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October 14, 2009

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GRAS Notice Review

Robert L. Martin, Ph.D.
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835

RE: GRAS Notice for Low Saturate, High Oleic, Low Linolenic Soybean Oil (LSHOLLSO)

Dear Dr. Martin:

Monsanto has developed and intends to market low saturate high oleic low linolenic soybean oil (LSHOLLSO). As defined in the attached GRAS Notice, LSHOLLSO is GRAS on the basis of scientific procedures under specific conditions of use as a food ingredient. Information setting forth the basis for the GRAS determination, which includes a comprehensive summary of the data available and reviewed by an independent panel of experts in support of the safety of LSHOLLSO under the intended conditions of use, as well as *curricula vitae* evidencing the qualifications of the members of the panel of experts for evaluating the safety of food ingredients, are enclosed. Based on this GRAS determination and consistent with proposed 21 CFR §170.36 [Notice of a claim for exemption based on a Generally Recognized As Safe (GRAS) determination] published in the *Federal Register* (62 FR 18938) of the *Federal Food, Drug, and Cosmetic Act,* the use of LSHOLLSO in food as described in the notice is exempt from the requirement of premarket approval.

Should you have questions concerning this GRAS Notice, please contact Dr. Russell Schneider, Regulatory Affairs Director, Washington D.C., (202-383-2866), or me (314-694-6587).

Sincerely,

(b) (6)

Aaron Crawford
Pipeline Regulatory Manager
(314) 694-6587

cc: R. Schneider, Ph.D., Monsanto, Washington, DC
Regulatory Files

enc.: Four paper copies of the GRAS Notice for LSHOLLSO
CD containing the GRAS Notice for LSHOLLSO and *Curricula vitae* for expert panel members

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Prepared for:

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Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
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5100 Paint Branch Parkway
College Park, MD 20740-3835

Prepared by:

Aaron W. Crawford, Ph.D.
Monsanto Company
800 North Lindbergh Blvd.
St. Louis, MO 63167
U.S.A.

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Monsanto 09-SY-199F

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Table of Contents

		Page
I	GRAS EXEMPTION CLAIM	1
	A. Claim of Exemption From the Requirement for Premarket Approval Pursuant to Proposed 21 CFR §170.36(c)(1) [62 FR 18938 (17 April 1997)] (U.S. FDA, 1997)	1
	B. Name and Address of Notifier	1
	C. Common Name of the Notified Substance	1
	D. Conditions of Intended Use in Food	1
	E. Basis for the GRAS Determination	2
	F. Availability of Information	2
II.	DETAILED INFORMATION ABOUT THE IDENTITY OF THE SUBSTANCE	3
	A. Identity	3
	B. Method of Manufacture	5
	C. Specifications for Food-Grade Material	8
III.	SELF-LIMITING LEVELS OF USE	10
IV.	BASIS FOR GRAS DETERMINATION	11
	A. Documentation to Support the Safety of LSHOLLSO	11
	B. Estimated Intake of LSHOLLSO	11
	C. Estimated baseline daily intake of total fat and selected fatty acids from the oil blend component of targeted foods	13
	D. Estimated consumption of LSHOLLSO from proposed food uses.	13
	E. Impact of replacement under proposed food uses in total diet.	14
	F. Animal studies	19
	G. Studies in Humans	19
	H. Other Data Pertaining to the Safety of LSHOLLSO	23
	Potential Allergenicity	23
V.	SUMMARY AND BASIS FOR GRAS CONCLUSION.	25
REFERENCES		26

List of Tables

Table 1. Fatty acid composition of LSHOLLSO compared to soybean, olive, canola, high oleic canola, and high oleic safflower oils, based on USDA Food Composition Database. 3

Table 2. Common or usual name, scientific name, CAS number, empirical formula, and molecular weight of oleic acid, the fatty acid present at higher levels in LSHOLLSO compared to commodity soybean oil. 4

Table 3. List of processing aids used in the manufacture of LSHOLLSO. 8

Table 4. Specifications and analytical methods for soybean oil (FCC) and LSHOLLSO (Monsanto). 9

Table 5. Fatty acid specifications for soybean oil (FCC) and LSHOLLSO (% total fatty acids). ..9

Table 6. Proposed food uses for LSHOLLSO. 12

Table 7. Fatty acid profile for LSHOLLSO used in dietary substitution analysis. 13

Table 8. Mean *per capita* fatty acid intake from oil blend in target foods pre- and post-LSHOLLSO replacement, U.S. population. 16

Table 9. 90th percentile per user fatty acid intake from oil blend in target foods pre- and post-LSHOLLSO replacement, U.S. population. 16

Table 10. Mean per capita fatty acid intake from total diet pre- and post- LSHOLLSO replacement, U.S. population. 16

