-linolenic (GLA)-enriched oils in Adults and Children Receiving Sole-Source Enteral Nutrition				
Reference	**Study Design and Population**	**Treatment and Number of Subjects**	**Duration**	**Safety Outcomes**
Grau-Carmona et al., 2011	Prospective, randomized, open-label, controlled, multicenter, parallel-group Adult with sepsis and acute lung injury or acute respiratory distress syndrome (ARDS) who were receiving mechanical ventilation and could be fed by enteral nutrition	Group 1: Ensure Plus HN® enteral nutrition by Abbott Labs (control diet); n= 71 Group 2: Oxepa enteral nutrition by Abbott Labs (contains borage oil; equiv. to up to 0.129 g GLA/d; mean intake of 0.113 g GLA/d); n= 61	12 d	The Oxepa-treated group experienced significantly fewer days in the Intensive Care Unit (ICU) vs. the control group. Physicians withdrew 28 patients, nine in the control group (1 did not receive any intervention; 7 did not receive mechanical ventilation; and 1 had a protocol violation), and 19 in the treatment group (7 did not receive any intervention; 8 did not receive mechanical intervention; 4 has a protocol violation), in the first 2 d of treatment. The study was stopped early due to inadequate statistical power to detect significant differences between groups. There was a non-significant trend for a lower SOFA score (indicative of altered organ function), fewer days on mechanical ventilation, and lower mortality in the treatment group vs. control. Diets were well-tolerated and gastrointestinal complications were unusual; the most frequent was high gastric residual volumes, with significantly less incidence in the treatment group vs. control. Diarrhea episodes were similar across groups.

Table 9. Clinical Trials of γ-linolenic (GLA)-enriched oils in Adults and Children Receiving Sole-Source Enteral Nutrition

Reference	Study Design and Population	Treatment and Number of Subjects	Duration	Safety Outcomes
Wills et al., 2014	Randomized, double-blind, placebo-controlled trial in adults with amyotrophic lateral sclerosis (ALS)	Group 1 (Placebo; isocaloric tube-fed diet): Jevity 1.0, Abbott; n=7 Group 2 (High-carbohydrate hypercaloric tube-fed diet): Jevity 1.5, Abbott ; n=9 Group 3 (High-fat hypercaloric tube-fed diet): Oxepa, Abbott; n=8 Goal was to replace 100% of estimated energy requirements, although some patients were still able to consume food by mouth.	4 months with a 5 month follow-up	28 patients were screened and 24 were randomized to the three treatments groups. Four participants (one in Group 1, one in Group 2, and two in Group 3) withdrew after randomization, but before starting study diet. More control participants discontinued the study diet because of adverse events than die the other groups (3 of six patients in the control group vs. none of eight in Group 2 and one of six in Group 3. Group 2 and 3 had fewer adverse events and serious adverse events compared to the control group. The most common adverse events were gastrointestinal (six patients in Group 1 had at least one gastrointestinal adverse event, four patients in Group 2, and six patients in Group 3. No patients in Group 2 or 3 had cardiovascular adverse events or cardiovascular serious adverse events. One of the eight patients in Group 3 died during the 5-month follow-compared to none in Group 2, and three in Group 1. All deaths occurred within 4 months of study diet initiation and all patients who died discontinued the study diet as a result of adverse events. In addition, all deaths were due to respiratory failure and none was considered to be related to study diet. Ingestion of the high-fat hypercaloric tube-fed diet was not associated with either increased cholesterol or hs-C reactive protein, or a change in fasting blood glucose and serum insulin over time. Patients in Group 1 consumed a mean of 1.21 +/- 0.26 times their estimated energy requirements. Patients in Group 2 consumed a mean of 1.54 +/- 0.33 times their estimated energy requirements. Patients in Group 1 consumed a mean of 1.51 +/- 0.33 times their estimated energy requirements.

Table 9. Clinical Trials of γ-linolenic (GLA)-enriched oils in Adults and Children Receiving Sole-Source Enteral Nutrition				
Reference	**Study Design and Population**	**Treatment and Number of Subjects**	**Duration**	**Safety Outcomes**
Kushta et al., 2011 (Abstract)	Patients with ALS	Group 1 (Placebo): Standard enteral nutrition formula (Nutrison, Nutricia; 30 kcal and 1.2 g proteins/kg bw/day Group 2: Respiratory Failure formula (Oxepa, Abbott)	1 year	Authors only noted that both formulas were well tolerated and reported that there were no alterations in routine biochemistry.

D. ANIMAL STUDIES

The safety of γ-linolenic acid intake has been evaluated in four toxicology studies using oils derived from transgenic plant sources and borage oil (Tso et al., 2012; Liu et al., 2004; Wainwright et al., 2003; Palombo et al., 2000). Importantly, all of these studies administered diets containing greater than 10% fat to rats, which are higher than recommended amount of 5% (National Research Council, 1982). As discussed in the response letter to GRN 41, the administration of a macronutrient, outside recommended levels in a toxicology study makes it difficult to distinguish whether an observed effect related to the test material is a normal physiological response to the high dietary load of the particular macronutrient, deficiency of another dietary ingredient resulting from the presence of a large amount of the test material in the diet, or a toxicological effect. Rats fed diets high in fats can develop non-alcoholic fatty liver disease, which is a pathological condition associated with increase blood levels of AST, ALT and insulin resistance (i.e., elevated blood glucose levels) (Li et al., 2013). Considering the anti-inflammatory activity of γ-linolenic acid (see Chapter 3), the increases in blood glucose, AST, ALT, and LDH levels noted in studies conducted by Tso et al. (2012) and Li et al., (2004) represent a generalized effect of consuming a high fat diet rather than a specific effect of consuming high levels of γ-linolenic acid. Importantly, these effects would not occur in humans because their recommended diet should contain approximately 20-40% fat (U.S. Department of Agriculture and U.S. Department of Health and Human Services, 2010).

1. Toxicology Studies

a. SONOVA

Pathogen-free male and female Sprague-Dawley rats were fed semi-synthetic, pelleted diets containing 10% fat (wt/wt) composed of either 47% SONOVA, 27% soybean oil, and 26% safflower oil, or 90% borage oil and 10% canola oil (Tso et al., 2012). Importantly, γ-linolenic acid levels were similar in both diets (approximately 21% of the total fat) and except for 10 times more gadoleic and nervonic acid, and 30 times more erucic acid in the borage oil-containing diet, abundance of the remaining fatty acids in the two diets was similar.

Rats were placed in one of two weight-matched groups for each gender and received either the SONOVA test diet (HGSO) or borage oil control diet for the entire 90-day study period. Clinical appearance, body weight and feed intake were monitored weekly. Body fat mass composition was determined using an EchoMRI at baseline and at week twelve. Unfasted total lipids in plasma, liver, kidney, spleen, mesenteric fat, and feces were determined at study termination.

All rats survived to the end of the 90-day feeding trial and scheduled termination. There was no evidence of adverse clinical, motor activity, or behavioral observations in any of the animals throughout the study period. Mean daily feed consumption by the borage diet male controls (18.1 g/rat/d) were similar to the HGSO-treated males (18.5 g/rat/d) ($P > 0.05$). Female rats had lower feed consumption versus males, but daily feed consumption was not different between the borage oil- (13.1 g/rat/d) and HGSO-treated (12.7 g/rat/d) ($P > 0.05$) groups.

Based on the average daily feed consumption data for males and females and analysis of diet samples to confirm γ-linolenic acid concentration, the mean daily intake of γ-linolenic acid for males was 888 mg γ-linolenic acid/kg bw/day for the borage group and 913 mg γ-linolenic acid/kg bw/day for rats given HGSO. Female rats had a mean daily intake of 1191 mg γ-linolenic acid/kg bw/day for the borage group and 1161 mg γ-linolenic acid/kg bw/day for the SONOVA rats.

Similar growth patterns and no significant differences in body weight changes were observed for both borage oil- and HGSO-treated male and female rats throughout the 12 week feeding period. At termination, mean body weights were: 420 ± 11 g and 438 ± 10 g for the males in the borage oil- and HGSO-treated groups, respectively, and; 234 ± 4 and 236 ± 7 g for the females in the borage oil- and HGSO-treated groups, respectively.

Fecal fat content was not significant different in females administered HGSO or borage oil. In contrast, the administration of borage oil to the males led to significantly higher amounts of fecal fat (1.1%) compared to the males receiving HGSO (0.47%). Notably, the authors speculated that because the percentage of fecal fat was below 1.5%, there were no abnormalities in digestion or absorption of either the HGSO or borage oil blends.

The absolute and relative mean weights of the liver, spleen, kidney, lung, and heart were similar among male and female rats in the borage oil- and HGSO-treated groups (Table 10). The evaluation of organ (kidney, spleen, and middle lobe of the liver) histology revealed very mild focal tubule dilation with protein and no histologic damage in the kidneys of some male and female rats given either borage oil or HGSO. Because these changes were not observed in all rats within the borage oil- and HGSO-treated groups, the findings were not considered to be borage oil- or HGSO-related. One female rat given borage oil and one female rat given HGSO had a mild diffuse hepatocellular vacuolization. This change was reported by the authors to be frequently observed in untreated rodents and was not considered a significant alteration. One male and one female rat from both the borage oil- and HGSO-treated groups also had mild diffuse subcapsular edema. Although edema is an abnormal finding, it was attributed by the authors to likely be due to osmolarity alterations during tissue fixation and processing and was considered to be artifacts unrelated to the diets.

Table 10. Absolute (abs) and Relative (rel) Major Organs Weights of Male and Female Sprague-Dawley Rats Fed a Diet Containing Either a Borage Oil Blend or a High γ-Linolenic Safflower Oil (HGSO) Blend for 12 Weeks[a]

Organ	Weight	Male		Female	
		Borage oil	HGSO	Borage oil	HGSO
Liver	Abs (g)	10.30 ± 0.30	10.20 ± 0.30	5.80 ± 0.10	5.60 ± 0.20
	Rel (%)	2.40 ± 0.04	2.30 ± 0.03	2.50 ± 0.03	2.40 ± 0.03
Spleen	Abs (g)	1.20 ± 0.04	1.10 ± 0.04	0.90 ± 0.02	0.90 ± 0.03
	Rel (%)	0.27 ± 0.00	0.25 ± 0.00	0.37 ± 0.00	0.36 ± 0.00
Kidney	Abs (g)	1.50 ± 0.03	1.50 ± 0.03	0.90 ± 0.01	0.90 ± 0.01
	Rel (%)	0.35 ± 0.00	0.34 ± 0.00	0.40 ± 0.00	0.40 ± 0.00
Mesenteric Fat	Abs (g)	4.00 ± 0.30	4.00 ± 0.20	1.70 ± 0.10	1.90 ± 0.20
	Rel (%)	0.95 ± 0.08	0.92 ± 0.04	0.72 ± 0.05	0.78 ± 0.07
Lung	Abs (g)	1.90 ± 0.04	1.80 ± 0.05	1.60 ± 0.04	1.50 ± 0.02
	Rel (%)	0.44 ± 0.00	0.42 ± 0.00	0.67 ± 0.02	0.65 ± 0.02
Heart	Abs (g)	1.70 ± 0.03	1.70 ± 0.04	1.20 ± 0.01	1.20 ± 0.03
	Rel (%)	0.40 ± 0.00	0.39 ± 0.00	0.50 ± 0.01	0.50 ± 0.01
Total Body Weight	(G)	420 ± 11	438 ± 10	234 ± 4	236 ± 7

Data represents mean +/- standard error of the mean (N=12/group). Relative weights of organs are expressed as percentages of total body weights.

[a]Adopted from Tso et al., 2012.

There were no adverse effects attributed to the ingestion of HGSO or borage oil on any of the hematology parameters for both male and female rats (Table 11). Statistically significant differences were not considered adverse because they were minor and within the normal range of variation for each particular hematological parameter (with the exception of basophils (% WBC) which was slightly above historical laboratory range).

Table 11. Hematological Parameters of Male and Female Sprague-Dawley Rats Fed a Diet Containing a Borage Oil Blend or a High γ-Linolenic Safflower oil (HGSO) Blend for 12 Weeks*, [b]

	Male		Female	
Parameter [normal range]	Borage Oil	HGSO	Borage Oil	HGSO
RBC ($\times 10^6/\mu L$) [5–10]	8.3 ± 0.1	8.6 ± 0.1	7.4 ± 0.1	8.1 ± 0.2[a]
TotalWBC ($\times 10^3/\mu L$) [3–17]	4.1 ± 0.4	4.6 ± 0.6	1.8 ± 0.2	2.2 ± 0.2
Neutrophils (% WBC) [13–26]	17.7 ± 2.5	21.9 ± 1.8	14.6 ± 1.3	13.5 ± 1.7
Lymphocytes(%WBC) [65–83]	76.8 ± 2.6	70.7 ± 2.3	79.9 ± 1.9	81.3 ± 2.0
Monocytes (% WBC) [0–4]	4.5 ± 0.8	3.0 ± 0.6	1.6 ± 0.8	1.0 ± 0.5
Eosinophils (% WBC) [0–4]	0.8 ± 0.2	2.5 ± 0.4[a]	4.1 ± 1.0	2.5 ± 0.6
Basophils (% WBC) [0–1]	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	1.5 ± 0.6[a]
Hemoglobin (gm/dl) [11–19]	15.7 ± 0.2	14.4 ± 1.3	14.3 ± 0.1	15.1 ± 0.2[a]
Hematocrit (%) [35–57]	53.1 ± 4.0	44.8 ± 0.4	38.8 ± 2.0	44.5 ± 1.1[a]
MCH (pg) [18–23]	18.5 ± 0.2	18.3 ± 0.1	19.2 ± 3.5	19.3 ± 0.2
MCHC (gm/dl) [31–40]	33.8 ± 0.1	34.5 ± 0.2[a]	28.7 ± 3.0	34.7 ± 01
MCV (fl) [46–65]	54.7 ± 0.6	52.9 ± 0.3	55.2 ± 0.4	55.7 ± 0.4
Platelets ($\times 10^3/\mu l$) [200–1500]	761 ± 14	832 ± 14[a]	858 ± 26	782 ± 15[a]
PT (sec) [19.3–32.6]	20.5 ± 0.5	18.7 ± 0.2	18.5 ± 0.1	18.5 ± 0.2

Values in brackets indicate the normal range for the laboratory rat.

*Data represent means +/- SEM (n=6 male borage oil, n=12 male HCSO, n=11 female borage oil, n=12 female HGSO).

RBC, Red Blood Cell; WBC, White Blood Cell; MCH, Mean Corpuscler Hemoglobin; MCHC, Mean Corpuscular Hemoglobin Concentration; MCV, Mean Corpuscular Volume; PT, Prothrombin time.

[a]Indicates statistically significant difference from borage oil blend diet ($P<0.05$).

[b]Adopted from Tso et al., 2012.

The analysis of the serum biochemistry profiles revealed that although the levels of glucose in the males of the borage oil-treated group, aspartate aminotransferase (AST) in males and females of the borage oil- and HGSO-treated groups, alanine aminotransferase (ALT) in the males of the borage oil- and HGSO-treated groups, and lactate dehydrogenase (LDH) in males and females of the borage oil- and HGSO-treated groups were elevated, the remaining serum components were all within the normal range for laboratory rats (Johnson et al., 2008; Table 12).

Table 12. Serum Biochemistry Profiles of Male and Female Sprague-Dawley Rats Fed on a Diet Containing Either the Borage Oil Blend or the High γ-Linolenic Safflower Oil (HSGO) Blend at the End of 12-Week Feeding*, [b]

Parameter [normal range]	Male		Female	
	Borage Oil	HGSO	Borage Oil	HGSO
Sodium (mEq/l) [142–154]	143 ± 0.8	143 ± 0.4	144 ± 0.3	142 ± 0.4[a]
Potassium (mEq/l) [3.6–9.2]	5.4 ± 0.2	4.5 ± 0.2[a]	4.1 ± 0.2	4.7 ± 0.3
Chloride (mEq/l) [84–110]	108 ± 0.6	106 ± 0.5[a]	107 ± 0.5	106 ± 0.3
Phosphorus (mg/dl) [4.7–16]	6.8 ± 0.2	6.2 ± 0.2	5.6 ± 0.2	5.8 ± 0.3
Calcium (mg/dl) [9.1–15.1]	10.2 ± 0.1	9.8 ± 0.1[a]	9.4 ± 0.1	9.3 ± 0.1
Bicarbonate (mEq/l) [12.6–32]	22.4 ± 0.8	22.8 ± 0.6	19.1 ± 0.7	22.1 ± 0.7
BUN (mg/dl) [11–23]	16.8 ± 0.5	16.8 ± 0.4	14.9 ± 0.5	18.0 ± 0.7[a]
Total Protein (gm/dl) [4.5–8.4]	5.9 ± 0.1	5.9 ± 0.1	6.0 ± 0.1	5.7 ± 0.0[a]
Albumin (gm/dl) [2.9–5.9]	3.2 ± 0.1	3.2 ± 0.0	3.5 ± 0.0	3.3 ± 0.0[a]
Glucose (mg/dl) [50–135]	153 ± 4	125 ± 5[a]	74.8 ± 1.4	86.2 ± 5.3
Globulin (gm/dl) [1.8–3.0]	2.6 ± 0.0	2.7 ± 0.0	2.5 ± 0.0	2.5 ± 0.0
Alb/Glob Ratio [0.72:1.21]	1.0 ± 0.0	1.0 ± 0.0	1.1 ± 0.1	1.0 ± 0.0
Uric Acid (mg/dl) [1.2–7.5]	0.8 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	0.9 ± 0.1
Cholesterol (mg/dl) [40–130]	92.6 ± 1.8	85.8 ± 3.6	103.4 ± 3.1	80.1 ± 3.4[a]
Triglycerides(mg/dl) [26–145]	34.4 ± 2.2	26.7 ± 1.2[a]	25.6 ± 1.8	26.7 ± 1.2
Liver Function Test				
AST (IU/l) [46–81]	159 ± 16	159 ± 43	98 ± 5	92 ± 5
ALT (IU/l) [17.5–30.2]	45.7 ± 3.7	46.1 ± 10.2	19.0 ± 1.1	27.1 ± 1.3[a]
LDH (IU/l) [61–121]	1350 ± 168	1426 ± 128	769 ± 39	800 ± 62
AP (IU/l) [57–128]	63.6 ± 3.1	72.8 ± 3.1	52.3 ± 1.8	53.9 ± 1.3
Total Bilirubin (mg/dl) [0– 0.64]	0.2 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0

ALT, alanine aminotransferase; AST aspartate aminotransferase; LDH, lactate dehydrogenase; BUN, blood urea nitrogen; AP, alkaline phosphatase; Alb/Glob albumin/globulin ratio.

Values in brackets indicate the normal range for the laboratory rat (Johnson et al., 2008).

*Data represent means +/- SEM. (n=10 male borage oil, n=11 male HGSO, n= 12 female borage oil, n= 12 female HGSO).

[a]Indicates statistically significant difference from borage oil blend diet ($P<0.05$).

[b]Adopted from Tso et al., 2012.

The levels of linoleic acid in phospholipids of plasma, liver, kidney, spleen and mesenteric fat in rats given HGSO were significantly higher than those given borage oil. Although this was not an unexpected effect because the HGSO diet contained higher amounts of linoleic acid than the borage oil diet (39.1% vs 34.1%), the higher ratio of n-3/n-6 fatty acids in the HGSO may have contributed to a reduction in linoleic acid desaturation because n-3 fatty acids are the preferred substrates for Δ6-desaturase. Notably, Tso et al. considered all changes in the percentage of γ-linolenic, di-homo γ-linolenic, arachidonic, adrenic (22:4n-6), and docopentanoic acid (22:5n-6) in plasma and tissue phospholipids to be minor in magnitude and did not reflect adverse or significant changes in fatty acid metabolism.

To assess whether the absorption and metabolism of fat from the HGSO diet was similar to the fat provided in the borage oil diet, total fecal fat and fatty acid composition of the phospholipids in plasma, liver, kidney and spleen were measured after 12 weeks. Total fecal fat content was similar among the diet groups for females. However, male rats given borage oil had

higher fecal fat levels compared to males given HGSO. Although the percentage of fecal fat was higher in borage oil fed males, the percentages of fecal fat for all four dietary groups in this study were lower when compared to similar studies assessing the metabolic effects of 30-day or 90-day feeding of diets containing either borage oil or SONOVA (Liu et al., 2004; Palombo et al., 2001). Thus, with a percentage of fecal fat below 1.5% for all groups, the data suggest that there were no abnormalities in the digestion and/or absorption of either SONOVA or borage oil. These data are also consistent with a study by Christensen and Hoy (1996), which found no significant differences in the absorption and metabolism of fatty acids from current commercial sources of γ-linolenic acid (borage, evening primrose, black currant seed oils).

In conclusion, these data show that compared to consuming a diet containing borage oil, consuming a diet containing SONOVA does not produce adverse effects on growth, body composition, hematology, organ weight or histology. Importantly, although the increases in ALT, AST, and LDH levels were noted in both the borage oil- and HGSO-treated groups relative to normal levels of these enzymes in laboratory rats, the magnitude of these increases were similar in both groups. Typical laboratory rat diets contain 5% fat and, moreover, studies have shown that rats consuming diets containing 20% fat develop non-alcoholic fatty liver disease, which is an inflammatory condition associated with increase blood levels of AST, ALT and insulin resistance (i.e., elevated blood glucose levels) (Li et al., 2013). In this study, the diets contained 10% fat, with similar levels of γ-linolenic acid. Thus, considering the anti-inflammatory activities associated with γ-linolenic acid (see Chapter 3), it is likely that the increases in ALT, AST, and LDH reported in this study are the result of the rats consuming high fat diets rather than a specific effect of consuming high levels of a particular fatty acid such as γ-linolenic acid. In addition, these increases would not occur in humans because their recommended diet should contain approximately 20-40% fat (U.S. Department of Agriculture and U.S. Department of Health and Human Services, 2010).

b. Other High γ-Linolenic Containing Oils

Liu et al. (2004) evaluated the effects of a high- γ-linolenic acid canola oil (HGCO), which was obtained from a genetically engineered strain of canola and borage oil on growth, hematology, serum biochemistry, and n-6 fatty acid metabolism in rats. Pathogen-free male Sprague-Dawley rats were randomly assigned (10 rats/diet) to receive one of four experimental diets for 12 weeks. The experimental diets were modified semisynthetic fat free powdered diets fortified with vitamins and supplemented with 5, 10, or 15% (w/w) of HGCO or 15% borage oil. Importantly, the levels of essential nutrients were similar in the different diets and the amount of carbohydrates was reduced to accommodate the increases in fat, resulting in higher energy

densities in the higher fat diet. Moreover, the HGCO-supplemented diets contained approximately 36% whereas the borage-oil supplemented diet contained 21.8% γ-linolenic acid. Assuming the rats ate approximately 1 g feed/day, the daily intake of γ-linolenic acid for the 5, 10, or 15% HGCO- or 15% borage oil-supplemented diets would be 1.8, 3.6, 5.4, and 3.1 mg/d.

The clinical appearances, body weights, and diet consumption were monitored weekly. At the end of 12 weeks, blood from each rat was collected for hematological studies and analysis of cholesterol and fatty acid composition; serum was collected for biochemical analysis, and feces were collected for analysis of total fat content. Liver, heart, kidneys, and spleen were excised and weighed. The kidney, spleen, and the middle lobe of the liver were fixed in formalin for histologic assessment. Aliquots of liver, skeletal muscle, and epididymal adipose tissue from all the rats were taken for lipid analyses (total triacylglycerol content, total cholesterol content, and phospholipid fatty acid composition). Hematological parameters included red blood cell count, white blood cell count, (monocytes, neutrophils, eosinphils, lymphocytes), platelet count, hematocrit, hemoglobin, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). Biochemical tests on serum included alkaline phosphatase, lactate dehydrogenase (LDH), aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, globulin, albumin/globulin ratio, total protein, blood urea nitrogen (BUN), creatinine, BUN/creatinine ratio, bilirubin (direct, indirect, and total), glucose, iron, sodium, potassium, chlorine, calcium, and phosphorus.

Although γ-linolenic acid intakes could not be calculated because the feed intakes were not correctly reported, rats fed the 5, 10, and 15% HGCO diets had similar growth patterns and their body weights were not significantly different from each other throughout the 12-week feeding period (Table 13). At termination, the mean body weights were 479, 487, and 478 g for the 5, 10, and 15% HGCO dietary groups, respectively, while the mean weight gains were 410, 417, and 405 g for the 5, 10, and 15% HGCO dietary groups, respectively. During the first 6 weeks, the three HGCO dietary groups collectively had similar body weights to the 15% borage oil dietary group. However, beyond week 6 and through the rest of study, rats fed the HGCO diets tended to have lower body weights and weight gains than those fed the 15% borage oil diet. At the end of study, rats fed the 15% borage oil diet had a mean body weight of 510 g, which was significantly higher than the 478 g of rats fed the 15% HGCO diet. Rats given 15% HGCO and 15% borage oil diets had similar fecal total fat contents of 5% (w/w), which was significantly higher than the 2.7 and 3.3% of rats given the 5 and 10% HGCO diets, respectively.

The absolute and relative weights of organs (heart, kidney, liver, and spleen) were similar among rats fed the 5, 10, and 15% HGCO and 15% borage oil diets (Table 13). Histopathology

revealed that the kidneys and spleens were comparable among the four dietary groups. There were localized fat infiltrations (focal fatty metamorphosis) in the peri-portal (triad) area of the livers in rats from all the dietary groups based on the visual examination of a subset (n=4) of rats from each dietary group. On average, the 5 and 10% HGCO and 15% borage oil dietary groups had fat infiltration in the peri-portal area of liver.

Table 13. Absolute (abs) and Relative (rel) Liver, Heart, Spleen and Kidney Weights in Male Sprague-Dawley Rats Fed a Diet Containing 5, 10, or 15% (w/w) of High-γ-Linolenic Acid Canola Oil (HGCO) or 15% (w/w) of Borage Oil for 12 Weeks[a, b]

	weight	5% HGCO	10% HGCO	15% HGCO	15% BO
liver	abs (g)	15.0 ± 0.6	15.7 ± 0.6	14.6 ± 0.5	15.8 ± 0.6
	rel (%)	3.1 ± 0.1	3.2 ± 0.1	3.1 ± 0.0	3.1 ± 0.1
heart	abs (g)	1.4 ± 0.0	1.4 ± 0.1	1.4 ± 0.1	1.4 ± 0.0
	rel (%)	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0
spleen	abs (g)	1.1 ± 0.1	1.1 ± 0.1	1.0 ± 0.1	1.2 ± 0.1
	rel (%)	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
kidney	abs (g)	3.0 ± 0.1	2.9 ± 0.1	2.8 ± 0.1	3.1 ± 0.1
	rel (%)	0.6 ± 0.0	0.6 ± 0.0	0.6 ± 0.0	0.6 ± 0.0
total body	abs (g)	479 ± 12.1	487 ± 17.3	478 ± 16.6	510 ± 10.3

[a]Data represent means +/- standard error of the mean (SEM) (n=10). Relative weights of organs are expressed as percentages of total body weights.

[b]Adopted from Liu et al., 2004.

There were no significant differences in any of the hematology parameters among dietary groups with the exception of the MCV value, which was slightly, but significantly lower in the 5% HGCO dietary group as compared with those in the 10 and 15% HGCO and 15% borage oil dietary groups. However, this difference was minor and within the normal historical range of variation (Table 14) and no dose response relationship was observed.

Table 14. Hematological Parameters of Male Sprague-Dawley Rats Fed a Diet Containing 5, 10, or 15% (w/w) of High-γ-Linolenic Acid Canola Oil (HGCO) or 15% (w/w) of Borage Oil (BO) for 12 Weeks[a, b]

	5% HGCO	10% HGCO	15% HGCO	15% BO
RBC ($10^6/\mu L$)	8.2 ± 0.1	8.1± 0.1	7.8 ± 0.1	7.8 ± 0.1
total WBC ($10^3/\mu L$)	4.7 ± 0.4	4.6 ± 0.6	3.8 ± 0.4	4.8 ± 0.4
monocytes (% WBC)	3.8 ± 0.5	4.2 ± 0.5	5.2 ± 0.7	5.3 ± 1.2
neutrophils (% WBC)	8.5 ± 0.7	7.7 ± 1.1	11.0 ± 1.5	9.6 ± 1.3
eosinophils (% WBC)	2.2 ± 0.4	1.6 ± 0.2	1.9 ± 0.3	1.7 ± 0.3
lymphocytes (% WBC)	86.3 ± 1.1	86.9 ± 1.3	82.2 ± 1.5	83.7 ± 2.2
platelet count ($10^3/\mu L$)	802 ± 43.9	692 ± 33.5	762 ± 24.8	795 ± 26.8
hematocrit (%)	39.7 ± 0.5	40.8 ± 0.4	39.1 ± 0.5	40.4 ± 0.7
hemoglobin (g/dL)	14.5 ± 0.2	14.9 ± 0.1	14.4 ± 0.2	14.8 ± 0.3
MCH (pg)	17.9 ± 0.4	18.5 ± 0.3	18.7 ± 0.2	18.9 ± 0.3
MCHC (g/dL)	36.7 ± 0.3	36.7 ± 0.3	37.3 ± 0.4	36.9 ± 0.2
MCV (fL)	48.2 ± 0.8^x	50.5 ± 0.4^y	49.7 ± 0.6^{xy}	51.2 ± 0.4^y

[a]Mean +/- SEM (n=10 except n=7 for eosinophils); RBC, red blood cell; WBC, white blood cell; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume. Data followed by the same letter are not significantly different ($p<0.05$).

[b]Adopted from Liu et al., 2004.

There were no significant differences in any of the clinical chemistry parameters among dietary groups with the exception of the calcium level which was slightly, but significantly lower in the 15% HGCO dietary group as compared with the 15% borage oil dietary group (Table 15). However, the difference was minor and the value was within the normal historical range of variation and was not considered to be physiologically relevant.

Table 15. Serum Biochemistry of Male Sprague-Dawley Rats Fed a Diet Containing 5, 10, or 15% (w/w) of High-γ-Linolenic Acid Canola Oil (HGCO) or 15% (w/w) of Borage Oil for 12-Weeks[a, b]

	5% HGCO	10% HGCO	15% HGCO	15% BO
ALT(U/L)	34.8 ± 5.7	39.1 ± 6.9	33.0 ± 2.3	36.2 ± 4.0
AST (U/L)	112 ± 14.8	149 ± 29.8	169 ± 42.2	178 ± 46.4
LDH (U/L)	882 ± 167	1001 ± 295	1014 ± 224	1377 ± 341
alkaline phosphatase (U/L)	91.9 ± 3.0	105 ± 5.4	104 ± 5.0	95.1 ± 7.1
direct bilirubin (mg/dL)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
indirect bilirubin (mg/dL)	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0
total bilirubin (mg/dL)	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.0
albumin (g/dL)	2.9 ± 0.1	3.0 ± 0.0	3.0 ± 0.0	3.1 ± 0.1
globulin (g/dL)	3.7 ± 0.1	3.6 ± 0.1	3.6 ± 0.1	3.5 ± 0.1
albumin/globulin ratio	0.8 ± 0.0	0.9 ± 0.0	0.8 ± 0.0	0.9 ± 0.1
total protein (g/dL)	6.7 ± 0.1	6.6 ± 0.1	6.5 ± 0.1	6.6 ± 0.1
BUN (mg/dL)	28.9 ± 1.6	30.0 ± 1.0	28.1 ± 2.1	29.3 ± 2.0
creatinine (mg/dL)	0.7 ± 0.0	0.7 ± 0.0	0.7 ± 0.0	1.1 ± 0.4
BUN/Creatinine Ratio	44.7 ± 2.1	46.0 ± 1.6	42.6 ± 3.3	45.6 ± 4.7
glucose (mg/L)	178 ± 11.0	173 ± 10.6	173 ± 7.7	190 ± 8.6
carbon dioxide (mEq/L)	25.8 ± 0.6	27.3 ± 0.3	27.4 ± 0.8	25.9 ± 0.8
anion gap	23.4 ± 1.1	22.7 ± 0.7	21.4 ± 1.1	22.7 ± 1.0
iron (μg/dL)	260 ± 25.2	281 ± 16.1	293 ± 18.4	313 ± 10.3
chloride (mEq/L)	98.9 ± 0.4	99.2 ± 0.5	99.6 ± 0.3	99.1 ± 0.5
potassium (mEq/L)	4.2 ± 0.1	4.2 ± 0.1	4.2 ± 0.1	4.5 ± 0.2
sodium (mEq/L)	148 ± 0.3	149 ± 0.5	148 ± 0.7	148 ± 0.5
phosphorus (mg/dL)	7.0 ± 0.2	7.3 ± 0.2	6.5 ± 0.2	6.7 ± 0.2
calcium (mg/dL)	10.7 ± 0.1^{xy}	10.7 ± 0.1^{xy}	10.5 ± 0.1^{x}	11.0 ± 0.1^{y}

[a]Data represent means +/- SEM (n=10). ALT, alanine aminotransferase; AST, aspartate aminotransferase; LDH, lactate dehydrogenase; BUN, blood urea nitrogen. Data followed by the same letter are not significantly different ($p<0.05$).

[b]Adopted from Liu et al., 2004.

The plasma cholesterol levels were similar in the HGCO-treated groups, although compared to the borage-oil treated group, there was a significant reduction in the plasma cholesterol levels in the 15% HGCO-treated group. In contrast, liver cholesterol increased dose-dependently in the HGCO-treated groups while only the 15% HGCO-supplement diet was significantly different than the liver cholesterol in the borage oil-treated group.

Analysis of different fatty acid levels in plasma, liver, and muscle revealed that linoleic and γ-linolenic acid were generally higher in the 10 and 15% HGCO-treated groups compared to the 5% HGCO-treated group whereas docosapentaenoic acid was lower in the 10 and 15% HGCO-treated groups than the 5% HGCO-treated groups. In the 15% HGCO treated group there were also significant increases in linoleic, adrenic, and docosapentaenoic acid, and significant reductions in γ-linolenic and di-homo γ-linolenic acid compared to the 15% borage oil-treated

group. Although the reasons for the reduction are unclear, it may have been due to a difference in the stereospecific distribution of γ-linolenic acid in HGCO and borage oil. The majority (75%) of γ-linolenic acid in HGCO was located at the sn-1, 3 positions and only 25% at sn-2 position of the triacylglycerol (TG) molecules (Liu et al., 2001, cited in Liu 2004), whereas less than half (45.8%) of 18:3n-6 in borage oil was located at sn-1 and sn-3 positions and 54.2% at sn-2 position (Lawson et al. 1988, cited in Liu et al., 2004). Because lipoprotein lipase more readily hydrolyzes the fatty acids at the sn-1 and sn-3 positions of triglyceride molecules, the greater distribution of γ-linolenic acid at these two positions in HGCO would allow the γ-linolenic acid molecules of HGCO to be more readily available to the tissues when compared with borage oil. Hempenius et al. (2000) have also reported the presence of fat vacuoles in the liver in rats fed a diet containing 13% of a highly unsaturated fat. The finding of hepatic fat infiltration in four dietary groups is likely due to the high levels of PUFA in the diets.

Overall, all groups had comparable liver weights and levels of liver enzymes (ALT, AST, LDH, and alkaline phosphatase) and bilirubins (direct, indirect, and total), indicating that liver function among the four dietary groups was similar. Interestingly, AST, ALT, LDH and blood glucose concentrations were outside the normal range for laboratory rats and resemble the biochemical aspects of non-alcoholic fatty liver disease, which was also observed in the 90-day toxicology study with HGSO conducted by Tso et al. (2012). As discussed previously, typical laboratory rat diets containing 5% fat (Johnson et al., 2008). Thus, although the increases in blood AST, ALT, LDH and glucose concentrations relative to their normal ranges are notable, it is unlikely that similar effects would occur in humans because their recommended diet should contain approximately 20-40% fat (U.S. Department of Agriculture and U.S. Department of Health and Human Services, 2010).

