Application for the Placing on the Market of Chia Seed Oil (*Salvia Hispanica* L.) as a Novel Foods and Novel Food Ingredient pursuant to Article 4 of Regulation 258/97/EC

By

Functional Products Trading S.A.

Application submitted by

K&L GATES

Brussels, October 18, 2012

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I. SUMMARY

Chia oil is obtained from Chia by cold-pressing process. Chia (*Salvia hispanica* L.) is a yearly herbaceous plant of the mint family (Labiatae). It is native to southern Mexico and northern Guatemala and is grown to produce oil for human consumption (Ayerza, 1995).

Chia seed contains about 250–390 g oil/kg of fresh matter (FM) (Ayerza, 1995; Ting *et al.*, 1990). The fatty acids (FA) in chia oil are highly unsaturated, their main components being linoleic acid (LA, C18:2 n–6; 170–260 g/kg of the total FA) and alpha-linolenic acid (ALA, C18:3n–3; 500–570 g/kg of the total FA).

Chia oil's main characteristic is its contribution of essential fatty acids, specially the alpha-linolenic C18:3 w3 acid in high percentages, over 60% of the total fatty acids present. Its contribution of the other essential fatty acid, Linoleic C18:2 w6 acid is lesser, around 18 – 20%.

This special fatty acid composition gives Chia seed oil an outstanding position among vegetal oils, as one of the best natural vegetal sources of alpha-linolenic acid, which is normally considered insufficient in the Western diet.

Data showing that the source organism and/or foods obtained thereof are not detrimental to human health are included in the different sections of this application.

Further evidence supporting the absence of detrimental effects can be drawn from the food safety regulatory approvals in North and South America, Asia and Australasia, as well as the multiple consumer products containing Chia seed and oil which are now consumed worldwide with no reported negative health impacts.

Further, despite the proliferation of Chia seed and Chia oil as food ingredients, and the consumption of whole Chia seed, no events of allergic or other adverse reactions to Chia has been reported and/or recorded to date.

It is anticipated that Chia oil will be consumed (i.e. by vegetarians) as an alternative to flaxseed oil, borage oil and other sources of Omega-3 fatty acids.

Chia oil products have been introduced to the market at an increasing pace and variety. This oil is currently added to a wide variety of food products such as multigrain bread, cereals, pasta, olive oils, and margarines. Furthermore, this oil is sold in grocery stores, naturist shops, supermarkets, and through the Internet, as nutritional supplements in soft capsules or as gourmet cooking oils.

Other than an acute toxicity study showing no adverse effects, no other toxicity in *in vivo* animal testing was considered justifiable in the present case, as derived from previous considerations on Chia oil and its source and from detailed studies on NF composition.

Chia seed and grounded chia has been authorized as novel food ingredient pursuant to Commission Decision 2009/827 of October 13, 2009[1] following a six year evaluation process[2].

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[1] Commission Decision of 13 October 2009 authorizing the placing on the market of Chia seed (*Salvia hispanica*) as novel food ingredient under Regulation (EC) No 258/97 of the European Parliament and of the Council (OJ L 294, 11.11.2009, p. 14). Available online: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:294:0014:0015:EN:PDF.

[2] An application for Chia as a novel food was first submitted by ROBERT CRAIG & SONS LTD before the UK authorities in June 2003 for use in soft grain bread (available online: http://www.acnfp.gov.uk/assess/fullapplics/chia). The European Food Safety Authority (hereinafter, the **"EFSA"**) published an initial opinion in October 2005 whereby it requested additional data to perform a full

Uses other than in bread products, as well as marketing of derived ingredients from Chia, such as oil, require fresh authorisations in accordance with article 4 of Regulation 258/97[3].

On May 27, 2011, THE CHIA COMPANY (Australia) submitted an application to the FSA, to *extend the currently authorised use of chia seeds* (*Salvia hispanica* L) to include a number of additional *food products that commonly contain other seeds* (packaged Chia seeds, baked products (muffins, cookies, crackers and biscuits), breakfast cereals and fruit, nut and seed mixes (sprinkles)). On March 16, 2012, the UK FSA informed DG SANCO on the extension of use of chia seeds to several food categories.

Following the initial approval of Chia as a novel food, FUNCTIONAL PRODUCTS TRADING (*Benexia*) is now hereby applying for an authorization for Chia oil as novel food ingredient pursuant to Article 4 of Regulation 258/97.

As it is developed herein below, Chia oil is considered safe and there is no reason to consider consumption of this novel food as nutritionally disadvantageous to the consumer. It is very unlikely that under the recommended use conditions consumption of Chia seed oil would have an adverse effect on public health.

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nutritional assessment (available online: http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620765844.htm). In 2006, the COLUMBUS PARADIGM INSTITUTE S.A. became responsible for the application, in collaboration with FUNCTIONAL PRODUCTS TRADING S.A., and submitted an amended file to enable EFSA to complete the assessment. EFSA published its second opinion on March 13, 2009 (available online: http://www.efsa.europa.eu/en/scdocs/doc/996.pdf) in which it concluded that it is unlikely that the use of Chia seeds in bread at a maximum of 5 % would have an adverse effect on public health.

[3] Regulation (EC) No 258/97 of the European Parliament and of the Council of 27 January 1997 concerning novel foods and novel food ingredients (OJ L 43, 14.2.1997, p. 1–6).

II. ADMINISTRATIVE INFORMATION

2.1 Applicant

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2.3 Name of the Novel Food Ingredient

Chia Oil

2.4 Date of Application

October 18, 2012

III. NOVEL FOOD SPECIFICATION

Pursuant to Commission Recommendation 97/618/EC concerning the scientific aspects and the presentation of information necessary to support applications for the placing on the market of novel foods [4] (hereinafter, the "**Commission Recommendation**"), the following questions pertaining to the specifications of the Novel Food must be addressed:

a. *"Is there an appropriate specification (including species, taxon, etc, for living organisms) to ensure that the NF marketed is the same as that evaluated?*

b. *"Is the information representative of the NF when produced at commercial scale?*

c. *"Depending on the derivation and composition of the NF, is appropriate analytical information available on potentially toxic inherent constituents, external contaminants and nutrients?"*

3.1 Chemical and Trade name

Chia oil is an alpha-linolenic acid-rich oil produced from the seeds of *Salvia Hispanica L.*

3.2 Novel Food Specification

The Specification for Chia Oil is presented in Table 1.

Table 1. Proposed Specification of Chia Oil

Analisys	Specification	Test Methods
Acid Value (FFA)	<2 % Oleic Acid	AOCS Ca 5a-40
Peroxide Value	< 5 mEq/kg	NF EN ISO 27107
Insoluble Impurities	<0,01 %	AOCS Ca 3a-46
Alfa- Linolenic Acid (ALA)	>60 %	AOCS Ce 1e-91
Linoleic Acid	>15 %	

3.3 Representative Commercial Scale Batch

[4] Commission Recommendation 97/618/EC of 29 July 1997 concerning the scientific aspects and the presentation of information necessary to support applications for the placing on the market of novel foods and novel food ingredients (Official Journal L 253 , 16/09/1997 P. 0001 - 0036).

Quality control analysis results for 7 batches of Chia Oil are provided in Table 2 for those parameters identified in the specification. These clearly show compliance to specification.