Table 11. 90th percentile per user fatty acid intake from total diet, pre- and post- LSHOLLSO replacement, U.S. population. 17

Table 12. Studies assessing the safe intake of dietary oleic acid. 22

Table 13. Levels of dietary oleic acid/monounsaturated fatty acids (% energy) in clinical studies with positive cardiovascular outcomes. 22

List of figures

Figure 1. Structural formula of oleic acid, which is present at higher levels in LSHOLLSO compared to commodity soybean oil. 4

Figure 2. Schematic overview of the manufacturing process for LSHOLLSO. 6

Figure 3. Mean and 90th percentile oleic acid intake in European populations (g/day) compared to U.S. intake pre- and post- LSHOLLSO replacement. 21

List of Appendices

Appendix A. Expert Panel Consensus Statement regarding the Generally Recognized as Safe (GRAS) status of Monsanto's Low Saturate, High Oleic, Low Linolenic acid Soybean Oil (LSHOLLSO) for use in foods. 30

Appendix B. Batch analyses of LSHOLLSO. 48

Appendix C. Storage stability data for LSHOLLSO. 50

Appendix D. Mean per capita and 90th percentile per user fatty acid intake from total diet, pre- and post- substitution with LSHOLLSO. 51

List of Abbreviations.

AI	Adequate intake
ALA	alpha-linolenic acid
AOCS	American Oil Chemists Society
CDC	Center for Disease Control
cGMP	current Good Manufacturing Practices
CODEX	Codex Alimentarius
CP4 EPSPS	5-enolpyruvylshikimate-3-phosphate synthase from *Agrobacterium* sp., strain CP4
CRF	U.S. Code of Federal Regulations
CSO	Conventional soybean oil
DHA	Docosahexaenoic acid
En	energy
EPA	Eicosapentaenoic acid
EPA	Environmental Protection Agency
FA	Fatty acid
FAD2	*Glycine max* gene for Δ-12 desaturase
Fad3-1b	*Glycine max* gene for Δ-15 desaturase
Fad3-1c	*Glycine max* gene for Δ-15 desaturase
FATB	*Glycine max* gene for Palmitoyl-ACP Thioesterase
FCC	Food Chemical Codex
GM	Genetically modified
GRAS	Generally Recognized as Safe
HOLLCO	High oleic low linolenic canola oil
HOSAFO	High oleic safflower oil
HOSFO	High oleic sunflower oil
IOM	Institute of Medicine
LSHOLL	Low saturated, high oleic, low linolenic
LSHOLLSO	Low saturated, high oleic, low linolenic soybean oil
MON 87705	soybean variety with suppression of FAD2 and FATB genes
MUFA	Monounsaturated fatty acid
NCEP	National Cholesterol Education Program
NHANES	National Health and Nutrition Examination study
PUFA	Polyunsaturated fatty acid
U.S.	United States
USDA	United States Department of Agriculture
WHO	World Health Organization

I GRAS EXEMPTION CLAIM

A. Claim of Exemption From the Requirement for Premarket Approval Pursuant to Proposed 21 CFR §170.36(c)(1) [62 FR 18938 (17 April 1997)] (U.S. FDA, 1997)

As defined herein, low saturated, high oleic, low linolenic soybean oil (LSHOLLSO) has been determined by Monsanto Company (hereafter referred to as Monsanto) to be Generally Recognized as Safe (GRAS) for use in a variety of traditional food products. This determination is based on scientific procedures, as described in the following sections, under the conditions of intended use in food. Therefore, consistent with Section 201(s) of the *Federal Food, Drug, and Cosmetic Act,* the use of LSHOLLSO in food as described below is exempt from the requirement of premarket approval.

Signed,

(b) (6)

Oct. 14, 2009

Raymond C. Dobert, PhD
Lead, U.S. Biotech Regulatory Affairs
Monsanto Company

Date

B. Name and Address of Notifier

Raymond C. Dobert, PhD
Lead, U.S. Biotech Regulatory Affairs
800 North Lindbergh Blvd.
St. Louis, MO 63167
U.S.A.

C. Common Name of the Notified Substance

Low saturated, high oleic, low linolenic soybean oil (LSHOLLSO)

D. Conditions of Intended Use in Food

Monsanto intends to market LSHOLLSO as a food ingredient for frying and spraying applications in the United States in a variety of food products including meat, poultry and fish dishes (commercial and restaurant), fried eggs (commercial and restaurant), French fries, potato chips and puffs, creamy salad dressings, salty snacks, and grain mixture dishes.