The effects of high intakes of γ-linolenic acid on reproduction, growth, and brain and behavioral development were evaluated in B6D2F_1 Sprague-Dawley mice using HGCO and borage oil (Wainwright et al., 2003). The primary objective was to evaluate the effects of diets containing equal amounts of γ-linolenic acid (23%), either from HGCO diluted with palm, corn and flaxseed oil, or borage oil to control diets containing undetectable levels of γ-linolenic acid. The second objective was to determine if there is dose-response to γ-linolenic acid by comparing control diet containing no γ-linolenic acid to a diet containing 23% γ-linolenic acid (HGCO diluted with palm, corn and flaxseed oil), and a diet containing 36% γ-linolenic acid (HGCO). The mice were 8 wk old at the start of the study and were assigned randomly to one of the diets; feeding began 2 wk prior to the commencement of breeding. All diets contained 10% fat and were fed to dams prior to conception and throughout pregnancy and lactation, as well as to the

pups after weaning; effects on reproduction, pup development and pup brain fatty acid composition were assessed.

The pregnant dams were weighed on days 0, 7, and 14. Starting on day 18 they were checked twice daily for births, and the number of live and dead pups recorded. Births occurred on days 19 or 20, and on day 21 dams and litters were weighed and litter size recorded again. At this time, litters were then culled to six pups (three males and three females where possible). Litters were weighed on days 25, 32, 39, and 46 (weaning). Pups were also weighed individually at weaning to compare animals by sex. On day 32 (approximately 12 d after birth), one male and one female from each litter was assessed by a battery of tests for sensorimotor development. All behavioral testing was done independently of knowledge of the treatment group. As a positive control, two additional females from each litter were assessed on day 30. Evidence of slowed development in animals on day 30 compared with day 32 supports the reliability of the behavioral measure. Animals were weaned between days 43 and 48 postconception (about 23 to 28 d after birth) and ear-notched for identification. One male and one female, selected randomly from each litter (excluding the animals previously tested), were weighed, then anesthetized using halothane, and decapitated. The brains were extracted, weighed, and frozen immediately in liquid nitrogen.

There were no significant differences in feed intake, body weight, number of successful pregnancies, gestation length, or maternal weight gain during pregnancy and lactation between the different groups. Although litter size did not differ significantly either at birth (day 19) or on day 21, there was a small difference in pup loss (Table 16). Litter from the dams consuming the diets that containing 23% and 36% γ-linolenic acid, lost slightly more pups, post-parturition, than controls; however, it did not differ significantly from the borage oil-treated group.

Table 16. Litter Size and Pup Weight[a, e]

	CON (*n* = 22)[b]	BO (*n* = 22)	GLA-23 (*n* = 21)	GLA-36 (*n* = 23)
Litter size at birth (day 19)	9.86 ± 1.73	9.27 ± 1.70	10.43 ± 1.03	10.13 ± 1.46
Litter size on day 21	9.45 ± 1.82	8.41 ± 1.87	8.95 ± 1.77	8.65 ± 1.82
Pup loss between birth and day 21	0.41 ± 0.59^{y}	0.86 ± 0.99^{x,y}	1.48 ± 1.47^{x}	1.48 ± 1.41^{x}
Pup weight day 21[c]	1.61 ± 0.18^{x}	1.54 ± 0.16	1.52 ± 0.18	1.45 ± 0.15^{y}
Pup weight day 46 (weaning)	16.75 ± 1.10^{x}	16.65 ± 1.27^{x}	15.31 ± 1.24^{z}	15.65 ± 1.27^{y,z}
Pup brain weight[d] (weaning)	0.349 ± 0.058	0.351 ± 0.053	0.363 ± 0.044	0.354 ± 0.069

[a]Values represent mean +/- SD (litter mean scores, three male and three female pups). Groups that do not share a superscript letter are significantly different [Student-Newman-Keuls (SNK) test, P<0.05].

[b]n=number of litters.

[c]Days postconception, day 0=conception; birth normally occurred on day 19-20.

[d]Based on one male and one female.

Abbreviations: Con, corn oil (92.7%) + soybean oil (7.3 %) = control; BO, borage oil (97.9%) + flaxseed oil (2.1%); GLA-23, palm oil (12%) + corn oil (29.5%) + flaxseed oil (0.5%) + high γ-linolenic acid canola oil (HCGO) (58%); GLA-36 HCGO (100%).

[e]Adopted from Wainwright et al., 2003.

Pups given the diet containing 36% γ-linolenic acid diet weighed less than controls at all times from birth to weaning. On days 39 and 46 (weaning) pups from dams consuming the diets derived from HGCO weighed less than those from dams fed the control and borage oil-supplemented diets, whereas there were no significant differences in the pup weights in the control and borage oil treated groups. There were also no significant differences in brain weight, pup behavioral development across all the groups.

Overall, supplementation with γ-linolenic acid increased levels of specific n-6 fatty acids and decreased those of n-3 fatty acids, predominantly docosahexanoic acid (22:6n-3). Although the effects of both borage oil and 23% γ-linolenic acid relative to control were in the same direction, the effects of 23% γ-linolenic acid were larger than borage oil (Tables 17, 18, and 19). For example, as seen in both arachidonic acid and DHA in the ethanolamine phosphoglycerides

fraction, although borage oil did not increase arachidonic acid relative to control, 23% γ-linolenic acid increased arachidonic acid relative to both control and borage oil. Similarly, the findings for DHA indicated that while borage oil decreased DHA relative to control in the ethanolamine phosphoglycerides fraction, 23% γ-linolenic acid from the HGCO decreased DHA relative to both control and borage oil.

Table 17. Selected Fatty Acid Composition of Brain Ethanolamine Phosphoglycerides (wt % Fatty Acids)[a, b]

	CON (n = 19)	BO (n = 21)	GLA-23 (n = 20)	GLA-36 (n = 21)
Total sat	37.25 ± 1.93	37.36 ± 1.94	37.69 ± 2.1	37.26 ± 2.59
Total mono	18.01 ± 1.40^{p}	17.59 ± 1.35^{p,q}	17.04 ±1.34^{p,q}	16.65 ± 1.29^{q}
Total poly	39.80 ± 2.68	40.30 ± 2.36	39.97 ± 2.22	41.37 ± 2.93
20:3n-6	0.69 ± 0.05^{y}	1.37 ± 0.15^{q}	1.15 ± 0.14^{x}	1.59 ± 0.20^{p}
20:4n-6	14.35 ± 1.47^{x}	14.67 ± 1.40^{x}	16.17 ± 0.97^{q}	17.73 ± 1.94^{p}
22:4n-6	4.43 ± 0.29^{y}	4.86 ± 0.40^{x}	5.97 ± 0.36^{q}	6.34 ± 0.60^{p}
22:5n-6	2.34 ± 0.30^{q}	2.87 ± 0.35^{p}	2.86 ± 0.42^{p}	2.80 ± 0.36^{p}
Total n-6	22.84 ± 1.47^{y}	24.48 ± 1.41^{x}	26.88 ± 1.39^{q}	29.12 ± 2.14^{p}
22:6n-3	16.70 ± 1.43^{p}	15.53 ± 1.34^{q}	12.33 ±1.37^{x}	11.19 ± 1.34^{y}
Total n-3	16.96 ±1.43^{p}	15.83 ± 1.31^{q}	13.09 ± 1.30^{x}	12.25 ± 1.29^{x}
Ratio n-6 to n-3	1.35 ± 0.08^{y}	1.55 ± 0.11^{x}	2.07 ± 0.18^{q}	2.40 ± 0.23^{p}

[a]Values represent mean ± SD (litter mean scores, one male and one female pup, where n = number of litters). Groups that do not share a superscript are significantly different (SNK). Total sat, total saturated FA; Total mono, total monounsaturated FA; Total poly, total PUFA.

[b]Adopted from Wainwright et al., 2003.

Table 18. Selected Fatty Acid Composition of Brain Choline Phosphoglycerides (wt % Fatty Acids)[a, b]

	CON (n = 19)	BO (n = 21)	GLA-23 (n = 20)	GLA-36 (n = 21)
Total sat	63.67 ± 1.25	64.14 ± 1.00	64.35 ± 1.67	64.55 ± 2.38
Total mono	26.47 ± 0.73	26.25 ± 0.76	26.32 ± 0.96	26.07 ± 1.71
Total poly	9.87 ± 1.20	9.61 ± 0.76	9.32 ± 1.16	9.37 ± 1.27
20:3n-6	0.21 ± 0.02^{y}	0.47 ± 0.03^{q}	0.38 ± 0.04^{x}	0.54 ± 0.08^{p}
20:4n-6	5.17 ± 0.65	5.21 ± 0.46	5.38 ± 0.69	5.53 ± 0.78
22:4n-6	0.33 ± 0.06^{y}	0.36 ± 0.04^{x}	0.44 ± 0.05^{q}	0.47 ± 0.05^{p}
22:5n-6	0.36 ± 0.06^{x}	0.49 ± 0.11^{p}	0.43 ± 0.08^{q}	0.42 ± 0.06^{q}
Total n-6	7.52 ± 0.76	7.45 ± 0.51	7.63 ± 0.85	7.82 ± 0.94
22:6n-3	2.31 ± 0.45^{p}	2.10 ± 0.33^{p}	1.56 ± 0.38^{q}	1.37 ± 0.36^{q}
Total n-3	2.35 ± 0.46^{p}	2.16 ± 0.31^{p}	1.70 ± 0.38^{q}	1.56 ± 0.37^{q}
Ratio n-6 to n-3	3.27 ± 0.41^{q}	3.50 ± 0.39^{q}	4.64 ± 0.75^{q}	5.19 ± 0.89^{p}

[a]Values represent mean ± SD (litter mean scores, one male and one female pup, where n = number of litters). Groups that do not share a superscript are significantly different (SNK).

[b]Adopted from Wainwright et al., 2003.

Table 19. Selected Fatty Acid Composition of Brain PS/PI (%wt FA)[a, b]

	CON (n = 19)	BO (n = 21)	GLA-23 (n = 20)	GLA-36 (n = 21)
Total sat	48.54 ± 3.69	49.42 ± 4.04	48.06 ± 3.48	49.18 ± 3.99
Total mono	18.31 ± 1.03	17.86 ± 1.25	18.04 ± 1.60	17.73 ± 1.23
Total poly	33.15 ± 3.88	32.72 ± 4.06	33.89 ± 3.86	33.09 ± 4.53
20:3n-6	0.58 ± 0.06^{y}	1.12 ± 0.11^{q}	0.97 ± 0.12^{x}	1.29 ± 0.17^{p}
20:4n-6	$11.79 \pm 1.23^{p,q}$	11.48 ± 1.20^{q}	12.74 ± 1.44^{p}	$12.17 \pm 1.73^{p,q}$
22:4n-6	2.52 ± 0.17^{y}	2.77 ± 0.23^{x}	3.45 ± 0.44^{q}	3.97 ± 0.27^{p}
22:5n-6	2.79 ± 0.43^{x}	3.37 ± 0.52^{p}	$3.59 \pm 0.69^{p,q}$	3.73 ± 0.42^{q}
Total n-6	18.31 ± 1.57^{q}	19.23 ± 1.71^{q}	21.33 ± 2.17^{p}	21.63 ± 2.08^{p}
22:6n-3	14.71 ± 2.40^{p}	$13.37 \pm 2.50^{p,q}$	$12.08 \pm 1.85^{q,x}$	10.74 ± 2.56^{x}
Total n-3	14.83 ± 2.41^{p}	$13.49 \pm 2.50^{p,q}$	$12.56 \pm 1.93^{q,x}$	11.46 ± 2.59^{x}
Ratio n-6 to n-3	1.25 ± 0.12^{y}	1.45 ± 0.15^{x}	1.72 ± 0.18^{q}	1.94 ± 0.28^{p}

[a]Values represent mean ± SD (litter mean scores, one male and one female pup, where n = number of litters). Groups that do not share a superscript are significantly different (SNK).

[b]Adopted from Wainwright et al., 2003.

In summary, Wainright et al. (2003) evaluated the bioequivalence and safety of HGCO with traditional γ-linolenic acid-rich borage oil on reproduction, growth, and brain and behavioral development in mice. The results indicate that some of the effects of γ-linolenic acid provided as HGCO differed from those of equivalent amounts of γ-linolenic acid provided as borage oil. Specifically, 23% γ-linolenic acid from HGCO reduced pup body weight and was associated with a slight increase in neonatal pup attrition. There were no significant effects on behavioral development or on physical performance. An increase in dietary γ-linolenic acid resulted in an increase in brain n-6 fatty acids and a corresponding decrease in brain n-3 fatty acids. Moreover, despite their similar levels of γ-linolenic acid, the effects on brain fatty acid composition were greater in the mice that received the diet containing 23% γ-linolenic acid derived for HGCO than with those receiving the same amount from borage oil. Comparison of the group receiving 23% γ-linolenic acid from HGCO with that receiving 36% γ-linolenic acid indicated that at the higher level the effects on growth were greater, as were those on brain fatty acid composition, particularly in the ethanolamine phosphoglycerides fraction. The intake of γ-linolenic acid from HGCO was not reported, however, using default assumptions of feed intake of 5 g/day and body weight of 20 g for the mice results in an estimation of intake of γ-linolenic acid by the dams of 5.75 and 9 g/kg/day in the 23% and 36% groups, respectively. This is equivalent to an intake of 345 or 540 g/day for a 60-kg person.

b. Dihomo-γ-linolenic Acid-Containing Oils

The safety of γ-linolenic acid ingestion is also corroborated by an Ames assay, and acute and subchronic oral toxicity studies conducted in rats with a dihomo-γ-linolenic acid-enriched oil (Kawahima et al., 2009).

In the Ames assay, the mutagenicity of 313, 625, 1250, and 5000 µg of a dihomo-γ-linolenic acid-enriched oil was assessed using *Salmonella typhimurium* strains TA100, TA1535, TA98, and TA1537, the *Escherichia coli* strain WP2uvrA/pKM101, and preincubation methods both with and without metabolic activation. In all strains treated with the dihomo-γ-linolenic acid-enriched oilthe number of revertant colonies was less than the solvent/negative control, both with and without metabolic activation,. In addition, no microbial toxicity was observed. Together these results indicate that a dihomo-γ-linolenic acid-enriched oil is neither mutagenic nor cytotoxic.

To assess the acute toxicity of a dihomo-γ-linolenic acid-enriched oil, 6-10 week old male (n=5) and female (n=5) Sprague-Dawley (Crj:CD) rats were administered 10 g/kg of either a dihomo-γ-linolenic acid-enriched oil or soybean oil by gavage. The rats were observed over the next 14 days, and clinical signs and body weights were recorded. On day 14, the rats were euthanized and examined by necropsy. No deaths occurred and loose stools and soiled periproctal regions were observed in both the dihomo-γ-linolenic acid-enriched oil- and soybean oil-treated groups. Body weight gains and clinical signs were also similar in both groups during the in-life portion of the study and at necropsy no macroscopic differences between the two groups were noted.

The subchronic toxicity of dihomo-γ-linolenic acid-enriched oil was determined in 90-day oral toxicity study conducted in rats. Either water, soybean oil, or 0.5 (0.056 g dihomo-γ-linolenic acid /kg bw/day), 1.0 (equivalent to 0.112 g dihomo-γ-linolenic acid/kg bw/day), or 2.0 g/kg bw/day (equivalent to 0.224 g dihomo-γ-linolenic acid /kg bw/day) of the dihomo-γ-linolenic acid-enriched oil was administered once a day by gavage for 13 weeks to 6 week-old male (n=20/group) and female (n=20/group) Sprague Dawley (Crj:CD) rats. The rats were observed over the course of the study and clinical signs, body weights, and feed consumption were recorded. At the end of the study, ophthalmological examinations, uninalysis, hematological examinations, blood biochemistries, necropsy, organ weight, and histopathalogical examinations were performed.

No deaths occurred and no treatment-related clinical signs were noted throughout the administration period. There were also no treatment-related ophthalmological abnormalities.

Although feed consumption was lower in the soybean oil- and dihomo-γ-linolenic acid-containing oil-treated groups compared to the water-treated groups, feed consumption was similar in the soybean oil- and dihomo-γ-linolenic acid-enriched oil-treated groups. Body weight gain was similar across all the groups. Relative weights of the thyroid and spleen were significantly lower in the males of the 1.0 g/kg/d dihomo-γ-linolenic acid-enriched oil- and soybean oil-treated groups compared to the water-treated group whereas the absolute weights of the submaxillary glands and relative weights for the spleen were significantly higher in the males of the 2.0 g/kg dihomo-γ-linolenic acid-containing oil-treated group and the absolute and relative weights of the uterus in the females of the 1.0 g/kg/d dihomo-γ-linolenic acid-enriched oil-treated group were significantly lower compared to the soybean oil-treated group. Importantly, Kawahima et al., reported that there were no treatment-related macroscopic or histopathological findings and that all observed histopathological changes occurred at comparable frequencies among the different treatment groups and were common findings in rats of the same strain and age.

For the blood biochemistries, total blood protein and albumin were lower in the soybean oil- and dihomo-γ-linolenic acid-enriched oil-treated groups compared to the water-treated group, AST was lower in the males of the 0.5 and 1.0 g/kg/d dihomo-γ-linolenic acid-enriched oil-treated groups and in the females of the soybean-oil treated group compared to the respective sex in water-treated group. BUN was also decreased in the males of the 0.5 and 1.0 g/kg/d dihomo-γ-linolenic acid-enriched oil- and soybean-oil-treated groups compared to the males in the water-treated group. Albumin to globulin ratio was decreased in the males of the 1.0 g/kg/d dihomo-γ-linolenic acid-enriched oil-treated group, but not in the soybean oil-treated group, compared to the males of the water-treated group. In contrast, between the dihomo-γ-linolenic acid-containing oil and soybean-oil treated groups, only lower triglyceride levels were observed in the males treated with 1.0 and 2.0 g/kg/d dihomo-γ-linolenic acid-containing oil.

For urine parameters, total sodium, potassium and chloride levels were lower in the males of the 0.5, 1.0, and 2.0 g/kg/d dihomo-γ-linolenic acid-enriched oil- and soybean oil-treated groups compared to the males in the water-treated group, whereas total excretion of sodium and potassium were lower in the females of the 1.0 g/kg dihomo-γ-linolenic acid-enriched oil-treated group, but not in the soybean oil-treated group, compared to the females in the water-treated group. Importantly, between the soybean oil- and dihomo-γ-linolenic acid-enriched oil-treated groups there were no significant differences in any of the urine parameters measured.

For hematologies, low erythrocyte counts were observed in females treated with 1.0 and 2.0 g/kg/d dihomo-γ-linolenic-enriched oil, high mean corpuscular hemoglobin concentration

(MCHC) was observed in the females treated with 2.0 g/kg/d dihomo-γ-linolenic-enriched oil, lower activated partial thromboplastin time (APTT) was observed in the females treated with 2.0 g/kg/d dihomo-γ-linolenic-enriched oil, low hemoglobin levels, prothrombin time (PT), and APTT were observed in the males treated with 2.0 g/kg/d dihomo-γ-linolenic-enriched oil compared to the respective sex in the water-treated group. Hematocrit was decreased in both males and females in the 2.0 g/kg dihomo-γ-linolenic-enriched oil group compared to the water-treated group. PT and APTT were also lower in the males of the soybean oil-treated group compared to the water-treated group. In contrast, between the dihomo-γ-linolenic acid-containing oil and soybean-oil treated groups, the hematocrits were significantly lower in both males and treated with 2.0 g/kg/d dihomo-γ-linolenic-enriched oil, and the PT was significantly higher in the males treated with the 2.0 g/kg/d dihomo-γ-linolenic-enriched oil.

In conclusion, although decreased feed consumption was observed in both the soybean oil- and the dihomo-γ-linolenic acid groups compared to the water-treated group, this decrease occurred in the absence of organ weight and histopathological abnormalities. Moreover, these results are consistent with the results reported in other studies where rats were fed diets high in corn oil, ARA and/or DHA (Lina et al., 2006). Thus, the decreased feed consumption ismost likely the result of consuming large amounts of oil, rather than high amounts of dihomo-γ-linolenic acid. In a similar manner, because the reductions in urinary sodium, potassium, and chloride levels, and the reductions in serum protein, albumin, BUN, and albumin/globulin ratio were not dose-dependent in the dihomo-γ-linolenic acid enriched oil-treated groups and occurred in both the soybean oil- and dihomo-γ-linolenic acid enriched oil-treated groups, the reductions are likely secondary to the decreased feed consumption that results from the increased intake of fat. For the hematological effects, the effects were mild, did not correlate with any perturbations in reticulocyte ratios or histopathological abnormalities in the hematopoietic organ, and thus are not toxicologically significant. Moreover, similar effects have been reported in other studies where in rats were fed other oils containing long chain n-3 fatty acids (Lina et a., 2006, Hempenius et al., 2000; Burns et al., 1999). Lastly, the significant decreases in triglycerides are consistent with the reported effects of long chain n-3 fatty acid-enriched oils (reviewed in Shearer et al., 2012). Therefore, the no-observed-adverse effect level for the ingestion of the dihomo-γ-linolenic acid-enriched oil can be considered to be 2.0 g/kg/day (equivalent to 0.224 g dihomo-γ-linolenic acid /kg bw/day).

2. Equivalence of Fatty Acid Metabolism

Palombo et al. (2000) evaluated the bioequivalency of γ-linolenic acid obtained from transgenic canola plants relative to γ-linolenic acid from borage oil in Sprague Dawley male rats

fed diets enriched with oils from each source to provide approximately 0.23 g γ-linolenic acid/g total fatty acids in each diet. A control group was fed diet containing corn oil. The total lipid content of each diet was 10% by weight. Growth, feed consumption, and fecal fat content (during the second week of study) were measured and after 3 weeks of feeding, fatty acid composition of the principal phospholipid fractions of liver and plasma and triglycerides in epididymal adipose were evaluated to determine effects on the metabolism of the principal n-6 fatty acids in these tissues.

No evidence of adverse effects on general appearance or behavior was reported based on daily observations. Growth of the rats from each dietary treatment group was similar; body weights and body weight gain were not significantly different. Total diet consumption was similar among groups. There were no significant differences among groups with respect to total fecal weight or fat content. Postmortem examination of the major organs did not reveal any adverse treatment effects; mean weights of liver, heart, kidney and spleen were not statistically different among treatment groups. There was no difference between diets for the percentage of total n-6 polyunsaturated fatty acids in total or individual phospholipid fractions. The composition of n-6 fatty acids within the individual liver phospholipids was primarily influenced by the n-6 fatty acids present in the diet. The liver phospholipids from rats fed the γ-linolenic acid from canola and borage oil had higher levels of γ-linolenic acid and dihomo γ-linolenic acid and lower levels of linoleic acid compared with rats given a control diet. The relative percentages of 18:2n-6 and 18:3n-6 in the liver total phospholipids and phosphatidylethanolamine fraction were also similar between the two γ-linolenic acid diet groups. The phosphatidylcholine fraction of liver from the group fed the diet supplemented with high γ-linolenic acid canola oil had a slightly greater percentage of γ-linolenic acid than the group fed the diet supplemented with borage oil. The percentages of dihomo γ-linolenic acid and arachidonic acid were similar between the γ-linolenic acid diet groups in the total and all individual phospholipid fractions. The percentage of linoleic acid liver phosphatidyl-inositol/serine was significantly higher in the γ-linolenic acid canola diet group as compared with the borage oil group.

Total n-6 polyunsaturated fatty acids present in the total and individual phospholipid fractions was similar across the three diet groups. As observed in the liver phospholipids, the composition of n-6 fatty acids within the plasma phospholipid fractions reflected the composition of n-6 fatty acids present in the diet. In general, the plasma phospholipids from rats fed the canola and borage oil diets tended to have higher levels of γ-linolenic acid and di-homo γ-linolenic acid and lower levels of linoleic acid in comparison to the phospholipids from rats

given the control diet. The relative percentages of 18:2n-6, 18:3n-6, 20:3n-6, and 20:4n-6 within the total and individual phospholipid fractions were similar for the γ-linolenic acid diet groups.

The fatty acid composition of the liver and plasma phospholipids largely reflected the fatty acid composition of the diets. The incorporation and metabolism of γ-linolenic acid within the liver and plasma phospholipid fractions was similar between the γ-linolenic acid groups. These results indicate that the metabolism of γ-linolenic acid in these tissues was dependent upon the content of γ-linolenic acid in the diet and not the source of the γ-linolenic acid.

Tso et al. (2002) evaluated the intestinal absorption and lymphatic transport of γ-linolenic acid from transgenic canola oil to borage oil using a lymph fistula rat model. After overnight recovery from surgery, rats received either 1 mL of canola oil (37% γ-linolenic acid; 26% linoleic acid; 22% oleic acid; with other fatty acids) or 1 mL of borage oil (23% γ-linolenic acid; 37% linoleic acid; 14% oleic acid; with other fatty acids). The fatty acid compositions of triglycerides in mesenteric lymph were compared over a 24 hr collection period. The digestion, uptake and lymphatic transport of γ-linolenic acid and the normal physiologic changes associated with fat absorption (e.g., lymph flow and an increase in lymphatic endogenous lipids outputs, triglycerides, cholesterol and phospholipids) were similar in the canola- and the borage oil-fed rats. The original differences in γ-linolenic acid, linoleic and oleic acid content in canola and borage oil were preserved in the fatty acid composition of the rats' lymph lipid. γ-Linolenic acid from canola was absorbed and transported into lymph similarly to borage oil.

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October 20, 2016

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Re: GRAS Notice No. GRN 000652; Response to FDA letter of October 18, 2016

Dear Dr. Zhu:

This letter provides Arcadia Biosciences' statement and certification to fulfill the GRAS notification procedure (21 CFR part 170, subpart E). Please note the following:

None of the data and information in GRAS Notice No. GRN 000652 are exempt from disclosure under the Freedom of Information Act, 5 U.S.C. 552 (FOIA).

GRN 000652 is a complete, representative, and balanced submission that includes unfavorable as well as favorable information known to Arcadia and pertinent to the evaluation of the safety and GRAS status of the use of the substance.

The data and information addressing parts 2 through 7 of a GRAS notice (21 CFR 170.230 to 170.255) are included in GRN 000652.

GRN 000652 contains no data and information that is exempt from disclosure under the FOIA. There was no non-public, safety-related data and information used in reaching a conclusion of GRAS status.

The GRAS panel for GRN 000652 did not have access to any non-public, safety-related data and information.

Please let us know if there are additional statements and/or certification necessary. Thank you.

Sincerely yours,

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November 8, 2016

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RE: Response to FDA's Questions on GRN 652

Dear Dr. Zhu:

In response to your request of October 25, 2016, following are our responses to FDA's Questions on GRN 652. FDA's questions are enumerated below in italicized text. Arcadia's responses are written in plain text. All revisions have been included in the attached/revised version of GRN 652.

1. *In your notice, you referenced GRN 306 for the estimated per capita mean and 90th percentile dietary intakes of γ-linolenic acid and made the assumption that approximately 10% of total n-6 polyunsaturated fatty acid intake is γ-linolenic acid. However, we note that the estimates of dietary intake of linolenic acid presented in GRN 306 are for α-linolenic acid. Please correct and make appropriate adjustments to your statements on consumption and exposure estimates of γ-linolenic acid in the notice.*

   Although soybean oil is widely known to contain high levels of the n-3 polyunsaturated fatty acid α-linolenic acid, GRN 306 does not specify whether the per capita intakes for linolenic acid were for α-linolenic acid or γ-linolenic acid, as was done in Table 8 and 10 of GRN 283. We have now corrected our discussions of GRN 306 (pages 24 and 32 of the revised Notification) and included the per capita γ-linolenic intakes specified in Table 8 and 10 of GRN 283 in our calculations for the background dietary intake, cumulative exposure, and per capita intakes of n-6 polyunsaturated fatty acids (pages 24, 28, and 32 of the revised Notification).

2. *On Page 27 and 28 (Table 6), you calculated an acceptable macronutrient distribution range (AMDR) for γ-linolenic acid based on information in GRN 306 (the last column of Table 6 and footnote e). These calculations include an assumption that 10% of the AMDR for total n-6 polyunsaturated fatty acids is γ-linolenic acid. We are unable to identify the information in GRN 306 that you are incorporating in this notice, specifically, the AMDR for total n-6 polyunsaturated fatty acids and the rationale for the 10% assumption. Please address.*

Our assumption that 10% of n-6 polyunsaturated fatty acid intake is γ-linolenic was based on the relative per capita intakes for linoleic acid and linolenic acid specified in GRN 306. As noted in question #1, it is now clear that the linolenic acid per capita intakes presented in GRN 306 are for α-linolenic acid, not γ-linolenic acid. We have now removed all references to our assumption in the revised Notification, added the γ-linolenic acid per capita intakes specified in GRN 283 (pages 24 and 32 of the revised Notification), and calculated the cumulative exposure to n-6 polyunsaturated fatty acids from the intended uses and background dietary intake (page 28 of the revised Notification). We have also corrected our calculations and discussion of the AMDR to represent total n-6 polyunsaturated fatty acids (pages 30 and 31 of the revised Notification).

3. *Based on the Adequate Intakes (AI) of n-6 polyunsaturated fatty acids in the IOM table of Dietary Reference Intakes for macronutrients, the daily consumption for individuals aged 4 and above is between 10 and 17 g/d for both male and female. If it is assumed that 10% of these totals are γ-linolenic acid, the AI becomes 1-1.7 g/d, which is lower than the 1 to 3 or 4 g/d stated in the revised version of the GRAS notice (Page 27). Please provide the source material for the 1 to 3 or 4 g/d estimates.*

Based on the information provided in question #1, our assumption that 10% of n-6 polyunsaturated fatty acid intake is α-linolenic is no longer valid. As noted in our response to question #2, we have now removed all references to our assumption in the revised Notification. We have also added the γ-linolenic acid per capita intakes specified in GRN 283 (pages 23 and 29 of the revised Notification) and edited our calculations of the AMDR to represent total n-6 polyunsaturated fatty acids (page 28 of the revised Notification). Importantly, the EDIs from the intended uses represent approximately 10% of total dietary intake of n-6 polyunsaturated fatty acids (specified on page 32 of the revised Notification). Moreover, the cumulative intakes of n-6 polyunsaturated fatty acids from the intended uses and background dietary intake fall below the upper boundary for n-6 polyunsaturated fatty acids (pages 30 and 31 of the revised Notification).

4. *You cited in your notice (Page 30 and Table 8) the study by Guivernau et al., 1994. This study reported that ingestion of evening primrose oil by hyperlipidemic men, which resulted in approximately 0.24 g γ-linolenic acid/d, significantly reduced platelet aggregation and function as well as thromboxane production, and also significantly increased prostacyclin production and the bleeding time. The changes in the platelet activity and eicosanoid profile are consistent with the increased bleeding time reported in the study. Therefore, the increased bleeding time cannot be dismissed as an isolated or sporadic observation. Because higher level of γ-linolenic acid consumption has been associated with increased bleeding time, please address how this published information is not in contradiction to the general recognition of safety of γ-linolenic acid for the intended use of up to 2.0 g/d.*

Because up to 100% of linoleic acid can be metabolized to γ-linolenic acid, it is reasonable to draw upon the publicly available literature on linoleic acid to evaluate the effect on n-6 polyunsaturated fatty acids on platelet aggregation and bleeding time. Seven studies that evaluated the effect of linoleic acid-enriched diets on platelet aggregation and/or bleeding time were retrieved. Four of these seven studies evaluated both platelet aggregation and bleeding time. In most of these studies the intake levels of n-6 polyunsaturated fatty acids exceed those expected from the intended uses by approximately 10-fold. Importantly, although the majority of the studies reported that the ingestion of linoleic acid-enriched diets reduced platelet aggregation *in vitro*, the majority of the studies that evaluated bleeding time *in vivo* showed no effect. It is therefore clear that the platelet aggregation assays used in the seven studies and those used by Guivernau et al. do not accurately reflect the effect of the diets on bleeding time, Moreover, the weight of the evidence indicates that the ingestion of n-6 polyunsaturated fatty acids from the intended uses will have no effect on bleeding time. To document these findings, we have now added an overarching summary of these findings and a table (Table 10) that parses out the experimental details of each study on page 33 and 75 of the revised Notification, respectively. Also, to accommodate these additions we have updated the reference list and all subsequent table titles and in-text table references. It is important to note that although these studies provide context for the study conducted by Guivernau et al. SONOVA is intended to be used in place of the γ-linolenic acid-enriched source currently used to formulate products sold in the marketplace. Therefore, the addition of SONOVA to the intended products will not increase the consumer's exposure to γ-linolenic acid or n-6 polyunsaturated fatty acids.

Should you have additional questions, please let us know.