Table 2. Analytical Data for Batches of Chia oil

SAMPLE ORIGIN	TRIO 2009-B1	TRIO 2009-B4	TRIO P01/2010-B1	TRIO P02/2010	TRIO P01/2011	TRIO P02/2011	TRIO P03/2011
Impurities (%)	0.05	0.05	0.01	0.02	0.05	0.01	0.01
Peroxides (mEq/kg)	1.4	1.1	1.1	0.21	0.50	0.52	0.5
FFA (% oleic)	0.43	0.85	1.4	1.82	0.23	0.26	0.27
Linoleic C18:2	17.38	17.4	17.2	17.1	18.8	18.6	18.9
Linolenic C18:3	67.04	67.3	66.4	67.8	65.0	66.3	64.3

Certificate of analysis TRIO 2009-B1, 2009-B4, P01/2010-B1, P02/2010, P01/2011, P02/2011, P03/2011 and test method are attached as **Dossier/Appendix.**

3.2.1 Additional Quality Control Testing for Chia Oil

Table 3. Additional Quality Control Testing for Chia Oil

TEST	TYPICAL VALUE
Refractory Index	1,4825 at 40º C
Specific Gravity	0.9330 g/cm^3 at 25º C
Freezing Point	-12º C
Cold Test	Negative >5.5 hours
Viscosity	60 Centipoises
Iodine Index	201
Saponification Index	194 mg/KOH/g
Moisture	<0.03 %
Waxes	296 ppm
Non-saponifiable matter	0,69%
Mineral Oil	Not Detected

Certificated of analysis and test methods on this matter are hereby attached as **Dossier/Appendix.**

3.3 Analytical information

3.3.1 Composition Analysis

a. Nutritional content

Table 4. Nutritional Content (100 g)

Energy (Kcal)	900
Energy (kj)	3700
Total Carbohydrates (g)	<0.1
Dietary Fiber (g)	<0.2
Sugars (g)	
Fructose	<0.2
Glucose	<0.2
Lactose	<0.2
Maltose	<0.2
Sucrose	<0.4
Proteins (g)	<0.5
Total fat (g)	100
Saturated fatty acids (g)	9.3
Monounsaturated fatty acids (g)	6.8
Polyunsaturated fatty acids (g)	79.4
Trans fatty acids (g)	0.1

Certificates of analysis and test method on this matter (EUROFINS AR-11-AA-064258-01/ E4-370-02540264) are hereby attached as **Dossier/Appendix.**

b. Fatty Acids Profile

The composition of fatty acids is evidently a most important parameter in all fats, as it determines its nutritional, physiological, functional and technological properties.

Chia oil's main characteristic is its contribution of essential fatty acids, specially the alpha-linolenic C18:3 w3 acid in high percentages, over 60% of the total fatty acids present. Its contribution of the other essential fatty acid, Linoleic C18:2 w6 acid is lesser, around 18 – 20%. This special fatty acid composition gives Chia seed oil an outstanding position among vegetal oils, as one of the best natural vegetal sources of alpha-linolenic acid, which is normally considered insufficient in the Western diet.

Table 5 shows the average fatty acids composition as extracted from Chia seed oil by the cold-pressing method.

Table 5. Fatty acid profile (%)

C4:0 Butyric acid	<0.05
C5:0 Pentanoic acid	<0.05
C 6:0 Caproic acid	<0.05
C7:0 Enanthic acid	<0.05
C 8:0 Caprylic acid	<0.05
C9:0 Nonanic acid	0.1
C 10:0 Capric acid	<0.05
C11:0 Undecylic acid	<0.05
C11:1 Undecenoic acid	<0.05
C12:0 Lauric acid	<0.05
C12:1 Dodecenoic acid	<0.05
C13:0 Tridecylic acid	<0.05
C13:1 Tridenenoic acid	<0.05
C14:0 Myristic acid	<0.05
C14:1 (n-5c) Myristoleic acid	<0.05
C14:1 (n-5t) Transmyritelaidic acid	<0.05
C 15:0 Pentadecanoic acid	<0.05
C15:1 (n-5c) Pentadecenoic acid	<0.05
C15:1 (n-5t) Transpentadecenoic acid	<0.05
C16:0 Palmitic acid	6.3
C16:1 (n-7c) Palmitoleic acid	0.1
C16:1 (n-7t) Palmitelaidic acid	<0.05
C17:0 Margaric acid	<0.05
C17:1 (n-7c) Heptadecenoic acid	0.1
C17:1 (n-7t) Transheptadecenoic acid	<0.05
C18:0 Stearic acid	3.1

C18:1 (n-6c) Methyl 12-Octadecenoate	<0.05
C18:1 (n-7c) Vaccenic acid	0.8
C18:1 (n-7t) Transvaccenic acid	<0.05
C18:1 (n-9c) Oleic acid	6.1
C18:1n-12t+C18:1n-9t A.Petroselaidic+A.Elaidic	<0.05
C18:2(10t,12c)+ C20:1n-9t + C20:1n-15c	<0.05
C18:2 (9c,11t + 9t,11c) Conj. Linol. Acid	<0.05
C18:2 (n-6c) Linoleic - ω6	19.3
C18:2 (n-6t) Linolelaidic acid	0.1
C18:3 (n-3) Alpha linolenic acid (ALA) - ω3	63.3
C18:3 (n-6) Gamma-linolenic acid GLA - ω6	0.3
C19:0 Nonanic acid	<0.05
C19:1 (n-12c) 7-Nonadecenoic acid	<0.05
C19:1 (n-12t) 7-transnonadecenoic acid	<0.05
C19:1 (n-9t) 10-Transnonadecenoic acid	<0.05
C 20:0 Arachidic acid	<0.05
C20:1 (n-12c) 8-eicosenoic acid	<0.05
C20:1 (n-9c) Gadoleic acid	0.1
C20:2 (n-6c) 11-14-eicosodienoic - ω6	<0.05
C20:3 (n-3c) Eicosatrienoic acid - ω3	0.1
C20:3 (n-6c) Eicosatrienoic acid DHGLA - ω6	<0.05
C20:4 (n-6c) Arachidonic acid- ω6	<0.05
C20:5 (n-3c) Eicosapentaenoic EPA - ω3	<0.05
C21:0 Heneicosanoic acid	<0.05
C22:0 Behenic acid	0.1
C22:1 (n-9c) Erucic acid	<0.05

C22:1 (n-9t) Brassidic acid	<0.05
C22:2 (n-6c) Docosadienoic acid - ω6	<0.05
C22:3 (n-3c) Docosatrienoic acid - ω3	<0.05
C22:4 (n-6c) Docosatetraenoic acid- ω6	<0.05
C22:5 (n-3c) Docosapentaenoic acid - DPA - ω3	<0.05
C22:6 (n-3c) Docosahexaenoic acid DHA - ω3	<0.05
C23:0 Tricosanoic acid	<0.05
C24:0 Lignoceric acid	0.1
C24:1 Nervonic acid	<0.05
Saturated fatty acids	9.7
Mono-unsaturated fatty acids	7.1
Polyunsaturated fatty acids	83.0

Certificates of analysis and test method on this matter (EUROFINS AR-11-AA-064258-01/ E4-370-02540264) are hereby attached as **Dossier/Appendix**.

c. Bio-active Components

Vegetal oils contain substances that naturally protect them from the chemical instability mentioned above. Main substances are antioxidants, among which are tocopherols and phenol compounds.

(i) *Tocopherols*

Table 6. Tocopherols (mg/kg)

Tocopherols	**mg/kg**
Alfa-Tocopherol	26
Alfa-tocotrienol	<10
Beta-Tocopherol	<10
Beta-tocotrienol	<10
Delta-Tocopherol	66
Delta- tocotrienol	<10

Gamma-Tocopherol	500
Gamma-tocotrienol	<10
Total tocopheroles	592
Total tocotrienoles	<10

Certificates of analysis and test method on this matter (EUROFINS AR-11-AA-064258-01/ E4-370-02540264) are hereby attached as **Dossier/Appendix**.

(ii) Antioxidant Activity.

The antioxidant activity of a specific food may be determined by different methods, although not all of them are equivalent. Among the most common methods is the ORAC Method expressed as Trolox (ORAC unit) equivalent micromoles per 100 g of sample.

Hydro, Lipo, and Total ORAC Test.

Chia Oil: ORAC hyro, micromoles TE/100g = 15.4

Chia Oil: ORAC lipo micromoles TE/100g = 946.5

Chia Oil: ORAC total, micromoles TE/100g = 962.2

Certificates of analysis PAM 081-09 and test method are attached as **Dossier/Appendix**.

(iii) Total Polyphenols

Chia Oil: 109.5 mg Eq. Gallic Acid /Kg

Results indicate that Chia seed oil contains bioactive components of anti-oxidative character that contribute to its oxidative stability in time.

Certificates of analysis PAM 081-09 and test method are attached as **Dossier/Appendix**.