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Monsanto Company

Date

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E. Basis for the GRAS Determination

Pursuant to 21 CFR § 170.30, LSHOLLSO has been determined by Monsanto to be GRAS on the basis of scientific procedures (FDA, 2007). This GRAS determination is based on data generally available in the public domain pertaining to the safety of LSHOLLSO, as discussed herein and in the accompanying documents, and on a consensus among a panel of experts[1] who are qualified by scientific training and experience to evaluate the safety of LSHOLLSO as a component of food [see Appendix A, entitled, "EXPERT PANEL CONSENSUS STATEMENT REGARDING THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF MONSANTO'S LOW SATURATED, HIGH OLEIC, LOW LINOLENIC SOYBEAN OIL (LSHOLLSO) FOR USE IN FOODS"].

F. Availability of Information

The data and information that serve as the basis for this GRAS Notification will be sent to the U.S. Food and Drug Administration (FDA) upon request, or will be available for review and copying at reasonable times at the offices of:

Monsanto Company
800 North Lindbergh Blvd.
St. Louis, MO 63167
U.S.A.

Should the FDA have any questions or additional information requests regarding this notification, Monsanto will supply these data and information.

[1] The panel of experts consisted of Joseph F. Borzelleca, Ph.D. (Virginia Commonwealth University School of Medicine), Dr. Robert J. Nicolosi (University of Massachusetts, Lowell) and Dr. John A. Thomas (University of Indiana School of Medicine)

II. DETAILED INFORMATION ABOUT THE IDENTITY OF THE SUBSTANCE

A. Identity

Monsanto proposes to market soybean varieties, developed through a combination of genetic modification and traditional breeding, that produce oil with increased concentrations of oleic acid and reduced levels of saturated fatty acids and linolenic acid. The new soybean varieties have been developed by crossing the biotechnology-derived soybean MON 87705 (low saturated, high oleic soybeans) with Vistive™ low linolenic soybean varieties. Soybean oil from these varieties, herein referred to as low saturated high oleic low linolenic soybean oil (LSHOLLSO), has the following specification: palmitic acid (< 4.0 %); stearic acid (< 4.0 %); oleic acid (55 - 85 %); linoleic acid (8 – 30 %); and linolenic acid (< 4.0 %). As a result of this fatty acid profile, LSHOLLSO has improved heat and oxidative stability relative to commodity soybean oil.

LSHOLLSO contains the same major fatty acids as commodity soybean oil, although not at the same levels. The major fatty acids in LSHOLLSO (palmitic, stearic, oleic, linoleic and linolenic acids) are present at similar levels in several other commonly consumed vegetable oils (Table 1). LSHOLLSO has a fatty acid profile that is comparable to other commercial high oleic vegetable oils (high oleic canola, high oleic safflower, high oleic sunflower), traditional oils such as olive oil that has a long history of consumption in the diet, and canola oil that has obtained GRAS status by the U.S. FDA (21 CFR §184.1555).

Table 1. Fatty acid composition of LSHOLLSO compared to soybean, olive, canola, high oleic canola, and high oleic safflower oils, based on USDA Food Composition Database.

Fatty Acids	LSHOLLSO	USDA Food Composition Database Values				
		Soybean Oil	Olive Oil	Canola Oil	High Oleic Canola Oil	High Oleic Safflower Oil
C16:0 (palmitic)	2.47	10.66	11.29	4.30	3.44	4.29
C18:0 (stearic)	3.15	4.00	1.95	2.09	1.92	1.92
C18:1 (oleic)	71.72	22.57	71.27	61.71	69.89	74.65
C18:2 (linoleic)	16.87	50.12	9.76	19.00	14.34	14.35
C18:3 (linolenic)	2.86	6.54	0.761	9.1	2.07	0.00
(palmitic + stearate)	5.62	15.3	13.81	7.36	5.36	6.21
Total *trans*	0.22	0.68	NR	2.00	0.84	NR

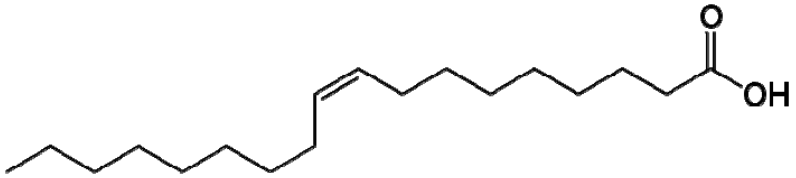
Source: USDA (2008).
NR=not reported

™ Vistive is a trademark of Monsanto Technology LLC.

There is no Chemical Abstracts Services (CAS) number for LSHOLLSO. Table 2 contains information pertaining to oleic acid, the fatty acid present at higher levels in LSHOLLSO compared to commercial soybean oil. The structural formula of oleic acid is presented in Figure 1.