Sincerely,

Claire L, Kruger, Ph.D., D.A.B.T.
President

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Generally Recognized as Safe (GRAS) Determination for GLA Safflower Oil (SONOVA®) in Conventional and Medical Foods

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TABLE OF CONTENTS

I. GRAS EXEMPTION CLAIM 1
A. NAME AND ADDRESS OF THE SPONSOR 1
B. COMMON OR USUAL NAME 1
C. INTENDED USE 1
D. BASIS FOR GRAS DETERMINATION 2
E. AVAILABILITY OF INFORMATION 6
F. SIGNATURE 7
II. DESCRIPTION OF SUBSTANCE 8
A. COMMON OR USUAL NAME 8
B. DESCRIPTION OF SONOVA 8
C. PRODUCTION PROCESS 11
1. Generation of Carthamus tinctorius L. GLA Safflower 11
2. Production of SONOVA 13
D. FINISHED PRODUCT DESCRIPTIONS AND SPECIFICATIONS 17
1. Product Specifications 17
2. Hexanes 19
3. Tocopherols 19
4. Pesticides 19
5. Allergens 20
6. Microbiologicals 20
7. Stability 20
III. INTENDED EFFECT 21
IV. HISTORY OF USE, INTENDED USE, AND ESTIMATED DAILY INTAKE 24
A. HISTORICAL EXPOSURE TO SONOVA 24
B. DIETARY INTAKES OF N-6 POLYUNSATURATED FATTY ACIDS 24
C. INTENDED USES AND ESTIMATED DAILY INTAKES OF SONOVA 25
D. CUMULATIVE EXPOSURE TO N-6 POLYUNSATURATED FATTY ACIDS 28
V. SAFETY OF SONOVA 29
A. ADEQUATE INTAKES AND ACCEPTABLE MACRONUTRIENT INTAKE LEVELS FOR N-6 POLYUNSATURATED FATTY ACIDS 30
B. PER CAPITA INTAKES OF N-6 POLYUNSATURATED FATTY ACIDS 32
C. HUMAN STUDIES 32
1. Orally Administered Products Containing γ-Linolenic Acid 32
2. Enterally Administered Products Containing γ-Linolenic Acid 34
3. γ-Linolenic Acid and Inflammation 36
4. Orally Administered Products Containing Dihomo-γ-Linolenic Acid 39
D. ANIMAL STUDIES 79
1. Toxicology Studies 79
2. Equivalence of Fatty Acid Metabolism 96
VI. REFERENCES 99

LIST OF TABLES

TABLE 1. FATTY ACID COMPOSITIONAL COMPARISON OF SONOVA, CONVENTIONAL FOODS, AND DIETARY SUPPLEMENTS....9

TABLE 2. TOCOPHEROL AND STEROL COMPOSITION OF SONOVA, COMMON EDIBLE OILS, BORAGE OIL, AND EVENING PRIMROSE OIL....10

TABLE 3. PRODUCT SPECIFICATIONS AND BATCH DATA FOR SONOVA....18

TABLE 4. TOCOPHEROL LEVELS IN SONOVA....19

TABLE 5. MAXIMUM DAILY INTAKE OF γ-LINOLENIC ACID (GLA) FROM THE INTENDED USES OF SONOVA IN NUTRITIONAL BEVERAGES AND MEDICAL FOODS27

TABLE 6. ACCEPTABLE MACRONUTRIENT DISTRIBUTION RANGE (AMDR) FOR LINOLENIC ACID IN CHILDREN AND ADULTS....31

TABLE 7. CLINICAL TRIALS OF γ-LINOLENIC ACID (GLA)-ENRICHED OILS IN INFANTS AND CHILDREN....41

TABLE 8. CLINICAL TRIALS OF γ-LINOLENIC (GLA)-ENRICHED OILS IN ADULTS....47

TABLE 9. CLINICAL TRIALS OF γ-LINOLENIC (GLA)-ENRICHED OILS IN ADULTS AND CHILDREN RECEIVING SOLE-SOURCE ENTERAL NUTRITION....64

TABLE 10. CLINICAL TRIALS EVALUATING THE EFFECT OF LINOLEIC ACID-ENRICHED DIETS ON PLATELET AGGREGATION AND BLEEDING TIME....75

TABLE 11. ABSOLUTE (ABS) AND RELATIVE (REL) MAJOR ORGANS WEIGHTS OF MALE AND FEMALE SPRAGUE-DAWLEY RATS FED A DIET CONTAINING EITHER A BORAGE OIL BLEND OR A HIGH γ-LINOLENIC SAFFLOWER OIL (HGSO) BLEND FOR 12 WEEKS....81

TABLE 12. HEMATOLOGICAL PARAMETERS OF MALE AND FEMALE SPRAGUE-DAWLEY RATS FED A DIET CONTAINING A BORAGE OIL BLEND OR A HIGH γ-LINOLENIC SAFFLOWER OIL (HGSO) BLEND FOR 12 WEEKS....82

TABLE 13. SERUM BIOCHEMISTRY PROFILES OF MALE AND FEMALE SPRAGUE-DAWLEY RATS FED ON A DIET CONTAINING EITHER THE BORAGE OIL BLEND OR THE HIGH γ-LINOLENIC SAFFLOWER OIL (HSGO) BLEND AT THE END OF 12-WEEK FEEDING....83

TABLE 14. ABSOLUTE (ABS) AND RELATIVE (REL) LIVER, HEART, SPLEEN AND KIDNEY WEIGHTS IN MALE SPRAGUE-DAWLEY RATS FED A DIET CONTAINING 5, 10, OR 15% (W/W) OF HIGH-γ-LINOLENIC ACID CANOLA OIL (HGCO) OR 15% (W/W) OF BORAGE OIL FOR 12 WEEKS....86

TABLE 15. HEMATOLOGICAL PARAMETERS OF MALE SPRAGUE-DAWLEY RATS FED A DIET CONTAINING 5, 10, OR 15% (W/W) OF HIGH-γ-LINOLENIC ACID CANOLA OIL (HGCO) OR 15% (W/W) OF BORAGE OIL (BO) FOR 12 WEEKS....87

TABLE 16. SERUM BIOCHEMISTRY OF MALE SPRAGUE-DAWLEY RATS FED A DIET CONTAINING 5, 10, OR 15% (W/W) OF HIGH-γ-LINOLENIC ACID CANOLA OIL (HGCO) OR 15% (W/W) OF BORAGE OIL FOR 12-WEEKS....88

TABLE 17. LITTER SIZE AND PUP WEIGHT....91
TABLE 18. SELECTED FATTY ACID COMPOSITION OF BRAIN ETHANOLAMINE PHOSPHOGLYCERIDES....92
TABLE 19. SELECTED FATTY ACID COMPOSITION OF BRAIN CHOLINE PHOSPHOGLYCERIDES....92
TABLE 20. SELECTED FATTY ACID COMPOSITION OF BRAIN PS/PI (%WT FA)....93

LIST OF FIGURES

FIGURE 1. PLASMID MAP OF PSBS4119....12
FIGURE 2. PRODUCTION PROCESS FOR SONOVA....16
FIGURE 3. METABOLISM TO γ-LINOLENIC ACID (GLA) TO THE EICOSANOIDS (PROSTAGLANDINS, THROMBOXANES), 15-(S)-HYDROXY-8, 11, 13-EICOSATRIENOIC ACID (15HETRE) AND PLATELET ACTIVATING FACTOR (PAF)....23

LIST OF ABBREVIATIONS

AA	Arachidonic Acid
AI	Adequate Intake
Alb/Glob	Albumin/Globulin Ratio
ALI	Acute Lung Injury
ALS	Amyotrophic Lateral Sclerosis
ALT	Alanine Aminotransferase
AMDR	Acceptable Macronutrient Distribution Range
AP	Alkaline Phosphatase
APHIS	Animal and Plant Health Inspection Service
araP	Arabidopsis Thaliana Oleosin Promoter
araT	Arabidopsis Thaliana Oleosin Terminator
ARDS	Acute Respiratory Distress Syndrome
AST	Aspartate Aminotransferase
BEE	Basal Energy Expenditure
BMI	Body Mass Index
BQMS	Biotechnology Quality Management System
BUN	Blood Urea Nitrogen
CFU	Colony Forming Unit
DGLA	Dihomo-γ-Linolenic Acid
EDI	Estimated Daily Intake
EER	Estimated Energy Requirement Range

ELISA	Enzyme-Linked Immunosorbant Assay
EPA	Environmental Protection Agency
FDA	Food and Drug Administration
FFDCA	Federal Food, Drug, and Cosmetic Act
GLA	γ-Linolenic Acid
GRN	GRAS Notification
HACCP	Hazard Analysis and Critical Control Point
HGCO	High- γ-Linolenic Acid Canola Oil
HGSO	SONOVA Test Diet (High- γ-Linolenic Acid Safflower Oil)
HHS	Health and Human Services
IOM	Institute of Medicine
KCS	Keratoconjunctivitis Sicca
LA	Linoleic Acid
LDH	Lactate Dehydrogenase
MCH	Mean Corpuscler Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
MCV	Mean Corpuscular Volume
NAFLD	Non-Alcoholic Fatty Liver Disease
NHANES	National Health and Nutrition Examination Survey
PAL	Physical Activity Level
PAT	Phosphinothricin-N-Acetyltransferase
PMN	Polymorphonuclear Granulocyte
PT	Prothrombin Time
PUFA	Polyunsaturated Fatty Acids
RBC	Red Blood Cell
SIRS	Systemic Inflammatory Response Syndrome
SNK	Student-Newman-Keuls
T-DNA	Transfer DNA
TG	Triacylglycerol
ubiP	Ubiquitin Promoter
ubiT	Ubiquitin Terminator
UL	Tolerable Upper Intake Level
USDA	United States Department of Agriculture
USP	United States Pharmacopeial Convention
WBC	White Blood Cell

I. GRAS EXEMPTION CLAIM

A. NAME AND ADDRESS OF THE SPONSOR

Arcadia Biosciences, Inc.
200 Cousteau Place
Suite 200
Davis, CA 95618

B. COMMON OR USUAL NAME

GLA (γ-linolenic acid) safflower oil, also known as SONOVA

C. INTENDED USE

GLA safflower oil will replace the γ-linolenic acid-enriched sources currently used in nutritional beverages and medical foods intended for children (>2 years old) and adults.

The intended uses in:

- Nutritional beverages and medical foods for children (>2 years old) and adults will result in the following intakes of γ-linolenic acid from the recommended servings per day:
    - 0.3 g GLA/day from pediatric supplemental or interim sole-source nutritional beverages;
    - 0.5 g GLA /day from adult supplemental or interim sole-source nutritional beverages;
    - 2.0 g GLA /day from adult performance nutrition products;
    - 1.0 g GLA /day in adult blood glucose management products.
- Sole-source therapeutic nutrition products containing 4 g γ-linolenic acid/L and an equivalent macronutrient and micronutrient content to the product were used in the study by Gadeck et al. (1999). γ-Linolenic acid intake will be based on the caloric need for each subject, which can be calculated using the Harris-Benedict equation to account for gender, weight, height, and age. The resulting basal

metabolic rate is multiplied by a factor to account for the energy requirement of the medical condition.

D. BASIS FOR GRAS DETERMINATION

This GRAS determination for the use of SONOVA for the intended uses specified above has been shown to be safe and GRAS, using scientific procedures, under the Federal Food, Drug, and Cosmetic Act (FFDCA), as described under 21 CFR §170.30(b). The safety of the intake of SONOVA has been determined to be GRAS by demonstrating that the safety of this level of intake is generally recognized by experts qualified by both scientific training and experience to evaluate the safety of substances directly added to food, and is based on generally available and accepted information.

The proposed use of SONOVA as an ingredient for the intended uses in foods has been determined to be safe through scientific procedures set forth under 21 CFR §170.30(b) based on the following:

1. SONOVA is a refined, bleached, and deodorized oil that contains 400 mg γ-linolenic acid /g of oil and a variety of other fatty acids, tocopherols, and sterols, all of which are already present in conventional foods and edible oils.

2. The unique feature of SONOVA is that it contains high levels of γ-linolenic acid, approximately 2 times higher than those found in borage oil and significantly higher than those found in other edible foods and oils.

3. γ-Linolenic acid is a fatty acid that can either be obtained from the diet or synthesized *in vivo* via the desaturation of linoleic acid by Δ6 desaturase.

4. SONOVA is produced from a genetically engineered strain of *Carthamus tinctorius* L. cv. Centennial safflower that produces high amounts of γ-linolenic acid and may be blended with oil extracted from non-genetically engineered *C. tinctorius* oleic safflower varieties.

5. The genetically engineered strain of *C. tinctorius* is grown by Arcadia Biosciences under USDA authorization (BP_Number 12-102-107n) and in accordance with clearly defined standards for containment.

6. SONOVA is manufactured in a manner similar to those used for edible oils like safflower, sunflower, canola and corn oil. All processing aids used to produce

SONOVA are similar to those used in the production of other edible oils (GRAS Notification (GRN) 283 and 306) and comply with appropriate federal regulations. Food grade safflower oleic acid-containing oil is used for blending and standardizing the γ-linolenic acid level in SONOVA.

7. SONOVA was introduced into the United States Market by Arcadia Biosciences in 2009 as a New Dietary Ingredient (FDA-1995S-0039). Labeling guidelines instructed consumers to limit their intake of SONOVA capsules to 4 capsules/d (approximately 800 mg γ-linolenic acid), SONOVA liquid to ¼ tsp/d (~280 mg γ-linolenic acid), SONOVA-containing omega 3-6-9 blend capsules to 3 capsules/d (~600 mg γ-linolenic acid), and SONOVA-containing omega 3-6-9 blend liquids to 3 tbsp/d (~1200 mg γ-linolenic acid).

8. The total mean and 90th percentile exposures to n-6 polyunsaturated fatty acids from the diet are 14.98 and 25.96 g/day, respectively, per GRN 283, GRN 306, Meyer et al. (2003) and Astorg et al. (2004), which are consistent with the adequate intakes for n-6 polyunsaturated acids established by the Institute of Medicine (IOM).

9. The intake of γ-linolenic acid from the intended uses represents a very small contribution (less than 10%) to the total dietary exposure of n-6 polyunsaturated fatty acids.

10. Because the intended uses include nutritional and medical food products, it is unlikely that a consumer would ingest different types of SONOVA-containing products in one day.

11. The mean cumulative daily exposure to n-6 polyunsaturated fatty acids will range from 15.01 to 16.98 g from ingestion of dietary n-6 polyunsaturated fatty acids and a SONOVA-containing product delivering either 0.3 or 2.0 g/d γ-linolenic acid, respectively. The 90th percentile daily exposure will range from 26.26 to 27.96 g n-6 polyunsaturated fatty acids from ingestion of dietary n-6 polyunsaturated fatty acids and a SONOVA-containing product delivering either 0.3 or 2.0 g/d γ-linolenic acid, respectively.

12. A comparison of the AMDR upper boundaries for n-6 polyunsaturated fatty acids of up to 41 g/day and the range of cumulative daily exposure to n-6 polyunsaturated fatty acids of 15.01 to 27.96 g confirms that the contribution of γ-linolenic acid

from use of SONOVA in selected foods will not increase n-6 polyunsaturated fatty acid intake beyond a level determined to be safe by authoritative bodies.

13. Studies that evaluated the effect of γ-linolenic acid and linolenic acid on platelet aggregation and bleeding time show that:

    a. The *in vitro* platelet aggregation assays used in the studies do not accurately reflect of the effect of the diets on bleeding time;

    b. There is no dose-response relationship or time-related effect of ingesting n-6 polyunsaturated fatty acid-enriched diets on bleeding time;

    c. The weight of the evidence indicates that the ingestion of n-6 polyunsaturated fatty acids at the intended use levels will not significantly or reproducibly affect blood biochemistries, electrolytes, liver enzymes, hematologies, or coagulation indices.

14. Ten studies were reviewed that orally administered γ-linolenic acid to both healthy or health-compromised (atopic dermatitis, asthma, and atopic eczema) infants (< 12 months old) and children (8 to 26 months old) at levels ranging from 0.1 g – 3 g γ-linolenic acid/d for up to 6 months and found that the higher dose and prolonged intake of γ-linolenic acid were well tolerated and did not result in any adverse effects on measures of health status such as hematology or clinical chemistry or reported adverse events.

15. Thirty-five clinical studies orally administered γ-linolenic acid to both healthy and health-compromised adults at levels ranging from 0.03 – 6 g γ-linolenic acid/d for up to 18 months and found that prolonged intakes of these high amounts of γ-linolenic acid were also well tolerated and did not adversely affects hematology or clinical chemistry parameters.

16. Eleven clinical studies enterally administered γ-linolenic acid-enriched formulas to adults with acute lung injury, acute respiratory distress syndrome, early sepsis, amyotrophic lateral sclerosis, or who had undergone subtotal esophagostomy and total gastroectomy.

    a. Nine of these eleven clinical studies administered 0.1 – 4.6 g γ-linolenic acid/d for up to 7 days and found that the γ-linolenic acid-enriched enteral

formulas were well tolerated, did not report adverse events, and were not associated with adverse effects on hematology and clinical chemistry. In some studies, the administration of the γ-linolenic acid-enriched diets improved the clinical endpoints.

b. Two of these eleven studies that administered γ-linolenic acid-enriched enteral formulas resulted in intake levels higher than 4.6 g γ-linolenic acid/d (Rice et al., 2011; Gadek et al., 1999).

   i. Study results from the trial reported by Rice et al. (2011) could not be used to assess safety of γ-linolenic acid-enriched enteral formulas because the study was stopped early after a futility analysis. In addition, there were confounding issues due to the macronutrient differences between test and control products, and differences in the baseline demographics and clinical characteristics between patients in the control and treatment groups.

   ii. Gadek et al. (1999) administered either a control enteral diet or γ-linolenic acid-enriched enteral diet to adults with acute respiratory distress syndrome at a constant rate to achieve a minimum of basal energy expenditure x 1.3. The diets differed only in lipid composition and level of antioxidant vitamins. The γ-linolenic acid-enriched enteral diet delivered a mean of approximately 5.8 g γ-linolenic acid/d, was well tolerated, and was associated with significant decreases in the number of adverse health effects. Adverse events that were reported were unrelated to the amount of γ-linolenic acid administered.

17. Four toxicology studies using oils derived from transgenic plant sources and borage oil determined the safety of γ-linolenic acid intake. All studies administered diets that contained greater than 10% fat to rats, which is higher than the recommended amount of 5 % (National Research Council, 1982). Rats fed diets high in fats can develop non-alcoholic fatty liver disease, which is a pathological condition associated with increase blood levels of AST, ALT and insulin resistance (i.e., elevated blood glucose levels). Thus, the increases in blood glucose, AST, ALT, and LDH levels in both the On HSGO- and borage oil-treated groups likely represent a generalized effect of consuming a high fat diet rather than a specific effect of consuming high levels of a particular fatty acid such as γ-linolenic acid.

Because of this species specific response to high fat diet, clinical studies were determined to be most relevant to assess the safety of ingesting diets high in γ-linolenic acid.

18. The Estimated Daily Intake (EDI) for γ-linolenic acid from the proposed uses have been determined safe and GRAS based studies evaluating its physiological effects, and clinical studies in infants, children, and adults, which are supported by animal toxicology studies.

Determination of the GRAS status of SONOVA under the intended conditions of use has been made through the deliberations of Roger Clemens, DrPH, CNS, CFS, FACN, FIFT, A. Wallace Hayes, PhD, DABT, FATS, ERT, CNS, FACN, and Thomas Sox PhD, JD. These individuals are qualified by scientific training and experience to evaluate the safety of food and food ingredients. These experts have carefully reviewed and evaluated the publicly available information summarized in this document, including the safety of SONOVA and the human exposure to of SONOVA resulting from its intended use as an ingredient in nutritional beverages and medical foods, and concluded:

> *There is no evidence in the available information on SONOVA that demonstrates, or suggests reasonable grounds to suspect, a hazard to the public when SONOVA is used at levels that might reasonably be expected from the proposed applications of SONOVA for use in nutritional beverages and medical foods intended for adults and children (>2 years old) as proposed by Arcadia Biosciences.*

Therefore, SONOVA is safe and GRAS at the proposed levels of addition to the intended foods. SONOVA is, therefore, excluded from the definition of a food additive, and may be used in the U.S. without the promulgation of a food additive regulation by the FDA under 21 CFR.

E. AVAILABILITY OF INFORMATION

The data and information that serve as the basis for this GRAS determination will be available for review and copying at reasonable times at the office of Claire L. Kruger, Ph.D., D.A.B.T., President, Spherix Consulting, A Division of ChromaDex, Inc., at 11821 Parklawn Drive, Suite 310, Rockville, MD 20852. Telephone: 301-230-2181; Facsimile: 301-230-2188; Email: clairek@chromadex.com, or be sent to FDA upon request.

F. SIGNATURE

Pursuant to the criteria provided in proposed 21 CFR 170.36, Arcadia Biosciences hereby notifies the United States Food and Drug Administration (FDA) that the use of SONOVA in foods under the intended conditions of use is exempt from the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act, because Arcadia Biosciences has determined that such use is Generally Recognized As Safe through scientific procedures.

Signature
Keith Redenbaugh, Ph.D.
Authorized Representative of Arcadia Biosciences, Inc.

MAY 26, 2016
Date

II. DESCRIPTION OF SUBSTANCE

A. COMMON OR USUAL NAME

GLA (γ-linolenic acid) safflower oil

B. DESCRIPTION OF SONOVA

GLA safflower oil, also referred to as SONOVA® and SONOVA 400, is a refined, bleached, and deodorized oil that contains 400 mg γ-linolenic acid (18:3Δ 6, 9, 12)/g oil and a variety of other fatty acids, tocopherols, and sterols, all of which are present in other edible oils and conventional foods (Tables 1 and 2). SONOVA 400 was the subject of the New Dietary Ingredient Notification (FDA-19955-0039) filed with the FDA by Arcadia Biosciences on August 27, 2009 without comment.

Table 1. Fatty Acid Compositional Comparison of SONOVA, Conventional Foods, and Dietary Supplements

Fatty Acids		SONOVA 400[1] (average % of oil)	Foods (%)									Dietary Supplements (%)			
			Oleic Safflower Oil[2]	Linoleic Safflower Oil[2]	Chicken Thigh Meat[2]	Chicken Wing Meat[2]	Atlantic Salmon Meat[4]	Sunflower Oil[3]	Peanut Oil[3]	Corn Oil[3]	Soybean Oil[3]	Evening Primrose Oil[2]	Black Currant Oil[2]	Borage Oil[2]	Hemp Seed Oil[2]
14:0	Myristic	0.1	0.1	0.1	0.8	0.5	4.6	0.1	0.1	0.0	0.1	0.1	0.0	0.0	0.0
16:0	Palmitic	7.1	6.2	7.2	21.8	23.5	14.8	6.4	10.4	12.3	10.8	6.5	9.7	9.7	6.3
18:0	Stearic	2.1	2.0	2.1	7.1	8.9	2.8	4.5	3.0	1.9	3.9	1.9	2.3	3.9	2.8
18.1	Oleic	35.0	80.9	13.1	38.9	35.8	15.2	22.1	47.9	27.7	23.9	7.9	12.9	18.2	12.1
18:2	Linoleic	12.2	11.1	73.6	20.2	19.4	4.4	65.6	30.3	56.1	52.1	73.7	51.1	40.4	55.9
18:2	18:2Δ6, 9	0.1	0.0	0.0	0.1	0.1	-	-	-	-	-	0.0	0.0	0.0	0.1
γ18:3	γ-linolenic	41.7	0.0	0.0	0.3	0.3	-	-	-	-	-	9.1	10.5	18.4	2.8
α18:3	α-linolenic	0.2	0.0	0.1	1.0	0.8	1.4	0.5	0.4	1.0	7.8	0.2	12.9	0.3	19.7
18:4	Stearidonic	0.1	0.0	0.0	0.1	0.0	1.7	-	-	-	-	0.0	1.9	0.1	0.0
20:0	Arachidic	0.6	0.5	0.4	0.1	0.1	-	0.3	1.2	0.3	0.3	0.3	0.3	0.3	0.7
20:2	Gadoleic	0.3	0.5	0.2	0.6	0.6	8.1	0.2	1.3	0.2	0.1	0.2	1.4	5.3	0.0
22:2	Behenic	0.4	0.4	0.3	0.1	0.1	-	0.8	2.3	0.8	0.2	0.1	0.1	0.2	0.3
24:2	Lignoceric	0.2	0.0	0.2	0.0	0.2	-	0.2	1.4	0.2	-	0.0	0.0	0.0	0.0

"-" denotes "not reported"; "0.0" indicates that amount was below the detection limit.

[1]Fatty acid composition for SONOVA was determined by gas chromatography and mass spectrometry. Values represent averages of 2 pilot and 2 laboratory scale lots.

[2]Data obtained from Arcadia Biosciences analysis of chicken, commercial refined, bleached, and deodorized oil (evening primrose, black currant, borage, and safflower oils), hempseed oil from crude oil, chicken meat purchase in June 2009.

[3]Reported in Dubois et al. (2007).

[4]Reported in Bell et al. (2003).

Table 2. Tocopherol and Sterol Composition of SONOVA, Common Edible Oils, Borage Oil, and Evening Primrose Oil

Parameter (mg/g)	Edible Oils							Dietary Supplements	
	Sonova 400[1]	Oleic Safflower Oil[2]	Linoleic Safflower Oil[3]	Sunflower Oil[3, 6]	Peanut Oil[4]	Corn Oil[3,7]	Soybean Oil[3]	Evening Primrose Oil[5]	Borage Oil[5]
Total Tocopherols (α, β, δ, and γ)	0.301	0.179	0.237	0.671	0.408	1.087	1.046	0.543	3.168
Total Sterols (Stigmasterol, Campesterol, and β-sitosterol)	2.338	1.227	6.242	3.915	1.876	9.520	3.500	15.957	6.460

[1]Tocopherol and sterol concentrations in SONOVA before the addition of antioxidants as determined by the modified version of AOAC method 992.04 (Holen, 1985). Values represent averages of 2 pilot and 2 laboratory scale lots.

[2]Tocopherol levels obtained from Ortega-García et al. (2006).

[3]Tocopherol levels obtained from Zilch (2000).

[4]Tocopherol levels obtained from Padley (1994).

[5]Tocopherol levels obtained from Clough (2001).

[6]Sterol levels obtained from Weber and Mukherjee (2006).

[7]Sterol levels obtained from Kritchevsky and Bonfield (1997).

C. PRODUCTION PROCESS

SONOVA is produced by extracting oil from the seeds of *Carthamus tinctorius* L. GLA safflower, a genetically engineered strain of *C. tinctorius* L. cv. Centennial safflower that ectopically expresses *Saprolegnia diclina* Δ6 desaturase and produces high amounts of γ-linolenic acid. To achieve γ-linolenic acid levels within the product specification of 380-475 mg/g, *Carthamus tinctorius* L. GLA safflower raw seed and/or crude degummed oil may be mixed with *C. tinctorius* oleic safflower seed or refined and bleached oleic safflower oil.

1. Generation of *Carthamus tinctorius* L. GLA Safflower

The *C. tinctorius* L. GLA safflower was generated by genetically engineering *Carthamus tinctorius* L. cv. Centennial to ectopically express *Saprolegnia diclina* Δ6 desaturase and produce high amounts of γ-linolenic acid using *Agrobacterium tumefaciens*-mediated transformation of two day-old cotyledons and pSBS4119, a plasmid containing cDNA encoding *Saprolegnia diclina* Humphrey Δ6 desaturase controlled by the *Arabidopsis thaliana* oleosin promoter (araP; GeneBank Accession X62353) and terminator (araT; GenBank Accession NM_118647). pSBS4119 also contains cDNA encoding *Streptomyces viridochromogenes* phosphinothricin-N-acetyltransferase (PAT; GenBank Accession M22827) controlled by the *Petroselinum crispum* ubiquitin promoter (ubiT; GenBank Accession X64345) and the *P. crispum* ubiquitin terminator (ubiT) located between the right border and left border regions of the transfer DNA (T-DNA) region obtained from the *A. tumefaciens* plasmid pTiC58 T-DNA (GeneBank Accession AJ237588) (Figure 1).

S. diclina Δ6 desaturase converts linoleic acid to γ-linolenic acid. *S. viridochromogenes* phosphinothricin-N-acetyltransferase is a selectable marker used for the glufosinate-mediated selection of the genetically modified two day-old cotyledons that had undergone genetic recombination following the transformation of *C. tinctorius* with pSBS4119. The right and left border regions are recognized by endonucleases that mediate the transfer of DNA from pSBS4119 to the genome of *C. tinctorius* during *Agrobacterium tumefaciens*-mediated transformation. Notably, Δ6 desaturases also desaturate oleic acid (18:1Δ 9 cis) and α-linolenic acid (18:3Δ 9, 12, 15) to octadecadienoic acid (18:2Δ 6, 9) and steridonic acid (18:4Δ 6, 9, 12, 15), although not as efficiently as γ-linolenic acid (Brenner, 1974).

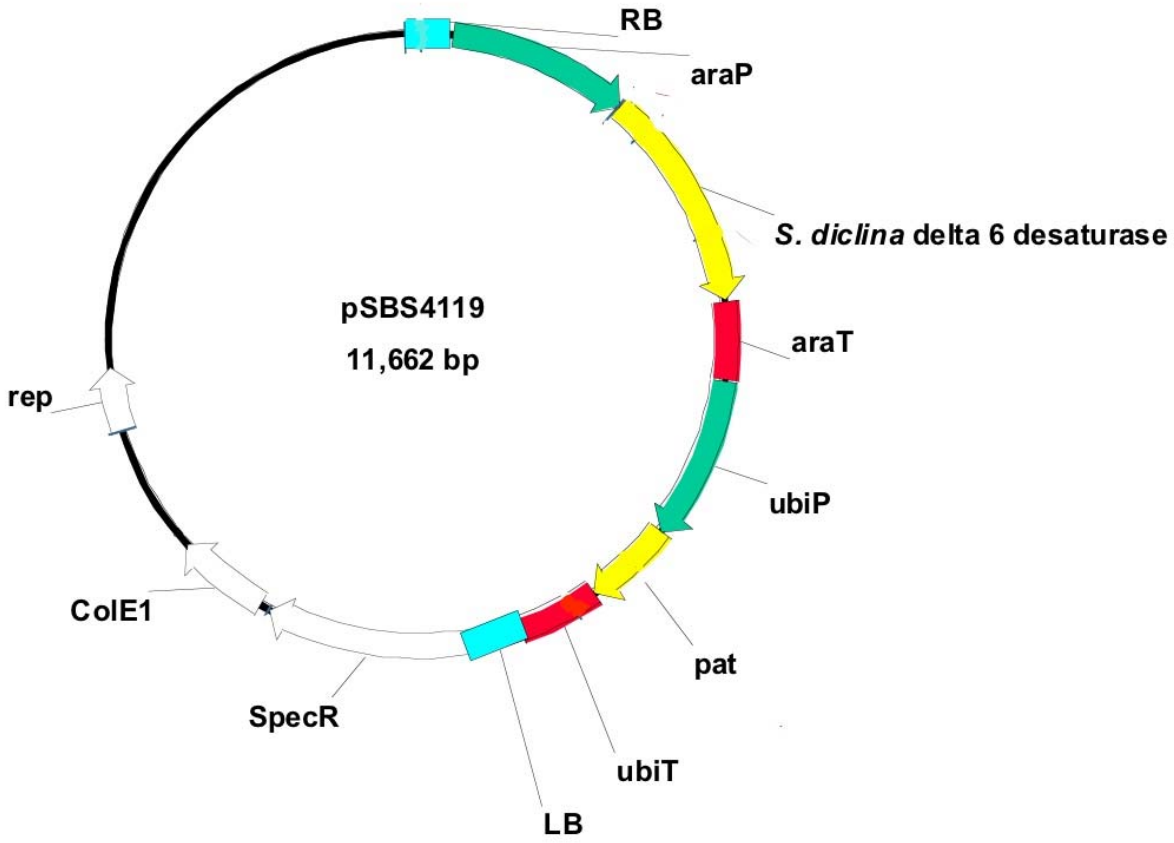

Figure 1. Plasmid Map of pSBS4119

pSBS4119 contains DNA sequences that define the extent of the T-DNA that are normally transferred into the plant genome. These are termed the right border (RB) and left border (LB) regions, and they contain sequences necessary for efficient transfer of the T-DNA into the plant cell. In pSBS4119, the RB region is located 5' to the delta 6 desaturase cassette (nucleotides 5 to 183) and the LB region is located adjacent the 3' end of the selectable marker cassette (nucleotides 5,193 to 5,503). ColE1 is the origin of replication, SpecR is the spectinomycin resistance marker, and rep is the origin of replication.

Southern blot analyses and subsequent direct sequencing confirmed that the genome of the genetically engineered *C. tinctorius* contained a single copy of the genetic material between the right and left border regions of pSBS4119 (Appendices 3 and 4). The single copy contained 41 base pairs of the right border region, intact araP, *S. declina* Δ6 desaturase, araT, ubiP and PAT cDNAs, and 423 base pairs of ubiT. There was no integration of either the left border region or the vector backbone. Subsequent functional analyses showed that the genetically engineered safflower produced high amounts of γ-linolenic acid and low amounts of linoleic acid (Nykiforuk et al., 2012).

2. Production of SONOVA

a. Cultivation of C. tinctorius GLA Safflower

C. tinctorius GLA safflower seed used for GLA safflower oil production and subsequent planting is grown on an annual basis in exactly the same manner (farming practices, equipment, and applied chemicals), in the same locations, and during the same growing season as conventional safflower grown for edible oil production. Conventional safflower requires 300 – 500 mm of moisture during the growing season and no moisture when seed is present because germination can occur. Moreover, the crop is ready for harvest approximately 45 days after full flower and requires 120 frost-free days (Smith, 1996).

The amount of *C. tinctorius* GLA safflower seed grown is based on the demands of oil production, future planting, unanticipated sales increases, crop failures, and inclement weather. To safeguard against catastrophic failure, a sufficient volume of planting seed is reserved to produce adequate quantities of oil and planting seed.

Importantly, Arcadia Biosciences grows *C. tinctorius* GLA safflower seed under strict Identity Preservation conditions and USDA authorization (BP_Number 12-102-107n). Arcadia Biosciences is also a participating member of the Animal and Plant Health Inspection Service (APHIS) Biotechnology Quality Management System (BQMS), which is an auditable quality system that provides external verification of compliance with 7 CFR 340, the *Introduction of Organisms and Products Altered or Produced Through Genetic Engineering Which Are Plant Pests or Which There is Reason to Believe Are Plant Pests*. The scope of BQMS audit includes the field release, importation, and movement of plant materials regulated by the USDA through procedures and records. Following harvest each truckload of seed is delivered to the seed handling facility at Arcadia Biosciences and sampled. All seed from a particular field or farm is pooled for analysis. Composite samples are tested for debris, moisture, oil content, and γ-linolenic acid concentration. Prior to storage, the seed is cleaned by screening out extraneous plant debris, such as stones, dirt, and sticks, and if necessary, seed moisture levels are adjusted to 8 to 10% by drying the seed. Seed earmarked for seed production is stored in nitrogen blanketed sealed containers and maintained in a warehouse under ambient storage conditions. Seed earmarked for SONOVA production is delivered to the vegetable oil processing facility.

b. Processing of C. tinctorius GLA Safflower Seed and Production of SONOVA

At the vegetable oil processing facility SONOVA is manufactured in a continuous process that occurs over days and in a manner that is similar to the production processes used for manufacturing other edible oils, such as safflower, sunflower, canola and corn oil. The different manufacturing stages such as crushing, refining, bleaching and deodorization are outlined in Figure 2.

C. tinctorius GLA safflower seed harvested from all the production fields are mixed and analyzed for γ-linolenic acid composition. If γ-linolenic acid levels are greater than 70%, *C. tinctorius* oleic safflower seed may be mixed with *C. tinctorius* GLA safflower seed to reduce γ-linolenic acid levels to less than 70%. The seeds are then cleaned with a mechanical sieve or by wind sifting, cracked with a roller mill, and crushed with an expeller press, producing "expeller oil" and an "expeller cake". The residual oil present in the expeller cake is extracted using recycled hexanes, which is removed via distillation. The extracted oil is mixed with the expeller oil, producing crude γ-linolenic acid safflower oil. Depending on the γ-linolenic acid concentration in the crude oil, the γ-linolenic acid level may be adjusted to approximately 50% with the addition of oleic safflower oil prior to refining. The crude γ-linolenic acid safflower oil is stored in a single tank (Figure 2).

During refining, crude γ-linolenic acid safflower oil is mixed with a 50% citric acid solution, heated at 60 – 65°C, agitated for 20 – 30 minutes in a process known as "degumming". The resulting water phase, which contains the phospholipids, free fatty acids, monoglycerides, and diglycerides from the oil, is decanted. The degummed oil is then neutralized with 50% sodium hydroxide, agitated and heated at 60 – 65°C until the alkaline fraction or soapstock, separates from the oil. The oil/soapstock mixture is heated to 85°C, mixed with water and centrifuged. The soapstock is then removed and the resulting oil is mixed with acid-activated clays, such as bentonite and possibly silica, heated to 100 - 120°C under a vacuum, and agitated for 20 to 30 minutes for bleaching. The oil/silica/clay mixture is vacuum-dried to remove residual moisture, filtered using silicone dioxide-imbedded filters to remove the acid activated clays and dispensed into a storage tank. The level of γ-linolenic acid is then standardized to 400 to 450 mg/g oil with the addition of food grade oleic oil extracted from *C. tinctorius* oleic safflower, which is also produced by the vegetable oil processing facility. The product is de-aerated at 85 - 90°C with a vacuum, heated to 240°C, and steam-stripped for approximately 20 minutes yielding the deodorized oil. The oil is then cooled to ambient temperature and mixed with citric acid to chelate any remaining trace metals and a mixture of antioxidants, to prevent oxidation. For storage, SONOVA is placed in air-tight polyethylene food-grade containers, which are sparged, blanketed with nitrogen, sealed, and placed at 4°C.

The vegetable oil processing facility meets the requirements of Level 2 Hazard Analysis and Critical Control Point (HACCP)-based food safety plan and utilizes segregation procedures to prevent cross contamination with other raw materials. Prior to delivery of *C. tinctorius* GLA safflower seeds to the vegetable oil processing facility, all grain-handling areas (silos, conveyors, etc.) are dry-cleaned and swept to remove all non-*C. tinctorius* materials. Ideally, SONOVA is produced immediately after the production of oleic safflower oil. If an oil or seed other than oleic safflower seed or oil was processed immediately prior to SONOVA production, the crush plant (elevators, augers, etc.), storage tanks, and refinery are cleaned and flushed with oleic safflower,

the production lines and hoses are flushed with nitrogen, and separate lines and hoses are used for crude and refined oil transfers.

Spherix Consulting reviewed the Certificates of Analysis and conditions of use for all the processing aids currently used in the production of SONOVA. Except for hexanes, all of the processing aids used in the production of SONOVA and the oleic acid extracted from *C. tinctorius* oleic safflower comply with 21 CFR. For hexanes, although there are no specific federal regulations stating that it can be used as a processing aid in the extraction of vegetable oils, GRN 306 determined it to be safe and similar to paraffinic hydrocarbons that have a long history of safe use in the production of food oils (pre-1958), GRN 94 and 326 determined it to be safe for use as an extraction solvent for edible oils used in infant formulas, and Directive 2009/32/EC establishes a maximum residue limit for hexanes in the production of fats and oils of 1 mg/kg (1 ppm) fat or oil. Importantly, vegetable oil processing facility occasionally changes their suppliers of the processing aids, and all future processing aids and their uses shall be either GRAS or comply with 21 CFR. In addition, all other manufacturers used to produce SONOVA will comply with the processing conditions described in this GRAS determination.

c. Quality Control

Quality control measures span both the agricultural and production processes. Prior to being unloaded, all oilseeds are tested for oil content, moisture, and fatty acid profile. During expelling, the oil is tested for free fatty acid content, peroxide value, fatty acid composition, refractive index, color (Gardner) and general appearance. The crude oil is analyzed for color, moisture, free fatty acids and peroxide value. Before the refining process is started, the crude *C. tinctorius* GLA safflower oil is blended down to 50% γ-linolenic acid using oleic safflower oil; γ-linolenic acid levels are confirmed by fatty acid composition analysis. During the alkali refining process, the oil is tested for free fatty acids and soaps. During the bleaching process, the oil is tested for color and fatty acid salts. During the deodorization process, the oil is tested for free fatty acids, peroxide value, color, clarity and flavor. After deodorization, the oil is blended with oleic safflower oil to meet the γ-linolenic acid specification of SONOVA and analyzed for fatty acid composition, free fatty acid content, peroxide value, moisture, color, appearance, odor, flavor, and iodine value. During the drumming process, a composite sample is taken and the same analyses performed.