(iv) Total Sterols

Table 7. Total Sterols (%)

Betasitosterol	65.4
Brasicasterol	<0.1
Campesterol	14.3
Cholesterol	0.2
Clerosterol	0.9
D5.23Stig+Clero+Bsito+Sit	77.3
Delta5 avenasterol	8.4
Delta 7 avenasterol	1.1
Delta 7 stigmasterol	2.2
Delta 7 Campesterol	0.5
Delta-5.23-stigmastadienol	<0.1
Delta-5.23-stigmastadienol	1.5

Sitostanol	1.1
Stigmasterol	4.4
Others Sterols	<0.1
Total Sterols	**100 %**
Total Sterols (mg/100g)	**565.4± 57**

Certificates of analysis and test method on this matter (EUROFINS AR-11-AA-064258-01/ E4-370-02540264) are hereby attached as **Dossier/Appendix**.

3.3.2 External Contaminants

(i) Heavy Metals

The presence of heavy metals in food is an issue of concern; lead and mercury are two of them which presence is important to determine in Chia seed oil extracted by cold-pressing process

Table 8. Heavy Metals (mg/kg)

Arsenic	<0.1
Cadmium	<0.01
Mercury	<0.03
Lead	<0.075

Certificates of analysis and Test method on this matter (EUROFINS AR-11-AA-064258-01/ E4-370-02540264) are hereby attached as **Dossier/Appendix**.

(ii) Pesticides (mg/kg)

OC/PY/PCB Pesticides < 0.005

Organophosphorus Pesticides < 0.02

Nitrogen Pesticides < 0.03

Certificates of analysis and Test method on this matter (EUROFINS AR-11-AA-064258-01/ E4-370-02540264) are hereby attached as **Dossier/Appendix**.

(iii) Hazardous Air Pollutants (HAPs)

Table 9. Hazardous Air Pollutants (HAPs)

5-Methilchrysene	<1
Benzo(a)anthracene	0.80
Benzo(a)pyrene	<0.5

Benzo(b)fluoranthene	<0.5
Benzo(c)fluorene	<1
Benzo(ghi)perylene	<0.5
Benzo(j)fluoranthen	<0.5
Benzo(k)fluoranthen	<0.
Chrysene	1.1
Cyclopenta(cd)pyrene	<1
Dibenzo(ae)pyrene	<1
Dibenzo(ah)antharacene	<0.5
Dibenzo(ah)pyrene	<1
Dibenzo(ai)pyrene	<1
Dibenzo(al)pyrene	<1
Indeno(123cd)pyrene	<0.5

Certificates of analysis and Test method on this matter (EUROFINS AR-11-AA-064258-01/ E4-370-02540264) are hereby attached as **Dossier/Appendix**.

(iv) Dioxins and Furans

Table 10. Dioxins and Furans (pg/g)

2,3,7,8-TCDD	<0.05
1,2,3,7,8-PeCDD	<0.04
1,2,3,4,7,8-HxCDD	<0.08
1,2,3,6,7,8-HxCDD	<0.08
1,2,3,7,8,9-HxCDD	<0.08
1,2,3,4,6,7,8-HpCDD	<0.55
OCDD	<1.78
2,3,7,8-TCDF	<0.07
1,2,3,7,8-PeCDF	<0.06
2,3,4,7,8-PeCDF	<0.06
1,2,3,4,7,8-HxCDF	<0.07
1,2,3,6,7,8-HxCDF	<0.06
1,2,3,7,8,9-HxCDF	<0.08
2,3,4,6,7,8-HxCDF	<0.06
1,2,3,4,6,7,8-HpCDF	<0.11
1,2,3,4,7,8,9-HpCDF	<0.09
OCDF	<0.51
Dioxins and Furans	ND
Dioxins and Furans	0.183

Certificates of analysis and Test method on this matter (EUROFINS AR-11-AA-064258-01/ E4-370-02540264) are hereby attached as **Dossier/Appendix**.

(v) PCBs

Table 11. PCB/ Dioxin (pg/g)

PCB 77	<2.37

PCB 81	<0.47
PCB 105	<4.94
PCB 114	<1.11
PCB 118	<18.6
PCB 123	<1.50
PCB 126	<0.63
PCB 156	<6.13
PCB 157	1.07
PCB 167	<2.37
PCB 169	<2.37
PCB 189	<1.56
Co-PCBs (>detection limit)	ND
Co-PCBs (<detection limit)	0.277

Certificates of analysis and Test method on this matter (EUROFINS AR-11-AA-064258-01/ E4-370-02540264) are hereby attached as **Dossier/Appendix**.

(vi) Inorganic metals in oil

Trace levels of metal ions (Cu, Fe) are known to have adverse effect on the oxidative stability of edible oils. Transition metals such as copper and iron catalyze the decomposition of hydroperoxides and lead to more rapid formation of undesirable substances. Taking into account the metabolic role of some toxic metals, the development of fast and accurate analytical methods for trace element determination in edible vegetable oils is important from the viewpoint of both production quality control and food analysis.

Table 12. Inorganic metals (mg/kg)

Iron	<0.1
Copper	<0.1

Certificates of analysis and Test method on this matter (EUROFINS AR-11-AA-064258-01/ E4-370-02540264) are hereby attached as **Dossier/Appendix**.

IV. EFFECTS OF THE PRODUCTION PROCESS APPLIED TO THE NOVEL FOOD

Pursuant to the Commission Recommendation, the following questions must be addressed relative to the production process of Chia oil:

a. *"Does Chia oil undergo a production process?"*

b. *"Is there a history of use of the production process for the food? If not, "does the process result in a significant change in the composition or structure of Chia oil compared to its traditional counterpart?"*

c. *"Is information available to enable identification of the possible toxicological, nutritional and microbiological hazards arising from use of the process?"*

d. *"Are the means identified for controlling the process to ensure that Chia oil complies with its specification?"*

e. *"Has the process the potential to alter the levels in Chia oil of substances with an adverse effect on public health?"*

f. *"After processing, is Chia oil likely to contain microorganisms of adverse public health significance?"*

4.1 Process Flow Chart

Raw material: Chia seeds (*Salvia hispanica L*.) are received as raw material at the pressing plant with a seed purity degree of over 99.9%. The seeds are produced and selected under strict standards of Good Agricultural Practices, Good Manufacturing Practices and HACCP.

The final product is stored in steel drums specially conditioned as oil containers. Natural tocopherols ensure oil stability in time.

At the end of the process, the drums are tightly sealed and labelled with production data and identifying batch information.

A detailed diagram of this production method is included in **Dossier/Appendix**.

4.2 History of production process

The cold-pressing extraction process is a standard technique used in the oil processing industry. As explained above, this process guarantees 100% pure and natural oil with no presence of pollutants, preserving the same characteristics as those in Chia seed.

4.3 Potential Hazard and Production Control

Information available on identification of potential toxicological, nutritional and microbiological hazards is included in sections X, VIII and IX respectively.

Sections X and IX provides information on inherent toxicological constituents, external and microbiological contaminants.

Chia seed cold-pressing process is made for FPT S.A. by TRIO S.A., which is independently certified in Hazards Analysis and Critical Control Points (HACCP).

TRIO S.A HACCP certificate is included in **Dossier/Appendix**

The HACCP-based control checklist is included in **Dossier/Appendix**.

V. HISTORY OF THE ORGANISM USED AS NOVEL FOOD SOURCE

Pursuant to the Commission Recommendation, the following questions must be addressed on the history of the source organism:

a. *"Is Chia oil obtained from a biological source, i.e., a plant, animal or microorganism?"*

b. *"Has the organism used as Chia oil source been derived using GM?"*

c. *"Is the source organism characterized?"*

d. *"Is there information to show that the source organism and/ or foods obtained from it are not detrimental to human health?*

5.1 Source of Chia Oil

In pre-Columbian times, Chia (*Salvia hispanica L.)* was one of the four basic foods of Central American civilizations. It was less common than corn and beans, but more important than amaranth. Chia seed was not just a food, but was also used for medical purposes, fed to animals, and often given as an offering to the Aztec gods.

The use of Chia in Aztec religious ceremonies led the Spanish *conquistadores* to try to eliminate it, and replace it with species brought from the old world. They came close to eradicating Chia as a crop, and it was pushed into obscurity for five hundred years, being grown only in small patches in scattered mountain areas of southern Mexico and northern Guatemala.