Table 2. Common or usual name, scientific name, CAS number, empirical formula, and molecular weight of oleic acid, the fatty acid present at higher levels in LSHOLLSO compared to commodity soybean oil.

Common or usual name	Scientific name	CAS number	Empirical formula	Molecular weight
Oleic acid	9-*cis*-octadecenoic acid; octadecenoic acid	8046-01-3	$C_{18}H_{34}O_2$	282.46



Oleic acid (C18:1)

Figure 1. Structural formula of oleic acid, which is present at higher levels in LSHOLLSO compared to commodity soybean oil.

B. Method of Manufacture

The fatty acid profile of oil from low saturate, high oleic, low linolenic soybeans has been purposefully altered to improve performance of the oil in frying applications, and consequently the fatty acid profile of LSHOLLSO is different from oil produced from other commodity soybean varieties. LSHOLLSO has significant increases in oleic acid, and corresponding decreases in saturated (palmitic, stearic) and polyunsaturated (linoleic and linolenic) fatty acids. LSHOLLSO is derived from soybean plants produced through a breeding cross of a biotechnology-derived low saturate, high-oleic soybean (MON 87705) crossed with Vistive™ low linolenic acid soybeans. FATB and FAD2 are two enzymes involved in the soybean seed fatty acid biosynthetic pathway. The improved fatty acid profile in MON 87705 soybean is achieved through the introduction of endogenous soybean *FATB-1A* and *FAD2-1A* gene segments configured to suppress FATB and FAD2 gene expression. Vistive™ low linolenic acid soybean was developed through conventional breeding; the low linolenic acid phenotype in these soybeans results from loss of function mutations in two genes, *Fad3-1b* and *Fad3-1c*, similar to other soybean lines described in the literature (Wilcox and Cavins, 1985)[2]. Analytical data on multiple lots of LSHOLLSO demonstrate consistent fatty acid composition across a wide variety of geographies where soybeans are cultivated commercially.

LSHOLLSO is processed using conventional industry standard processing methods, which include extrusion methods (Erickson et al., 1980). Following cracking and aspiration of the soybeans to separate the hulls, the hulls are screened to recover the fines generated during cracking, and the cracked soybean meats are conditioned and flaked to rupture oil cells and prepare a thin flake with a large surface area for solvent extraction. The soybean flakes then undergo solvent extraction with iso-hexane/hexane to yield crude soybean oil and soybean meal. The crude oil is processed through a series of steps known as refining. In the first step, phospholipids are removed through a process known as “degumming”. This involves mixing with water or an acid solution to form “gums”, followed by centrifugation. The fatty acids in the degummed oil are neutralized through the addition of a caustic solution (sodium hydroxide). The resulting soap solution is removed by centrifugation. Water washing and centrifugation or treatment with a suitable adsorbent followed by filtration removes soaps to levels compatible with bleaching. In the next step, the oil is “bleached’ by mixing with a citric acid solution,

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[2] FAD2 (delta-12 desaturase) facilitates the conversion of oleic acid (C18:1) to linoleic acid (C18:2) in the endoplasmic reticulum. FATB (acyl-ACP-thioesterase) allows the release of palmitic acid (C16:0) and stearic acid (C18:0) from the plastid into the cytoplasm. *Fad3-1c* and *Fad3-1b* encode delta-15 desaturases that catalyzes the production of α-linolenic acid. Suppression of FATB and FAD2 gene expression is contributed through MON 87705 soybeans, while the naturally occurring mutations in *Fad3-1c* and *Fad3-1b* are derived from Vistive™ low linolenic soybean varieties. Conventional breeding is used to cross MON 87705 with Vistive™ low linolenic soybean lines to generate LSHOLL soybean varieties.

followed by treatment with adsorbent clay to remove the peroxides, phosphatides, color bodies and traces of soap. Under vacuum to inhibit oxidation, the pigments are adsorbed and removed by filtration. In the final refining step, odoriferous components, flavor components and additional free fatty acids are removed by steam distillation. This process of deodorization is carried out at high temperatures (typically 225°C to 255°C) under vacuum. Permitted anti-oxidants (*e.g.*, Tenox-20) are utilized to limit oxidation of the oil.

LSHOLLSO is produced using commercial food-grade soybean oil manufacturing practices with food-grade raw materials and processing agents consistent with current Good Manufacturing Practices (cGMP). Because commercial soybean oil manufacturing processes used to produce LSHOLLSO are well-characterized, the altered fatty acid profile of LSHOLLSO is a reflection of the LSHOLL soybean variety and not due to or impacted by the manufacturing process. A schematic diagram of the manufacturing process for LSHOLLSO is presented in Figure 2. All processing aids used in the manufacture of LSHOLLSO are used in compliance with appropriate federal regulations (see Table 3).