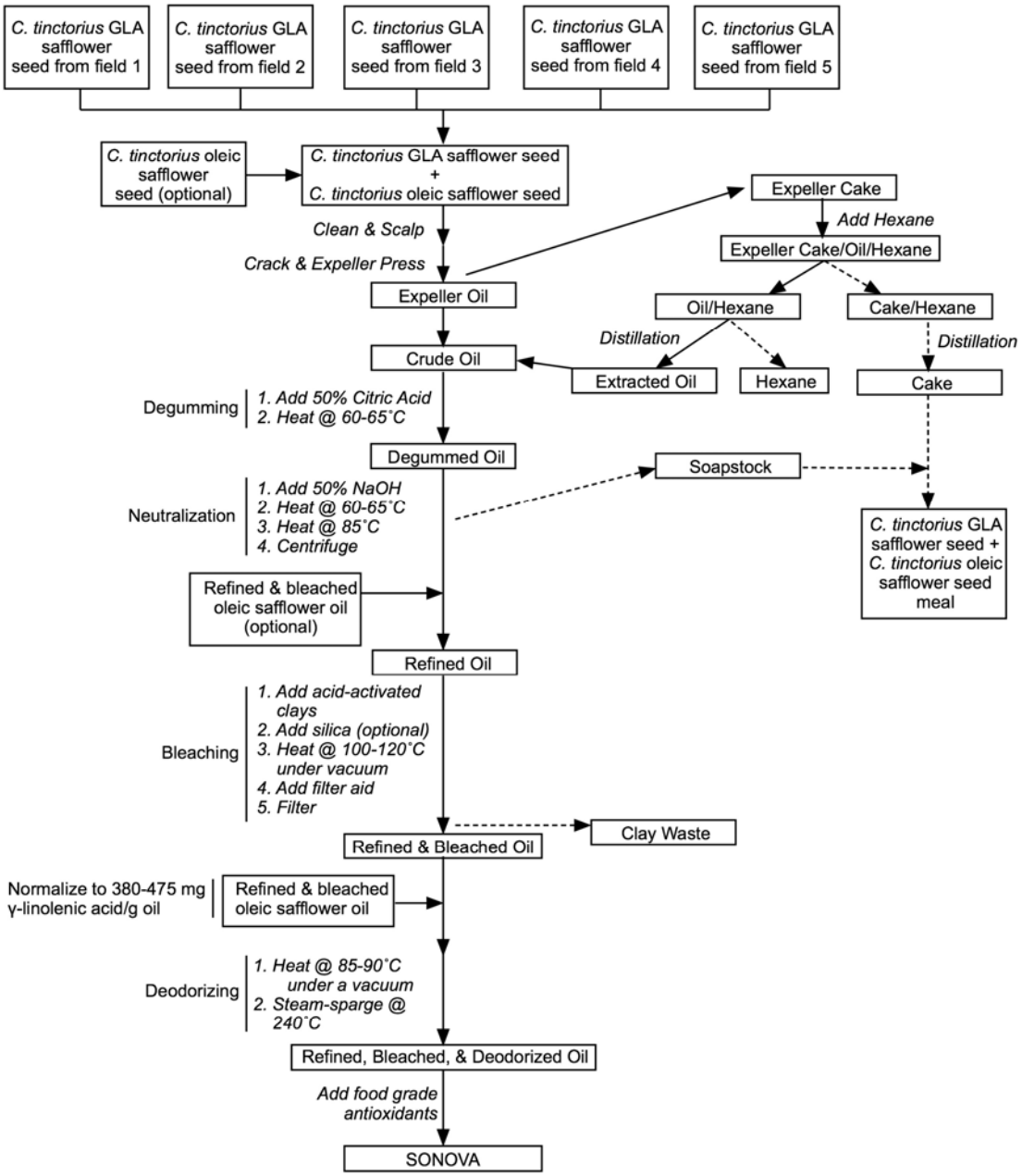

Figure 2. Production Process for SONOVA

SONOVA is produced from C. tinctorius GLA safflower seed obtained from multiple fields. To obtain a γ-linolenic acid levels within the product specification, raw seed and/or crude degummed oil may be mixed with C. tinctorius oleic safflower seed or refined and bleached oleic safflower oil. Mixing with C. tinctorius oleic safflower seed or refined and bleached oleic safflower oil does not occur when starting γ-linolenic acid levels in the γ-linolenic acid safflower seed are less than 70% or the amount of γ-linolenic acid levels in the crude degummed oil are less than 50%. Waste streams are highlighted with dashed lines.

D. FINISHED PRODUCT DESCRIPTIONS AND SPECIFICATIONS

1. Product Specifications

From crushing the seeds to packaging the oil, the production of SONOVA is a continuous process that occurs over days. All seed delivered to vegetable oil processing facility is mixed and expeller pressed. The resulting expeller oil and solvent-extracted oil are combined and stored in a single tank, resulting in a lot of crude *C. tinctorius* GLA safflower oil, which is then refined, bleached, and deodorized to produce SONOVA (also referred to as SONOVA 400). During normalization to 400 mg γ-linolenic acid/g oil with oleic safflower oil, one lot of SONOVA may be subdivided. If normalization occurs in a single tank, one third of the volume constitutes a lot of finished product. If more than one tank is required for normalization, each tank is treated as a single lot of finished product and, if necessary, subdivided to produce at least three lots of finished product. During drumming, samples are taken and analyzed to produce the certificate of analysis for each lot of finished product. Each drum is clearly labeled with the lot number. To date, three crushes have been completed yielding a total of 14 lots (Table 3). In 2012, Arcadia Biosciences separated the expeller oil from the solvent oil. Batch analyses for the 14 lots produced either from the expeller oil, solvent oil, or combination of the two demonstrates that Arcadia Biosciences and the vegetable oil processing facility have tight control over the manufacturing processes.

Table 3. Product Specifications and Batch Data for SONOVA

Parameter	Method	Spec.	Year:	2011					2012						2014			Mean +/- St. Dev.
			Lot:	1	2	3	4	5	6	7	8	9	10	11	12	13	14	
			Crude Source:	Exp. +Sol.[1]	Exp. +Sol.	Exp. +Sol.	Exp. +Sol.	Exp. +Sol.	Exp.	Sol.	Exp.	Exp.	Exp.	Exp.	Exp. +Sol.	Exp. +Sol.	Exp. +Sol.	
Fatty Acids																		
Palmitic Acid (C16:0)	AOCS Ce 1e-91	50-200 mg/g		66.34	69.33	67.33	66.88	65.54	69.70	69.80	65.80	67.90	67.30	67.30	73.90	74.00	73.70	68.92 +/- 3.01
Stearic Acid (C18:0)	AOCS Ce 1e-91	10-100 mg/g		20.56	21.06	20.46	20.41	20.36	19.30	19.80	19.30	19.70	19.70	19.40	20.90	20.90	20.90	20.20 +/- 0.66
Oleic Acid (C18:1n-9)	AOCS Ce 1e-91	100-500 mg/g		297.36	285.45	285.80	290.17	288.86	292.00	288.00	331.00	309.00	309.00	310.00	240.00	240.00	240.00	286.19 +/- 28.96
Linoleic Acid (C18:2n-6)	AOCS Ce 1e-91	100-300 mg/g		146.26	146.63	147.45	147.28	146.74	132.00	136.00	140.00	137.00	138.00	138.00	180.00	180.00	180.00	149.67 +/- 17.83
γ-Linolenic Acid (C18:3n-6)	AOCS Ce 1e-91	380-475 mg/g		435.21	442.96	445.60	438.71	441.00	443.00	451.00	383.00	422.00	421.00	432.00	464.00	464.00	465.00	439.17 +/- 22.26
Heavy Metals																		
Lead	ICP-AOCS Ca 17-01[3]	<0.2 ppm		ND[2]	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Arsenic	ICP-AOCS Ca 17-01[3]	<0.2 ppm		ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Cadmium	ICP-AOCS Ca 17-01[3]	<0.2 ppm		ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Mercury	ICP-AOCS Ca 17-01[3]	<0.2 ppm		ND	ND	ND		ND	ND		ND	ND	ND	ND	ND	ND	ND	ND
Other																		
Color	5 ¼" Lovibond	≤ 20 Red		6.1	6.1	ND 6.8	7.2	3.4	ND 10.8	20.0	7.8	8.1	7.6	8.3	8.0	7.9	7.7	8.27 +/- 3.84
Peroxide Value	AOCS Cd 8-53	≤ 10 meq/kg		1.2	1.0	1.1	1.0	1.2	1.1	2.2	1.1	1.2	1.3	1.7	1.3	1.6	1.9	1.35 +/- 0.38
Acid Value	AOCS Cd 3a-63	< 4.0 mg KOH/g		0.12	0.08	0.10	0.10	0.10	0.03	0.10	0.05	0.10	0.10	0.10	0.04	0.02	0.04	0.08 +/- 0.03
Moisture	AOCS Ca 2c-25	≤ 0.2%		0.06	0.07	0.06	0.06	0.08	0.06	0.05	0.05	0.04	0.05	0.04	0.04	0.04	0.02	0.05 +/- 0.02

[1]Exp = Expeller extracted oil; Solvent extracted oil.
[2]ND=None detected. Limit of detection = 0.1 ppm.

2. Hexanes

Hexane is the most commonly used solvent in edible oil processing (Williams, 2010). During the production of SONOVA, the hexane used to extract the residual amount of oil in the expeller cake is removed by distillation. To confirm that SONOVA does not contain hexanes or other solvents, Arcadia Biosciences analyzed four lots of SONOVA using the United States Pharmacopeial (USP) Convention method for detecting residual solvents in pharmaceutical products (USP <476>). Hexanes were undetectable at a limit of quantitation of 1 ppm, which is the maximum residue limit for hexane in fats and oils in the European Union (Directive 2009/32/EC).

3. Tocopherols

Tocopherols are fat soluble compounds produced by plants that are commonly found in vegetable-derived edible oils, and play an important role in minimizing fatty acid oxidation (Papas, 2006; Weber and Mukherjee, 2006). Because a majority of tocopherols are removed during the production process (O'Brien, 2000; Ortega-García et al., 2006), the vegetable oil processing facility supplements SONOVA with a mixture of α- and γ-tocopherols, citric acid, ascorbyl palmitate, and rosemary extract, to reduce the rate of oxidation. All the components in the mixture comply with 21 CFR 182 and 184. Although the analysis of tocopherol levels in SONOVA is not routine, an analysis of α- and γ-tocopherol levels in five samples were taken during the production of a pilot scale lot of SONOVA revealed that the average amount of α- and γ-tocopherol was 294 mg and 307 mg/kg SONOVA, respectively (Table 4).

Table 4. Tocopherol Levels in SONOVA

Tocopherol[1]	Sample Number					Average
	1	2	3	4	5	
α (mg/kg)	328	294	298	279	272	294
γ (mg/kg)	256	266	265	247	505	307

[1]Determined according to Total Tocopherol (TTLC_S:8) at Covance Laboratories.

4. Pesticides

Only safflower-specific pesticides registered by the Environmental Protection Agency (EPA), such as Dow Sonalan® HFP, Gowan Eptam® 7E, DuPont™ Harmony® SG, Syngenta Quadris® and Monsanto Roundup®, are used according to label guidance during the farming of *C. tinctorius* GLA safflower seed. Although the analysis of pesticide residues is not routine, Arcadia Biosciences has also analyzed SONOVA for the presence of pesticides not approved for use on safflower for due diligence purposes. All pesticides that were analyzed were undetectable.

5. Allergens

There are no reports of food hypersensitivity responses to safflower, safflower oils, or any of the genetic elements used to generate the genetically engineered *C. tinctorius* GLA safflower. However, SONOVA and the oleic safflower oil used to standardize γ-linolenic acid content are produced in a facility and on a manufacturing line that is used for the production of peanuts and peanut products, soybean and soybean products, sesame seed and sesame seed products, tree and tree nut products and other vegetable oils. As described in Section II.C.2.b appropriate containment and washing procedures are in place at the vegetable oil processing facility to prevent cross-contamination. Furthermore, Arcadia Biosciences determined that the protein concentration in the finished products is approximately 30 µg/kg (0.03 ppm). In addition, *S. diclina* Δ6 desaturase and *S. viridochromogenes* PAT gene products were undetectable by mass spectrometry and an enzyme-linked immunosorbant assay (ELISA; detection limit 0.5 ng), respectively. Assuming that the maximum intake of γ-linolenic acid would be approximately 2 g through the ingestion of performance nutrition products (Table 5), the resulting protein intake from SONOVA would be 0.15 µg, which is approximately 1000-fold lower that the lowest reported eliciting dose (0.1 mg) to peanut protein, one of the most potent allergens (Wensing et al., 2002).

6. Microbiologicals

Although microbiological contamination is not routinely monitored, *Escherichia coli*, *Salmonella*, total aerobes, yeast and mold colony forming units (CFUs) were determined in 5 samples harvested during a five day production run of a pilot-scale lot of SONOVA. No *E. coli*, *Salmonella*, yeast, mold, or other aerobic bacteria CFUs were detected. It is noteworthy that refining edible oils involves incubations at extreme temperatures for extended periods of time (i.e., 240°C for 20 min), thus greatly reducing the potential for microbial contamination.

7. Stability

SONOVA stability was determined when the material was stored frozen or at 23°C for 13 months. All drums were nitrogen purged, samples were taken monthly from previously unopened drums, and oil quality was evaluated using the peroxide value. When stored at both temperatures, the peroxide values were always less than the product specification limit of 10 meq/kg. Furthermore, SONOVA met all product specifications when drums were maintained at 23°C for 15 months.

III. INTENDED EFFECT

The intended effect of adding SONOVA to conventional and medical foods is to provide an enriched source of γ-linolenic acid. Although it contains high levels of γ-linolenic acid, SONOVA is composed of a variety of saturated and polyunsaturated fatty acids, sterols, and tocopherols that are present at levels equal to or less than those in other edible oils and conventional foods (Tables 1 and 2). Therefore, the absorption, distribution, metabolism, and excretion of these components, including the increased levels of γ-linolenic acid, will be the same as the absorption, distribution, metabolism, and excretion of these components when obtained from other dietary sources.

Fatty acids are a major source of fuel for the body and play integral roles in cell signaling, lipid and carbohydrate metabolism, inflammation, neurological function, and cell membrane structural integrity and dynamics (reviewed in IOM (US) Panel on Macronutrients and IOM (US) Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, 2005). Fatty acids can be either obtained from the diet or synthesized *in vivo*, and can be classified into 5 major categories: saturated fatty acids; cis-monounsaturated fatty acids; n-*6* polyunsaturated fatty acids; n-3 polyunsaturated fatty acids; and trans-fatty acids. Saturated fatty acids [caprylic acid (8:0), caproic acid (10:0), lauric acid (12:0), myristic acid (14:0), palmitic acid (16:0), stearic acid (18:0)] contain no double bonds. Cis-monounsaturated fatty acids [oleic acid (18:1), myristoleic acid (14:1), palmitoleic acid (16:1), vaccenic acid (18:1), eicosenoic acid (20:1), erucic acid (22:1)] have one double bond located 7 (n-7) or 9 (n-9) carbon atoms from the methyl tail of the fatty acid and the hydrogen atoms in double bond are present on the same side. n-*6* Polyunsaturated fatty acids [linoleic acid, γ-linolenic acid, dihomo γ-linolenic acid (C20:3Δ 8, 11, 14), arachidonic acid (C20:4Δ 4, 8, 11, 14), adrenic acid (22:4), docosapentaenoic acid (22:5)] contain more than one double bond with the most terminal bond located 6 (n-6) carbons atoms from the methyl end. n-3 Polyunsaturated fatty acids [α–linolenic acid (18:3), eicoapentanoic acid (20:5), docosapentaenoic acid (22:5), and docosahexaenoic acid (22:6)] also contain more than one double, but the most terminal double bond is located 3 carbon atoms from the methyl end. Trans unsaturated fatty acids contain at least one double bond with the hydrogen atoms present on opposite sides of the double bond.

Functionally, saturated fatty acids are used for fuel and are components of cell membranes. Monounsaturated fatty acids are components of cell membranes. Polyunsaturated fatty acids are components of cell membranes, activate signal transduction pathways, and are precursors for the production of eicosanoids and platelet activating factor (PAF), which are key modulators of inflammation, muscle tone, hemostasis, thrombosis, partuition, and gastrointestinal secretions. Trans fatty acids, in contrast, are not essential and do not provide health benefits.

Mammalian cells do not have the ability to insert a cis double bond at the *n*-6 position of a fatty acid chain. As a result, *n*-6 polyunsaturated fatty acids, specifically linoleic acid, can only be obtained from the diet. *In vivo,* linoleic acid is desaturated by Δ6 desaturase in a rate-limiting reaction to generate γ-linolenic acid (Figure 3). γ-Linolenic acid is then elongated to dihomo γ-linolenic acid (C20:3Δ 8, 11, 14) and further desaturated by Δ5 desaturase in another rate-limiting reaction to form arachidonic acid (C20:4Δ 4, 8, 11, 14), which is the primary precursor for eicosanoids and PAF (for review Smyth et al., 2009). Importantly, a diet deficient in linoleic acid result in adverse clinical symptoms such as scaly rashes and reduced growth rate (IOM (US) Panel on Macronutrients and IOM (US) Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, 2005). Moreover, reduced rates of γ-linolenic acid synthesis have been associated with aging, diabetes, alcoholism, rheumatoid arthritis, viral infections, hypercholesterolemia, and stress (reviewed in Horrobin, 1992). In contrast, diets containing high amounts of γ-linolenic acid (0.8 to 3 g/d) have been reported to improve symptoms associated with several inflammatory disorders (see Chapter 5, Section C.3; Biagi et al., 1994; Kawamura et al., 2011; Stewart et al., 1991; Schalin-Karrila et al., 1987; Wright and Burton, 1982; Belch et al., 1988; Brzeski et al., 1991; Leventhal et al., 1993; Leventhal et al., 1994; Zurier et al., 1996; Barabino et al., 2003; Ranieri et al., 2009; Jamal and Carmichael, 1990; Keen et al., 1993; Pacht et al., 2003; Gadek et al., 1999; Surette et al., 2003). Although the mechanism(s) by which this occurs is unclear, evidence in human and animal studies suggest that the ingestion of γ-linolenic acid can result in an accumulation of dihomo-γ-linolenic acid, the subsequent production of series-1 prostaglandins and 15-(S)-hydroxy-8,11,13-eicosatrienoic aid (15-HETrE), and inhibition of the inflammatory process (reviewed in Fan and Chapkin, 1998). Thus, in addition to provide an additional source of essential n-6 polyunsaturated fatty acids, the ingestion of products containing high amounts of γ-linolenic acid may increase endogenous dihomo γ-linolenic acid levels, reduce anti-inflammatory eicosanoid production, and provide some benefit to consumers with inflammatory conditions.

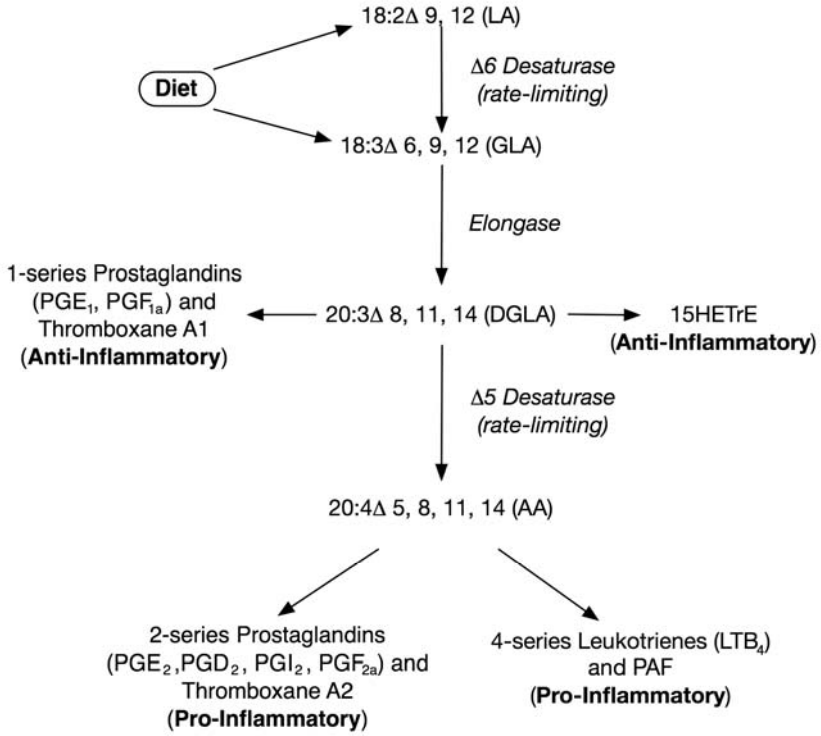

Figure 3. Metabolism to γ-linolenic Acid (GLA) to the Eicosanoids (Prostaglandins, Thromboxanes), 15-(S)-hydroxy-8, 11, 13-eicosatrienoic Acid (15HETrE) and Platelet Activating Factor (PAF)

Linoleic (LA) and GLA are obtained from the diet, but GLA can also be synthesized from LA via a rate-limiting reaction involving Δ6 desaturase. GLA is then elongated to dihomo-γ-linolenic acid (DGLA), which is desaturated to arachidonic acid (AA) via another rate limiting reaction involving Δ5 desaturase. DGLA is used to produce 1-series prostaglandins, thromboxane A1 and 15-(S)-hydroxy-8, 11, 13-eicosatrienoic acid (15HETrE), which are predominantly anti-inflammatory. Arachidonic acid is used to produce 2-series prostaglandins, thromboxane A2, 4-series leukotrienes, and platelet activating factor, which are predominantly pro-inflammatory.

Adopted from Fan et al., 1998.

IV. HISTORY OF USE, INTENDED USE, AND ESTIMATED DAILY INTAKE

A. HISTORICAL EXPOSURE TO SONOVA

SONOVA was introduced into the United States market in 2009 as a dietary supplement (FDA-1995S-0039). Labeling guidelines instructed consumers to limit their intake of SONOVA capsules to 4 capsules/d (approximately 800 mg γ-linolenic acid), SONOVA liquid to ¼ tsp/d (~280 mg γ-linolenic acid), SONOVA-containing omega 3-6-9 blend capsules to 3 capsules/d (~600 mg γ-linolenic acid), and SONOVA-containing Omega 3-6-9 blend liquids to 3 tbsp/d (~1200 mg γ-linolenic acid).

Borage seed oil contains approximately half the amount of γ-linolenic acid as SONOVA and has been commercially available as a dietary supplement since the early- to mid-1980s (Clough, 2001).

B. DIETARY INTAKES OF N-6 POLYUNSATURATED FATTY ACIDS

γ-Linolenic acid, its precursor linoleic acid, and downstream metabolites dihomo-γ-linolenic acid and arachidonic acid are n-6 polyunsaturated fatty acids. Because humans lack the ability to insert a cis double bond at the n-6 position of a fatty acid chain, linoleic acid must be obtained from the diet whereas γ-linolenic acid, dihomo-γ-linolenic acid and arachidonic acid can be either obtained from the diet or synthesized *in vivo* from linoleic acid. Linoleic, γ-linolenic acid, and arachidonic acid are found in a wide variety of foods and edible oils (Table 1), although the levels of γ-linolenic acid and arachidonic acids in foods are much lower than those for linoleic acid ((IOM (US) Panel on Macronutrients and IOM (US) Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, 2005; GRN 283; Meyer et al., 2003; Astorg et al., 2004). Sources of linoleic and γ-linolenic acid include nuts, seeds, certain vegetables, and vegetable oils such as soybean, safflower oil, and corn oil. In contrast, arachidonic acid is found in meat, poultry, and eggs.

Exposure to n-6 polyunsaturated fatty acids results from the ingestion of linoleic acid, γ-linolenic acid and arachidonic acid from foods and endogenous metabolism of linoleic acid to γ-linolenic acid and other downstream metabolites, such as dihomo-γ-linolenic acid and arachidonic acid. Per GRN 283, which provides the most recent per capita intakes for γ-linolenic acid, the mean and 90th percentile per capita intakes for γ-linolenic acid are 0.03 and 0.1 g/d, respectively. Meyer et al. (2003) and Astorg et al. (2004) reported arachidonic acid intakes are

extremely small, ranging from 0.01 and 0.2 g/day. Per GRN 306, which provides the most recent per capita intakes for linoleic acid, the mean and 90th percentile per capita intakes for linoleic acid are approximately 14.75 and 25.66 g/d. Thus, the total mean and 90th percentile exposures to n-6 polyunsaturated fatty acids from diet are 14.98 and 25.96 g/day, respectively.

C. INTENDED USES AND ESTIMATED DAILY INTAKES OF SONOVA

SONOVA will be used as substitute for other γ-linolenic-enriched sources that are currently used in nutritional beverages and medical foods. Therefore, dietary exposure to γ-linolenic from the ingestion of SONOVA-containing products will not exceed those currently resulting from the intake of nutritional beverages and medical foods enriched with other γ-linolenic-enriched sources.

Nutritional beverage and medical foods include meal replacement drinks, pediatric supplemental beverages, adult nutritional beverages, blood glucose management products, performance nutrition products, and tube-feeding or sole-source nutrition products (Table 5). The levels of addition range from 0.05 to 4 g γ-linolenic acid/serving (Table 5). Importantly, products that will be formulated with SONOVA as a source of γ-linolenic acid for use in these foods are not widely consumed by the U.S. population, have recommended directions for consumer use and some may be used as a sole source of nutrition. Assuming that these products are used as a sole source of nutrition, the resulting maximum γ-linolenic acid estimated daily intakes (EDIs) will range from 0.3 to 2 g γ-linolenic acid/d from ingested products and up to approximately 6 g/d from adult enterally-fed nutrition products.

The pediatric supplemental beverage products are intended for children 1 to 13 years of age, although they are primarily consumed by children of 3-6 years old at 1-2 servings/d (8 fl oz per serving). They may be used as a sole source of nutrition to provide approximately 946 ml/d for children of 1-8 years old and approximately 1420 ml/d for children of 9-13 years old.

The adult nutritional beverage products provide a source of nutrients to help fill nutritional gaps. It is used mostly as supplemental nutrition (1-2 servings/d), but can also be used as interim sole source nutrition, at 4-6 servings/d (providing approximately 1000-2000 kcal/d), for patients who have special nutritional needs.

Blood glucose management products are designed specifically to help adults with diabetes manage their blood glucose levels as part of an overall diabetes management plan. They are mainly used as a supplemental source of nutrition, up to 3 servings/d.

Performance nutrition products are used by fitness enthusiasts and athletes at up to 2 servings/d.

Tube-feeding or sole-source nutrition products may be high caloric (1.5 Kcal/ml) products intended for critically ill, mechanically ventilated patients, especially those with SIRS (systemic inflammatory response syndrome, e.g., sepsis, trauma, burns), ALI (acute lung injury), or ARDS (acute respiratory distress syndrome). Importantly, intake is dependent upon caloric need.

Table 5. Maximum Daily Intake of γ-Linolenic Acid (GLA) from the Intended Uses of SONOVA in Nutritional Beverages and Medical Foods****

Intended population	Product type	Intended use	Maximum amount of GLA	Recommended servings/d	GLA intake from product (g/d)
Pediatric	Supplemental or interim sole-source nutritional beverage	Nutritional beverage use	0.15 g/serving	2	0.3
		Medical food use (1 – 8 years old)	0.075 g/serving	4	0.3
		Medical food use (9 – 13 years old)	0.05 g/serving	6	0.3
Adult	Supplemental or interim sole-source nutritional beverage	Nutritional beverage use	0.25 g/serving	2	0.5
		Medical food use	0.075 g/serving	6	0. 5
	Performance nutrition product	Nutritional beverage use	1.0 g/serving	2	2.0
	Blood glucose management product	Medical food use	0.33 g/serving	3	1.0
	Tube-feeding or sole-source nutrition product for critically-ill, mechanically ventilated patients, especially those with SIRS (systemic inflammatory response syndrome, e.g., sepsis, trauma, burns), ALI (acute lung injury), or ARDS (acute respiratory distress syndrome)**	Medical food use	4 g/L	NA*	Based on caloric goal***

*NA=not applicable.
**These products contain approximately 1.5 Kcal/L and are administered according to the caloric need of the patient.
***Caloric goal for each subject can be calculated using the Harris-Benedict equation, which accounts for the gender, weight, height, and age. The resulting basal metabolic rate is multiplied by a factor that accounts for the energy requirement of the medical condition.
****SONOVA will be used as a substitute for the currently used sources of GLA in the nutritional beverages and medical foods. Therefore, GLA intake from the intended use will not exceed those that currently occur.

D. CUMULATIVE EXPOSURE TO N-6 POLYUNSATURATED FATTY ACIDS

To calculate mean and 90th percentile cumulative exposures to n-6 polyunsaturated fatty acids, the daily intake of γ-linolenic acid from the SONOVA-containing products was added to the mean and 90th percentile exposures to dietary n-6 polyunsaturated fatty acids (See Chapter IV, Section B). The mean cumulative daily exposure to n-6 polyunsaturated fatty acids will range from 15.01 to 16.98 g from ingestion of a SONOVA-containing product delivering either 0.3 or 2.0 g/d γ-linolenic acid, respectively. The 90th percentile daily exposure will range from 26.26 to 27.96 g n-6 polyunsaturated fatty acids from ingestion of a SONOVA-containing product delivering either 0.3 or 2.0 g/d γ-linolenic acid, respectively. It is important to note that the intended uses include nutritional and medical food products. Therefore, it is unlikely that a consumer would ingest different types of SONOVA-containing products in one day.

V. SAFETY OF SONOVA

The safe ingestion of γ-linolenic acid from the intended uses is supported by:

1. Adequate Intakes (AI; the recommended average daily intake levels of n-6 polyunsaturated fatty acids based on observed or experimentally determined approximations or estimates of nutrient intake by a group (or groups) of apparently, healthy people that are assumed to be adequate) and the Acceptable Macronutrient Distribution Range (AMDR; the range of intakes that are associated with reduced risk of chronic diseases while providing an adequate level of intake) for n-6 polyunsaturated fatty acids established by IOM (Table 6);

2. Calculation of per capital intakes of n-6 polyunsaturated fatty acids using the intake data from GRN 283, GRN 306, Astorg et al. 2004, and Meyer et al. (2003);

3. A wide variety of clinical studies involving healthy and health-compromised adults and children (i.e. subjects suffering for atopic dermatitis, acute lung injury, acute respiratory distress syndrome, etc.) (Tables 8, 9, and 10);

4. Four animal studies that administered diets containing up to 15% of γ-linolenic acid (Tso et al., 2012; Liu et al., 2004; Wainwright et al., 2003; Palombo et al., 2001).

5. Corroborative clinical and animal studies evaluating the safety of dihomo-γ-linolenic acid.

Although the animal studies provide data that supports the safety of consuming diets high in γ-linolenic acid, the previous GRNs, AIs, AMDRs, and clinical studies on γ-linolenic and dihomo-γ-linolenic acid provide the most relevant and compelling safety data because the diets used in the animals studies where the safety of γ-linolenic was evaluated contained 10% fat (Tso et al., 2012; Liu et al., 2004; Palombo et al., 2001; Wainwright et al., 2003), which is approximately double the amount that is typically in diets fed to laboratory rats (Johnson et al., 2008).

A. ADEQUATE INTAKES AND ACCEPTABLE MACRONUTRIENT INTAKE LEVELS FOR N-6 POLYUNSATURATED FATTY ACIDS

A Tolerable Upper Intake Level (UL), or the intake level at which adverse effects occur, for n-6 polyunsaturated fatty acids could not be set by the IOM because a defined intake level at which an adverse effect occurs has not been determined (IOM, 2005). However, IOM did establish an AMDR for n-6 polyunsaturated fatty acids based on the adverse effects of consuming a diet low or high in n-6 polyunsaturated fatty acids. According to IOM, the AMDR for n-6 polyunsaturated fatty acids is estimated to be 5 to 10% of total energy intake (Table 6). The minimum intake of 5 % of energy through the ingestion of linoleic acid would be needed to meet the AI for n-6 polyunsaturated fatty acids whereas the upper boundary of 10% is based on three factors: 1) individual dietary intakes in the North American population rarely exceeding 10% of energy; 2) epidemiological evidence for the safety of intakes greater than 10% of energy is generally lacking; and 3) high intakes of linoleic acid create a pro-oxidant state that may predispose consumers to severe chronic diseases such as coronary heart disease and cancer. The AMDRs for n-6 polyunsaturated fatty acids for males and females 1-year old are approximately 4 – 9 g/d (Table 6). For males and females older than 1-year old, the lower boundaries increase to approximately 11 g/d and the upper boundaries increase to 41 g/d (Table 6). In addition, the AMDRs for males are generally higher than those for females after 8 years-of-age. For reference, the AIs for linoleic acid range from 7 – 10 g/d for children ages 1 – 8 years old, 12 – 16 g/d for boys 9 – 18 years old, 10 – 11 g/d for girls 9 – 18 years old, 17 – 14 g/d for men 19 years and older, and 12 – 11 g/d for women 19 years and older.

Importantly, a comparison of the AMDR upper boundaries for n-6 polyunsaturated fatty acids of up to 41 g/day (Table 6) and the range of cumulative daily exposure to n-6 polyunsaturated fatty acids of 15.01 to 27.96 g confirms that the contribution of γ-linolenic acid from use of SONOVA in selected foods will not increase n-6 polyunsaturated fatty acid intake beyond a level determined to be safe by authoritative bodies.

Table 6. Acceptable Macronutrient Distribution Range (AMDR) for N-6 Polyunsaturated Fatty Acids in Children and Adults

Population	Age (years)	EER Range[a] (kcal/d)	AMDR for N-6 Polyunsaturated Fatty Acids (g/d)	
			Lower boundary	Upper Boundary
Females	1	768	4.3[b]	8.5[c]
	8	1360 - 2173	7.5[d]	24.1[e]
	14	1718 - 2831	9.5[d]	31.5[e]
	Adults (BMI[a]=18.5 kg/m^2; height =67 inches)	1881 - 2662	10.5[f]	29.6[g]
	Adults (BMI of 24.99 kg/m^2; height =67 inches)	2057 - 2916	11.4[f]	32.4[g]
Males	1	844	4.7[b]	9.4[c]
	8	1453 - 2225	8.0[d]	24.7[e]
	14	2090 - 3283	11.6[d]	36.5[e]
	Adults (BMI[a]=18.5 kg/m^2; height =71 inches)	2301 - 3225	12.8[f]	35.8[g]
	Adults (BMI of 24.99 kg/m^2; height =71 inches)	2636 - 3720	14.6[f]	41.3[g]

All data except AMDRs were obtained from IOM (US) Panel on Macronutrients and IOM (US) Standing Committee on the Scientific Evaluation of Dietary Reference Intakes (2005).

[a] EER = Estimated Energy Requirement Range for the indicated sex and age group; BMI = Body Mass Index.

[b]Calculated by multiplying the EER of girls and boys 1 year old by the minimum percentage of dietary fat recommended by IOM (5%) and the caloric density of fat (9 kcal/g).

[c]Calculated by multiplying the EER of girls and boys 1 year old by the maximum percentage of dietary fat recommended by IOM (10%) and the caloric density of fat (9 kcal/g).

[d]Calculated by multiplying the EER of sedentary (Physical Activity Level (PAL) $\geq 1.0 < 1.4$) girls and boys 8 and 14 years old by the minimum percentage of dietary fat recommended by IOM (5%) and the caloric density of fat (9 kcal/g).

[e]Calculated by multiplying the EER of very active (PAL $\geq 1.9 < 2.5$) girls and boys 8 and 14 years old by the maximum percentage of dietary fat recommended by IOM (10%) and the caloric density of fat (9 kcal/g).

[f]Calculated by multiplying the EER of sedentary (Physical Activity Level (PAL) $\geq 1.0 < 1.4$) women and men, at the indicated BMI and height by the minimum percentage of dietary fat recommended by IOM (5%) and the caloric density of fat (9 kcal/g).