This was the situation until the North-western Argentina Regional Project began researching Chia in 1991. At that time, growers, commercial entities and scientific personnel began collaborating with the production of Chia under the direction of Dr. Wayne Coates and Ing. Ricardo Ayerza. The idea was to provide growers with an alternative crop that would improve human health, by reintroducing Chia into western diets as a source of omega-3 fatty acids, antioxidants, protein and fiber, and Chia oil as a source of omega-3 fatty acids and antioxidants.

5.2 GM Status of Salvia Hispanica L.

Salvia Hispanica L. is not a genetically modified organism.

The GMO analysis of *Salvia Hispanica L.* is included in **Dossier/Appendix**.

5.3. Characterization of *Salvia Hispanica L.*

The taxonomy of the plant source of Chia oil is as follows:

Division: *Magnoliophyta- Angiosperma*
Class: *Magnoliopsida- Dicotiledonea*
Subclass: *Asteridae*
Order: *Lamiales*
Family: *Lamiaceae*
Genus: *Salvia L*
Species: *Salvia Hispanica L*

5.4 Information Showing that the Source Organism is not Detrimental to Human Health

Data showing that the source organism and/or foods obtained thereof are not detrimental to human health are included in the different sections of this dossier. Further evidence supporting the absence of detrimental effects can be drawn from the food safety regulatory approvals in North and South America, Asia and Australasia, as well as the multiple consumer products containing Chia seed and oil which are now consumed worldwide with no reported negative health impacts.

The EFSA Scientific Opinion on Safety of Chia Seed, under which the R. CRAIG AND SON application was approved, and the ACNFP opinion under which TCC Subsequent Equivalent application was approved, both conclude that the inclusion of Chia seed up to 5% in bread is unlikely to have "*an adverse effect on public health*" (EFSA 2009).

On May 27, 2011, THE CHIA COMPANY (Australia) submitted an application to the FSA, to *extend the currently authorised use of chia seeds* (*Salvia hispanica* L) to include a number of additional *food products that commonly contain other seeds* (packaged Chia seeds, baked products (muffins, cookies, crackers and biscuits), breakfast cereals and fruit, nut and seed mixes (sprinkles)). On March 16, 2012, the UK FSA informed DG SANCO on the extension of use of chia seeds to several food categories.

Documentation of recent Chia consumption can be found *infra* in Section VII "*Information on Previous Human Exposure to Chia oil and/or its source*".

VI. ANTICIPATED NOVEL FOOD INTAKE/EXTENT OF USE

Pursuant to the Commission Recommendation, the following questions must be addressed on intake/extent of use of the novel food:

a. *"Is there information on the anticipated uses of Chia oil based on its properties?"*

b. *"Is there information to show anticipated intake for groups predicted to be at risk?"*

c. *"Will introduction of the novel Food be restricted geographically?"*

d. *"Will the novel food replace other foods in the diet?*

6.1. Proposed Uses

The proposed food use of Chia oil is detailed in Table 13 below.
Chia oil levels in proposed are based in the *Opinion on Labelling Reference Intake values for Omega-3 fatty acids; Commission Regulation (EU) No 116/2010 of 9 February 2010 amending Regulation (EC) No 1924/2006.*

Table 13. Proposed Used of Chia Oil

PROPOSED FOOD USES / CATEGORY	% INCLUSION/ RECOMMENDED DOSE/DAILY INTAKE	CHIA OIL CONSUMPTION PER PRODUCT CATEGORY
Fats & oils	10%	10 g per 100 g total oil
Non-Alcoholic Beverage (Fruit Juice and Milk)	0,25%	0,25%
Food Supplements	2 g/day	2 g/day

6.2.1. Anticipated intake by age groups

(i) United Kingdom
United Kingdom Food Standards Agency's, Dietary Survey Programme (DSP) provides consumption data for the average UK consumers (aged 19 to 64 years) (Hoare, Henderson et al, 2004). Estimates for the intake of Chia Oil (Table 14.) in the EU were based on the proposed use-levels (Table 13.) and food consumption data collected as part of the DPS. Calculations for the mean all-person and all-user intakes, and percent consuming were performed for each of the individual proposed food-uses for Chia Oil.

Table 14. Average Potential Intake of Chia Oil as calculated by the UK NDNS for Fats and Oils, Non-Alcoholic Beverage

ALL RESPONDENTS	MEAN CONSUMPTION (GRAMS PER DAY)				ALL CONSU-MERS	% ALL CONSUMERS	% CHIA OIL	GRAMS CHIA OIL CONSUMED/ DAY
PRODUCT CATEGORIES	AGE GROUPS							
	19-24	25-34	34-49	50-64				
Fats & oils	11.4	11.1	12	13.9	12.3	93%	10%	1.2
Non-Alcoholic Beverage								

Fruit Juice	43.8	41	54.7	53.1	47.5	45%	0.25%	0.1
Milk	142.7	191.8	221.5	220.8	204.2	94%	0.25%	0.5
SUM								**1.8**

Source: Table 2.1.a, 2.1.b, and 2.1.c of The National Diet & Nutrition Survey: adults aged 19 to 64 years, Summary Report Volume 5 (2004)

Similar calculations were used to determine the estimated total intake of ALA from chia oil from all proposed food-uses combined. Calculations for the high-level (97.5th percentile) all-person and all-user intakes, and percent consuming were performed for each of the individual proposed food-uses for ALA from Chia Oil.

Table 15. 97.5 Percentile Potential higher intake of Chia oil as calculated by the UK NDNS for, Fats and Oils, Non-Alcoholic Beverage

ALL RESPONDENTS	**97.5 PERCENTILE (GRAMS PER DAY)**							
PRODUCT CATEGORIES	**AGE GROUPS**				**ALL CONSUMERS**	**% CHIA OIL INCLU-SION**	**GRAMS CHIA OIL CONSUMED/DAY**	**GRAMS ALA CONSUMED/DAY**
	19-24	**25-34**	**34-49**	**50-64**				
Fats & oils	22.8	22.2	24	27.8	24.6	10%	2.5	1.5
Non-Alcoholic Beverage								
Fruit Juice	85.4	79.9	106.6	103.5	92.6	0.25%	0.2	0.1
Milk	278.2	374	431.9	430.5	398.1	0.25%	0.9	0.6
SUM							**3.6**	**2.3**

Source: Table 2.1.a, 2.1.b, and 2.1.c of The National Diet & Nutrition Survey: adults aged 19 to 64 years, Summary Report Volume 5 (2004)

In summary, on all-user basis, the 97.5th percentile, the high-level intake of ALA by the UK population from all proposed food-used of Chia Oil in the EU, observed in all age groups were estimated to be 2 g/person/day.

These calculations are inside the EFSA recommendation for ALA 2-3 g/ day for energy intake of 1800 - 2700 kcal/ day, and the EFSA-NDA-panel-accepted (EFSA, 2009) labeling reference intake of ALA 2 g/day.

6.2 Groups At Risk

Despite the proliferation of Chia seed and Chia oil as food ingredients, and the consumption of whole Chia seed, no events of allergic or other adverse reactions to Chia has been reported and/or recorded to date. For further information see Section X.

6.3 Geographical Restrictions

There are no anticipated geographical restrictions within the European Union for introduction of this product.

6.4. Replacement of Other Foods

It is anticipated that Chia oil will be consumed (i.e. by vegetarians) as an alternative to flaxseed oil, canola oil and other sources of Omega-3 fatty acids.

VII. INFORMATION FROM PREVIOUS HUMAN EXPOSURE TO THE NOVEL FOOD OR ITS SOURCE

Pursuant to the Commission Recommendation, the following questions must be addressed regarding the previous human exposure to Chia Oil.

a. *"Is there available information from previous direct, indirect, intended, or unintended situations with respect to production, preparation, population, lifestyles, and intakes?"*

b. *"Is there information showing that exposure to the Novel Food is unlikely to generate any nutritional, microbiological, toxicological and/or allergenicity problems?"*

7.1 Information from previous human exposure to Chia oil source

Section V provides information on pre-colonial consumption of Chia by Central and Southern America indigenous populations.

Chia seed products have been introduced to the market at an increasing pace and variety.