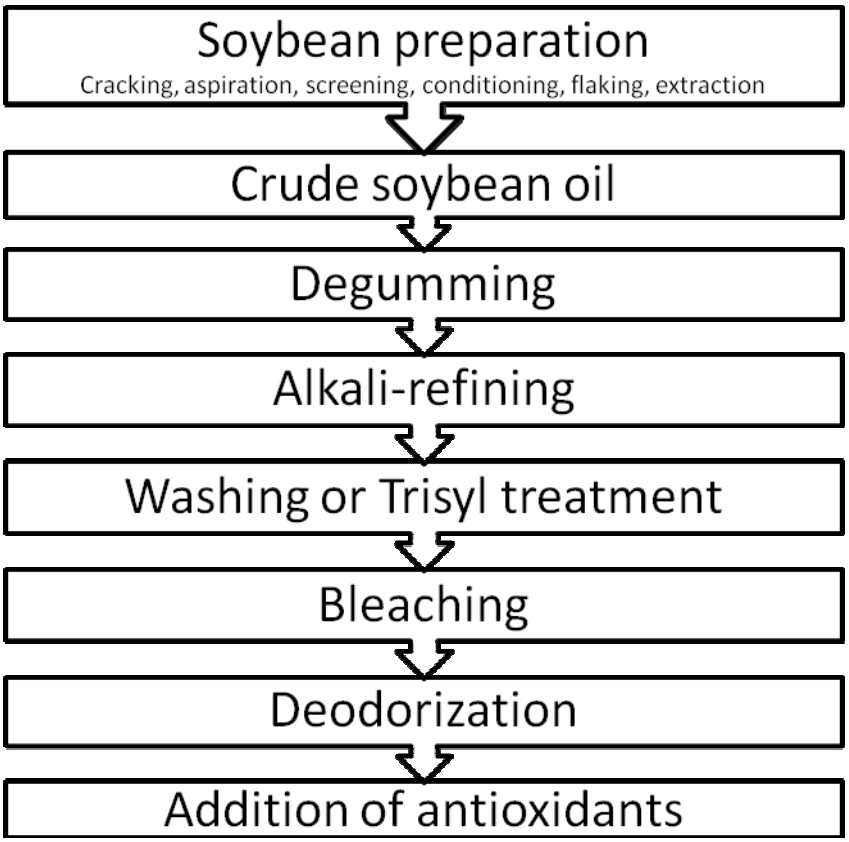

Figure 2. Schematic overview of the manufacturing process for LSHOLLSO.

Table 3. List of processing aids used in the manufacture of LSHOLLSO.

Processing Aids	Reference to Appropriate Use in Food
Iso Hexane/Hexane	21 CFR §172.340 Fish protein isolate (hexane is cleared as a solvent in the removal of lipids from fish protein isolate) 21 CFR §173.270 (hexane is cleared for use when present as a residue from the extraction of spices and in the production of hop extracts) No federal regulations pertaining to the use of hexane as a processing agent for use in the extraction of vegetable oils exist. However, hexane and similar paraffinic hydrocarbons have a long history (pre-1958) of use in the manufacture of food oils in this regard; therefore these uses would be considered GRAS. The use of isohexane/ hexane should be limited to current Good Manufacturing Practices (cGMP).
Anhydrous Citric Acid (diluted to 50% solution)	21 CFR §184.1033 Citric acid.
Sodium Hydroxide	21 CFR §184.1763 Sodium hydroxide.
Phosphoric Acid	21 CFR §182.1073 Phosphoric acid.
Trisyl S165	21 CFR §177.2250 Filters, microporous polymeric.
Tonsil 167 FF Bleaching Clay (Darco Carbon)	21 CFR §186.1256 Clay (kaolinin).
Antioxidants (Tenox™-20)	Tenox-20 consists of propylene glycol, citric acid, and tertiary butylhydroquinone (TBHQ). 21 CFR §184.1666 Propylene glycol. 21 CFR §184.1033 Citric acid. 21 CFR §172.185 TBHQ.

CFR = Code of Federal Regulations

C. Specifications for Food-Grade Material

The product specifications and methods of analysis for LSHOLLSO are presented in Tables 4 and 5. Food Chemicals Codex specifications for commercial soybean oil (FCC, 2008) also are included for comparative purposes. The specification set for LSHOLLSO is consistent with that established for commodity soybean oil, with the exception of those parameters that are related to the improved oil stability of LSHOLLSO (iodine value and AOM stability). LSHOLLSO has a lower iodine value and has a higher AOM stability value; these differences indicate a more stable oil.

Table 4. Specifications and analytical methods for soybean oil (FCC) and LSHOLLSO (Monsanto).