[g]Calculated by multiplying the EER of very active (PAL $\geq 1.9 < 2.5$) women and men, at the indicated BMI and height by the maximum percentage of dietary fat recommended by IOM (10%) and the caloric density of fat (9 kcal/g). AMDRs for women and men with heights and BMIs greater or smaller than those shown will be larger or smaller, respectively.

B. PER CAPITA INTAKES OF N-6 POLYUNSATURATED FATTY ACIDS

In GRN 286, Monsanto Company determined that the mean and 90th percentile per capita intake of γ-linolenic acid are 0.03 and 0.1 g/d, respectively using the 1999-2002 National Health and Nutrition Examination Surveys (NHANES). In GRN 306, Monsanto Company determined that mean and 90th percentile per capita intake of linoleic acid are 14.75 and 25.66 g/d using the 2003-2006 NHANES. Meyer et al. (2003) and Astorg et al. (2004) reported arachidonic acid intakes are extremely small, ranging from 0.01 and 0.2 g/day. Therefore, the total mean and 90th percentile per capita intake of n-6 polyunsaturated fatty acid are 14.98 and 25.96 g/day. Importantly, the intake of γ-linolenic acid from the intended uses represents a very small contribution (less than 10%) to the total dietary exposure of n-6 polyunsaturated fatty acids.

C. HUMAN STUDIES

To evaluate the safety of consuming products containing high levels of γ-linolenic acid, publicly available clinical studies where γ-linolenic and dihomo-γ-linolenic acid, the direct metabolite γ-linolenic acid, were administered to health and health-compromised subjects were retrieved, critically reviewed, and evaluated against the AMDRs set by IOM in 2005. Although the majority of these studies were not designed to directly evaluate the safety of γ-linolenic or dihomo-γ-linolenic acid and only a few evaluated the effects on hematology and clinical chemistry parameters, the weight of the evidence shows that prolonged intakes of high amounts of γ-linolenic and dihomo-γ-linolenic acid are well tolerated and do not result in adverse effects on measures of health status, such as hematology or clinical chemistries.

1. Orally Administered Products Containing γ-Linolenic Acid

Ten studies orally administered products containing γ-linolenic acid to healthy or health-compromised [atopic dermatitis (n=7), asthma (n=1), and atopic eczema (n=2)] infants and children at levels ranging from 0.1 g – 3 g γ-linolenic acid/d for up to 6 months (Table 7). The highest dose of 3 g γ-linolenic acid/d was administered for 4 weeks to children with atopic dermatitis who were 8 – 26 months old. In general, prolonged intakes of high amounts of γ-linolenic acid were well tolerated.

Thirty-five studies orally administered γ-linolenic acid to either healthy (n=6) and health-compromised adults such as those with hyperlipidemia (n=1), eczema (n=5), atopic dermatitis

(n=5), transitional cell carcinoma (n=1), rheumatoid arthritis (n=7), diabetic neuropathy (n=2), osteoporosis (n=1), periodontitis (n=1), compressive root syndrome (n=1), ulcerative colitis (n=1), metastatic breast cancer (n=1), asthma (n=2) and aqueous-deficient keratoconjunctivitis (n=1) at levels ranging from 0.03 – 6 g γ-linolenic acid/day for up to 18 months (Table 8). The highest dose of 6 g/day was administered to healthy adults for 21 days.

Ten studies evaluated clinical parameters, including blood biochemistries, electrolytes, liver enzymes, hematologies, and coagulation indices (Covar et al., 2010; Hederos et al., 1996; Surrette et al., 2003; Surrett et al., 2008; Rilliet et al., 1988; Keen et al., 1993; Kruger et al., 1998; Guivernau et al., 1994; Gadek et a., 1999; Jacobs et al., 2013). Three studies were conducted in children who received up to 3 g γ-linolenic acid/day for up to 16 weeks from γ-linolenic acid-enriched sources (Covar et al., 2010 and Hederos et al., 1996; Jacobs et al., 2013). One of the three studies in children was in critically-ill, mechanically-ventilated children receiving sole source enteral nutrition that were diagnosed with either acute lung injury or acute respiratory distress syndrome (Jacobs et al., 2013). The remaining eight studies were conducted in adults who received up to 5.8 g γ-linolenic acid/day for up to 18 months from γ-linolenic acid-enriched sources (Surrette et al., 2003; Surrett et al., 2008; Rilliet et al., 1988; Keen et al., 1993; Kruger et al., 1998; Guivernau et al., 1994; Gadek et a., 1999; Jacobs et al., 2013). One of the eight adult studies was conducted in adults receiving sole source enteral nutrition that were diagnosed with acute respiratory distress syndrome (Gadek et al., 2013).

It is noteworthy that Guivernau et al. (1994) reported that the ingestion of evening primrose oil by hyperlipidemic men, which resulted in an intake of approximately 0.24 g γ-linolenic acid/d, significantly decreased serum triglycerides and cholesterol, increased HDL-cholesterol, reduced platelet aggregation and function, and increased bleeding time. Currently, reductions in serum triglycerides and cholesterol, and increases in HDL-cholesterol are widely accepted as beneficial effects, whereas reductions in platelet aggregation and function, and increases in bleeding time are considered to be adverse effects that may pose risks to consumers. Considering that up to 100% of ingested linoleic acid is metabolized γ-linolenic acid, it is therefore reasonable to draw upon the publicly available literature on linoleic acid to evaluate the safety of n-6 polyunsaturated fatty acids on platelet aggregation and bleeding time.

Seven studies investigated the effect of n-6 polyunsaturated fatty acids, notably linoleic acid on platelet aggregation (Hornstra et al., 1973; Fleischman et al., 1975; Fleischman et al., 1974; O'Brien et al., 1976; Challen et al., 1983; Vericel et al., 1986; Boberg et al., 1986). Four of these studies also evaluated bleeding time (Hornstra et al., 1972; O'Brien et al., 1976; Challen et al., 1983; Vericel et al., 1986). In seven of the eight studies, n-6 polyunsaturated fatty acids were

delivered via the ingestion of controlled diets for period up to 6 years. Boberg et al. (1986) administered the highest amount of linoleic acid (approximately 45 g/d) for 1 week whereas, Hornstra et al. (1973) administered the highest amount of n-6 polyunsaturated fatty acids (approx. 33 g/d) for the longest period of time (approx. 6 years). Importantly, although a majority of the studies reported that the linoleic acid-enriched diets reduced platelet aggregation *in vitro* (Hornstra et al., 1973; Fleischman et al., 1975; Fleischman et al., 1974; O'Brien et al., 1976; Challen et al., 1983; Vericel et al., 1986), there was no consistent effect on bleeding time *in vivo* (Hornstra et al., 1973; O'Brien et al., 1976; Challen et al., 1983; Vericel et al., 1986). Specifically, Hornstra et al. (1973), Challen et al. (1983), and Vericel et al. (1986) found that diets containing upwards of 52 g linoleic acid/d for up to 6 years had no effect on bleeding time despite reduced platelet aggregation whereas O'Brien et al. (1976) found that the administration of a diet containing approximately 19 g linoleic acid/day for 5 weeks increased both platelet aggregation and bleeding time.

Taken together, the studies evaluating the effect of γ-linolenic acid and linoleic acid on platelet aggregation and bleeding time collectively show that: 1) the *in vitro* platelet aggregation assays used in the studies do not accurately reflect of the effect of the diets on bleeding time *in vivo*; 2) there is no dose-response relationship or time-related effect of ingesting the n-6 polyunsaturated fatty acid-enriched diets on bleeding time; and 3) the weight of the evidence indicates that the ingestion of n-6 polyunsaturated fatty acids at the intended use levels will not significantly or reproducibly affect blood biochemistries, electrolytes, liver enzymes, hematologies, or coagulation indices.

2. Enterally Administered Products Containing γ-Linolenic Acid

Eleven studies administered γ-linolenic acid-enriched enteral diets to subjects with acute lung injury, acute respiratory distress syndrome, early sepsis, amyotrophic lateral sclerosis (ALS) or who had undergone subtotal oesophagectomy and total gastroectomy (Table 9).

The level of γ-linolenic acid administered and duration of administration in nine of these studies ranged from 0.1 – 4.6 g /day and for up to 7 days (Pontes-Arruda et al., 2006; Pontes-Arruda et al., 2011; Singer et al., 2006; Jacobs et al., 2013; Grau-Carmona et al., 2011; Sultan et al., 2012, Wills et al., 2014, Kushta et al., 2011; Pacht et al., 2003). Compared to the control formulas, the γ-linolenic acid-enriched formulas were well-tolerated, did not produce adverse events, and were not associated with adverse effects on hematology or clinical chemistries. In some studies, the administration of the γ-linolenic acid-enriched diets improved the clinical endpoints, such as survival rate, gas exchange, ventilator-free days, and organ dysfunction.

The remaining two studies administered γ-linolenic acid-enriched enteral diets at levels higher than 4.6 g γ-linolenic acid/day (Gadek et al., 1999; Rice et al., 2011). Gadek et al. (1999) administered enteral diet to adults with acute respiratory distress at a constant rate to achieve a minimum of 50% of basal energy expenditure (BEE), calculated using the Harris-Benedict equation, x 1.3 within the first 24 hr. Continuous enteral nutrition was then advanced as tolerated with the goal of achieving a minimum of 75% of BEE x 1.3 within 72 hours of initiation of enteral feeding. Enteral nutrition was delivered continuously for a minimum of four study days ± 1 day at a rate not to exceed BEE x 1.3. Daily enteral intake was recorded for total volume and calories delivered to the patient. The average intake of γ-linolenic acid was 5.8 +/- 0.3 g/day. Consistent with the results of the other studies (Pontes-Arruda et al., 2006; Singer et al., 2006), γ-linolenic acid-enriched enteral diets were well tolerated and associated with statistically significant decreases in the number of adverse health effects. Adverse events that were reported were unrelated to the amount of GLA administered. In contrast, Rice et al. (2011) administered an enteral diet containing high amounts of γ-linolenic acid to adults with developing acute lung injury in twice daily bolus doses for 3 weeks resulting in a γ-linolenic acid intake of 5.9 g/day. Compared to the control group, which received a high carbohydrate/high protein enteral diet, administration of the γ-linolenic acid-enriched diet was associated with significant increases in mortality and diarrhea, and the study was terminated after the first interim analysis because the primary end point (ventilator free days) and the major secondary end point (mortality) crossed the predefined futility boundaries. Notably, the statistically significant effect on mortality, which was identified in the futility analysis, could not be confidently attributed to the γ-linolenic acid - enriched formula.

Other differences in the Gadek et al. (1999) and Rice et al. (2011) studies may have also accounted for the disparity in the results such as the types of formula used and the age of the subjects. Although the formulas used by Rice et al. were isocaloric, the γ-linolenic acid-enriched formula contained 5-times less protein, 12-times less carbohydrate, and higher amounts of vitamin C, vitamin E, zinc, selenium, L-carnitine, and taurine than the control formula. In a study conducted by Gadek et al., the test and control formulas were similar in protein, carbohydrate, and lipid content and the only differences were in the amount of γ-linolenic acid, vitamin C, vitamin E, β-carotine, L-carnitine, and taurine. In addition, there was a slight imbalance in age, APACHE (Acute Physiology, Age, Chronic Health Evaluation) III score, Pa_{O2}:FI_{O2} ratio, and minute ventilation in the study conducted by Rice et al., which may have favored the control group. Thus, due to confounding factors in the design of the Rice et al. study, it is not possible to determine the safety of administration of γ-linolenic acid enriched enteral diets to critically-ill patients when given a bolus administration of the enteral formula containing GLA used in that

study. However, continuous administration of enteral formula containing 4 g/liter of GLA in critically-ill patients demonstrated safety and tolerance (Gadek et al., 1999, Wills et al., 2014; Sultan et al., 2012; Kushta et al., 2011).

It is also noteworthy that seven of the 11 studies that administered γ-linolenic acid-enriched enteral diets to humans, used a γ-linolenic acid-containing enteral diet by Abbott Laboratories (Pontes-Arruda et al., 2011; Singer et al., 2006; Jacobs et al., 2013; Grau-Carmona et al., 2011; Gadek et al., 1999, Sultan et al., 2012, Wills et al., 2014, Kushta et al., 2011). This diet has been distributed in the United States for more than 10 years and approximately 511,000 L were distributed from April 1, 2012 to March 31, 2014. During this time the adverse event reports were primarily related to gastrointestinal symptoms that are within the expected safety profile of this product when fed to the intended population according to directions provided on the label or as instructed by a health care professional. Further analysis of these and all other adverse events do not suggest atypical trends in the frequency and/or severity of the adverse events reported. Based on the adverse event reports received, when aligned with patient exposure, the likelihood of a patient experiencing any specific adverse event coincident with the use of γ-linolenic acid-enriched enteral diets is unlikely (i.e. <1 report/100,000 L distributed).

3. γ-Linolenic Acid and Inflammation

The effects of diets containing γ-linolenic acid on inflammation have been investigated in a wide variety of clinical studies involving healthy and health-compromised subjects.

In healthy subjects, three studies have shown that the ingestion of γ-linolenic acid at levels ranging from 0.8 g/day to 3.0 g/d for periods ranging from 3 to 4 weeks (Table 7; Johnson et al., 1997; Chilton et al., 2008; Weaver et al., 2009) can attenuate inflammatory metabolites. Johnson et al. (1997) orally administered 3.0 g γ-linolenic acid/day for 3 weeks to healthy adults and evaluated its effect on serum and neutrophil membrane γ-linolenic acid, dihomo-γ-linolenic acid, and arachidonic acid levels. Circulating neutrophils were also harvested from the subjects before and after the dihomo-γ-linolenic acid treatment. The production of the proinflammatory leukotriene, LTB_4, and PAF by the cells was determined following their stimulation with the calcium ionophore A23187. Although γ-linolenic acid administration led to significant, dose-dependent increases in arachidonic, dihomo-γ-linolenic, and γ-linolenic acid serum levels relative to baseline, it significantly and dose-dependently increased only dihomo-γ-linolenic acid levels in neutrophil membranes. *Ex vivo* stimulation of the neutrophils showed that the γ-linolenic acid administration reduced LTB_4 and PAF production. Chilton et al. (2008) administered 1.5 g γ-linolenic acid/day in diets supplemented with borage oil for 3 weeks. Plasma fatty acids and *ex*

vivo stimulated whole-blood LTB_4 production were measured in whole blood samples collected at baseline, weekly for 3 weeks and after a 2-week washout period. γ-Linolenic acid supplementation resulted in increased circulating plasma arachidonic acid concentrations, but because dihomo-γ-linolenic acid is efficiently incorporated into the polymorphonuclear granulocyte (PMN) lipids and PMNs lack Δ5 desaturase activity, the PMN content of arachidonic acid did not increase. Furthermore, *ex vivo* stimulation of whole blood leukocytes with the calcium ionophore, A23187, revealed that 2 weeks of γ-linolenic acid supplementation significantly decreased LTB_4 production, which returned to baseline levels after the 2-week washout period. Weaver et al. (2009) obtained similar results administering 0.8 g γ-linolenic acid/day for 4 weeks.

To evaluate the effect of ingesting γ-linolenic acid on inflammation, formulations containing γ-linolenic acid alone or in combination with other polyunsaturated fatty acids, such as eicosapentanoic acid, have been administered to subjects with inflammatory conditions such as atopic dermatitis and eczema, lung injury, respiratory distress syndrome, rheumatoid arthritis, aqueous-deficient keratoconjunctivitis sicca (KCS; dry eye syndrome), and cancer (Tables 8 and 9). Although the effects are not entirely reproducible, the evidence suggests that consuming a diet containing γ-linolenic acid may be beneficial.

Atopic dermatitis is associated with a number of abnormalities of non-immunological nature, including changes in the fatty acid composition of blood lipids, particularly an increase in linoleic acid and a decrease in arachidonic acid and other linolenic metabolites (Manku et al., 1984; Biagi et al., 1994; van Gool et al., 2003). The increase in linolenic acid and decrease in arachidonic acid suggest that the Δ6 desaturase may be defective in subjects with atopic dermatitis, and result in a decrease in the amount γ-linolenic acid and an imbalance in prostaglandin production. Other groups have speculated that administering high amounts of γ-linolenic acid may improve inflammatory skin disease by increasing the relative amount of non-inflammatory prostaglandins and leukotrienes (Berth-Jones and Graham-Brown, 1993; Hederos and Berg, 1996). Five studies have reported that direct administration of γ-linolenic acid (from evening primrose oil or γ-linolenic acid-enriched oil extracted from the fungus *Mucor circinelloides*) led to an improvement in the symptoms associated with atopic dermatitis (Table 7 and 9; Biagi et al., 1994; Kawamura et al., 2011; Stewart et al., 1991; Schalin-Karrila et al., 1987; Wright and Burton, 1982). In contrast, a meta-analysis of nineteen placebo-controlled trials of γ-linolenic acid from borage oil, evening primrose oil and blackcurrant seed oil and five trials of fish oil did not confirm that supplementation with γ-linolenic acid exerts a large effect

on reducing severity of atopic dermatitis (van Gool et al., 2004), although the study had a low power to detect a moderate effect of supplementation with γ-linolenic acid on atopic dermatitis.

γ-Linolenic acid administration has also been evaluated in subjects with rheumatoid arthritis (Table 8; Belch et al., 1988; Brzeski et al., 1991; Leventhal et al., 1993; Leventhal et al., 1994; Zurier et al., 1996). In five studies, the administration of formulations containing γ-linolenic acid ranging from 0.54 g to 2.8 g/d resulted in significantly and clinically relevant reductions in the signs and symptoms of disease activity in patients with rheumatoid arthritis were noted (Belch et al., 1988; Brzeski et al., 1991; Leventhal et al., 1993; Leventhal et al., 1994; Zurier et al., 1996). In contrast, two studies that administered 0.36 g and 1 g γ-linolenic acid/d reported no improvements in the signs and symptoms of disease (Hansen et al., 1983; Pullman-Mooar et al., 1990).

Barabino et al. (2003) administered capsules delivering approximately 30 mg γ-linolenic acid/d to subjects with aqueous-deficient keratoconjunctivitis sicca (dry eye syndrome) and reported that the γ-linolenic acid-containing formulation reduced ocular surface inflammation and improved the associated symptoms (Table 9).

Ranieri et al., 2009 administered a combination of 0.36 g γ-linolenic acid and 0.6 g α-lipoic acid/d to patients with compressive radiculopathy syndrome undergoing rehabilitation for 6 weeks. Compared to a group that received the rehabilitation program alone, the linolenic acid α-lipoic acid combination improved the associated neuropathic symptoms of paresthesia, stabbing and back pain, possibly through the modulation of prostaglandins (Table 8).

Two studies conducted in patients with diabetic neuropathy showed that the administration of formulation containing 0.3 and 0.48 g γ-linolenic acid/d significantly improved a variety of symptoms, such as neuropathy symptom scores, median nerve motor conduction velocity and compound muscle action potential amplitude, peroneal nerve motor conduction velocity and compound muscle action potential amplitude, median and sural sensory nerve action potential amplitude and ankle heat and cold threshold values (Table 8; Jamal and Carmichael, 1990; Keen et al., 1993).

One study found that a borage seed oil delivering approximately 1.4 g γ-linolenic acid/d to patients with ulcerative colitis provided no benefit (Table 8; Middleton et al., 2002).

Three studies evaluated the effect of γ-linolenic and eicosapentanoic acid-enriched formulations in modulating the inflammatory responses in subjects with acute lung injury (Table 9; Rice et al., 2011; Pacht et al., 2003; Gadek et al., 1999). As discussed above, Rice et al. was

prematurely terminated because the primary and major secondary endpoints crossed predefined futility boundaries. In the remaining two studies, Pacht et al. (2003) and Gadek et al. (1999) both found that the ingestion of the γ-linolenic and eicosapentanoic acid-containing formulations significantly increased arterial oxygenation and reduced the number of neutrophils in the bronchoalveolar lavage fluid.

Two studies evaluated the effects of a γ-linolenic acid-containing formulation in the dietary management of asthmatic subjects (Table 8). One study found that the administration of 0.75 and 1.13 g γ-linolenic acid/d to asthmatic patients reduced zymosan-induced LTB_4 production from ex vivo neutrophils (Surette et al., 2003). The other found that formulation delivering 0.75 g γ-linolenic acid/d improved quality of life measurements, determined using validated asthma questionnaires, and reduced bronchodilator use (Surette et al., 2008).

4. Orally Administered Products Containing Dihomo-γ-Linolenic Acid

Two studies orally administered products containing dihomo-γ-linoleic acid to healthy adults (Teraoka et al., 2009; Tanaka et al., 2012).

In a randomized, double-blind, placebo-controlled study, Tanaka et al. (2012) evaluated the safety and tolerability of ingesting dihomo-γ-linoleic acid by administering either a dihomo-γ-linoleic acid-enriched oil, delivering approximately 450 mg dihomo-γ-linoleic acid, or placebo (olive oil) to healthy males and females for 4 weeks, followed by a 4-week washout period. Eleven subjects received dihomo-γ-linolenic acid and ten subjects received placebo. Only one subject in the dihomo-γ-linolenic acid-treated group withdrew due to having a cold. There were no side effects and all adverse events observed were considered unrelated to supplementation. After 4 weeks of supplementation, significant increases in dihomo-γ-linolenic and arachidonic acid levels serum phospholipids were observed in the dihomo-γ-linoleic acid-treated group, both of which returned to baseline levels after the 4-week washout. Importantly, although significant differences in the levels of lactate dehydrogenase, albumin, total cholesterol, the number of red blood cells, hematocrits relative to their respective levels at baseline occurred in both dihomo-γ-linoleic acid- and placebo-treated groups, all values were with the normal ranges for the enrolled subjects. Moreover, diastolic and systolic blood pressures, coagulation (prothrombin time, activated partial thromboplastin time and antithrombin III) and urinalysis parameters were all within the normal ranges throughout the study and did not significantly different between the two groups.

To evaluate the effects of dietary dihomo-γ-linolenic acid on physical characteristics, blood and urine chemistries, platelet aggregation, and serum phospholipids, Teraoka et al. (2009)

administered either placebo (olive oil; n=10) or a dihomo-γ-linoleic acid-enriched oil, delivering 50 (n=10) and 150 mg dihomoγ-linolenic acid/day (n=10) to healthy men for 4 weeks followed by a 4 week washout period in a randomized, double-blind, placebo-controlled study. One subject in the 50 mg-treated group withdrew due to personal reasons. No side effects were reported and all adverse events that occurred were considered unrelated to ingestion of the dihomo-γ-linoleic acid-enriched oil. After 4 weeks, dihomo-γ-linoleic acid levels increased in a dose-dependent fashion in the serum phospholipids in the dihomo-γ-linoleic acid-treated subjects, and the increase was significant ($p<0.05$) in 150 mg/day-treated group compared to the placebo-treated group. After the 4-week washout period, the significant increase in serum dihomo-γ-linoleic acid levels in 150 mg/day-treated group returned to baseline. There were no significant effects of dihomo-γ-linoleic acid supplementation on platelet aggregation and, although significant differences in serum albumin in the low-dose group compared to the placebo group, creatinine in the high dose group relative to baseline, and prothrombin time in the high-dose group compared to the placebo group were reported, all effects on blood and urine chemistries were not dose-dependent and within the normal range for the males enrolled in the study.

Table 7. Clinical Trials of γ-linolenic Acid (GLA)-enriched Oils in Infants and Children

Reference	Study Design and Population	Treatments and Number of Subjects	Duration	Safety Outcomes
Fiocchi et al., 1994	Open-label, uncontrolled Children between 8-26 mo with atopic dermatitis	3 g GLA/d; n=10 (5M/5F)	4 wk	Relative to baseline, GLA supplementation significantly increased percent of CD8+ lymphocytes at 4 wk. Relative to baseline, GLA supplementation had no effect on IgE levels. Relative to baseline, GLA supplementation significantly reduced the presence, intensity and site of itching at 4 wk.
Covar et al., 2010	Randomized, double-blind, placebo-controlled Children, aged 6-14 years old, with mild to moderate persistent asthma. Of 43 randomized, 19 receiving test formula (NNF) and 18 receiving control formula completed the study.	Group 1: Control, given complete ready-to-drink nutritional formula with an oil blend (100% high-oleic safflower oil); n=18 Group 2: NNF, complete ready-to-drink nutritional formula with a blend of oils (fish oil and borage oil), and a mix of antioxidant vitamins and minerals. Equivalent to 3 g GLA/d; n=19 Of those who did not complete: in the NNF group, four withdrew, one was due to hospitalization for asthma immediately after randomization, one disliked the taste of formula, one due to physician discretion and one withdrew consent. In the control group, two withdrew, one due to formula taste intolerance and one due to elevated serum potassium.	12 wk	At baseline and at 4 and 12 weeks of study, the control and NNF groups had similar mean values for serum chemistries, electrolytes, liver function, hematology and blood coagulation indices, with all variable remaining within the normal range. No significant differences in body weight, height, or body mass index changes between study groups. Adverse events were reported in 16 NNF and 10 control patients; of the 42 adverse events reported, 26 were in the NNF group and 16 in the control group. Only three of these events were considered possible related to study formula: one in the NNF group (mild elevation of liver enzymes) and two in the control group (one mild elevated potassium and one moderate episode of vomiting). The only severe event, an upper respiratory infection, probably not related to the study formula, occurred in the control group. No difference in asthma free days between active treatment and control.

Table 7. Clinical Trials of γ-linolenic Acid (GLA)-enriched Oils in Infants and Children

Reference	Study Design and Population	Treatments and Number of Subjects	Duration	Safety Outcomes
Biagi et al., 1994	Double-blind, placebo-controlled Children aged 2.2-8.5 yr with atopic dermatitis and abnormalities of IgE-mediated immune responses	Subjects were sorted into allergic and non-allergic groups and then randomized to three Different groups Group 1: Olive oil + 10 mg Vitamin E (Placebo); n=16 Group 2: Olive oil mixed with evening primrose oil (Low GLA; equivalent to 0.02 g GLA/kg•d); n=16 Group 3: Evening primrose oil (High GLA; equivalent to 0.4 g GLA/kg•d); n=16	8 wk	Three children did not attend for follow-up (reasons not given). Overall severity of atopic dermatitis was improved at 8 wk vs. baseline. Individual fatty acid content of erythrocyte membranes was altered more after treatment in children without concomitant allergies, in comparison with those having allergies. GLA was only increased in erythrocyte membranes of non-allergic children receiving the higher dose of oil. Overall *n*-6/*n*-3 ratio was significantly increased from baseline to study's end in the allergic children but not in non-allergic children. GLA supplementation had no effect on erythrocyte membrane fluidity. The clinical significance of these findings was unclear.

Table 7. Clinical Trials of γ-linolenic Acid (GLA)-enriched Oils in Infants and Children

Reference	Study Design and Population	Treatments and Number of Subjects	Duration	Safety Outcomes
Berth-Jones and Graham-Brown, 1993	Double-blind, placebo-controlled, parallel-group Children and adults with atopic dermatitis	Group 1: Paraffin and olive oil (Placebo); n=41 (25M/16F; 20 subjects were 12-yr old or younger) Group 2: Capsules containing evening primrose oil (0.48 g GLA/d); n=41 (22M/19F; 21 subjects were 12-yr old or less) Group 3: Capsules containing evening primrose oil and fish oil (0.4 g GLA/d); n=20 (22M/19F; 20 subjects were 12-yr or less)	16 wk	At 4 weeks, 9 subjects had defaulted or been withdrawn. By 18 weeks, 14 had defaulted or been withdrawn. By 16 weeks, 21 had defaulted or been withdrawn. In the placebo group, four subjects experienced deterioration of eczema, two were non-compliant, and 1 defaulted. In the evening primrose oil -treated group, one subject experienced deterioration of eczema, two were excluded due to non-compliance, three withdrew consent, and two experienced nausea. In the evening primrose oil + fish oil group, three subjects experienced deterioration of eczema, two defaulted, and one had diarrhea. Baseline symptoms were similar across all groups. With the exception of patients withdrawn due to deterioration, there were no apparent differences in response to treatment.

Table 7. Clinical Trials of γ-linolenic Acid (GLA)-enriched Oils in Infants and Children

Reference	Study Design and Population	Treatments and Number of Subjects	Duration	Safety Outcomes
Hederos and Berg, 1996	Double-blind, randomized, placebo-controlled, parallel Children (1-16 yr old) with atopic dermatitis	Dosage was according to age Group 1: Sunflower oil + vitamin E (Placebo); n = 30 (13M/17F). Group 2: 1-12 yr received 4 capsules containing evening primrose oil (equiv. to 0.3 g GLA/d); > 12 yr received 6 capsules containing evening primrose oil (equiv. to 0.4 g GLA/d)	16 wk	Routine hematological and biochemical analyses showed one significant difference between treatments and that was for urate, but all serum urate concentrations remained within the normal range. Five patients in the evening primrose oil group and six patients in the placebo group reported seven adverse events. None were considered serious and only one in each group was considered a possible effect of treatment.
Guenther and Wexler, 1987	Double-blind, crossover Children with atopic dermatitis	Treatment 1: Placebo Treatment 2: evening primrose oil Dosage was according to age. < 7 yr received 4 capsules of evening primrose oil/d 7-14 yr received 6 capsules of evening primrose oil /d > 14 yr received 8 capsules of evening primrose oil /d GLA content of Efamol is not reported; calculated estimated doses at 0.18, 0.27, and 0.36 g GLA/d, based on estimated 9% GLA per 0.5 g capsule.	12 wk	Fourteen patients completed the study and three withdrew after the first visit. Both groups showed improvement, but no statistically significant differences in the severity of eczema between the two groups. No other safety parameters reported; fidgeting was reduced versus placebo and versus baseline.

Table 7. Clinical Trials of γ-linolenic Acid (GLA)-enriched Oils in Infants and Children

Reference	Study Design and Population	Treatments and Number of Subjects	Duration	Safety Outcomes
Bordoni et al., 1988 [Note: authors mention that data suggest that in atopic eczema there is a reduced ability to convert linoleic acid to GLA and DGLA]	Randomized Children 2-4 yr old with atopic eczema	Group 1: Olive oil (placebo); n =12 Group 2: Evening primrose oil (equiv. to 0.3 g GLA/d); n=12	4 wk	Relative to baseline, the percent of DGLA in plasma increased significantly in the evening primrose oil -treated group. Adrenic acid (22:4 *n*-6) levels also increased significantly in neutrophils relative to baseline levels in the EPO-treated group. Authors reported that no side effects were apparent in either group.
Shimasaki, 1995	Intervention-only Japanese children (3-10 yr; 30M/21F) with and without atopic dermatitis	Group 1: Japanese children without atopic dermatitis; n =30 Group 2: Japanese children with atopic dermatitis that received candy-type jellies containing GLA (equiv. to 0.18 g GLA/d); n=51	8 wk	GLA as plasma levels increased in Group 2 relative to Group 1 Other fatty acids, which were increased in the plasma of treated subjects, included 15:0, DGLA (20:3 *n*-6), ARA (20:4 *n*-6), DPA (22:5 *n*-3) and α-LA (18:3 *n*-3). Adverse effects were not mentioned by the authors.

Table 7. Clinical Trials of γ-linolenic Acid (GLA)-enriched Oils in Infants and Children

Reference	Study Design and Population	Treatments and Number of Subjects	Duration	Safety Outcomes
Lovell et al., 1981	Randomized, double-blind, cross-over Children and adults with atopic eczema	Treatment 1: Liquid paraffin Treatment 2: Evening primrose oil (equiv. 0.2 g GLA/d) n=32	3 wk	Efamol caused no adverse reactions in either adults or children. Subjects treated with evening primrose oil exhibited a modest, but statistically significant, improvement on both the doctor's and self-assessments.
van Gool et al., 2003	Randomized, double-blind, placebo-controlled Formula-fed infants at high risk for atopic dermatitis 121 infants were included, 118 completed follow-up; gestational age of ≥ 38 wk, birth weight > 2500 g, uncomplicated perinatal period, exclusive formula-feeding from 2 wk of age	Group 1: Sunflower oil (Placebo); n=60 Group 2: Borage oil (equiv. to 0.1 g GLA/d); n=61	6 mo	Two infants in Group 1 were lost to follow-up due to lack of time to parent and due to abdominal cramps). One infant in Group 2 was lost to follow-up due to lack of time of parent. Adverse events were similar across two groups and among the subjects that completed the study, there were 4 reports of milk reflux and 1 report of lactose intolerance in Group 1, and 1 report of food aversion, 2 reports of abdominal cramps, 1 report of milk reflux, 1 report of reflux oesophagitis, and 1 report of obstipation in Group 2. At 1-yr follow up, there was a non-significant reduction in the severity of atopic dermatitis.

Table 8. Clinical Trials of γ-linolenic (GLA)-enriched oils in Adults

Reference	Study Design and Population	Treatment and Number of Subjects	Duration	Safety Outcomes
Johnson et al., 1997	GLA supplementation (4 studies involving healthy adults).	Study 1: Capsules containing a proprietary blend of oil (Ultra GLA or BIO-EFA; equiv to 3.0 g GLA/d); n=9 Study 2: Capsules containing a proprietary blend of oil (Ultra GLA or BIO-EFA; equiv to 3.0 g GLA/d); n=5. Study 3: 4 groups Group 1: no supplementation; n =3 Group 2: Equiv. to 1.5 g GLA/d; n=3 Group 3: Equiv. to 3.0 g GLA/d; n=3 Group 4: Equiv. to 6 g GLA/d; n=3 Study 4: Equiv. to 5 g GLA/d; n=3.	Study 1, 2, and 3 - 3 wk Study 4 - 12 wk	GLA supplementation significantly increased serum concentrations of AA at all doses whereas only 3 and 6 g GLA/day significantly increased the serum concentrations of DGLA and GLA; led to significant increases in GLA levels in serum phospholipids, and cholesterol esters, and DGLA and AA levels in serum phospholipids, but had no effect on GLA, DGLA, or AA levels in diglyceride, free fatty acid, or triglyceride fractions; increased DGLA concentrations in the glycerolipid fraction of neutrophil membranes, specifically in the neutral lipid and phosphatidylethanolamine but not the phosphatidylinositol/phosphatidylserine and phosphatidylcholine fractions; and increased the amount of DGLA release from A23187-stimulated neutrophils harvested from the different groups in study 4 (increased appeared to be dose-dependent) Significant increases in serum concentrations of AA and DGLA were seen as early as 2 weeks after supplementation began and continued until supplementation was stopped. GLA levels were also significantly increased at 2, 8 and 12 weeks but were not increased at 4 and 10 week. AA, DGLA, and GLA levels returned to normal when GLA supplementation was stopped. GLA supplementation reduced LTB_4 and PAF production from A23187-stimulated neutrophils harvested from subjects consuming 3 g GLA/day for 3 weeks. The authors stated that no adverse effects were reported before or after supplementation.