Functional foods are successful in the consumers' market when they are attractive, nutritive and with good flavour. Both Chia seed and oil, with their pleasant walnut flavour, oxidative stability and alpha-linolenic fatty acid content, fit very well in this market niche.

MINTEL is a world leader in market intelligence for food, beverage, and FMCG products. Mintel´s Global New Product Database (GNPD) collects and records information on new product releases, allowing companies to identify which products are being created in their category globally.

Mintel GNPD lists more 350 new products containing Chia seed or oil that have been launched into the global market from November 2009 through November 2011.

7.2. Worldwide Consumption Recent History

Chia oil products have been introduced to the market at an increasing pace and variety. This oil is currently added to a wide variety of food products such as multigrain bread, cereals, pasta, olive oils, and margarines. Furthermore, this oil is sold in grocery stores, naturist shops, supermarkets, and through the Internet, as nutritional supplements in soft capsules or as gourmet cooking oils.

A list of Food Products and Nutritional Supplements with chia seed and chia oil, existing on the world market, are included in table 16.

Table 16. Worldwide Consumption

LATINAMERICA				
COMPANY NAME	WEB SITE	CONSUMPTION/ PRODUCTION	APPLICATIONS	PICTURE OR DOCUMENTS
FPT SA, Chile	www.benexia.com	MT/year Chia seed, MT/year Chia oil for its final product (Chia seed growers: 1400 MT/ year for Food Industry)	Nutritional Supplement Industry: Chia oil soft gel caps and Chia oil	
Empresas Carrozzi SA, Chile	www.carrozzi.cl	3 MT/month	Pastas (Chia Oil) Cereal Breakfast (Chia seed)	
TerraMater	www.terramater.cl	600 kg/year	Olive & Chia oil gourmet	
Fuentenatura,Mexico	www.fuentenatura.cl	500 kg/year	Nutritional Supplement: Chia oil	
Madaus & CO,Argentina	www.drmadaus.com.ar	500 kg/year	Nutritional Supplement	

LATINAMERICA				
COMPANY NAME	WEB SITE	CONSUMPTION/ PRODUCTION	APPLICATIONS	PICTURE OR DOCUMENTS
Alimentos Sturla S.A	www.alimentossturla.com.ar	100 kg/ year	Nutritional Supplement Industry: Chia oil	
Cattivelli Hnos S:A	www.cattivelli.com	50 kg/year	Frankfurters Chia oil	
Nestlé, Chile	www.nestlenutricion.cl	200 kg/ month	Crackers (chia oil)	
Castaño, Chile	www.castaño.cl	1,5 MT/ month	Bread (chia seed)	
Pepsico, Chile	http://www.quaker.cl/trailmixnuts-y-semillasquaker	600 kg/ year	Cereals Bar (chia seed)	
Terrum, Chile	www.terrium .cl		Cookies (Chia seed)	

ASIA				
COMPANY NAME	**WEB SITE**	**CONSUMPTION/ PRODUCTION**	**APPLICATIONS**	**PICTURE OR DOCUMENTS**
Latina Inc, Japon	www.latina-inc.com	500 kg/year	Nutritional Supplement Chia oil	
K-Squares, Korea	www.ksquares.com	500 kg/month	Nutritional Supplements	
AUTRALIA AND NEW ZEALAND				
The Chia Co. AU	www.thechiaco.com.au	Chia growers 1000 MT/year	Nutritional Supplement Industry: Chia seed and Chia oil	
Pepsico	www.pepsicocareers.com.au	10 MT/year	Healthy Snacks (Chia seed)	
Sunrice	www.sunrice.com.au	19 MT/year	Food Industry (chia seed, Chia oil)	
Fonterra	www.fonterra.com	500 kg/month	Yogurt (chia oil)	Fonterra Dairy for life
Centenary Bakery	www.madebybikini.com	2 MT/month	Bread (Chia seed)	
Campbells Arnotts	www.arnotts.con.au	10 MT/year	Biscuits	

EUROPEAN UNION

COMPANY NAME	WEB SITE	CONSUMPTION/ PRODUCTION	APPLICATIONS	PICTURE OR DOCUMENTS
Naturkost Übelhör GmbH & Co. KG, Germany	http://www.sachia.de/home.htm http://www.shop.sachia.de/	ND	Nutritional Supplement Industry	

USA AND CANADA

COMPANY NAME	WEB SITE	CONSUMPTION/ PRODUCTION	APPLICATIONS	PICTURE OR DOCUMENTS
Valensa Int.	www.valensa.com	10 MT/ month	Nutritional Supplement	valensa
PNI	http://www.pharmachemlabs.com/divisions/pni/	940 MT/year Chia seed, 7 MT/year Chia oil	Nutritional Supplement	
Green Plus	www.greenplus.com	17 MT/ month	Nutritional Supplement	
Ruth´s Hempfood	www.ruthshempfood.com/chia.html	1,5 MT/ month	Cereal Breakfast	
Salba	www.sourcealba.com	300 MT/ year	Nutritional supplement	

Chia oil is widely available worldwide and no adverse effects from consumption of Chia oil have been reported. Based on the experience from previous and current use of Chia seed and Chia seed oil, together with the considerations included in sections XI, XII and XIII below, it can be expected that, provide the recommended intakes are followed, the Novel Food is unlikely to cause any nutritional, microbiological, toxicological and/or allergenicity issues.

VIII. NUTRITIONAL AND METABOLIC DATA

Pursuant to the Commission Recommendation, the following questions must be addressed pertaining to nutritional information available on the novel food

a. *"Is there information to show that the novel food is nutritionally equivalent to existing foods that it might replace in the diet?"*

8.1 Nutritional Equivalent information

Chia oil obtained by cold-pressing process is an excellent natural source of Alfa-Linolenic Omega-3 fatty acid.

Of all Omega-3 fatty acid sources, flax, Chia and canola come from land crops. The first two are the plant species with the highest concentration of alpha-linolenic fatty acids known to date, that are different from and an exception to common vegetal oils.

Chia Oil is not proposed as an alternative to fish oil having a very different composition. Chia oil will be consumed (i.e. by vegetarians) as an alternative to flaxseed oil, canola oil and other vegetal oils, sources of Omega-3 fatty acids.

8.1.1 *Table 17. Comparison of Omega-3 fatty acid content in different oils*

	CHIA OIL	CANOLA OIL	FLAX OIL
% Fatty Acid			
Saturated	7,2	7	6,9
Monounsaturated	7,2	61	19,5
Polyunsaturated			
Omega-6	18,8	21	15
Omega-3			
ALA	**62,9**	**11**	**54,2**
DHA	0	0	0
EPA	0	0	0
Omega-3 Total	**62,9**	**11**	**54,2**

Comparative data for Novel Food and Traditional Counterpart

The information included in the document "*Opinion on the equivalence between chia seed oil and flaxseed oil*" prepared by Professor Lilia Masson, based on analytical report of EUROFINS labs, compare Chia seed oil with flax seed oil and it is hereby attached as **Dossier/Appendix**.

This comprehensive study includes physical and chemical parameters to assess the identity, fatty acid composition, quality, stability, and shelf life of Chia seed oil, using official analytical methods, and compares it to flax seed oil, considering Chia seed oil as equivalent to flaxseed oil.

Both elements present the highest Alpha Linolenic Omega-3 fatty acid content, and are both the best vegetable and natural sources of this essential fatty acid. Compared with Flax seed oil, Chia seed oil has some advantages, because it does not have any toxicant compounds and chia seed oil is more stable than flaxseed oil regarding oxidative behaviour. Both oils, and particularly Chia seed oil, present a beneficial effect on HDL, and LDL cholesterol, and serum levels of triacylglycerols and other health indicators.

8.1.2 Alpha-Linolenic Omega-3 fatty acid vegetal source

Alpha-linolenic acid (ALA, 18:3n-3) is a polyunsaturated fatty acid (PUFA) abundant in some vegetable oils (*Table 17*). ALA is the essential fatty acid originator of the series n3 fatty acids (or Omega 3), and is the precursor of the longer chain n-3 PUFAs . The n-3 fatty acid family is defined by a double bound beginning at the third carbon from the methyl end. Because mammals cannot insert double bounds more proximal to the methyl end than the ninth carbon atom (D-9 desaturase), n-3 fatty acids cannot be synthesized *de novo;* consequently n-3 fatty acids have to be present in the diet. These features are shared with another family of fatty acids, the n-6 fatty acids which precursor is linoleic acid (C18:2w6) (LNA). As in the case of n-3 fatty acids, the n-6 fatty acids are defined by a double bound beginning at the sixth carbon from the methyl end (Fig. 1).