Parameter	FCC Soybean Oil Specification	LSHOLLSO Specification	Analysis Method
Cold test	Passes test	Passes test	AOCS Cc 11-53
Fatty acid composition	Please see Table 5	Please see Table 5	AOCS Ch 2a-94, Ce 1f-96
Lovibond color (Yellow)	NMT 20	NMT 20	AOCS Cc 13j-97 (Lovibond)
Lovibond color (Red)	NMT 2.0	NMT 2.0	AOCS Cc 13j-97 (Lovibond)
Free fatty acids	NMT 0.1%	NMT 0.1%	AOCS Ca 5a-40
Iodine value	120 – 143	90 - 100	AOCS Tg 1a-64 (modified: POS Method IN-LS-26)
Lead	NMT 0.1 mg/kg	NMT 0.1 mg/kg	FCC Lead Limit Test, Atomic Absorption Spectrophotometric Graphite Furnace Method II
Peroxide value	NMT 10 meq/kg	NMT 10 meq/kg	AOCS Cd 8-53
Stability, Active oxygen method (AOM)	NLT 7 h	NLT 30h	AOCS Cd 12-57
Unsaponifiable matter	NMT 1.5 %	NMT 1.5%	AOCS Ca 6a-40
Water	NMT 0.1%	NMT 0.1%	AOCS Ca 2c-25

NLT = not less than; NMT = not more than

Table 5. Fatty acid specifications for soybean oil (FCC) and LSHOLLSO (% total fatty acids).

Fatty acid	FCC Soybean Oil Specification	LSHOLLSO Specification
C < 14:0	<0.1	<0.1
C14:0 (myristic)	<0.5	<0.5
C16:0 (palmitic)	7.0 – 12.0	< 4.0
C16:1 (palmitoleic)	<0.5	<0.5
C18:0 (stearic)	2.0 – 5.5	< 4.0
C18:1 (oleic)	19 – 30	55 – 85
C18:2 (linoleic)	48 – 65	8 – 30
C18:3 (α-linolenic)	5 – 10	< 4.0
C20:0 (arachidic)	<1.0	<1.0
C20:1 (eicosenoic)	<1.0	<1.0
C22:0 (behenic)	<0.5	<0.5
C24:0 (lignoceric)	<0.3	<0.3

Product Analysis

Batch analyses data were generated for eight non-consecutive lots of LSHOLLSO produced from seeds grown during the 2008 harvest season at various U.S. locations. These data are presented in Appendix B, and include chemical and physical characteristics (Table B-1), fatty acid composition (Table B-2), and tocopherol and sterol content (Table B-3).

Storage stability of LSHOLLSO

The storage stability of LSHOLLSO was evaluated at ambient (25 °C) and elevated (40 °C) temperatures for 30 days. Results of storage stability testing demonstrate that LSHOLLSO is stable, with respect to peroxide value, iodine value and fatty acid content, for at least 30 days when stored at either room temperature (25 °C), or under accelerated conditions (40 °C). See Appendix C for details on storage stability of LSHOLLSO.

Comparative stability profiles were reported for high oleic low linolenic canola oil (HOLLCO) and LSHOLLSO throughout the 30-day study interval; peroxide and iodine values in particular for these oils distinguish their stability compared to commodity soybean oil. Collectively, these data demonstrate that LSHOLLSO has excellent storage stability that is comparable to other commercial oils with high oxidative and heat stability, such as HOLLCO.

III. SELF-LIMITING LEVELS OF USE

The use of LSHOLLSO is limited by the level of fat that can be added to the proposed foods.

IV. BASIS FOR GRAS DETERMINATION

A. Documentation to Support the Safety of LSHOLLSO

The safety and GRAS status of the intended uses of Monsanto's LSHOLLSO in food applications is based on the following:

- LSHOLLSO contains the same major fatty acids as commodity soybean oil, although not at the same levels. The major fatty acids in LSHOLLSO are palmitic, stearic, oleic, linoleic and linolenic acids.
- The concentration ranges of the remaining fatty acids, total tocopherols and plant sterols were within the range for soybean oil, or the CODEX standards for other vegetable oils
- The fatty acid profile of LSHOLLSO is similar to many other commercial vegetable food oils currently used for deep-frying or spraying applications. LSHOLLSO has a fatty acid profile that is comparable to other commodity high oleic vegetable oils (high oleic canola, high oleic safflower, high oleic sunflower), traditional oils such as olive oil that has a long history of consumption in the diet, and canola oil that has obtained GRAS status by the U.S. FDA.
- Published studies indicate that high oleic acid vegetable oils with fatty acid compositions comparable to LSHOLLSO are suitable for use in deep-frying food applications.
- LSHOLLSO is a refined vegetable oil produced through standard soybean oil manufacturing and refining practices under cGMP.
- Published studies indicate that refined vegetable oils derived from common allergenic foods (soybeans, peanuts) do not pose an allergenicity risk to sensitive individuals.
- A dietary intake assessment reveals no change in total fat intake when LSHOLLSO replaces current oils and oil blends used in intended food categories. In addition, replacement with LSHOLLSO supports the currently established adequate intake for the essential fatty acids, linolenic acid and linoleic acid.
- Following replacement of current oils and oil blends with LSHOLLSO in intended food categories, the estimated U.S. *per capita* daily intake of oleic acid does not exceed the daily intake in many European countries, is within the range used in multiple clinical studies, and is consistent with WHO, USDA and AHA dietary recommendations.