Table 8. Clinical Trials of γ-linolenic (GLA)-enriched oils in Adults

Reference	Study Design and Population	Treatment and Number of Subjects	Duration	Safety Outcomes
Laidlaw and Holub, 2003	Placebo-controlled clinical trial involving healthy women	Group 1: Fish oil concentrate (no GLA); n=8 Group 2: Fish oil conc. + borage oil conc. (equiv. 1 g GLA/d); n=8 Group 3: Fish oil conc. + borage oil conc. (equiv to 2 g GLA/d); n=7 Group 4: Fish oil conc. + borage oil conc. (equiv. 4 g GLA/d); n=8	28 d	No adverse side effects were reported by the investigators. The mixture of EPA + DHA and GLA favorably altered blood lipid and fatty acid profiles.
Chilton-Lopez et al., 1996	Healthy subjects	Capsules containing borage oil (equiv. to 3.0 g GLA/d); n=12	3 wk	No significant side effects were reported by any of the subjects before or after the supplementation period. GLA supplementation significantly increased the amount of DGLA in the membranes of neutrophils and had no effect on the amount of AA.
Zurier et al., 1996	Randomized, double-blinded, placebo-controlled trial followed by single-blinded trial. Adults with active rheumatoid arthritis.	Group 1: Capsules containing sunflower seed oil (placebo); n=28 Group 2: Capsules containing modified borage oil (equiv. to 2.8 g GLA/d); n=28	6-mo randomized double-blind comparison of GLA and placebo, followed by 6-mo GLA administration for all patients. GLA stopped at 12 mo, and patients were evaluated again at 15 mo.	Twenty-three patients in the placebo group and 20 patients in the modified borage oil-treated group were being treated with NSAIDs. Thirteen women patients in the placebo group and 15 in the borage oil-treated group were taking corticosteroids (5.1 and 5.2 mean daily dose, respectively). In the placebo group, 5 patients were taking methotrexate, 5 were taking gold salts, and 5 were taking hydroxychloroquine. In the borage oil-treated group, 10 patients were taking methotrexate, 3 were taking gold salts, and 4 were taking hydroxychloroquine. During the first 6 months, 6 patients in the modified borage oil-treated group withdrew (2 due to lack of

Table 8. Clinical Trials of γ-linolenic (GLA)-enriched oils in Adults				
Reference	**Study Design and Population**	**Treatment and Number of Subjects**	**Duration**	**Safety Outcomes**
				efficacy, 1 due to diarrhea, 1 due to surgery, 1 due to unrelated illness, and one due to protocol violation) and 9 patients in the placebo withdrew (4 due to lack of efficacy, 1 due to constipation, 2 due to unrelated illness, 1 due to a rash, and 1 due to protocol violation). The GLA group demonstrated statistically significant and clinically relevant reductions in the signs and symptoms of disease activity (physician's global assessment of disease activity, patient's global assessment of disease activity and a pain assessment, the number of joints with tenderness and/or pain on pressure or passive motion, the number of joints with swelling, joint pain/tenderness score; joint swelling score, duration of morning stiffness, grip strength, and degree of disability). Patients taking GLA during the entire 12 mo showed progressive improvement during the second 6 mo. GLA was reported to be well-tolerated. Representative analyses on selected patients showed enrichment of plasma and platelet lipids with GLA and DGLA during borage-oil but not placebo treatment.

Table 8. Clinical Trials of γ-linolenic (GLA)-enriched oils in Adults				
Reference	**Study Design and Population**	**Treatment and Number of Subjects**	**Duration**	**Safety Outcomes**
Kenny et al., 2000	Clinical trial of GLA supplementation compared to control cases selected on the basis of matching disease stage and initial estrogen receptor expression. Adults with locally advanced or metastatic breast cancer all of whom primary endocrine therapy was indicated as appropriate initial treatment.	Group 1: 20 mg of Tamoxifan/day; n=47 Group 2: 20 g tamoxifan + capsules containing GLA (equiv. to 2.8 g GLA/d); n=38 2 patients in each group received monthly subcutaneous depot injections of 3.6 mg goserelin	6 mo	GLA was well tolerated with no major side effects reported. 16 patients reported no side effects; no adverse effects results in discontinuation of treatment. 22 patients reported minor side effects: 13 patients reported mild alteration of bowel habit with tendency towards loose stool; several patients found the mild laxative effect beneficial. 2 patients had difficulty swallowing the capsules. 2 patients reported of a bitter aftertaste. 2 patients reported sleepiness, although researchers attributed this more likely to the concurrent tamoxifen administration. Faster clinical response of GLA + tamoxifen compared to tamoxifen; apparent enhancement of tamoxifen-induced estrogen receptor down-regulation by GLA was noted.

Table 8. Clinical Trials of γ-linolenic (GLA)-enriched oils in Adults				
Reference	**Study Design and Population**	**Treatment and Number of Subjects**	**Duration**	**Safety Outcomes**
Leventhal et al., 1994	Randomized, double-blind, placebo-controlled trial. Adults with rheumatoid arthritis and active synovitis.	Group 1: Capsules containing soybean oil; n=14 Group 2: Capsules containing black currant seed oil (equiv. to 1.99 g GLA/d; n=11	6 mo	7 patients in the treatment group and 13 in the placebo group withdrew from the study No patients in the treatment group withdrew because of adverse reactions. The vast majority withdrew because of the large size of the capsules. Treated subjects showed reduction in overall pain and joint tenderness, other measures of disease activity did not change.
Middleton et al., 2002	Double-blind, randomized, placebo-controlled trial. Adults with quiescent ulcerative colitis 63 patients randomized, 31 received treatment and 32 placebo (sunflower oil).	Group 1: Capsules containing sunflower oil; n=32 Group 2: Capsules containing 1.6 g GLA/d, EPA, and DHA.	12 mo	No statistically significant difference in adverse events between the groups. Disease relapse rates were similar at 12 months as were changes in sigmoidoscopic grade from baseline in both groups.
Leventhal et al., 1993	Randomized, double-blind, placebo-controlled study. Adults with rheumatoid arthritis and active synovitis. 37 patients enrolled, 19 received treatment and 18 placebo.	Group 1: Cotton seed oil (placebo); n=18 Group 2: Capsules containing borage seed oil (equiv. to 1.4 g GLA/day); n=19	6 mo	Adverse reactions included soft stools (2 patients in the borage seed oil-treated group, 1 in the placebo-treated group), constipation (1 patient in placebo group), flatulence (1 patient in borage seed oil-treated group), belching (1 patient in borage seed oil-treated group); no patients in the borage seed oil-treated group withdrew from the study because of adverse reactions. Treatment with borage seed oil resulted in clinically relevant and statistically significant reduction in signs and symptoms of disease activity compared to placebo.

Table 8. Clinical Trials of γ-linolenic (GLA)-enriched oils in Adults

Reference	Study Design and Population	Treatment and Number of Subjects	Duration	Safety Outcomes
Surette et al., 2008	Trial 1: Randomized, prospective, double-blind, placebo-controlled, parallel group trial. Atopic adults with mild-to-moderate asthma *Follow-on study to Surette et al., 2003 Trial 2: Open-label study Adults with a history of asthma	Trial 1: Group 1: A medical food emulsion containing olive oil (placebo); n=8 Group 2: A medical food emulsion containing borage seed oil and fish oil (equiv. to 0.75 g GLA/d); n=12 Group 3: A medical food emulsion containing borage seed oil and fish oil (equiv. to 1.13 g GLA/d) delivered in a medical food emulsion; n=15 Trial 2: A medical food emulsion containing borage seed oil and fish oil (equiv. to 0.75 g GLA/d); n=65	Trial 1: 4 wk Trial 2: 4 wk	Trial 1: Although the administration of the GLA containing formulas did not improve self-reported asthma status and bronchodilator use compared to placebo, the administration of the GLA-containing product reduced zymosan-induced neutrophil leukotriene B_4 (LTB_4) biosynthesis, which was reported in Surette et al., 2003. Trial 2: Asthma management scores, as determined by the validated Mini Asthma Quality of Life Questionnaire (MiniAQLQ) and the Asthma Control Questionnaire (ACQ), improved in the GLA-treated groups. No serious adverse events occurred during either trial. As reported in Surette et al., 2003, there were no significant between-group differences in adverse events or mean clinical chemistry values in Trial 1. In trial 2, two subjects reports gastrointestinal upset.
Surette et al., 2003	Randomized, double-blind, placebo-controlled, parallel group, prospective trial. Adults with mild to moderate atopic asthma	Group 1: A medical food emulsion containing olive oil (placebo); n=8 Group 2: A medical food emulsion containing borage seed oil and fish oil (equiv. to 0.75 g GLA/d); n=10 Group 3: A medical food emulsion containing borage seed oil and fish oil (equiv. to 1.13 g GLA/d) delivered in a medical food emulsion; n=11	4 wk	No clinically significant changes in vital signs were observed and there were no significant between-group differences in treatment-emergent adverse events or mean clinical laboratory values (hematology and clinical chemistry panels). None of the patients had elevations >2 times the upper limit of normal for any clinical laboratory measurements. GLA inhibited leukotriene biosynthesis.

Table 8. Clinical Trials of γ-linolenic (GLA)-enriched oils in Adults				
Reference	**Study Design and Population**	**Treatment and Number of Subjects**	**Duration**	**Safety Outcomes**
Pullman-Mooar et al., 1990	Open, uncontrolled study involving patients with active rheumatoid arthritis	All patients received in the form of borage seed oil capsules (equiv. of 1.062 g GLA/d); n=7 Patients were also treated with nonsteroidal anti-inflammatory drugs and analgesics and may have taken up to 10 mg prednisone/day.	12 wk	There were no adverse events other than softening of stools and occasional sensation of bloatedness. In general, administration of borage oil resulted in apparent clinical improvement in 6 patients (sleep patterns, joint scores, morning stiffness and the patients overall assessment of disease activity. No changes were seen in 50-foot walk time, grip strength, complete blood count, platelet count, erythrocyte sedimentation rate, or blood chemistry. One patients joint score worsened after borage oil supplementation was discontinued and when placed back on borage oil, the joint score improved.
Weaver et al., 2009	Uncontrolled study involving healthy volunteers. Volunteers were provided a background diet for five weeks and after the first week were given dietary supplements containing fish oil and borage oil for four weeks.	Capsules containing borage oil and fish oil (equiv 0.8 g GLA/d); n=27.	4 wk	Although fish oil and borage oil were combined and adverse events were not mentioned, there appeared to be a small and significant increase in the number of lymphocytes and neutrophils in the blood. Borage/fish oil supplementation also significantly reduced A23187-induced LTB_4 production from whole blood neutrophils.
Brosche and Platt, 2000	Open uncontrolled study involving 29 elderly people (mean age of 68.6 years) showing no signs of skin disease.	Capsules containing borage oil (equiv. to 0.36 and 0.72 g GLA/d); n=27	8 wk	Adverse events were not mentioned. Borage oil induced a statistically significant improvement in cutaneous barrier function.

Table 8. Clinical Trials of γ-linolenic (GLA)-enriched oils in Adults				
Reference	**Study Design and Population**	**Treatment and Number of Subjects**	**Duration**	**Safety Outcomes**
Bamford et al., 1985	Randomized, double-blind, blocked, cross-over trail involving patients (children and adults) with active eczema. *During the trial patients were allowed to continue the use of emollients, topical steroids, or oral antihistamines.	Test substance was capsules containing evening primrose oil and placebo was capsules containing liquid paraffin Children: Treatment 1: Capsules containing liquid paraffin (placebo); n=49 Treatment 2: Capsules containing evening primrose oil (equiv. 0.18 g GLA/d); n=33 Treatment 3: Capsules containing evening primrose oil (equivalent of 0.36 g GLA/d); n=16 Adults: Treatment 1: Capsules containing liquid paraffin (placebo); n=74 Treatment 2: Capsules containing evening primrose oil (equiv. to 0.54 g GLA/d); n=40 Treatment 3: Capsules containing evening primrose oil (equiv. to 0.72 GLA/d); n=34	12 wk	31 subjects dropped out (14 while taking evening primrose oil and 17 while taking placebo). 29 subjects dropped out for personal reasons and the remaining 3 believed that they had a reaction to the capsules (one stopped taking the evening primrose oil because of increased dermatitis; one child discontinued placebo because of hyperactivity developed). 80 subjects achieved 50% compliance and 56 achieved 75% compliance. Side effects, which were minor and temporary, were noted by 26 of the subjects The total number of complaints was equal between the evening primrose oil and placebo groups. Nausea and bloating occurred in five of the subjects taking evening primrose oil and one taking the placebo. Hyperactivity developed in three children taking the placebo and one taking evening primrose oil. There was no difference in weight, triceps weight, skin-fold thickness, or blood pressure, or in the ratings of appetite and stress.

Table 8. Clinical Trials of γ-linolenic (GLA)-enriched oils in Adults				
Reference	**Study Design and Population**	**Treatment and Number of Subjects**	**Duration**	**Safety Outcomes**
Rilliet et al., 1988(abstract)	Randomized; double-blind; placebo-controlled trial of adults with atopic dermatitis	Group 1: Grape seed oil (placebo); n=13 Group 2: Blackcurrant seed oil (equiv. to 0.6 g GLA/d); n=11	13 wk	Adverse events were not mentioned. Clinical parameters (such as transepidermal water loss, hematology, total IgE levels) in both groups improved substantially.
Andreassi et al., 1997	Placebo-controlled study involving patients with atopic dermatitis	Group 1: Placebo; n=30 Group 2: Borage oil (equiv. to 0.5 g GLA/d); n=30	12 wk	There were no side effects associated with the borage oil treatment.
Henz et al., 1999	Randomized, double-blind, placebo-controlled multicenter study involving otherwise healthy patients with atopic eczema. *Patients were allowed to use difucortolone 21-valerate cream during the trial if necessary.	Group 1: Bland oil (placebo); n=80 Group 2: Borage oil (equivalent to 0.5 g GLA/d assuming that borage oil contains approximately 18% GLA); n=80	24 wk	Nineteen and 17 patients dropped out of the placebo and borage oil groups. No significant differences were found between the groups with regard to corticosteroid dosage until response. Borage oil decreased serum IgE levels although the difference was not significant. Two patients in the placebo group withdrew because of hyperlipidemia and one patient in the borage oil group withdrew because of headache, diarrhea, and vomiting. Adverse events were reported by 27 patients in the placebo group and 29 patients in the borage oil group, and were mostly were mostly influenza-like symptoms

Table 8. Clinical Trials of γ-linolenic (GLA)-enriched oils in Adults				
Reference	**Study Design and Population**	**Treatment and Number of Subjects**	**Duration**	**Safety Outcomes**
Rosenstein et al., 2003	Single-site, randomized, placebo-controlled, double blind study involving patients with periodontitis (mean age of 41) 30 patients	Group 1: Capsules containing a mixture of olive and corn oil (placebo); n=6 Group 2: Capsules containing fish oil; n=5 Group 3: Capsules containing borage oil (equiv. to 0.5 g GLA assuming that borage oil is 18% GLA); n=7 Group 4: Capsules containing fish oil and borage oil; n=6	12 wk	6 patients dropped out due to adverse effects (gastrointestinal distress in three subjects and rest were due to non-compliance). The treatments these patients received were not noted.
Belch et al., 1988	Double-blind, placebo-controlled trial. Adults with classical or definite rheumatoid arthritis as defined by American Rheumatism Association criteria	Group 1: Liquid paraffin (placebo); n=18 Group 2: Capsules containing evening primrose oil and fish oil (equiv. to 0.45 g GLA/d); n= 15 Group 3: Capsules containing evening primrose oil (equiv. to 0.54 mg GLA/d); n= 16	15 mo	Four patients experienced side effects; 2 in Group 3, one with nausea and one with diarrhea that required withdrawal from the study. Two patients in Group 2 experienced nausea and headache but neither required withdrawal from the study. At 12 mo, significant subjective improvement in the condition of the subjects compared to placebo was reported for both 94% and 93% of Group 3 and Group 2, respectively.
Brzeski et al., 1991	Prospective, double-blind, placebo-controlled study. Adults with classical or definite rheumatoid arthritis	Group 1: Olive oil (placebo); n=21 Group 2: evening primrose oil (equiv. to 0.54 g GLA/day); n=19	6 mo	6/19 treated patients withdrew from the study, 2 with nausea, 2 for joint surgery, 1 with deteriorating arthritis and 1 due to flu-like symptoms. 4/21 from the placebo group withdrew, 3 with nausea and 1 leaving the area. Of the 13 completing treatment, 10 showed a significant rise in plasma DGLA, suggesting good compliance. In the evening primrose oil-treated group, arthritis was reduced at 3 months with a trend to reduction at 6 months as measured by indices of pain, articular index and morning stiffness.

Table 8. Clinical Trials of γ-linolenic (GLA)-enriched oils in Adults				
Reference	**Study Design and Population**	**Treatment and Number of Subjects**	**Duration**	**Safety Outcomes**
Wright and Burton, 1982	Double-blind, placebo-controlled, cross-over study Adults and children with atopic eczema	<u>Adults</u> Treatment 1: Liquid paraffin (placebo) Treatment 2: Evening primrose oil Group 1: Evening primrose oil (equiv. to 0.18 g GLA/d); n=20 Group 2: Evening primrose oil (equiv. to 0.36 g GLA/d); n=20 Group 3: Evening primrose oil (equiv. to 0.54 g GLA/d); n=20 <u>Children</u> Treatment 1: Liquid paraffin (placebo) Treatment 2: Evening primrose oil Group 1: Evening primrose oil (equiv. to 0.09 g GLA/d); n=20 Group 2: Evening primrose oil (equiv. to 0.18 g GLA/d); n =18	12 wk treatment followed by 12 wk placebo in random order.	16/60 adults dropped out before completing the 6 months; 8 in the treated group and 8 in placebo. 11/16 were lost to follow-up or moved out of the area and 2 with previous psychiatric illness became ill. One patient felt faint; 1 patient felt his eczema was getting worse, both were on placebo. One patient became pregnant and stopped. 3/39 children dropped out. Mean symptom scores showed an improvement in symptoms that was greatest in the high dose groups; adults responded better than children.

Table 8. Clinical Trials of γ-linolenic (GLA)-enriched oils in Adults				
Reference	**Study Design and Population**	**Treatment and Number of Subjects**	**Duration**	**Safety Outcomes**
Keen et al., 1993	Double-blind, placebo-controlled, parallel study Adults with mild diabetic neuropathy	Group 1: Liquid paraffin (placebo); n=57 (12M/45F) Group 2: Capsules containing evening primrose oil (equiv. to 0.48 g GLA/d); n=54 (18M/36F)	1 yr	10 patients withdrew from the evening primrose oil -treated group; 17 withdrew from the placebo group. 4 adverse events were reported in the evening primrose oil -treated group and 6 in the placebo group; only 2 cases of nausea and vomiting in each group were thought to be treatment-related. No clinically important changes in hematological parameters, urea, electrolytes, protein, albumin liver enzymes or fructosamine were observed. Substantial rises in aspartate aminotransferase appeared in 2 patients, but in both cases, the rise was found to be related to acute ingestion of alcohol. Neurophysiological and neurological parameters showed improvement with evening primrose oil -treatment.
Kruger et al., 1998	Placebo-controlled study Women with confirmed osteoporosis or who were osteopenic	Group 1: Coconut oil (placebo); n=31 Group 2: Evening primrose oil + Fish oil (equiv. 0.48 g GLA/d); n=29	18 mo; 21 patients continued for an additional 18 mo treatment period.	6 patients (3 from the evening primrose oil +fish oil treatment group and 1 from the placebo and 2 from the second evening primrose oil + fish oil treatment period withdrew due to non-compliance). No side effects were recorded, except soft stools in a few patients. Clinical chemistry values for hematology, kidney and liver functions were normal in all patients. Beneficial effects on bone density were seen in evening primrose oil + fish oil-treated patients.

Table 8. Clinical Trials of γ-linolenic (GLA)-enriched oils in Adults				
Reference	**Study Design and Population**	**Treatment and Number of Subjects**	**Duration**	**Safety Outcomes**
Berth-Jones and Graham-Brown, 1993	Double-blind, placebo-controlled, parallel-group Children and adults with atopic dermatitis	Group 1: Paraffin and olive oil (Placebo); n=41 (25M/16F; 20 subjects were 12-yr old or younger) Group 2: Capsules containing evening primrose oil (0.48 g GLA/d); n=41 (22M/19F; 21 subjects were 12-yr old or less) Group 3: Capsules containing evening primrose oil and fish oil (0.4 g GLA/d); n=20 (22M/19F; 20 subjects were 12-yr or less)	16 wk	At 4 weeks, 9 subjects had defaulted or been withdrawn. By 18 weeks, 14 had defaulted or been withdrawn. By 16 weeks, 21 had defaulted or been withdrawn. In the placebo group, four subjects experienced deterioration of eczema, two were non-compliant, and 1 defaulted. In the evening primrose oil -treated group, one subject experienced deterioration of eczema, two were excluded due to non-compliance, three withdrew consent, and two experienced nausea. In the evening primrose oil + fish oil group, three subjects experienced deterioration of eczema, two defaulted, and one had diarrhea. Baseline symptoms were similar across all groups. With the exception of patients withdrawn due to deterioration, there were no apparent differences in response to treatment.
Jamal and Carmichael, 1990	Double-blind, placebo-controlled, randomized, parallel Patients with distal diabetic polyneuropathy (21-74 yr)	Group 1: Placebo Group 2: EPO (equiv. 0.36 g GLA/d)	6 mo	Statistically significant improvements in various compound muscle action potential measurements, sensory nerve action potentials, and heat thresholds were observed in the treatment group vs. placebo. There was no mention of any adverse effects.

Table 8. Clinical Trials of γ-linolenic (GLA)-enriched oils in Adults				
Reference	**Study Design and Population**	**Treatment and Number of Subjects**	**Duration**	**Safety Outcomes**
Schalin-Karrila et al., 1987	Double-blind, placebo-controlled, randomized Young adults (19-31 yr) with moderate to severe eczema	Group 1: Liquid paraffin (placebo); n=11 Group 2: Evening primrose oil (equiv. to 0.36 g GLA/d); n=14	12 wk	One subject in the treatment group dropped out due to a severe allergic reaction to topical dequaline chloride. No side effects due to evening primrose oil were observed. Less topical steroid use was reported in the evening primrose oil -treated group vs. placebo. There was improvement in the overall severity of symptoms in all but one subject in the treatment group. However, the mean initial status of the eczema was worse in the evening primrose oil -treated group at baseline.
Hansen et al., 1983	Open-label, uncontrolled study Patients (39-81 yr) with definite or classical rheumatoid arthritis	A proprietary evening primrose oil formulation with zinc, ascorbic acid, niacin, and pyridoxine (equiv. to 0.36 g GLA/d); n=20	12 wk	Seventeen subjects completed the study; one died suddenly (81 yr old) but the cause of death was not considered treatment-related. One patient was unable to complete the treatment without non-steroidal anti-inflammatory drugs, and one patient was started on glucocorticosteroids and cyclophosphamide after 8 wk due to vasculitis and neuropathy. Only 3 patients reported definite improvement during the study.
Ranieri et al., 2009	Observational, two-arm Patients with compressive root syndromes from conflict disk root	Group 1: No treatment other than a rehabilitation program Group 2: A rehabilitation program + 0.36 g GLA and 0.6 g ALA	6 wk	Thirteen subjects were excluded during follow-up as they had not completed the trial. Of these, seven were in the treatment group and five were in the control group. One person discontinued treatment due to an adverse reaction to an excipient (polyvinylpyrrolidone), manifesting as a skin rash in the thoracoabdominal region.

Table 8. Clinical Trials of γ-linolenic (GLA)-enriched oils in Adults

Reference	Study Design and Population	Treatment and Number of Subjects	Duration	Safety Outcomes
Lovell et al., 1981	Randomized, double-blind, cross-over Children and adults with atopic eczema	Treatment 1: Liquid paraffin Treatment 2: Evening primrose oil (equiv. 0.2 g GLA/d) n=32	3 wk	Efamol caused no adverse reactions in either adults or children. Subjects treated with evening primrose oil exhibited a modest, but statistically significant, improvement on both the doctor's and self-assessments.
Stewart et al., 1991	Open, uncontrolled Patients with moderate to severe atopic dermatitis	Capsules containing a proprietary evening primrose oil (equiv. to 0.32g GLA/d); n=179	Subjects took the formulation for 12 wk and had the option to continue afterward. Median duration of treatment among responders was 11 mo.	Relative to baseline, 111 subjects improved and 68 did not improve (improvement was primarily noted for parameters such as erythema, dryness, scaling, and itch). Two adverse events were noted: stomach pains and mild fluid retention. It is unknown whether these were related to evening primrose oil.
Guivernau et al., 1994	Placebo-controlled, double-blinded, crossover Hyperlipidemic men with known family history of premature coronary artery disease	Treatment 1: Liquid paraffin (placebo); n=12 Treatment 2: Evening primrose oil (equiv. to 240 mg GLA/d); n=12	4 mo each treatment, with a 4 wk washout in-between	Relative to baseline, evening primrose oil supplementation caused a significant decrease in serum triglyceride levels, total serum cholesterol, LDL, and a significant increase in HDL. Some *in vitro* assays of platelet aggregation exhibited decreased values in the evening primrose oil -treated group, versus control (endpoints included adenosine diphosphate- and adrenaline-aggregated platelet assays and production of platelet-derived thromboxane B_2). Bleeding time was not affected after 1 mo of treatment (versus baseline). After 3-4 mo supplementation, there was a significant mean 40% increase in bleeding time.

Table 8. Clinical Trials of γ-linolenic (GLA)-enriched oils in Adults				
Reference	**Study Design and Population**	**Treatment and Number of Subjects**	**Duration**	**Safety Outcomes**
Kawamura et al., 2011	Double-blind, placebo-controlled Adults with dry skin and mild to moderate atopic dermatitis	Group 1: A mixture of rapeseed and soybean oil (placebo; equiv. to 0.00006 g GLA/d); n= 61 Group 2: Oil extracted from the fungus *Mucor circinelloides* (equiv. to 0.2 g GLA/d); n=61 [*Note: This is the only GLA preparation which is derived from a fungus, rather than from evening primrose oil]	4 wk observation period; 12 wk test period; 4 wk follow-up period	Transepidermal water loss at the cheek and forearm was improved at 4 wk in the *M. circinelloides* oil-treated vs. placebo-treated group. Systolic and diastolic blood pressure and heart rate were not significantly different between groups. The authors mention that there were some changes in parameters measured, but they were either slight and within normal reference ranges, or temporary. The monitoring physician judged them as not being clinically important. No adverse events attributable to the test diet were noted by the monitoring physician.
Harris et al., 2002	Uncontrolled study Adults with non-muscle-invasive transitional cell carcinoma (TCC) Fifteen patients received the low dose therapy; 15 additional patients received the high dose therapy.	Group 1: 0.05 g of a novel intravesical formulation of meglumine gamma-linolenic acid (GLA content unknown) Group 2: 0.125 g of a novel intravesical formulation of meglumine gamma-linolenic acid (GLA content unknown)	A single intravesical instillation was administered and retained for 1 h	There were no significant local or systemic side effects. There were four complete responses, nine partial responses and 17 non-responders. Histology indicated no evidence of damage to the urothelium.
Schubert et al., 2007	Randomized, placebo-controlled double-blind, parallel study Healthy volunteers (20-38 yr)	Group 1: Mixture of olive oil and a fat blend rich in AA; n= 15 Group 2: Fat blend enriched with GLA (equiv. to 0.073 g GLA/d); n= 15	2 wk, plus 2 wk follow-up	Prostaglandins E_1, E_2, leukotriene B_4 production from lipopolysaccharide stimulated whole blood cells was significantly reduced in the GLA-treated group 2 wk after stopping supplementation. Other cytokines were not significantly affected. Authors did not mention adverse effects.

Table 8. Clinical Trials of γ-linolenic (GLA)-enriched oils in Adults				
Reference	**Study Design and Population**	**Treatment and Number of Subjects**	**Duration**	**Safety Outcomes**
Barabino et al., 2003	Randomized 26 patients with aqueous-deficient keratoconjunctivitis sicca	Group 1: Preservative-free substitute tears and the oral administration of a capsule containing a low amount of sugar; n=13 Group 2: Preservative-free substitute tears and the oral administration capsules containing GLA (Medilar tablets, Fidia Oftal-Bausch & Lomb Pharmaceuticals; equiv. to 30 mg GLA/d); n=13	45 d	There was a significant reduction in the percentage of HLA-DR conjunctival epithelial cells, symptoms score, and lissamine green staining score in the GLA-treated group by the end of the study. The authors did not mention adverse effects.

Table 9. Clinical Trials of γ-linolenic (GLA)-enriched oils in Adults and Children Receiving Sole-Source Enteral Nutrition

Reference	Study Design and Population	Treatment and Number of Subjects	Duration	Safety Outcomes
Rice et al., 2011	Randomized, double-blind, placebo-controlled, multicenter trial. Adults within 48 hours of developing acute lung injury resulting from pneumonia, sepsis, aspiration, or trauma.	Group 1: An isocaloric-isovolemic carbohydrate-rich control (control); n=129 Group 2: Enteral supplementation that contained EPA, DHA, GLA, and antioxidants (equiv. to 5.92 g GLA/d); n=143 *Both study diets were administered enterally as twice-daily boluses of 120 ml beginning within 6 hr of randomization.	3 wk	Study was stopped early as a result of the futility analysis. Enteral supplementation with n-3 fatty acids, GLA and antioxidants did not improve lung physiology or clinical outcomes and did not protect from nosocomial infections or improve nonpulmonary organ function. Increased incidence of diarrhea was noted and may have been caused by the bolus administration. The enteral-supplementation-treated group had fewer ventilator free days ($p= 0.02$) and higher mortality ($p=0.054$), however this may have been a chance observation as a result of the futility analysis. If stopping boundaries for efficacy and futility had been the same, $p < 0.001$ would have been required to establish statistical significance. More patients in Group 2 were receiving vasorepressors at enrollment and through day 7.

Table 9. Clinical Trials of γ-linolenic (GLA)-enriched oils in Adults and Children Receiving Sole-Source Enteral Nutrition

Reference	Study Design and Population	Treatment and Number of Subjects	Duration	Safety Outcomes
Gadek et al., 1999	Prospective, multicentered, double-blind, randomized controlled trial. Adults with acute respiratory distress syndrome (ARDS, as defined by the American-European Consensus Conference) caused by sepsis/pneumonia, trauma or aspiration injury.	Group 1: Isonitrogenous, isocaloric standard diet without these fatty acids; n=47 Group 2: Mixture of fish and borage oil (equiv. to 5.8 ± 0.3 g GLA/d); n=51. *Both diets were delivered at a constant rate to achieve a minimum of 50% basal energy expenditure (BEE) x 1.3 within the first 24 hrs. Continuous enteral nutrition was advanced as tolerated with the goal of achieving a minimum of 75% of BEE x 1.3 within 72 hrs of initiation. Enteral nutrition was delivered continuously for a minimum of 4+/- 1 study days at a rate not to exceed BEE x 1.3.	At least 4-7 d	No statistically significant changes in the treatment group compared to control were noted in values for serum chemistries, electrolytes, liver function, hematology, and blood coagulation. Modulation of plasma phospholipid fatty acids indicated increases in DGLA and EPA and increases in the ratio of EPA to ARA in Group 2 compared to Group 1. Formula tolerance compared to control was demonstrated by similar time to caloric goal, decrease in serum triglycerides indicating better ability to clear circulating lipids in the treatment group and fewer adverse events, particularly gastrointestinal complications in the treatment group. Ingestion of the GLA-containing diet was associated with a significant decrease in the number of total cells and neutrophils in the alveolar fluid, an increase in arterial oxygenation, and significant decreases in ventilator, intensive care unit, and supplemental oxygen days.

Table 9. Clinical Trials of γ-linolenic (GLA)-enriched oils in Adults and Children Receiving Sole-Source Enteral Nutrition

Reference	Study Design and Population	Treatment and Number of Subjects	Duration	Safety Outcomes
Pontes-Arruda et al., 2006	Prospective, single-center, randomized, double-blinded, placebo controlled trial. Adults requiring mechanical ventilation, having enteral access, and having a clinical diagnosis of either sepsis or septic shock	Group 1 (Placebo): High fat, low carbohydrate enteral formulation for patients with pulmonary diseases; n=48. Group 2 (EPA + GLA enriched diet): High fat, low carbohydrate enteral formulation for patients with pulmonary diseases enriched with 4.3 g GLA/L, 4.5 g EPA/L, and 2.0 g DHA/L; n=55. *Both diets were delivered at a constant rate to achieve a minimum of 50% basal energy expenditure (BEE) x 1.3 within the first 24 hrs. Continuous enteral nutrition was advanced as tolerated with the goal of achieving a minimum of 75% of BEE x 1.3 within 72 hrs of initiation. Enteral nutrition was delivered continuously for a minimum of 7 days or until they were interrupted at the physician's discretion or because of the development of any adverse event that could be related to the enteral feeding.	Diets were delivered until patients were extubated or until interrupted at physician's discretion or due to the development of any adverse event that could be related to the enteral feeding (4 – 7 days)	Sixty-two patients were excluded from the final analysis before unblinding because of protocol violations such as unable to meet caloric goal; extubation; pulling out the feeding tube; withdrawal by the attending physician; and death. Total number and type of adverse events was similar in both groups (Group 1: n=48; Group 2: n=55). Mean dietary intake was similar between groups for total calories. Group 2 received an additional 4.6 g +/- 0.13 g GLA/day, 4.9 +/- 0.14 g EPA/day, and 2.2 +/- 0.06 g DHA/day and higher amounts of vitamin C and E. Group 2 had a higher survival rate ($p=0.037$); statistically significant increase in Pao_2/FIo_2 ratios on study days 4 and 7; more ventilator-free days (13.4 +/- 1.2 vs 5.8 +/- 1.0; $p<0.001$; developed less new organ dysfunction.

Table 9. Clinical Trials of γ-linolenic (GLA)-enriched oils in Adults and Children Receiving Sole-Source Enteral Nutrition

Reference	Study Design and Population	Treatment and Number of Subjects	Duration	Safety Outcomes
Pontes-Arruda et al., 2011	Randomized, multicenter, double-blinded, placebo-controlled trial Adults with clinically diagnoses early sepsis that required enteral nutrition	Group 1 (Control): Ensure Plus HN (higher carbohydrates than the study formula); n=53 Group 2 (Study Formula): Enteral nutrition enriched with EPA, GLA and elevated levels of antioxidants (Oxepa, Abbott Nutrition); n=53 *Both diets were delivered at a constant rate to achieve a minimum of 50% basal energy expenditure (BEE) x 1.3 within the first 24 hrs. Continuous enteral nutrition was advanced as tolerated with the goal of achieving a minimum of 75% of BEE x 1.3 within 72 hrs of initiation. Enteral nutrition was delivered continuously for a minimum of 7 days or until they were interrupted at the physician's discretion or because of the development of any adverse event that could be related to the enteral feeding. Resulting intake of GLA was equiv. to 4.4 g/day	7 days	No serious adverse events were recorded over the course of the study. Although some patients experienced diarrhea and vomiting, there were no statistically significant differences between the two groups. Group 2 developed less severe sepsis and septic shock than Group 1. Less patients in Group 2 developed respiratory failure (14 vs. 23) and cardiovascular failure (12 vs. 21). No differences between the groups in the development of coagulation failure, renal failure, metabolic failure, or hepatic failure.

Table 9. Clinical Trials of γ-linolenic (GLA)-enriched oils in Adults and Children Receiving Sole-Source Enteral Nutrition

Reference	Study Design and Population	Treatment and Number of Subjects	Duration	Safety Outcomes
Pacht et al., 2003	Prospective, randomized, double-blind, controlled trial. Adults with illnesses known to be associated with acute lung injury, pulmonary inflammation and ARDS.	Group 1: An isonitrogenous, isocaloric standard diet without fatty acids; n=22 Group 2: Mixture of fish and borage oil (equiv. to 4.4 g GLA/d); n= 21 *Both diets were delivered at a constant rate to achieve a minimum of 50% basal energy expenditure (BEE) x 1.3 within the first 24 hrs. Continuous enteral nutrition was advanced as tolerated with the goal of achieving a minimum of 75% of BEE x 1.3 within 72 hrs of initiation. Enteral nutrition was delivered continuously for a minimum of 4+/- 1 study days at a rate not to exceed BEE x 1.3.	At least 4-7 d	43 patients were deemed evaluable. A total of 17 patients did not complete from study day 4 through study day 7 (seven patients in Group 1 and 10 patients in Group 2). 60% of the patients in Group 2 were extubated whereas only 14 % of patients in Group 1 were weaned from the ventilator. Although ingestion of the mixture of fish and borage oil had no affect on total protein, neutrophil count, and leukotriene B_4 levels in the bronchoalveolar fluid, the mixture significantly reduced brochoalveolar lavage fluid ceruloplasmin and IL-8 levels, reduced pulmonary inflammation, increased oxygenation, and improved clinical outcomes.