Because neither of these fatty acids can be synthesized *de novo*, ALA and LNA are referred to as essential fatty acids for mammals.

Hence, ALA and LNA serve as the precursor molecules from which the rest of fatty acids of the n-3 or n-6 fatty acid family can be synthesized through a series of elongation and desaturation reactions.

The requirements/recommendations for these essential fatty acids are expressed as percentage of total calories. For linoleic acid (C18:2w6), a daily average of 3% of total calories is estimated for a healthy adult. For alpha-linolenic acid (C18:3w3), a daily average of 0,5% of total calories is estimated. These percentages typically increase in the growth and development stages and with special physiological conditions. There is no general agreement but it is estimated that the best dietary ratio between both fatty acids for optimal physiological benefits is about 6:1 or lower, which is not easily obtainable in a regular western diet.

This is due to the fact that most vegetal oils produced at industrial scale for human consumption, such as sunflower, corn, cotton, grape pip, sesamo, rice, safflower, are good sources of linoleic acid (C18:2 w6), among 55 and over 80%; however, the content of alpha-linolenic acid (C18:3W3) is very low, between 0.2-2%.

Another recommendation relative to the proportion of these fatty acids in the diet indicates that the currently recommended maximum percentage of fat calories is around 30% for a 2000kcal diet. This 30% of calories contributed by the diet must be equally balanced among saturated,

monounsaturated and polyunsaturated fatty acids, with approximately 10% for each group. In addition, for the polyunsaturated group, the already mentioned w6:w3 ratio is recommended.

As an example, only by calculation, without considering bio-availability, digestibility, etc, the 3% of total calories of linoleic acid for a 2000kcal diet corresponds to 60 Kcal, and if divided by 9 it corresponds to 6.7 g. of daily linoleic acid contributed by approximately 10 g of sunflower oil.

The 0.5% of total calories equally based on a 2000 kcal diet to be contributed daily as alpha-linolenic acid corresponds to approximately 10 kcal, or 1 g of daily alpha-linolenic acid. To reach this figure, we need about 12.5 g of soy oil. The larger the natural contribution of alpha linolenic acid in oil, the lesser the amount required to satisfy this daily requirement. In the case of chia seed oil, with about 60% of alpha linolenic acid, only 1.6 g of oil is needed to comply with the referenced requirement.

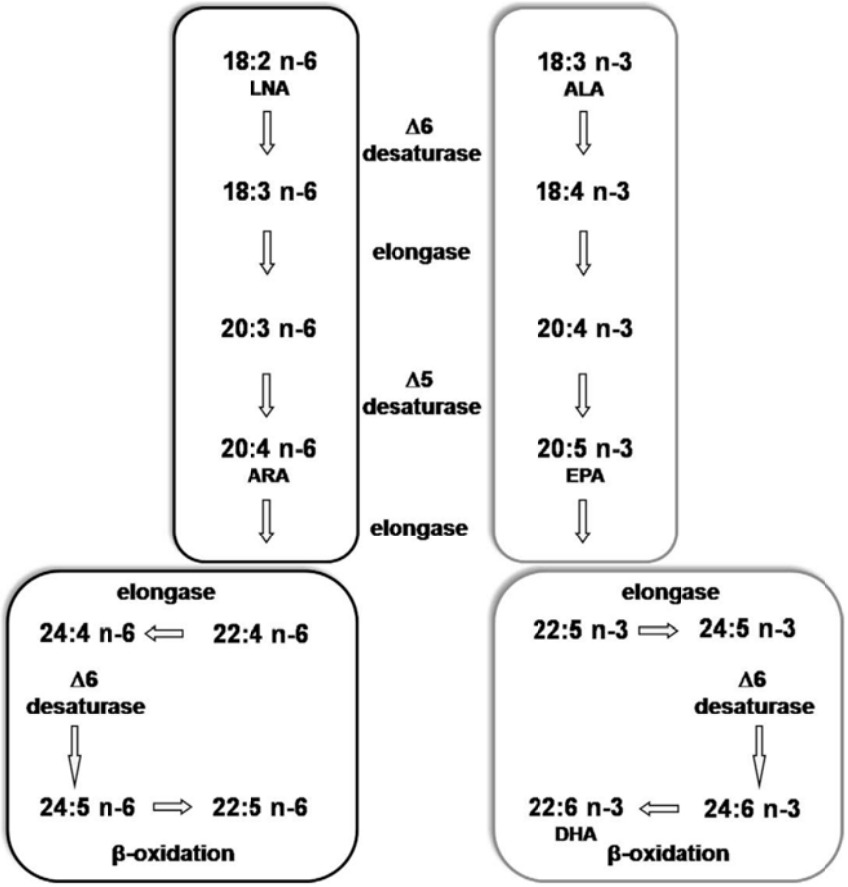
EFSA recommended for ALA (based on cardiovascular health and neurodevelopment) 2-3 g/day for energy intakes of 1800 – 2700 Kcal/ day, and the Panel (EFSA, 2009b) accepted labelling reference intake of 2 g/day, as proposed by the European Commission.

8.2 Bioavailability of ALA from Chia Oil

Figure 1 shows a general overview of the n-6 and n-3 fatty acid elongation and desaturation pathways. ALA is essential in human nutrition as precursor for the n-3 LCPUFA. EPA, DPA (docosapentaenoic acid) and, to a lesser degree DHA, are synthesised from ALA through the sequential action of various desaturases and elongases in animal tissues, but not in plants. Estimates for the conversion of ALA into EPA are around 8 to 12%, while the conversion into DHA may be less than 1% (EFSA, 2010).

Linoleic acid and ALA are converted into their respective LCPUFA by the same enzymes. In fact, the conversion of ALA into EPA and DHA decreases when the amount of linoleic acid in the diet increases (and vice versa). For this reason, some dietary recommendations also include guidelines for the n-3/n-6 ratio in the diet.

Figure 1. Overview of the n-6 and n-3 fatty acid elongation and desaturation pathway.



A recently published investigation (Valenzuela, R; Masson, L; Valenzuela, A, 2011) determined the ALA bioconversion of vegetable oils (chia and rose mosqueta) in EPA and DHA.

Male rats (n=8, for group) Sprague Dawley, (50-70 g), with normal diets for 21 days, divided in four experimental groups. The contribution of total fats came from different oils: Rosehip oil (group iii), Chia oil (group ii), sunflower oil (group i) and extra-virgin olive oil with addition of marine oil (group iv).

In the (ii) group the bioconversion increased significantly with levels of EPA and DHA similar to those of the group (iv), while the (iii) group reached only half of the level observed in (iv) group .

This study concludes that chia oil presented the higher bioconversion in EPA and DHA.

IX. MICROBIOLOGICAL INFORMATION

Pursuant to the Commission Recommendation, the following questions must be addressed pertaining to available Chia oil microbiological information:

a. *"Is the presence of any microorganism or their metabolites due to the novelty of the product/process?"*

Vegetal oils are not a good substrate for microorganism growth, mostly due to their very low moisture content. Nevertheless, the presence of adverse microorganisms is controlled. Tables 18 and 19 summarize the microbiological tests included in **Dossier/Appendix**, respectively.

Table 18. Microbiology Test Result

Aerobic Plate Count (35°)	<10	TRIO/2008-2009
Yeasts – Moulds (5 days)	<10	**TRIO/2008-2009**
Staphylococcus aureus	<10	**TRIO/2008-2009**
E. Coli	Absent	**TRIO/2008-2009**
Salmonella	Absent	TRIO/2008-2009

Certificates of analysis and test method are attached as **Dossier/Appendix**.

Table 19. Presence of microbiological metabolites (µg/kg)

Aflatoxine B1	<0.1
Aflatoxine B2	<01
Aflatoxine G1	<0.1
Aflatoxine G2	<0.1
Total Aflatoxines	<0.5

Certificates of analysis and test method on this matter (EUROFINS AR-11-AA-064258-01/ E4-370-02540264) are hereby attached as **Dossier/Appendix**.