Moreover, these data were reviewed by a panel of experts, qualified by scientific training and experience to evaluate the safety of ingredients as components of food, who concluded that the proposed uses of LSHOLLSO are safe and suitable and are GRAS based on scientific procedures (see Appendix A). A summary of the data is presented herein.

B. Estimated Intake of LSHOLLSO

LSHOLLSO is intended for use as low saturated, *trans*-fat free replacement for hydrogenated oils and liquid oils currently used in the food categories defined in Table 6. Major food categories include fried meat, poultry and fish dishes (commercial and restaurant), fried eggs (commercial and restaurant), French fries, potato chips and puffs, creamy salad dressings, salty snacks, and grain mixture dishes. The consumption of LSHOLLSO from all proposed food uses

was estimated by Exponent, Inc. (Washington, D.C.) using the proposed food uses and use levels in conjunction with food consumption data included in the National Health and Nutrition Examination Surveys, or NHANES 2003-2006 (CDC, 2007). The current intake of total fat and five major fatty acids in the diet pre-substitution and post-substitution with LSHOLLSO was calculated, assuming 100% replacement with LSHOLLSO in all identified food applications. Based on the market research, the oils in the target foods that would be replaced with LSHOLLSO are: 1) soybean, 2) corn, 3) sunflower, 4) palm, 5) canola, 6) cottonseed, and 7) rice bran oils. As commercial frying oils are blends of several oils, a computer-based program was developed to extract and combine the oil ingredient portions in each target food into a blended oil component (henceforth referred to as the 'oil blend'); in each target food, the oil blend was replaced with LSHOLLSO.

Table 6. Proposed food uses for LSHOLLSO.

FDA Food Classifications (21 CFR §170.3(n))[1]	Proposed food uses for LSHOLLSO
Egg Products	Fried Egg Dishes (Commercial/Restaurant)
Fats and Oils	Creamy Salad Dressings
	Tub Margarine
Fish Products	Fried Fish Dishes (Commercial/Restaurant)
Grain Products and Pastas	Grain Based Dishes (Burritos, Enchiladas, Tacos, Other Ethnic Mixed Dishes)
Meat Products	Fried Meat Dishes (Commercial/Restaurant)
Poultry Products	Fried Poultry Dishes (Commercial/Restaurant)
Processed Vegetables and Vegetable Juices	French Fries (Fast Food and Home Use)
Snack Foods	Crackers
	Potato Chips and Puffs
	Salty Snacks from Grain Products

[1] Categories as defined in Food and Drug Administration's Reference amounts customarily consumed per eating occasion (21 CFR §101.12).

The fatty acid profile for LSHOLLSO used in the dietary replacement model is summarized in Table 7. The substitution modeling assumed 100% replacement of the oil blend with LSHOLLSO, except for crackers, where 50% replacement with LSHOLLSO was assumed, to account for the spray oil portion only of this food category.

Table 7. Fatty acid profile for LSHOLLSO used in dietary substitution analysis.

Fatty Acids	Composition (% by weight)
16:0	2.47
18:0	3.15
18:1	71.72
18:2	16.87
18:3	2.86
Trans fatty acids (total)	0.22

C. Estimated baseline daily intake of total fat and selected fatty acids from the oil blend component of targeted foods

The mean and 90th percentile intakes of total fat and five selected FA from the oil blend in target foods (prior to substitution with LSHOLLSO) were estimated and are reported in Tables 8 and 9. For the U.S. population, the oil blend component of the target foods contributes an average of 14.02 g/day *per capita* of total fat (Table 8); this amounts to approximately 17.6% of total dietary fat intake (total fat intake = 79.58 g/day). At the 90th percentile, *per user*, the total fat contribution from the soybean oil component of the target foods is 30.05 g/day (Table 9), approximately 24% of total fat from the whole diet (total fat intake = 129.40 g/day). On a percent energy (% en) basis, the mean *per capita* contribution from the oil blend component of the target foods is 6.61%, and 12.94% in the 90th percentile per user group.