Table 9. Clinical Trials of γ-linolenic (GLA)-enriched oils in Adults and Children Receiving Sole-Source Enteral Nutrition				
Reference	**Study Design and Population**	**Treatment and Number of Subjects**	**Duration**	**Safety Outcomes**
Sultan et al., 2012	Prospective, double-blind randomized in patients undergoing subtotal oesphagectomy and total gastrectomy	Group 1 (control): Osmolite (1 kcal/ml); n=66 Group 2 (Standard enteral nutrition (SEN)): Ensure (1.5 kcal/ml); n=66 Group 3 (Immuno-enhancing diet (IED)): Oxepa, Abbott (1.5 kcal/ml; 4 g GLA/L); n=63 Supplementation was performed 7 days before surgery in the SEN and IED groups at a fixed rate of 1000 kcal/day, resulting in a daily desired volume of 675 mg/day. On the second day after surgery, feeding was commenced at 25 ml/hr and was increased to 50 ml/hr on the third day, reaching the maximum at some point during that day. Feeding was continued at the maximum rate between 4 and 7 days after surgery. The maximum rate of administration for all diets and for all patients was calculated according to the predicted proteins and calories that the individual thought to require based on Schofield equations for estimating basal metabolic rate, adjusting for stress or weight loss, and adding a combined factor for activity and diet-induced thermogenesis.	14 days (7 days before and after surgery)	21 patients were eligible and 195 patients were randomized and were used in the analysis. There was no difference between the three groups in the infective complications, proportion of patients who develop an infective complication, those requiring therapeutic antibiotics, other complications, critical care stay, hospital stay or mortality rate. There was no difference in the volume of feed received ether before or after surgery. Only 55 and 63% of the patients in the IED and SEN groups, respectively, achieved the desired maximum hourly feed. Only 22% of the patients in the control group achieved the maximum feed rate. GLA intake was not specifically noted, although based on overall pre- and post-operative feed volumes, and durations of feeding, the pre- and post-operative GLA intakes are estimated to be 2.4 +/- 0.5 g/day and 3.2 +/- 1.4 g/day, respectively.

Table 9. Clinical Trials of γ-linolenic (GLA)-enriched oils in Adults and Children Receiving Sole-Source Enteral Nutrition

Reference	Study Design and Population	Treatment and Number of Subjects	Duration	Safety Outcomes
Singer et al., 2006	Open, prospective, randomized, unblinded, and placebo-controlled Patients with acute lung injury (ALI)/acute respiratory distress syndrome (ARDS)	Group 1 (Control): ready-to-feed, high-fat, low-carbohydrate, enteral formula; n=49. Group 2 (EPA + GLA diet): Oxepa (differed from the control formula only in lipid composition) and in the level of antioxidants; n=46. Diet feeding in both groups was adjusted to achieve greater or equal to 50% of the resting energy expenditure (REE) x 1.2 on the first day and 70% REE x 1.2 on the second day.	Enteral feeding was administered continuously' patients received the same enteral formula throughout their stay in the ICU (approx. 7 days)	All patients included in the study were fed successfully for greater than or equal to 14 days through the gastric, duodenal, or jejunal route at a rate that did not exceed REE X 1.25. No patients were dropped because of formula-related safety concerns. Nutritional intake (kcal/day) as similar between the two groups on day 1 (1055 +/- 378 vs. 1053 +/- 351) and day 7 (1420 +/- 437 vs 1624 +/- 512). Borage oil accounted for 20% of the fat fraction EPA + GLA diet. Assuming that 18% of borage oil is GLA, the resulting intake of GLA at day 1 was 2.4 vs 2.4 g/day and at day 7, 3.05 vs 3.6 g/day. There were no significant differences in nutritional variables (weight variations, plasma albumin, and prealbumin).

Table 9. Clinical Trials of γ-linolenic (GLA)-enriched oils in Adults and Children Receiving Sole-Source Enteral Nutrition

Reference	Study Design and Population	Treatment and Number of Subjects	Duration	Safety Outcomes
Jacobs et al., 2013	Prospective, blinded, randomized, controlled, multicenter trial. Critically-ill mechanically ventilated children with a diagnosis of acute lung injury or acute respiratory distress syndrome	Group 1 (PEF): Ready-to-feed, concentrated version of a pediatric enteral nutrition formula (Pediasure, Abbott Nutrition); n=12. Group 2 (Experimental diet): A commercially available adult formula (Oxepa, Abbott Nutrition) that was isocaloric to the PEF diet, differing only in terms of its lipid composition and level of antioxidants; n=14. Diet feeding in both groups was adjusted to achieve greater or equal to 50% of the estimated energy expenditure (EEE) x 1.3 on the first day and advanced to 75% REE x 1.3 within 48 hr of initiating the enteral feeding. Enteral feeding was delivered continuously for a minimum of 4 +/- 1 study days at a rate not to exceed EEE x 1.3.	4 days	Thirty-seven patients were enrolled and 11 dropped out due to non-compliance such as unable to meet caloric goal; extubation; pulling out the feeding tube; withdrawal by the attending physician; and death. There were no significant differences in the daily intakes of calories or total lipids. Group 2 consumed approximately 2.6 g GLA/day. Except for statistically significant increases in serum blood urea nitrogen (BUN) and urine osmolality, which were within their normal ranges, there were no statistically significant differences noted in serum biochemistries, renal and hepatic function, blood coagulation parameters, and creatine clearance. Although there was a greater number of adverse events in Group 2 vs. Group 1 (n=14 vs. n=12), none was severe or directly related to the study formula, and there were no significant differences between the two groups with regard to specific categories (e.g., gastrointestinal, cardiac, hematological, respiratory, skin, and infections). One patient died in groups 1 and 2.

Table 9. Clinical Trials of γ-linolenic (GLA)-enriched oils in Adults and Children Receiving Sole-Source Enteral Nutrition				
Reference	**Study Design and Population**	**Treatment and Number of Subjects**	**Duration**	**Safety Outcomes**
Grau-Carmona et al., 2011	Prospective, randomized, open-label, controlled, multicenter, parallel-group Adult with sepsis and acute lung injury or acute respiratory distress syndrome (ARDS) who were receiving mechanical ventilation and could be fed by enteral nutrition	Group 1: Ensure Plus HN® enteral nutrition by Abbott Labs (control diet); n= 71 Group 2: Oxepa enteral nutrition by Abbott Labs (contains borage oil; equiv. to up to 0.129 g GLA/d; mean intake of 0.113 g GLA/d); n= 61	12 d	The Oxepa-treated group experienced significantly fewer days in the Intensive Care Unit (ICU) vs. the control group. Physicians withdrew 28 patients, nine in the control group (1 did not receive any intervention; 7 did not receive mechanical ventilation; and 1 had a protocol violation), and 19 in the treatment group (7 did not receive any intervention; 8 did not receive mechanical intervention; 4 has a protocol violation), in the first 2 d of treatment. The study was stopped early due to inadequate statistical power to detect significant differences between groups. There was a non-significant trend for a lower SOFA score (indicative of altered organ function), fewer days on mechanical ventilation, and lower mortality in the treatment group vs. control. Diets were well-tolerated and gastrointestinal complications were unusual; the most frequent was high gastric residual volumes, with significantly less incidence in the treatment group vs. control. Diarrhea episodes were similar across groups.

Table 9. Clinical Trials of γ-linolenic (GLA)-enriched oils in Adults and Children Receiving Sole-Source Enteral Nutrition

Reference	Study Design and Population	Treatment and Number of Subjects	Duration	Safety Outcomes
Wills et al., 2014	Randomized, double-blind, placebo-controlled trial in adults with amyotrophic lateral sclerosis (ALS)	Group 1 (Placebo; isocaloric tube-fed diet): Jevity 1.0, Abbott; n=7 Group 2 (High-carbohydrate hypercaloric tube-fed diet): Jevity 1.5, Abbott ; n=9 Group 3 (High-fat hypercaloric tube-fed diet): Oxepa, Abbott; n=8 Goal was to replace 100% of estimated energy requirements, although some patients were still able to consume food by mouth.	4 months with a 5 month follow-up	28 patients were screened and 24 were randomized to the three treatments groups. Four participants (one in Group 1, one in Group 2, and two in Group 3) withdrew after randomization, but before starting study diet. More control participants discontinued the study diet because of adverse events than die the other groups (3 of six patients in the control group vs. none of eight in Group 2 and one of six in Group 3. Group 2 and 3 had fewer adverse events and serious adverse events compared to the control group. The most common adverse events were gastrointestinal (six patients in Group 1 had at least one gastrointestinal adverse event, four patients in Group 2, and six patients in Group 3. No patients in Group 2 or 3 had cardiovascular adverse events or cardiovascular serious adverse events. One of the eight patients in Group 3 died during the 5-month follow-compared to none in Group 2, and three in Group 1. All deaths occurred within 4 months of study diet initiation and all patients who died discontinued the study diet as a result of adverse events. In addition, all deaths were due to respiratory failure and none was considered to be related to study diet. Ingestion of the high-fat hypercaloric tube-fed diet was not associated with either increased cholesterol or hs-C reactive protein, or a change in fasting blood glucose and serum insulin over time. Patients in Group 1 consumed a mean of 1.21 +/- 0.26 times their estimated energy requirements. Patients in Group 2 consumed a mean of 1.54 +/- 0.33 times their estimated energy requirements. Patients in Group 1 consumed a mean of 1.51 +/- 0.33 times their estimated energy requirements.

Table 9. Clinical Trials of γ-linolenic (GLA)-enriched oils in Adults and Children Receiving Sole-Source Enteral Nutrition				
Reference	**Study Design and Population**	**Treatment and Number of Subjects**	**Duration**	**Safety Outcomes**
Kushta et al., 2011 (Abstract)	Patients with ALS	Group 1 (Placebo): Standard enteral nutrition formula (Nutrison, Nutricia; 30 kcal and 1.2 g proteins/kg bw/day Group 2: Respiratory Failure formula (Oxepa, Abbott)	1 year	Authors only noted that both formulas were well tolerated and reported that there were no alterations in routine biochemistry.

Table 10. Clinical Trials Evaluating the Effect of Linoleic Acid-enriched Diets on Platelet Aggregation and Bleeding Time

Reference	Study Design and Population	Treatment and Number of Subjects	Duration	Safety Outcomes
Hornstra et al., 1973	Controlled crossover study Mentally-ill adults (n=68 – 77)	Treatment 1: control diet containing 4% linoleic acid (equiv. to approx. 11 g linoleic acid/d based on diet containing 2500 cal/d and 9 cal/g fat) Treatment 2: experimental diet containing 12% linoleic acid (equiv. to approx. 33 g linoleic acid/d based on diet containing 2500 cal/d and 9 cal/g fat)	6 years/ treatment	The experimental diet significantly ($p<0.001$) reduced platelet aggregation time. Platelet retention was reduced, but considered not significant ($p=0.05$). There were no significant differences between the two treatments on bleeding time, red blood cell counts, and platelet counts.
O'Brien et al., 1976	Blinded, controlled study Healthy men	Group 1 (n=20): normal diet (containing 1 – 2% linoleic acid; equivalent to approx. 5 g linoleic acid/day based on a diet containing 2500 cal/d and 9 cal/g fat) Group 2 (n=19): experimental diet (containing up to 65% linoleic acid; equivalent to approx. 19 g linoleic acid/day based on a diet containing 2500 cal/d and 9 cal/g fat)	5 weeks	The experimental diet significantly ($p<0.05$) decreased platelet aggregation time, platelet counts, cholesterol and increased bleeding time, fibrinogen levels, and antithrombic activity compared to the control diet.
Vericel et al., 1986	Single blinded study Old adults (74-95 years old)	Group 1 (n=8): Treatment Period 1: A supplement containing 1 g γ-linolenic acid and 1 g linoleic acid Treatment Period 2: A supplement containing 2 g linoleic acid Group 2 (n=8): Treatment Period 1: A supplement containing 2 g linoleic acid Treatment Period 2: A supplement containing 1 g γ-linolenic acid	2 x 2 month treatment periods	Platelet aggregation in the presence of plasma was significantly reduced after linoleic acid intake as opposed to γ-linolenic acid intake. Platelet aggregation in the absence of plasma was not affected by linoleic acid intake as opposed to γ-linolenic acid intake. γ-linolenic acid intake reduced thrombin-induced thromboxane B2 production compared to linoleic acid intake. Both treatments had no effect bleeding time, levels of anti-thrombin III, plasminogen, and plasma β-TG and PF4.

Table 10. Clinical Trials Evaluating the Effect of Linoleic Acid-enriched Diets on Platelet Aggregation and Bleeding Time

Reference	Study Design and Population	Treatment and Number of Subjects	Duration	Safety Outcomes
Fleischman et al., 1975	2-phase diet study Geriatric adults (60-109 years old) with documented coronary artery disease, diabetes mellitus, or cerebrovascular accident (n=66)	Phase 1: Saturated diet containing approx. 2.89 +/- 0.11% linoleic acid (equiv. to approx.. 5 g linoleic acid/day based on the ingestion of 1650 cal/day and 9 cal/g of fat) Phase 2: Unsaturated diet containing approx.. 5.0 +/- 0.26% linoleic acid (equiv. to approx.. 9 g linoleic acid/day based on the ingestion of 1650 cal/day and 9 cal/g of fat)	2 weeks/phase	The increase in dietary linoleic acid intake was associated with a significant ($p<0.01$) 2-fold increase in platelet aggregation time, and decrease in disaggregation time, indicating a decrease in platelet aggregation. Serum cholesterol also decreased serum cholesterol. Bleeding time was not evaluated.
Challen et al., 1983	3 phase diet study Healthy male volunteers (n=6)	Phase 1: Control diet delivering approximately 9.5 g linoleic acid/day Phase 2: Experimental diet delivering approximately 52.7 g linoleic acid/day Phase 3: Control diet delivering approximately 9.5 g linoleic acid/day Note: Diets were not isogenic; total fat increased from 127 g/d in the control diet to 181 g/d; polyunsaturated/saturated fat ratio increased from 0.3 to 1.13.	3 weeks/phase Note: Following the treatment phase of the experiment, the subjects returned to their normal diet. On the last day, while fasting, 5 of the six subjects took 900 mg aspirin.	Primary platelet aggregation was decreased with ingestion of the experimental diet; secondary aggregation to ADP, but not collagen and thrombin was also decreased. Dilute clot lysis time and bleeding time were not affected by the experimental diet. In contrast bleeding time was significantly increased in the subjects after they returned to their normal diet and took aspirin. Serum cholesterol concentrations were significantly reduced by the experimental diet.

Table 10. Clinical Trials Evaluating the Effect of Linoleic Acid-enriched Diets on Platelet Aggregation and Bleeding Time

Reference	Study Design and Population	Treatment and Number of Subjects	Duration	Safety Outcomes
Fleischman et al., 1979	2-phase diet study Mild hypertensive adults (n=28)	Phase 1: Linoleic acid-enriched diet (increase of the ingestion of linoleic acid by approx. 1.2% of total calories; equiv. an additional 4g linoleic acid/day) Phase 2: Normal diet Note: Other than increasing the amount of linoleic acid in the diet, no attempt was made to alter other normal eating habits, particularly in relation to sodium chloride intake.	1 month/phase	The linoleic acid-enriched diet: • Significantly ($p<0.01$) decreased systolic and diastolic blood pressure, which remained unchanged following the return to the normal diet; • Significantly increased platelet aggregation time ($p<0.05$), which returned to pre-experimental levels after the return to the normal diet; • Significantly decreased serum cholesterol ($p<0.01$), which returned to pre-experimental levels after the return to the normal diet; • Significantly decreased serum sodium ($p<0.01$). Bleeding time was not evaluated.

Table 10. Clinical Trials Evaluating the Effect of Linoleic Acid-enriched Diets on Platelet Aggregation and Bleeding Time

Reference	Study Design and Population	Treatment and Number of Subjects	Duration	Safety Outcomes
Boberg et al., 1986	2-phase diet study Healthy male and female adults (ages 33-7 years old; n=17)	Baseline diet: Contained 12.5 g linoleic acid/d (based on the ingestion of 2500 calories/d; a caloric value of 9 cal/g fat; and linoleic content in the diet of 4.5%) Treatment diet: Contained 45 g linoleic acid/d (based on the ingestion of 2500 calories/d; a caloric value of 9 cal/g fat; and linoleic content in the diet of 16.5 %)	1 week/diet	The treatment diet significantly ($p<0.05$) decreased cholesterol and triglycerides in all lipoprotein fractions. The treatment diet decreased the amount of saturated and monosaturated fatty acids, and increased the amount of linoleic acid in all plasma lipid esters. The treatment diet reduced the amount of 18:1 n-9, significant increased the amount of linoleic and arachidonic acid, and had no effect on saturated fatty acids in platelet phospholipids. The treatment diet had no effect on platelet aggregation. Bleeding time was not evaluated.

D. ANIMAL STUDIES

The safety of γ-linolenic acid intake has been evaluated in four toxicology studies using oils derived from transgenic plant sources and borage oil (Tso et al., 2012; Liu et al., 2004; Wainwright et al., 2003; Palombo et al., 2000). Importantly, all of these studies administered diets containing greater than 10% fat to rats, which are higher than recommended amount of 5% (National Research Council, 1982). As discussed in the response letter to GRN 41, the administration of a macronutrient, outside recommended levels in a toxicology study makes it difficult to distinguish whether an observed effect related to the test material is a normal physiological response to the high dietary load of the particular macronutrient, deficiency of another dietary ingredient resulting from the presence of a large amount of the test material in the diet, or a toxicological effect. Rats fed diets high in fats can develop non-alcoholic fatty liver disease, which is a pathological condition associated with increase blood levels of AST, ALT and insulin resistance (i.e., elevated blood glucose levels) (Li et al., 2013). Considering the anti-inflammatory activity of γ-linolenic acid (see Chapter 3), the increases in blood glucose, AST, ALT, and LDH levels noted in studies conducted by Tso et al. (2012) and Li et al., (2004) represent a generalized effect of consuming a high fat diet rather than a specific effect of consuming high levels of γ-linolenic acid. Importantly, these effects would not occur in humans because their recommended diet should contain approximately 20-40% fat (U.S. Department of Agriculture and U.S. Department of Health and Human Services, 2010).

1. Toxicology Studies

a. SONOVA

Pathogen-free male and female Sprague-Dawley rats were fed semi-synthetic, pelleted diets containing 10% fat (wt/wt) composed of either 47% SONOVA, 27% soybean oil, and 26% safflower oil, or 90% borage oil and 10% canola oil (Tso et al., 2012). Importantly, γ-linolenic acid levels were similar in both diets (approximately 21% of the total fat) and except for 10 times more gadoleic and nervonic acid, and 30 times more erucic acid in the borage oil-containing diet, abundance of the remaining fatty acids in the two diets was similar.

Rats were placed in one of two weight-matched groups for each gender and received either the SONOVA test diet (HGSO) or borage oil control diet for the entire 90-day study period. Clinical appearance, body weight and feed intake were monitored weekly. Body fat mass composition was determined using an EchoMRI at baseline and at week twelve. Unfasted total lipids in plasma, liver, kidney, spleen, mesenteric fat, and feces were determined at study termination.

All rats survived to the end of the 90-day feeding trial and scheduled termination. There was no evidence of adverse clinical, motor activity, or behavioral observations in any of the animals throughout the study period. Mean daily feed consumption by the borage diet male controls (18.1 g/rat/d) were similar to the HGSO-treated males (18.5 g/rat/d) ($P > 0.05$). Female rats had lower feed consumption versus males, but daily feed consumption was not different between the borage oil- (13.1 g/rat/d) and HGSO-treated (12.7 g/rat/d) ($P > 0.05$) groups.

Based on the average daily feed consumption data for males and females and analysis of diet samples to confirm γ-linolenic acid concentration, the mean daily intake of γ-linolenic acid for males was 888 mg γ-linolenic acid/kg bw/day for the borage group and 913 mg γ-linolenic acid/kg bw/day for rats given HGSO. Female rats had a mean daily intake of 1191 mg γ-linolenic acid/kg bw/day for the borage group and 1161 mg γ-linolenic acid/kg bw/day for the SONOVA rats.

Similar growth patterns and no significant differences in body weight changes were observed for both borage oil- and HGSO-treated male and female rats throughout the 12 week feeding period. At termination, mean body weights were: 420 ± 11 g and 438 ± 10 g for the males in the borage oil- and HGSO-treated groups, respectively, and; 234 ± 4 and 236 ± 7 g for the females in the borage oil- and HGSO-treated groups, respectively.

Fecal fat content was not significant different in females administered HGSO or borage oil. In contrast, the administration of borage oil to the males led to significantly higher amounts of fecal fat (1.1%) compared to the males receiving HGSO (0.47%). Notably, the authors speculated that because the percentage of fecal fat was below 1.5%, there were no abnormalities in digestion or absorption of either the HGSO or borage oil blends.

The absolute and relative mean weights of the liver, spleen, kidney, lung, and heart were similar among male and female rats in the borage oil- and HGSO-treated groups (Table 11). The evaluation of organ (kidney, spleen, and middle lobe of the liver) histology revealed very mild focal tubule dilation with protein and no histologic damage in the kidneys of some male and female rats given either borage oil or HGSO. Because these changes were not observed in all rats within the borage oil- and HGSO-treated groups, the findings were not considered to be borage oil- or HGSO-related. One female rat given borage oil and one female rat given HGSO had a mild diffuse hepatocellular vacuolization. This change was reported by the authors to be frequently observed in untreated rodents and was not considered a significant alteration. One male and one female rat from both the borage oil- and HGSO-treated groups also had mild diffuse subcapsular edema. Although edema is an abnormal finding, it was attributed by the authors to likely be due to osmolarity alterations during tissue fixation and processing and was considered to be artifacts unrelated to the diets.

Table 11. Absolute (abs) and Relative (rel) Major Organs Weights of Male and Female Sprague-Dawley Rats Fed a Diet Containing Either a Borage Oil Blend or a High γ-Linolenic Safflower Oil (HGSO) Blend for 12 Weeks[a]

Organ	Weight	Male		Female	
		Borage oil	HGSO	Borage oil	HGSO
Liver	Abs (g)	10.30 ± 0.30	10.20 ± 0.30	5.80 ± 0.10	5.60 ± 0.20
	Rel (%)	2.40 ± 0.04	2.30 ± 0.03	2.50 ± 0.03	2.40 ± 0.03
Spleen	Abs (g)	1.20 ± 0.04	1.10 ± 0.04	0.90 ± 0.02	0.90 ± 0.03
	Rel (%)	0.27 ± 0.00	0.25 ± 0.00	0.37 ± 0.00	0.36 ± 0.00
Kidney	Abs (g)	1.50 ± 0.03	1.50 ± 0.03	0.90 ± 0.01	0.90 ± 0.01
	Rel (%)	0.35 ± 0.00	0.34 ± 0.00	0.40 ± 0.00	0.40 ± 0.00
Mesenteric Fat	Abs (g)	4.00 ± 0.30	4.00 ± 0.20	1.70 ± 0.10	1.90 ± 0.20
	Rel (%)	0.95 ± 0.08	0.92 ± 0.04	0.72 ± 0.05	0.78 ± 0.07
Lung	Abs (g)	1.90 ± 0.04	1.80 ± 0.05	1.60 ± 0.04	1.50 ± 0.02
	Rel (%)	0.44 ± 0.00	0.42 ± 0.00	0.67 ± 0.02	0.65 ± 0.02
Heart	Abs (g)	1.70 ± 0.03	1.70 ± 0.04	1.20 ± 0.01	1.20 ± 0.03
	Rel (%)	0.40 ± 0.00	0.39 ± 0.00	0.50 ± 0.01	0.50 ± 0.01
Total Body Weight	(G)	420 ± 11	438 ± 10	234 ± 4	236 ± 7

Data represents mean +/- standard error of the mean (N=12/group). Relative weights of organs are expressed as percentages of total body weights.

[a]Adopted from Tso et al., 2012.

There were no adverse effects attributed to the ingestion of HGSO or borage oil on any of the hematology parameters for both male and female rats (Table 12). Statistically significant differences were not considered adverse because they were minor and within the normal range of variation for each particular hematological parameter (with the exception of basophils (% WBC) which was slightly above historical laboratory range).

Table 12. Hematological Parameters of Male and Female Sprague-Dawley Rats Fed a Diet Containing a Borage Oil Blend or a High γ-Linolenic Safflower oil (HGSO) Blend for 12 Weeks*, [b]

	Male		Female	
Parameter [normal range]	Borage Oil	HGSO	Borage Oil	HGSO
RBC ($\times 10^6/\mu L$) [5–10]	8.3 ± 0.1	8.6 ± 0.1	7.4 ± 0.1	8.1 ± 0.2[a]
TotalWBC ($\times 10^3/\mu L$) [3–17]	4.1 ± 0.4	4.6 ± 0.6	1.8 ± 0.2	2.2 ± 0.2
Neutrophils (% WBC) [13–26]	17.7 ± 2.5	21.9 ± 1.8	14.6 ± 1.3	13.5 ± 1.7
Lymphocytes(%WBC) [65–83]	76.8 ± 2.6	70.7 ± 2.3	79.9 ± 1.9	81.3 ± 2.0
Monocytes (% WBC) [0–4]	4.5 ± 0.8	3.0 ± 0.6	1.6 ± 0.8	1.0 ± 0.5
Eosinophils (% WBC) [0–4]	0.8 ± 0.2	2.5 ± 0.4[a]	4.1 ± 1.0	2.5 ± 0.6
Basophils (% WBC) [0–1]	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	1.5 ± 0.6[a]
Hemoglobin (gm/dl) [11–19]	15.7 ± 0.2	14.4 ± 1.3	14.3 ± 0.1	15.1 ± 0.2[a]
Hematocrit (%) [35–57]	53.1 ± 4.0	44.8 ± 0.4	38.8 ± 2.0	44.5 ± 1.1[a]
MCH (pg) [18–23]	18.5 ± 0.2	18.3 ± 0.1	19.2 ± 3.5	19.3 ± 0.2
MCHC (gm/dl) [31–40]	33.8 ± 0.1	34.5 ± 0.2[a]	28.7 ± 3.0	34.7 ± 01
MCV (fl) [46–65]	54.7 ± 0.6	52.9 ± 0.3	55.2 ± 0.4	55.7 ± 0.4
Platelets ($\times 10^3/\mu l$) [200–1500]	761 ± 14	832 ± 14[a]	858 ± 26	782 ± 15[a]
PT (sec) [19.3–32.6]	20.5 ± 0.5	18.7 ± 0.2	18.5 ± 0.1	18.5 ± 0.2

Values in brackets indicate the normal range for the laboratory rat.

*Data represent means +/- SEM (n=6 male borage oil, n=12 male HCSO, n=11 female borage oil, n=12 female HGSO).

RBC, Red Blood Cell; WBC, White Blood Cell; MCH, Mean Corpuscler Hemoglobin; MCHC, Mean Corpuscular Hemoglobin Concentration; MCV, Mean Corpuscular Volume; PT, Prothrombin time.

[a]Indicates statistically significant difference from borage oil blend diet ($P<0.05$).

[b]Adopted from Tso et al., 2012.

The analysis of the serum biochemistry profiles revealed that although the levels of glucose in the males of the borage oil-treated group, aspartate aminotransferase (AST) in males and females of the borage oil- and HGSO-treated groups, alanine aminotransferase (ALT) in the males of the borage oil- and HGSO-treated groups, and lactate dehydrogenase (LDH) in males and females of the borage oil- and HGSO-treated groups were elevated, the remaining serum components were all within the normal range for laboratory rats (Johnson et al., 2008; Table 13).

Table 13. Serum Biochemistry Profiles of Male and Female Sprague-Dawley Rats Fed on a Diet Containing Either the Borage Oil Blend or the High γ-Linolenic Safflower Oil (HSGO) Blend at the End of 12-Week Feeding*, [b]

Parameter [normal range]	Male		Female	
	Borage Oil	HGSO	Borage Oil	HGSO
Sodium (mEq/l) [142–154]	143 ± 0.8	143 ± 0.4	144 ± 0.3	142 ± 0.4[a]
Potassium (mEq/l) [3.6–9.2]	5.4 ± 0.2	4.5 ± 0.2[a]	4.1 ± 0.2	4.7 ± 0.3
Chloride (mEq/l) [84–110]	108 ± 0.6	106 ± 0.5[a]	107 ± 0.5	106 ± 0.3
Phosphorus (mg/dl) [4.7–16]	6.8 ± 0.2	6.2 ± 0.2	5.6 ± 0.2	5.8 ± 0.3
Calcium (mg/dl) [9.1–15.1]	10.2 ± 0.1	9.8 ± 0.1[a]	9.4 ± 0.1	9.3 ± 0.1
Bicarbonate (mEq/l) [12.6–32]	22.4 ± 0.8	22.8 ± 0.6	19.1 ± 0.7	22.1 ± 0.7
BUN (mg/dl) [11–23]	16.8 ± 0.5	16.8 ± 0.4	14.9 ± 0.5	18.0 ± 0.7[a]
Total Protein (gm/dl) [4.5–8.4]	5.9 ± 0.1	5.9 ± 0.1	6.0 ± 0.1	5.7 ± 0.0[a]
Albumin (gm/dl) [2.9–5.9]	3.2 ± 0.1	3.2 ± 0.0	3.5 ± 0.0	3.3 ± 0.0[a]
Glucose (mg/dl) [50–135]	153 ± 4	125 ± 5[a]	74.8 ± 1.4	86.2 ± 5.3
Globulin (gm/dl) [1.8–3.0]	2.6 ± 0.0	2.7 ± 0.0	2.5 ± 0.0	2.5 ± 0.0
Alb/Glob Ratio [0.72:1.21]	1.0 ± 0.0	1.0 ± 0.0	1.1 ± 0.1	1.0 ± 0.0
Uric Acid (mg/dl) [1.2–7.5]	0.8 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	0.9 ± 0.1
Cholesterol (mg/dl) [40–130]	92.6 ± 1.8	85.8 ± 3.6	103.4 ± 3.1	80.1 ± 3.4[a]
Triglycerides(mg/dl) [26–145]	34.4 ± 2.2	26.7 ± 1.2[a]	25.6 ± 1.8	26.7 ± 1.2
Liver Function Test				
AST (IU/l) [46–81]	159 ± 16	159 ± 43	98 ± 5	92 ± 5
ALT (IU/l) [17.5–30.2]	45.7 ± 3.7	46.1 ± 10.2	19.0 ± 1.1	27.1 ± 1.3[a]
LDH (IU/l) [61–121]	1350 ± 168	1426 ± 128	769 ± 39	800 ± 62
AP (IU/l) [57–128]	63.6 ± 3.1	72.8 ± 3.1	52.3 ± 1.8	53.9 ± 1.3
Total Bilirubin (mg/dl) [0– 0.64]	0.2 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0

ALT, alanine aminotransferase; AST aspartate aminotransferase; LDH, lactate dehydrogenase; BUN, blood urea nitrogen; AP, alkaline phosphatase; Alb/Glob albumin/globulin ratio.

Values in brackets indicate the normal range for the laboratory rat (Johnson et al., 2008).

*Data represent means +/- SEM. (n=10 male borage oil, n=11 male HGSO, n= 12 female borage oil, n= 12 female HGSO).

[a]Indicates statistically significant difference from borage oil blend diet ($P<0.05$).

[b]Adopted from Tso et al., 2012.

The levels of linoleic acid in phospholipids of plasma, liver, kidney, spleen and mesenteric fat in rats given HGSO were significantly higher than those given borage oil. Although this was not an unexpected effect because the HGSO diet contained higher amounts of linoleic acid than the borage oil diet (39.1% vs 34.1%), the higher ratio of n-3/n-6 fatty acids in the HGSO may have contributed to a reduction in linoleic acid desaturation because n-3 fatty acids are the preferred substrates for Δ6-desaturase. Notably, Tso et al. considered all changes in the percentage of γ-linolenic, di-homo γ-linolenic, arachidonic, adrenic (22:4n-6), and docopentanoic acid (22:5n-6) in plasma and tissue phospholipids to be minor in magnitude and did not reflect adverse or significant changes in fatty acid metabolism.

To assess whether the absorption and metabolism of fat from the HGSO diet was similar to the fat provided in the borage oil diet, total fecal fat and fatty acid composition of the phospholipids in plasma, liver, kidney and spleen were measured after 12 weeks. Total fecal fat content was similar among the diet groups for females. However, male rats given borage oil had

higher fecal fat levels compared to males given HGSO. Although the percentage of fecal fat was higher in borage oil fed males, the percentages of fecal fat for all four dietary groups in this study were lower when compared to similar studies assessing the metabolic effects of 30-day or 90-day feeding of diets containing either borage oil or SONOVA (Liu et al., 2004; Palombo et al., 2001). Thus, with a percentage of fecal fat below 1.5% for all groups, the data suggest that there were no abnormalities in the digestion and/or absorption of either SONOVA or borage oil. These data are also consistent with a study by Christensen and Hoy (1996), which found no significant differences in the absorption and metabolism of fatty acids from current commercial sources of γ-linolenic acid (borage, evening primrose, black currant seed oils).

In conclusion, these data show that compared to consuming a diet containing borage oil, consuming a diet containing SONOVA does not produce adverse effects on growth, body composition, hematology, organ weight or histology. Importantly, although the increases in ALT, AST, and LDH levels were noted in both the borage oil- and HGSO-treated groups relative to normal levels of these enzymes in laboratory rats, the magnitude of these increases were similar in both groups. Typical laboratory rat diets contain 5% fat and, moreover, studies have shown that rats consuming diets containing 20% fat develop non-alcoholic fatty liver disease, which is an inflammatory condition associated with increase blood levels of AST, ALT and insulin resistance (i.e., elevated blood glucose levels) (Li et al., 2013). In this study, the diets contained 10% fat, with similar levels of γ-linolenic acid. Thus, considering the anti-inflammatory activities associated with γ-linolenic acid (see Chapter 3), it is likely that the increases in ALT, AST, and LDH reported in this study are the result of the rats consuming high fat diets rather than a specific effect of consuming high levels of a particular fatty acid such as γ-linolenic acid. In addition, these increases would not occur in humans because their recommended diet should contain approximately 20-40% fat (U.S. Department of Agriculture and U.S. Department of Health and Human Services, 2010).

b. Other High γ-Linolenic Containing Oils

Liu et al. (2004) evaluated the effects of a high- γ-linolenic acid canola oil (HGCO), which was obtained from a genetically engineered strain of canola and borage oil on growth, hematology, serum biochemistry, and n-6 fatty acid metabolism in rats. Pathogen-free male Sprague-Dawley rats were randomly assigned (10 rats/diet) to receive one of four experimental diets for 12 weeks. The experimental diets were modified semisynthetic fat free powdered diets fortified with vitamins and supplemented with 5, 10, or 15% (w/w) of HGCO or 15% borage oil. Importantly, the levels of essential nutrients were similar in the different diets and the amount of carbohydrates was reduced to accommodate the increases in fat, resulting in higher energy

densities in the higher fat diet. Moreover, the HGCO-supplemented diets contained approximately 36% whereas the borage-oil supplemented diet contained 21.8% γ-linolenic acid. Assuming the rats ate approximately 1 g feed/day, the daily intake of γ-linolenic acid for the 5, 10, or 15% HGCO- or 15% borage oil-supplemented diets would be 1.8, 3.6, 5.4, and 3.1 mg/d.

The clinical appearances, body weights, and diet consumption were monitored weekly. At the end of 12 weeks, blood from each rat was collected for hematological studies and analysis of cholesterol and fatty acid composition; serum was collected for biochemical analysis, and feces were collected for analysis of total fat content. Liver, heart, kidneys, and spleen were excised and weighed. The kidney, spleen, and the middle lobe of the liver were fixed in formalin for histologic assessment. Aliquots of liver, skeletal muscle, and epididymal adipose tissue from all the rats were taken for lipid analyses (total triacylglycerol content, total cholesterol content, and phospholipid fatty acid composition). Hematological parameters included red blood cell count, white blood cell count, (monocytes, neutrophils, eosinphils, lymphocytes), platelet count, hematocrit, hemoglobin, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). Biochemical tests on serum included alkaline phosphatase, lactate dehydrogenase (LDH), aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, globulin, albumin/globulin ratio, total protein, blood urea nitrogen (BUN), creatinine, BUN/creatinine ratio, bilirubin (direct, indirect, and total), glucose, iron, sodium, potassium, chlorine, calcium, and phosphorus.

Although γ-linolenic acid intakes could not be calculated because the feed intakes were not correctly reported, rats fed the 5, 10, and 15% HGCO diets had similar growth patterns and their body weights were not significantly different from each other throughout the 12-week feeding period (Table 14). At termination, the mean body weights were 479, 487, and 478 g for the 5, 10, and 15% HGCO dietary groups, respectively, while the mean weight gains were 410, 417, and 405 g for the 5, 10, and 15% HGCO dietary groups, respectively. During the first 6 weeks, the three HGCO dietary groups collectively had similar body weights to the 15% borage oil dietary group. However, beyond week 6 and through the rest of study, rats fed the HGCO diets tended to have lower body weights and weight gains than those fed the 15% borage oil diet. At the end of study, rats fed the 15% borage oil diet had a mean body weight of 510 g, which was significantly higher than the 478 g of rats fed the 15% HGCO diet. Rats given 15% HGCO and 15% borage oil diets had similar fecal total fat contents of 5% (w/w), which was significantly higher than the 2.7 and 3.3% of rats given the 5 and 10% HGCO diets, respectively.