X. TOXICOLOGICAL INFORMATION

Pursuant to the Commission Recommendation, the following questions must be addressed pertaining to available Chia oil toxicological information:

a. *"Is there a traditional counterpart to Chia oil that can be used as a baseline to facilitate the toxicological assessment?"*

b. *"Compared to the traditional counterpart, does Chia oil contain any new toxicants or changed levels of existing toxicants?"*

c. *"Is there information from a range of toxicological studies appropriate to Chia oil to show that Chia oil is safe under anticipated conditions of preparation and use?"*

d. *"Is there information suggesting that Chia oil might pose an allergenic risk to humans?"*

10.1 Toxicological Information

In addition to normal consumption of Chia oil products present in the market at an increasing pace and variety, as described in section VII, various recent studies have tested the effects of Chia oil in animals and humans under specific conditions, which, while not specifically designed for toxicological evaluation, have shown no adverse effects were observed under the recommended use conditions.

10.1.1 Animal Studies

30 days Dietary exposure in rat

Two studies on feeding Chia to rats have been conducted to assess its effect on plasma composition. Chia oil reduced serum triacylglycerols levels in rats by 66% and 60% for diets of 15% ground chia seed and 5% chia oil, respectively; and significantly higher serum HDL (22% and 51% for 15% ground chia seed and 5% chia oil, respectively) (Ricardo Ayerza, Wayne Coates, 2005/ 2007).

The triacylglycerols/HDL, LDL/HDL, total cholesterol/HDL, TC/LDL, and TG/HDL ratios tended to improve when feeding ground chia seed and chia oil to rats.

Non Cardio/Hepatic injury after supplementation with 5% chia seeds 8 weeks

A recent study (Brown, et al; 2011) assessed if whether intake of chia seeds could attenuate the metabolic, cardiovascular and hepatic signs of a high-carbohydrate, high-fat diet (H diet) in rats.

The diet of the treatment groups was supplemented with 5% chia seeds (1,5 % chia oil) after 8 weeks on H diet for a further 8 weeks. Compared with the H rats, chia seed-supplemented rats had improved their insulin sensitivity and glucose tolerance, reduced visceral adiposity, decreased hepatic steatosis and reduced cardiac and hepatic inflammation and fibrosis without changes in plasma lipids or blood pressure. Chia seeds induced lipid redistribution with lipid moving away from the visceral fat and liver with increased accumulation in the heart.

The stearoyl-CoA desaturase-1 products were depleted in the heart, liver and the adipose tissue of chia seed- supplemented rats together with an increase in the substrate concentrations. The C18:1 trans-7 was mostly stored in the adipose tissue; the relatively inert C18:1n-9 was stored in sensitive organs such as liver and heart, and C18:2n-6, the parent fatty acid of the n-6 pathway, was preferentially metabolized. (Brown, et al; 2011)

Up to 2 months dietary exposure in rats

Seventy-two rats were randomised into three groups and fed an assigned diet for 3 weeks The first group of rats received a semi-synthetic diet containing maize starch (60 % energy), protein (17 % energy) and maize oil as the source of fat (23% energy) (control diet). The other two groups received the same semi- synthetic diet with sucrose as the carbohydrate and fat provided by maize oil (SRD) or by chia seed (SRD+ chia -362 g/kg-).

Ninety-six rats were divided into two groups and fed for 3 months with the control diet or SRD previously described in Experimental design 1. At that time, rats in the SRD groups were randomly subdivided into three subgroups. The first subgroup was immediately killed for each procedure as described below. The rats in the second subgroup continued with the SRD up to 5

months of feeding. The third subgroup received the SRD + chia seed as the source of dietary fat for the next 2 months. The control group was fed with the control diet throughout the experimental period.

The results showed that: (i) intake of dietary chia seed prevented the onset of dyslipidaemia and IR in rats fed the SRD for 3 weeks – glycaemia did not change; (ii) dyslipidaemia and IR in long-term SRD-fed rats were normalised with no change in insulinaemia when chia seed provided the dietary fat during the last 2 months of the testing period. Dietary chia seed reduced the visceral adiposity present in the SRD rats. (Chicco, et al; 2009).

10.1.2 Human studies

Dietary supplementation in athletes

The purpose of this study was to determine if Omega 3 Chia seed loading is a viable option for enhancing sports performance in events lasting >90 minutes and allow athletes to decrease their dietary intake of sugar while increasing their intake of Omega 3 fatty acids. It has been well documented that a high dietary carbohydrate (CHO) intake for several days before competition is known to increase muscle glycogen stores resulting in performance improvements in events lasting >90 minutes. This study compared performance testing results between 2 different CHO-loading treatments. The traditional CHO-loading treatment served as the control (100% cals from Gatorade). The Omega 3 Chia drink (50% of calories from Greens Plus Omega 3 Chia seeds, 50% Gatorade) served as the Omega 3 Chia loading drink. Both CHO-loading treatments were based on the subject's body weight and were thus isocaloric. Six highly trained male subjects ($\dot{V}O_2$max 47.8-84.2 ml·kg^{-1}; mean (***SD***) of $\dot{V}O_2$max 70.3 ml·kg^{-1} (13.3) performed a 1-hour run at ~65% of their $\dot{V}O_2$max on a treadmill, followed by a 10k time trial on a track. There were 2 trials in a crossover counterbalanced repeated-measures design with a 2-week washout between testing sessions to allow the participants to recover from the intense exercise and any effects of the treatment. There was no statistical difference (***p*** = 0.83) between Omega 3 Chia loading (mean 10k time = 37 minutes 49 seconds) and CHO loading (mean = 37 minutes 43 seconds). Under these conditions, Omega 3 Chia loading appears a viable option for enhancing performance for endurance events lasting >90 minutes and allows athletes to decrease their dietary intake of sugar while increasing their intake of Omega 3 fatty acids but offered no performance advantages. No adverse effects were reported. (Illian, Casey, Bishop; et al. 2011)

2 weeks dietary supplementation including Nopal, Chia Seed, Soy Protein, and Oat Reduces Serum Triglycerides and Glucose Intolerance in Patients with Metabolic Syndrome.

The purpose of this work was to evaluate the effects of a dietary pattern (DP; soy protein, nopal, chia seed, and oat) on the biochemical variables of MetS, the AUC for glucose and insulin, glucose intolerance (GI), the relationship of the presence of certain polymorphisms related to MetS, and the response to the DP. In this randomized trial, the participants consumed their habitual diet but reduced by 500 kcal for 2 wk.

They were then assigned to the placebo (P; n = 35) or DP (n = 32) group and consumed the reduced energy diet plus the P or DP beverage (235 kcal) minus the energy provided by these for 2 mo. All participants had decreases in body weight (BW), BMI, and waist circumference during the 2-mo treatment ($P < 0.0001$); however, only the DP group had decreases in serum TG, C-reactive protein (CRP), and AUC for insulin and GI after a glucose tolerance test. (Guevara-Cruz; et al. 2011)

12 weeks Dietary exposure in Individuals with type-2 diabetes

In a research on the long term effects of supplementing the diet with Chia seed 37 g/day (11 g chia oil), it was concluded that Chia seed attenuated major cardiovascular risk factors, i.e., systolic pressure and emerging factors such as ultrasensitive CRP (inflammation) and vWF (coagulation), while maintaining good glycemia and lipidemia control in individuals with controlled type-2 diabetes.

In addition, this study showed that the levels of ALA and Eicosapentaenoic (EPA) fatty acids in plasma rose by up to 87% after consumption of Chia seed (Vuksan, 2007)

A Systematic Review by the Natural Standard Research of Chia seed

This review evaluated the scientific evidence on chia (Salvia hispanica) including history, folkloric precedent, expert opinion, pharmacology, dosing, interactions, adverse effects, and toxicology.

The available human and non-human studies show possible effectiveness for allergies, angina, athletic performance enhancement, cancer, coronary heart disease (CHD), heart attack, hormonal/endocrine disorders, hyperlipidemia, hypertension, stroke, and vasodilatation. Some evidence also suggests possible anticoagulant, antioxidant, and antiviral effects of Salvia hispanica (Chia).

There is limited evidence supporting the efficacy of Salvia hispanica for any indication; thus far, only two clinical studies have examined the effects of Salvia hispanica on cardiovascular disease (CVD) risk factors (including body weight). One study showed some effects on some CVD risk factors, while the other did not. Neither study showed any effects of Salvia hispanica on weight loss.

However, the historical use of Salvia hispanica suggests that it is safe for consumption by nonallergic individuals. (Ulbrick, Chao; et al 2009)

10.1.3 Pre- clinical Studies

14-day toxicity study in rats

A 14 day repeated dose dietary toxicity study in rats was conducted on Chia Oil (FPT S.A., lot Nº TRIO/P01/2009). This study was developed by the Deparment of Biologic, Toxicologic and Biotic Systems studies. Study´s Code Nº RO-549349. This study is an assessment of the Acute Oral Toxicity of Chia Oil to rats (Rattus norvergicus)

This study was developed in accordance with the resolution Nº 350/99 and Nº 617/02 of Servicio Nacional de Sanidad y Calidad Agroalimentaria (SENASA) and development based on Acute Toxic Class Method OECD Nº423, 2001 (3), EPA, Health Effects Test Guideliness, OPPTS 870.1300, EPA 712-C-98-193, 1998.

One hundred healthy rats (Rattus norvergicus) were selected for the tests and equally distributed into 2 groups. Dietary levels of Chia oil: 9000 mg/ kg body weight. Clinical examinations and behavior observations were made and results registered, during 14 days, at least once each day. Individual weights of animals were determined shortly before the test substance was administered, weekly thereafter, and at death. At the end of the test, surviving animals were sacrificed and a gross necropsy was performed.

Further, rats were placed in an incubation room at 22° C (± 3° C), 30-70% RH and a photoperiod of LH=12, DH=12 during 14 days. Records of mortality and clinic observations were taken during 14 days, and records of body weight were taken at the beginning, 7th and 14th days.

According to the results obtained: DL50 Chia Oil >9000 mg/ Kg of body weight.

This toxicity study is hereby attached as **Dossier/Appendix**.

10.2 Allergy Potential

Allergy potential of Chia has been previously assessed by EFSA for Chia seed as a novel food-ingredient in bread (EFSA, 2009), and by the relevant UK authority (ACNFP, 2012) in relation to extending the application of chia to a number of foods. It was considered that allergy is practically the only issue of concern and that risk management measures (labelling) already in place are adequate to address said concerns amongst known at-risk individuals. In the case of Chia seed oil compared with Chia seed, given its very low protein content, it should be expected that the risk would be much lower.

Concerning studies other than those previously considered for Chia seed (EFSA, 2009; ACNFP, 2012), Chia's safety has been further investigated by Fernandez, Vidueiros, Ayerza, Coates and Pallardo (Fernandez, Vidueiros et al; 2008). This study was intended to analyse the effect of Chia on some aspects of the immune system, such as the thymus and serum IgE concentration. Weanling male Wistar rats (23 d of age) from the Department of Nutrition at the School of Pharmacy and Biochemistry of the University of Buenos Aires were divided in three groups (6 rats each) receiving for one month (g/kg diet) 150 ground Chia seed (T1); 50 Chia oil (T2); no Chia (T3; control group). Diets T1 and T2 provided equal amounts of alpha-linolenic acid from chia. All the experimental diets were isoenergetic, contained (g/kg) 200 protein and 70 oil, and were prepared according to the intake, body weight, thymus weight, total thymocyte number and IgE levels when Chia was added to experimental diets as seed (T1) or as oil (T2) when compared with control (T3) group. Moreover, no symptoms such as dermatitis, diarrhea and abnormal growth and behaviour were observed. Adding Chia seed or oil to experimental diets produced none of the problems associated with other n-3 fatty acids sources such as flaxseed or marine products, e.g. fishy flavor, weight loss, digestive problems, diarrhea and allergies.

On the other hand, allergy adverse effects of Chia oil are not expected based on its composition, with very low levels of protein (Table 4. Section III.3.1 *Nutritional Composition*). Also, the experience gained from previous considerations of EFSA and the European legislation (Directive 2003/89/EC)[5] on the potential allergenicity and its exemption from labelling of different products derived from well known allergenic sources as soybean, may suggest that oils or products derived therefrom are less likely to trigger allergic adverse effects (Directive 2007/68/EC)[6].

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[5] Directive 2003/89/EC of the European Parliament and of the Council of 10 November 2003 amending Directive 2000/13/EC as regards indication of the ingredients present in foodstuffs (OJ L 308, 25.11.2003, p. 15–18).

[6] Commission Directive 2007/68/EC of 27 November 2007 amending Annex IIIa to Directive 2000/13/EC of the European Parliament and of the Council as regards certain food ingredients (OJ L 310, 28.11.2007, p. 11–14).

10.3 Effects on Immune System

The effect of Chia on some aspects of the immune system as thymus and serum IgE concentration was studied in rats (Ayerza and Coates, 2007). Male Wistar rats (23 days of age), were divided in three groups (6 rats each) that received during a month 15% ground chia seed (T1), 5% chia oil (T2) and no chia (T3 = control group). T1 and T2 diets were formulated to provide equal quantities of alpha linolenic acid from the chia.

No significant differences were observed in FI, BW, TW, TN and IgE levels when chia is added to experimental diets as seeds (T1) as well as oil (T2) when compared to control (T3). Moreover, no symptoms like dermatitis, diarrheas and abnormal animal growth and behavior were observed. Adding chia seeds or oil to experimental diets had not produced any of the problems caused by other O-3 sources such as flaxseed or marine product, i.e., fishy flavor, animal weight loss, digestive problems, diarrhea and allergies.

XI. CONCLUSION

There is no evidence of adverse effects of Chia oil from all studies, including those on physical and chemical characterization, fatty acid composition, bioactive component contents, microbiological, nutritional, toxicological and other information obtained in different lots of Chia seed oil extracted by cold-pressing process. Methods used in the characterization and evaluation of Chia seed oil, of physical, chemical, microbiological and toxicological nature, are official and have been carried out by well-known, reputable laboratories.

Chia seed oil obtained in Chile by cold-pressing process is presented, from a nutritional viewpoint, as an excellent natural source of alpha-linolenic acid, contributing antioxidant bioactive components, such as tocopherols and polyphenols. It has excellent chemical and microbiological quality and contains no toxic heavy metals or other potential contaminants.

The EFSA Panel (EFSA, 2009) and the FSA (ACNFP, 2012) recognise the difficulty of predicting the potential allergenicity of Chia seed with currently available methodologies, and considers that these concerns could be reasonably addressed by adequate management measures. Compared to whole Chia seeds, it is less likely that adverse effects be derived from Chia oil consumption, based on its low-protein level composition , and on previous considerations from EFSA and European authorities regarding the potential allergenicity of products derived from well-known allergenic sources.

In conclusion, Chia oil is considered safe and there is no reason to consider consumption of this novel food as nutritionally disadvantageous to the consumer. It is very unlikely that under the recommended use conditions consumption of Chia seed oil would have an adverse effect on public health.

REFERENCES are hereby attached as **Dossier/Appendix**

APPENDICES

- APPENDIX # 1 EUROFINS AR 11-AA-064258-01 / E4-370-02540264
- APPENDIX # 2 CoA TRIO 2006
- APPENDIX # 3 SGS CT802462
- APPENDIX # 4 SGS AG702217
- APPENDIX # 5 GCL 176.128
- APPENDIX # 6 PAM 081-09
- APPENDIX # 7 TRIO 2009-B1,TRIO 2009-B2,TRIOP01/2010 B1,TRIOP02/2010,TRIOP01/2011,TRIOP02/2011,TRIO P03/2011
- APPENDIX # 8 Input on equivalence between Chia oil and Linseed oil
- APPENDIX # 9 Production Flowchart
- APPENDIX # 10 HACCP Control Checklist
- APPENDIX # 11 TRIO S.A HACCP Certificate
- APPENDIX # 12 GMO Analysis
- APPENDIX # 13 CoA Microbiological Test
- APPENDIX # 14 LD-50 Report

TABLES AND FIGURES