The absolute and relative weights of organs (heart, kidney, liver, and spleen) were similar among rats fed the 5, 10, and 15% HGCO and 15% borage oil diets (Table 14). Histopathology revealed that the kidneys and spleens were comparable among the four dietary groups. There were localized fat infiltrations (focal fatty metamorphosis) in the peri-portal (triad) area of the livers in rats from all the dietary groups based on the visual examination of a subset (n=4) of rats from each dietary group. On average, the 5 and 10% HGCO and 15% borage oil dietary groups had fat infiltration in the peri-portal area of liver.

Table 14. Absolute (abs) and Relative (rel) Liver, Heart, Spleen and Kidney Weights in Male Sprague-Dawley Rats Fed a Diet Containing 5, 10, or 15% (w/w) of High-γ-Linolenic Acid Canola Oil (HGCO) or 15% (w/w) of Borage Oil for 12 Weeks[a, b]

	weight	5% HGCO	10% HGCO	15% HGCO	15% BO
liver	abs (g)	15.0 ± 0.6	15.7 ± 0.6	14.6 ± 0.5	15.8 ± 0.6
	rel (%)	3.1 ± 0.1	3.2 ± 0.1	3.1 ± 0.0	3.1 ± 0.1
heart	abs (g)	1.4 ± 0.0	1.4 ± 0.1	1.4 ± 0.1	1.4 ± 0.0
	rel (%)	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0
spleen	abs (g)	1.1 ± 0.1	1.1 ± 0.1	1.0 ± 0.1	1.2 ± 0.1
	rel (%)	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
kidney	abs (g)	3.0 ± 0.1	2.9 ± 0.1	2.8 ± 0.1	3.1 ± 0.1
	rel (%)	0.6 ± 0.0	0.6 ± 0.0	0.6 ± 0.0	0.6 ± 0.0
total body	abs (g)	479 ± 12.1	487 ± 17.3	478 ± 16.6	510 ± 10.3

[a]Data represent means +/- standard error of the mean (SEM) (n=10). Relative weights of organs are expressed as percentages of total body weights.

[b]Adopted from Liu et al., 2004.

There were no significant differences in any of the hematology parameters among dietary groups with the exception of the MCV value, which was slightly, but significantly lower in the 5% HGCO dietary group as compared with those in the 10 and 15% HGCO and 15% borage oil dietary groups. However, this difference was minor and within the normal historical range of variation (Table 15) and no dose response relationship was observed.

Table 15. Hematological Parameters of Male Sprague-Dawley Rats Fed a Diet Containing 5, 10, or 15% (w/w) of High-γ-Linolenic Acid Canola Oil (HGCO) or 15% (w/w) of Borage Oil (BO) for 12 Weeks[a, b]

	5% HGCO	10% HGCO	15% HGCO	15% BO
RBC ($10^6/\mu L$)	8.2 ± 0.1	8.1± 0.1	7.8 ± 0.1	7.8 ± 0.1
total WBC ($10^3/\mu L$)	4.7 ± 0.4	4.6 ± 0.6	3.8 ± 0.4	4.8 ± 0.4
monocytes (% WBC)	3.8 ± 0.5	4.2 ± 0.5	5.2 ± 0.7	5.3 ± 1.2
neutrophils (% WBC)	8.5 ± 0.7	7.7 ± 1.1	11.0 ± 1.5	9.6 ± 1.3
eosinophils (% WBC)	2.2 ± 0.4	1.6 ± 0.2	1.9 ± 0.3	1.7 ± 0.3
lymphocytes (% WBC)	86.3 ± 1.1	86.9 ± 1.3	82.2 ± 1.5	83.7 ± 2.2
platelet count ($10^3/\mu L$)	802 ± 43.9	692 ± 33.5	762 ± 24.8	795 ± 26.8
hematocrit (%)	39.7 ± 0.5	40.8 ± 0.4	39.1 ± 0.5	40.4 ± 0.7
hemoglobin (g/dL)	14.5 ± 0.2	14.9 ± 0.1	14.4 ± 0.2	14.8 ± 0.3
MCH (pg)	17.9 ± 0.4	18.5 ± 0.3	18.7 ± 0.2	18.9 ± 0.3
MCHC (g/dL)	36.7 ± 0.3	36.7 ± 0.3	37.3 ± 0.4	36.9 ± 0.2
MCV (fL)	48.2 ± 0.8^x	50.5 ± 0.4^y	49.7 ± 0.6^{xy}	51.2 ± 0.4^y

[a]Mean +/- SEM (n=10 except n=7 for eosinophils); RBC, red blood cell; WBC, white blood cell; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume. Data followed by the same letter are not significantly different ($p<0.05$).

[b]Adopted from Liu et al., 2004.

There were no significant differences in any of the clinical chemistry parameters among dietary groups with the exception of the calcium level which was slightly, but significantly lower in the 15% HGCO dietary group as compared with the 15% borage oil dietary group (Table 16). However, the difference was minor and the value was within the normal historical range of variation and was not considered to be physiologically relevant.

Table 16. Serum Biochemistry of Male Sprague-Dawley Rats Fed a Diet Containing 5, 10, or 15% (w/w) of High-γ-Linolenic Acid Canola Oil (HGCO) or 15% (w/w) of Borage Oil for 12-Weeks[a, b]

	5% HGCO	10% HGCO	15% HGCO	15% BO
ALT(U/L)	34.8 ± 5.7	39.1 ± 6.9	33.0 ± 2.3	36.2 ± 4.0
AST (U/L)	112 ± 14.8	149 ± 29.8	169 ± 42.2	178 ± 46.4
LDH (U/L)	882 ± 167	1001 ± 295	1014 ± 224	1377 ± 341
alkaline phosphatase (U/L)	91.9 ± 3.0	105 ± 5.4	104 ± 5.0	95.1 ± 7.1
direct bilirubin (mg/dL)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
indirect bilirubin (mg/dL)	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0
total bilirubin (mg/dL)	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.0
albumin (g/dL)	2.9 ± 0.1	3.0 ± 0.0	3.0 ± 0.0	3.1 ± 0.1
globulin (g/dL)	3.7 ± 0.1	3.6 ± 0.1	3.6 ± 0.1	3.5 ± 0.1
albumin/globulin ratio	0.8 ± 0.0	0.9 ± 0.0	0.8 ± 0.0	0.9 ± 0.1
total protein (g/dL)	6.7 ± 0.1	6.6 ± 0.1	6.5 ± 0.1	6.6 ± 0.1
BUN (mg/dL)	28.9 ± 1.6	30.0 ± 1.0	28.1 ± 2.1	29.3 ± 2.0
creatinine (mg/dL)	0.7 ± 0.0	0.7 ± 0.0	0.7 ± 0.0	1.1 ± 0.4
BUN/Creatinine Ratio	44.7 ± 2.1	46.0 ± 1.6	42.6 ± 3.3	45.6 ± 4.7
glucose (mg/L)	178 ± 11.0	173 ± 10.6	173 ± 7.7	190 ± 8.6
carbon dioxide (mEq/L)	25.8 ± 0.6	27.3 ± 0.3	27.4 ± 0.8	25.9 ± 0.8
anion gap	23.4 ± 1.1	22.7 ± 0.7	21.4 ± 1.1	22.7 ± 1.0
iron (μg/dL)	260 ± 25.2	281 ± 16.1	293 ± 18.4	313 ± 10.3
chloride (mEq/L)	98.9 ± 0.4	99.2 ± 0.5	99.6 ± 0.3	99.1 ± 0.5
potassium (mEq/L)	4.2 ± 0.1	4.2 ± 0.1	4.2 ± 0.1	4.5 ± 0.2
sodium (mEq/L)	148 ± 0.3	149 ± 0.5	148 ± 0.7	148 ± 0.5
phosphorus (mg/dL)	7.0 ± 0.2	7.3 ± 0.2	6.5 ± 0.2	6.7 ± 0.2
calcium (mg/dL)	10.7 ± 0.1^{xy}	10.7 ± 0.1^{xy}	10.5 ± 0.1^{x}	11.0 ± 0.1^{y}

[a]Data represent means +/- SEM (n=10). ALT, alanine aminotransferase; AST, aspartate aminotransferase; LDH, lactate dehydrogenase; BUN, blood urea nitrogen. Data followed by the same letter are not significantly different ($p<0.05$).

[b]Adopted from Liu et al., 2004.

The plasma cholesterol levels were similar in the HGCO-treated groups, although compared to the borage-oil treated group, there was a significant reduction in the plasma cholesterol levels in the 15% HGCO-treated group. In contrast, liver cholesterol increased dose-dependently in the HGCO-treated groups while only the 15% HGCO-supplement diet was significantly different than the liver cholesterol in the borage oil-treated group.

Analysis of different fatty acid levels in plasma, liver, and muscle revealed that linoleic and γ-linolenic acid were generally higher in the 10 and 15% HGCO-treated groups compared to the 5% HGCO-treated group whereas docosapentaenoic acid was lower in the 10 and 15% HGCO-treated groups than the 5% HGCO-treated groups. In the 15% HGCO treated group there were also significant increases in linoleic, adrenic, and docosapentaenoic acid, and significant reductions in γ-linolenic and di-homo γ-linolenic acid compared to the 15% borage oil-treated

group. Although the reasons for the reduction are unclear, it may have been due to a difference in the stereospecific distribution of γ-linolenic acid in HGCO and borage oil. The majority (75%) of γ-linolenic acid in HGCO was located at the sn-1, 3 positions and only 25% at sn-2 position of the triacylglycerol (TG) molecules (Liu et al., 2001, cited in Liu 2004), whereas less than half (45.8%) of 18:3n-6 in borage oil was located at sn-1 and sn-3 positions and 54.2% at sn-2 position (Lawson et al. 1988, cited in Liu et al., 2004). Because lipoprotein lipase more readily hydrolyzes the fatty acids at the sn-1 and sn-3 positions of triglyceride molecules, the greater distribution of γ-linolenic acid at these two positions in HGCO would allow the γ-linolenic acid molecules of HGCO to be more readily available to the tissues when compared with borage oil. Hempenius et al. (2000) have also reported the presence of fat vacuoles in the liver in rats fed a diet containing 13% of a highly unsaturated fat. The finding of hepatic fat infiltration in four dietary groups is likely due to the high levels of PUFA in the diets.

Overall, all groups had comparable liver weights and levels of liver enzymes (ALT, AST, LDH, and alkaline phosphatase) and bilirubins (direct, indirect, and total), indicating that liver function among the four dietary groups was similar. Interestingly, AST, ALT, LDH and blood glucose concentrations were outside the normal range for laboratory rats and resemble the biochemical aspects of non-alcoholic fatty liver disease, which was also observed in the 90-day toxicology study with HGSO conducted by Tso et al. (2012). As discussed previously, typical laboratory rat diets containing 5% fat (Johnson et al., 2008). Thus, although the increases in blood AST, ALT, LDH and glucose concentrations relative to their normal ranges are notable, it is unlikely that similar effects would occur in humans because their recommended diet should contain approximately 20-40% fat (U.S. Department of Agriculture and U.S. Department of Health and Human Services, 2010).

The effects of high intakes of γ-linolenic acid on reproduction, growth, and brain and behavioral development were evaluated in B6D2F_1 Sprague-Dawley mice using HGCO and borage oil (Wainwright et al., 2003). The primary objective was to evaluate the effects of diets containing equal amounts of γ-linolenic acid (23%), either from HGCO diluted with palm, corn and flaxseed oil, or borage oil to control diets containing undetectable levels of γ-linolenic acid. The second objective was to determine if there is dose-response to γ-linolenic acid by comparing control diet containing no γ-linolenic acid to a diet containing 23% γ-linolenic acid (HGCO diluted with palm, corn and flaxseed oil), and a diet containing 36% γ-linolenic acid (HGCO). The mice were 8 wk old at the start of the study and were assigned randomly to one of the diets; feeding began 2 wk prior to the commencement of breeding. All diets contained 10% fat and were fed to dams prior to conception and throughout pregnancy and lactation, as well as to the

pups after weaning; effects on reproduction, pup development and pup brain fatty acid composition were assessed.

The pregnant dams were weighed on days 0, 7, and 14. Starting on day 18 they were checked twice daily for births, and the number of live and dead pups recorded. Births occurred on days 19 or 20, and on day 21 dams and litters were weighed and litter size recorded again. At this time, litters were then culled to six pups (three males and three females where possible). Litters were weighed on days 25, 32, 39, and 46 (weaning). Pups were also weighed individually at weaning to compare animals by sex. On day 32 (approximately 12 d after birth), one male and one female from each litter was assessed by a battery of tests for sensorimotor development. All behavioral testing was done independently of knowledge of the treatment group. As a positive control, two additional females from each litter were assessed on day 30. Evidence of slowed development in animals on day 30 compared with day 32 supports the reliability of the behavioral measure. Animals were weaned between days 43 and 48 postconception (about 23 to 28 d after birth) and ear-notched for identification. One male and one female, selected randomly from each litter (excluding the animals previously tested), were weighed, then anesthetized using halothane, and decapitated. The brains were extracted, weighed, and frozen immediately in liquid nitrogen.

There were no significant differences in feed intake, body weight, number of successful pregnancies, gestation length, or maternal weight gain during pregnancy and lactation between the different groups. Although litter size did not differ significantly either at birth (day 19) or on day 21, there was a small difference in pup loss (Table 17). Litter from the dams consuming the diets that containing 23% and 36% γ-linolenic acid, lost slightly more pups, post-parturition, than controls; however, it did not differ significantly from the borage oil-treated group.

Table 17. Litter Size and Pup Weight[a, e]

	CON (*n* = 22)[b]	BO (*n* = 22)	GLA-23 (*n* = 21)	GLA-36 (*n* = 23)
Litter size at birth (day 19)	9.86 ± 1.73	9.27 ± 1.70	10.43 ± 1.03	10.13 ± 1.46
Litter size on day 21	9.45 ± 1.82	8.41 ± 1.87	8.95 ± 1.77	8.65 ± 1.82
Pup loss between birth and day 21	0.41 ± 0.59^{y}	0.86 ± 0.99^{x,y}	1.48 ± 1.47^{x}	1.48 ± 1.41^{x}
Pup weight day 21[c]	1.61 ± 0.18^{x}	1.54 ± 0.16	1.52 ± 0.18	1.45 ± 0.15^{y}
Pup weight day 46 (weaning)	16.75 ± 1.10^{x}	16.65 ± 1.27^{x}	15.31 ± 1.24^{z}	15.65 ± 1.27^{y,z}
Pup brain weight[d] (weaning)	0.349 ± 0.058	0.351 ± 0.053	0.363 ± 0.044	0.354 ± 0.069

[a]Values represent mean +/- SD (litter mean scores, three male and three female pups). Groups that do not share a superscript letter are significantly different [Student-Newman-Keuls (SNK) test, P<0.05].

[b]n=number of litters.

[c]Days postconception, day 0=conception; birth normally occurred on day 19-20.

[d]Based on one male and one female.

Abbreviations: Con, corn oil (92.7%) + soybean oil (7.3 %) = control; BO, borage oil (97.9%) + flaxseed oil (2.1%); GLA-23, palm oil (12%) + corn oil (29.5%) + flaxseed oil (0.5%) + high γ-linolenic acid canola oil (HCGO) (58%); GLA-36 HCGO (100%).

[e]Adopted from Wainwright et al., 2003.

Pups given the diet containing 36% γ-linolenic acid diet weighed less than controls at all times from birth to weaning. On days 39 and 46 (weaning) pups from dams consuming the diets derived from HGCO weighed less than those from dams fed the control and borage oil-supplemented diets, whereas there were no significant differences in the pup weights in the control and borage oil treated groups. There were also no significant differences in brain weight, pup behavioral development across all the groups.

Overall, supplementation with γ-linolenic acid increased levels of specific n-6 fatty acids and decreased those of n-3 fatty acids, predominantly docosahexanoic acid (22:6n-3). Although the effects of both borage oil and 23% γ-linolenic acid relative to control were in the same direction, the effects of 23% γ-linolenic acid were larger than borage oil (Tables 18, 19, and 20). For example, as seen in both arachidonic acid and DHA in the ethanolamine phosphoglycerides

fraction, although borage oil did not increase arachidonic acid relative to control, 23% γ-linolenic acid increased arachidonic acid relative to both control and borage oil. Similarly, the findings for DHA indicated that while borage oil decreased DHA relative to control in the ethanolamine phosphoglycerides fraction, 23% γ-linolenic acid from the HGCO decreased DHA relative to both control and borage oil.

Table 18. Selected Fatty Acid Composition of Brain Ethanolamine Phosphoglycerides (wt % Fatty Acids)[a, b]

	CON (n = 19)	BO (n = 21)	GLA-23 (n = 20)	GLA-36 (n = 21)
Total sat	37.25 ± 1.93	37.36 ± 1.94	37.69 ± 2.1	37.26 ± 2.59
Total mono	18.01 ± 1.40^{p}	17.59 ± 1.35^{p,q}	17.04 ±1.34^{p,q}	16.65 ± 1.29^{q}
Total poly	39.80 ± 2.68	40.30 ± 2.36	39.97 ± 2.22	41.37 ± 2.93
20:3n-6	0.69 ± 0.05^{y}	1.37 ± 0.15^{q}	1.15 ± 0.14^{x}	1.59 ± 0.20^{p}
20:4n-6	14.35 ± 1.47^{x}	14.67 ± 1.40^{x}	16.17 ± 0.97^{q}	17.73 ± 1.94^{p}
22:4n-6	4.43 ± 0.29^{y}	4.86 ± 0.40^{x}	5.97 ± 0.36^{q}	6.34 ± 0.60^{p}
22:5n-6	2.34 ± 0.30^{q}	2.87 ± 0.35^{p}	2.86 ± 0.42^{p}	2.80 ± 0.36^{p}
Total n-6	22.84 ± 1.47^{y}	24.48 ± 1.41^{x}	26.88 ± 1.39^{q}	29.12 ± 2.14^{p}
22:6n-3	16.70 ± 1.43^{p}	15.53 ± 1.34^{q}	12.33 ±1.37^{x}	11.19 ± 1.34^{y}
Total n-3	16.96 ±1.43^{p}	15.83 ± 1.31^{q}	13.09 ± 1.30^{x}	12.25 ± 1.29^{x}
Ratio n-6 to n-3	1.35 ± 0.08^{y}	1.55 ± 0.11^{x}	2.07 ± 0.18^{q}	2.40 ± 0.23^{p}

[a]Values represent mean ± SD (litter mean scores, one male and one female pup, where n = number of litters). Groups that do not share a superscript are significantly different (SNK). Total sat, total saturated FA; Total mono, total monounsaturated FA; Total poly, total PUFA.

[b]Adopted from Wainwright et al., 2003.

Table 19. Selected Fatty Acid Composition of Brain Choline Phosphoglycerides (wt % Fatty Acids)[a, b]

	CON (n = 19)	BO (n = 21)	GLA-23 (n = 20)	GLA-36 (n = 21)
Total sat	63.67 ± 1.25	64.14 ± 1.00	64.35 ± 1.67	64.55 ± 2.38
Total mono	26.47 ± 0.73	26.25 ± 0.76	26.32 ± 0.96	26.07 ± 1.71
Total poly	9.87 ± 1.20	9.61 ± 0.76	9.32 ± 1.16	9.37 ± 1.27
20:3n-6	0.21 ± 0.02^{y}	0.47 ± 0.03^{q}	0.38 ± 0.04^{x}	0.54 ± 0.08^{p}
20:4n-6	5.17 ± 0.65	5.21 ± 0.46	5.38 ± 0.69	5.53 ± 0.78
22:4n-6	0.33 ± 0.06^{y}	0.36 ± 0.04^{x}	0.44 ± 0.05^{q}	0.47 ± 0.05^{p}
22:5n-6	0.36 ± 0.06^{x}	0.49 ± 0.11^{p}	0.43 ± 0.08^{q}	0.42 ± 0.06^{q}
Total n-6	7.52 ± 0.76	7.45 ± 0.51	7.63 ± 0.85	7.82 ± 0.94
22:6n-3	2.31 ± 0.45^{p}	2.10 ± 0.33^{p}	1.56 ± 0.38^{q}	1.37 ± 0.36^{q}
Total n-3	2.35 ± 0.46^{p}	2.16 ± 0.31^{p}	1.70 ± 0.38^{q}	1.56 ± 0.37^{q}
Ratio n-6 to n-3	3.27 ± 0.41^{q}	3.50 ± 0.39^{q}	4.64 ± 0.75^{q}	5.19 ± 0.89^{p}

[a]Values represent mean ± SD (litter mean scores, one male and one female pup, where n = number of litters). Groups that do not share a superscript are significantly different (SNK).

[b]Adopted from Wainwright et al., 2003.

Table 20. Selected Fatty Acid Composition of Brain PS/PI (%wt FA)[a, b]

	CON (n = 19)	BO (n = 21)	GLA-23 (n = 20)	GLA-36 (n = 21)
Total sat	48.54 ± 3.69	49.42 ± 4.04	48.06 ± 3.48	49.18 ± 3.99
Total mono	18.31 ± 1.03	17.86 ± 1.25	18.04 ± 1.60	17.73 ± 1.23
Total poly	33.15 ± 3.88	32.72 ± 4.06	33.89 ± 3.86	33.09 ± 4.53
20:3n-6	0.58 ± 0.06^{y}	1.12 ± 0.11^{q}	0.97 ± 0.12^{x}	1.29 ± 0.17^{p}
20:4n-6	$11.79 \pm 1.23^{p,q}$	11.48 ± 1.20^{q}	12.74 ± 1.44^{p}	$12.17 \pm 1.73^{p,q}$
22:4n-6	2.52 ± 0.17^{y}	2.77 ± 0.23^{x}	3.45 ± 0.44^{q}	3.97 ± 0.27^{p}
22:5n-6	2.79 ± 0.43^{x}	3.37 ± 0.52^{p}	$3.59 \pm 0.69^{p,q}$	3.73 ± 0.42^{q}
Total n-6	18.31 ± 1.57^{q}	19.23 ± 1.71^{q}	21.33 ± 2.17^{p}	21.63 ± 2.08^{p}
22:6n-3	14.71 ± 2.40^{p}	$13.37 \pm 2.50^{p,q}$	$12.08 \pm 1.85^{q,x}$	10.74 ± 2.56^{x}
Total n-3	14.83 ± 2.41^{p}	$13.49 \pm 2.50^{p,q}$	$12.56 \pm 1.93^{q,x}$	11.46 ± 2.59^{x}
Ratio n-6 to n-3	1.25 ± 0.12^{y}	1.45 ± 0.15^{x}	1.72 ± 0.18^{q}	1.94 ± 0.28^{p}

[a]Values represent mean ± SD (litter mean scores, one male and one female pup, where n = number of litters). Groups that do not share a superscript are significantly different (SNK).

[b]Adopted from Wainwright et al., 2003.

In summary, Wainright et al. (2003) evaluated the bioequivalence and safety of HGCO with traditional γ-linolenic acid-rich borage oil on reproduction, growth, and brain and behavioral development in mice. The results indicate that some of the effects of γ-linolenic acid provided as HGCO differed from those of equivalent amounts of γ-linolenic acid provided as borage oil. Specifically, 23% γ-linolenic acid from HGCO reduced pup body weight and was associated with a slight increase in neonatal pup attrition. There were no significant effects on behavioral development or on physical performance. An increase in dietary γ-linolenic acid resulted in an increase in brain n-6 fatty acids and a corresponding decrease in brain n-3 fatty acids. Moreover, despite their similar levels of γ-linolenic acid, the effects on brain fatty acid composition were greater in the mice that received the diet containing 23% γ-linolenic acid derived for HGCO than with those receiving the same amount from borage oil. Comparison of the group receiving 23% γ-linolenic acid from HGCO with that receiving 36% γ-linolenic acid indicated that at the higher level the effects on growth were greater, as were those on brain fatty acid composition, particularly in the ethanolamine phosphoglycerides fraction. The intake of γ-linolenic acid from HGCO was not reported, however, using default assumptions of feed intake of 5 g/day and body weight of 20 g for the mice results in an estimation of intake of γ-linolenic acid by the dams of 5.75 and 9 g/kg/day in the 23% and 36% groups, respectively. This is equivalent to an intake of 345 or 540 g/day for a 60-kg person.

b. Dihomo-γ-linolenic Acid-Containing Oils

The safety of γ-linolenic acid ingestion is also corroborated by an Ames assay, and acute and subchronic oral toxicity studies conducted in rats with a dihomo-γ-linolenic acid-enriched oil (Kawahima et al., 2009).

In the Ames assay, the mutagenicity of 313, 625, 1250, and 5000 µg of a dihomo-γ-linolenic acid-enriched oil was assessed using *Salmonella typhimurium* strains TA100, TA1535, TA98, and TA1537, the *Escherichia coli* strain WP2uvrA/pKM101, and preincubation methods both with and without metabolic activation. In all strains treated with the dihomo-γ-linolenic acid-enriched oil, the number of revertant colonies was less than the solvent/negative control, both with and without metabolic activation,. In addition, no microbial toxicity was observed. Together these results indicate that a dihomo-γ-linolenic acid-enriched oil is neither mutagenic nor cytotoxic.

To assess the acute toxicity of a dihomo-γ-linolenic acid-enriched oil, 6-10 week old male (n=5) and female (n=5) Sprague-Dawley (Crj:CD) rats were administered 10 g/kg of either a dihomo-γ-linolenic acid-enriched oil or soybean oil by gavage. The rats were observed over the next 14 days, and clinical signs and body weights were recorded. On day 14, the rats were euthanized and examined by necropsy. No deaths occurred and loose stools and soiled periproctal regions were observed in both the dihomo-γ-linolenic acid-enriched oil- and soybean oil-treated groups. Body weight gains and clinical signs were also similar in both groups during the in-life portion of the study and at necropsy no macroscopic differences between the two groups were noted.

The subchronic toxicity of dihomo-γ-linolenic acid-enriched oil was determined in 90-day oral toxicity study conducted in rats. Either water, soybean oil, or 0.5 (0.056 g dihomo-γ-linolenic acid /kg bw/day), 1.0 (equivalent to 0.112 g dihomo-γ-linolenic acid/kg bw/day), or 2.0 g/kg bw/day (equivalent to 0.224 g dihomo-γ-linolenic acid /kg bw/day) of the dihomo-γ-linolenic acid-enriched oil was administered once a day by gavage for 13 weeks to 6 week-old male (n=20/group) and female (n=20/group) Sprague Dawley (Crj:CD) rats. The rats were observed over the course of the study and clinical signs, body weights, and feed consumption were recorded. At the end of the study, ophthalmological examinations, urinalysis, hematological examinations, blood biochemistries, necropsy, organ weight, and histopathological examinations were performed.

No deaths occurred and no treatment-related clinical signs were noted throughout the administration period. There were also no treatment-related ophthalmological abnormalities.

Although feed consumption was lower in the soybean oil- and dihomo-γ-linolenic acid-containing oil-treated groups compared to the water-treated groups, feed consumption was similar in the soybean oil- and dihomo-γ-linolenic acid-enriched oil-treated groups. Body weight gain was similar across all the groups. Relative weights of the thyroid and spleen were significantly lower in the males of the 1.0 g/kg/d dihomo-γ-linolenic acid-enriched oil- and soybean oil-treated groups compared to the water-treated group whereas the absolute weights of the submaxillary glands and relative weights for the spleen were significantly higher in the males of the 2.0 g/kg dihomo-γ-linolenic acid-containing oil-treated group and the absolute and relative weights of the uterus in the females of the 1.0 g/kg/d dihomo-γ-linolenic acid-enriched oil-treated group were significantly lower compared to the soybean oil-treated group. Importantly, Kawahima et al., reported that there were no treatment-related macroscopic or histopathological findings and that all observed histopathological changes occurred at comparable frequencies among the different treatment groups and were common findings in rats of the same strain and age.

For the blood biochemistries, total blood protein and albumin were lower in the soybean oil- and dihomo-γ-linolenic acid-enriched oil-treated groups compared to the water-treated group, AST was lower in the males of the 0.5 and 1.0 g/kg/d dihomo-γ-linolenic acid-enriched oil-treated groups and in the females of the soybean-oil treated group compared to the respective sex in water-treated group. BUN was also decreased in the males of the 0.5 and 1.0 g/kg/d dihomo-γ-linolenic acid-enriched oil- and soybean-oil-treated groups compared to the males in the water-treated group. Albumin to globulin ratio was decreased in the males of the 1.0 g/kg/d dihomo-γ-linolenic acid-enriched oil-treated group, but not in the soybean oil-treated group, compared to the males of the water-treated group. In contrast, between the dihomo-γ-linolenic acid-containing oil and soybean-oil treated groups, only lower triglyceride levels were observed in the males treated with 1.0 and 2.0 g/kg/d dihomo-γ-linolenic acid-containing oil.

For urine parameters, total sodium, potassium and chloride levels were lower in the males of the 0.5, 1.0, and 2.0 g/kg/d dihomo-γ-linolenic acid-enriched oil- and soybean oil-treated groups compared to the males in the water-treated group, whereas total excretion of sodium and potassium were lower in the females of the 1.0 g/kg dihomo-γ-linolenic acid-enriched oil-treated group, but not in the soybean oil-treated group, compared to the females in the water-treated group. Importantly, between the soybean oil- and dihomo-γ-linolenic acid-enriched oil-treated groups there were no significant differences in any of the urine parameters measured.

For hematologies, low erythrocyte counts were observed in females treated with 1.0 and 2.0 g/kg/d dihomo-γ-linolenic-enriched oil, high mean corpuscular hemoglobin concentration

(MCHC) was observed in the females treated with 2.0 g/kg/d dihomo-γ-linolenic-enriched oil, lower activated partial thromboplastin time (APTT) was observed in the females treated with 2.0 g/kg/d dihomo-γ-linolenic-enriched oil, low hemoglobin levels, prothrombin time (PT), and APTT were observed in the males treated with 2.0 g/kg/d dihomo-γ-linolenic-enriched oil compared to the respective sex in the water-treated group. Hematocrit was decreased in both males and females in the 2.0 g/kg dihomo-γ-linolenic-enriched oil group compared to the water-treated group. PT and APTT were also lower in the males of the soybean oil-treated group compared to the water-treated group. In contrast, between the dihomo-γ-linolenic acid-containing oil and soybean-oil treated groups, the hematocrits were significantly lower in both males and treated with 2.0 g/kg/d dihomo-γ-linolenic-enriched oil, and the PT was significantly higher in the males treated with the 2.0 g/kg/d dihomo-γ-linolenic-enriched oil.

In conclusion, although decreased feed consumption was observed in both the soybean oil- and the dihomo-γ-linolenic acid groups compared to the water-treated group, this decrease occurred in the absence of organ weight and histopathological abnormalities. Moreover, these results are consistent with the results reported in other studies where rats were fed diets high in corn oil, ARA and/or DHA (Lina et al., 2006). Thus, the decreased feed consumption is most likely the result of consuming large amounts of oil, rather than high amounts of dihomo-γ-linolenic acid. In a similar manner, because the reductions in urinary sodium, potassium, and chloride levels, and the reductions in serum protein, albumin, BUN, and albumin/globulin ratio were not dose-dependent in the dihomo-γ-linolenic acid enriched oil-treated groups and occurred in both the soybean oil- and dihomo-γ-linolenic acid enriched oil-treated groups, the reductions are likely secondary to the decreased feed consumption that results from the increased intake of fat. For the hematological effects, the effects were mild, did not correlate with any perturbations in reticulocyte ratios or histopathological abnormalities in the hematopoietic organ, and thus are not toxicologically significant. Moreover, similar effects have been reported in other studies where in rats were fed other oils containing long chain n-3 fatty acids (Lina et a., 2006, Hempenius et al., 2000; Burns et al., 1999). Lastly, the significant decreases in triglycerides are consistent with the reported effects of long chain n-3 fatty acid-enriched oils (reviewed in Shearer et al., 2012). Therefore, the no-observed-adverse effect level for the ingestion of the dihomo-γ-linolenic acid-enriched oil can be considered to be 2.0 g/kg/day (equivalent to 0.224 g dihomo-γ-linolenic acid /kg bw/day).

2. Equivalence of Fatty Acid Metabolism

Palombo et al. (2000) evaluated the bioequivalency of γ-linolenic acid obtained from transgenic canola plants relative to γ-linolenic acid from borage oil in Sprague Dawley male rats

fed diets enriched with oils from each source to provide approximately 0.23 g γ-linolenic acid/g total fatty acids in each diet. A control group was fed diet containing corn oil. The total lipid content of each diet was 10% by weight. Growth, feed consumption, and fecal fat content (during the second week of study) were measured and after 3 weeks of feeding, fatty acid composition of the principal phospholipid fractions of liver and plasma and triglycerides in epididymal adipose were evaluated to determine effects on the metabolism of the principal n-6 fatty acids in these tissues.

No evidence of adverse effects on general appearance or behavior was reported based on daily observations. Growth of the rats from each dietary treatment group was similar; body weights and body weight gain were not significantly different. Total diet consumption was similar among groups. There were no significant differences among groups with respect to total fecal weight or fat content. Postmortem examination of the major organs did not reveal any adverse treatment effects; mean weights of liver, heart, kidney and spleen were not statistically different among treatment groups. There was no difference between diets for the percentage of total n-6 polyunsaturated fatty acids in total or individual phospholipid fractions. The composition of n-6 fatty acids within the individual liver phospholipids was primarily influenced by the n-6 fatty acids present in the diet. The liver phospholipids from rats fed the γ-linolenic acid from canola and borage oil had higher levels of γ-linolenic acid and dihomo γ-linolenic acid and lower levels of linoleic acid compared with rats given a control diet. The relative percentages of 18:2n-6 and 18:3n-6 in the liver total phospholipids and phosphatidylethanolamine fraction were also similar between the two γ-linolenic acid diet groups. The phosphatidylcholine fraction of liver from the group fed the diet supplemented with high γ-linolenic acid canola oil had a slightly greater percentage of γ-linolenic acid than the group fed the diet supplemented with borage oil. The percentages of dihomo γ-linolenic acid and arachidonic acid were similar between the γ-linolenic acid diet groups in the total and all individual phospholipid fractions. The percentage of linoleic acid liver phosphatidyl-inositol/serine was significantly higher in the γ-linolenic acid canola diet group as compared with the borage oil group.

Total n-6 polyunsaturated fatty acids present in the total and individual phospholipid fractions was similar across the three diet groups. As observed in the liver phospholipids, the composition of n-6 fatty acids within the plasma phospholipid fractions reflected the composition of n-6 fatty acids present in the diet. In general, the plasma phospholipids from rats fed the canola and borage oil diets tended to have higher levels of γ-linolenic acid and di-homo γ-linolenic acid and lower levels of linoleic acid in comparison to the phospholipids from rats

given the control diet. The relative percentages of 18:2n-6, 18:3n-6, 20:3n-6, and 20:4n-6 within the total and individual phospholipid fractions were similar for the γ-linolenic acid diet groups.

The fatty acid composition of the liver and plasma phospholipids largely reflected the fatty acid composition of the diets. The incorporation and metabolism of γ-linolenic acid within the liver and plasma phospholipid fractions was similar between the γ-linolenic acid groups. These results indicate that the metabolism of γ-linolenic acid in these tissues was dependent upon the content of γ-linolenic acid in the diet and not the source of the γ-linolenic acid.

Tso et al. (2002) evaluated the intestinal absorption and lymphatic transport of γ-linolenic acid from transgenic canola oil to borage oil using a lymph fistula rat model. After overnight recovery from surgery, rats received either 1 mL of canola oil (37% γ-linolenic acid; 26% linoleic acid; 22% oleic acid; with other fatty acids) or 1 mL of borage oil (23% γ-linolenic acid; 37% linoleic acid; 14% oleic acid; with other fatty acids). The fatty acid compositions of triglycerides in mesenteric lymph were compared over a 24 hr collection period. The digestion, uptake and lymphatic transport of γ-linolenic acid and the normal physiologic changes associated with fat absorption (e.g., lymph flow and an increase in lymphatic endogenous lipids outputs, triglycerides, cholesterol and phospholipids) were similar in the canola- and the borage oil-fed rats. The original differences in γ-linolenic acid, linoleic and oleic acid content in canola and borage oil were preserved in the fatty acid composition of the rats' lymph lipid. γ-Linolenic acid from canola was absorbed and transported into lymph similarly to borage oil.

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