D. Estimated consumption of LSHOLLSO from proposed food uses.

The mean and 90th percentile intakes of total fat, total *trans*-fat and five selected fatty acids from the introduction of LSHOLLSO under the proposed food uses were estimated for the target foods. The *per capita* and per user intakes for the U.S. population are presented in Tables 8 and 9.

Consistent with the fatty acid profile associated with LSHOLLSO, intake of oleic acid is increased the most among the five fatty acids evaluated when replacement with LSHOLLSO was carried out. For the U.S. population, the mean *per capita* intake of oleic 18:1 from the new soybean oil component from proposed foods is 8.86 g/day (4.18% en). At the 90th percentile, *per capita* intake of oleic acid from the new soybean oil component of the proposed foods is 19.33 g/day (8.21% en) (Table 9). There is essentially no impact on *trans* fatty acid levels in the target foods, as many of the products in these food categories have recently been reformulated to remove *trans* fats in accordance with current labelling requirements. For

example, the pre-substitution level is 0.07 g/day *per capita*, and this level drops to 0.04 g/day post-substitution. Importantly, the new soybean oil component of the proposed foods does not contribute to the *trans* fatty acid intake to the diet under the proposed uses, and offers an alternative to maintain low *trans* fatty acid levels in these categories of food products.

E. Impact of replacement under proposed food uses in total diet.

The impact of oil replacement under the proposed food uses with LSHOLLSO on the total daily intake (g/day) of the five major fatty acids (palmitic, stearic, oleic, linoleic, linolenic) in the diet of the U.S. population are summarized in Table 8 (*per capita*, target foods), Table 9 (90th percentile, target foods), Table 10 (*per capita*, total diet) and Table 11 (90th percentile, total diet). As expected, the replacement with LSHOLLSO does not change the total daily fat intake, which remains at 79.58 g/day on the mean *per capita* basis, and at 129.4 g/day in the 90th percentile per user group.

Following replacement with LSHOLLSO, there is an overall increase in the total daily intake of oleic acid from 27.34 g/day to 30.92 g/day on a mean *per capita* basis (Table 10) and an increase from 45.2 g/day to 53.34 g/day at the 90th percentile per user (Table 11). These oleic acid intake increases post-replacement are expected, since the oleic acid fraction in targeted replacement oils, such as hydrogenated soybean oil (25-38% oleic acid), commercial soybean oil (17-30%), corn oil (20-42%), sunflower oil (14-39%), palm oil (36-44%), canola oil (51-70%) and cottonseed oil (15-22%) (CODEX, 2005) are much lower compared to the 71.7% oleic acid observed in LSHOLLSO. The pattern of total daily intake of fatty acids in sub-groups stratified by age and gender is similar to that of the overall U.S. population, with oleic acid having the highest increase following replacement of target oils with LSHOLLSO.

Total daily intakes of palmitic acid and linoleic acid decreased as a consequence of dietary replacement with LSHOLLSO (Tables 10 and 11). On a mean *per capita* basis, palmitic acid intake decreased from 14.38 g/day to 12.84 g/day, while the decrease was predictably greater in the 90th percentile group, where daily intake decreased from 23.62 g/day to 20.24 g/day. These values represent a decrease in palmitic acid of 10.7% and 14.3% respectively. A decrease in dietary saturated fat intake is consistent with current dietary recommendations (Lichtenstein et al., 2006; USDA, 2005; WHO, 2003).

Total dietary intake of linoleic acid decreased by a similar rate, from 14.75 g/day to 13.45 g/day on a mean *per capita* basis, and from 25.66 g/day to 22.63 g/day in the 90th percentile per user group. These changes represent decreases of 8.8% and 11.8%, respectively. An adequate intake (AI) for linoleic acid of 17 g/day for men (19-50 years) and 12 g/day for women (19-50 years) has been established by the Institute of Medicine (IOM, 2002). While the post-replacement value for the entire U.S. population is slightly below this AI, the values observed

post-replacement for the respective subgroups are consistent with these recommendations (see Appendix D). For example, mean *per capita* post replacement values are 17.11 g/day (males 20-49; Table D-1) and 12.73 g/day (females 20-49; Table D-2).

On a mean *per capita* basis, there was negligible change in total dietary linolenic acid intake (1.42 g/day vs. 1.38 g/day) before and after replacement with LSHOLLSO (Table 10). Consistent with this, there was a very minor decrease (from 2.49 g/day to 2.35 g/day) pre- and post- replacement in the 90th percentile per user group (Table 11). Total dietary intakes of stearic acid were similar in pre- and post- LSHOLLSO replacement analysis as well, in both mean *per capita* (6.96 g/day and 6.83 g/day, respectively) and 90th percentile per user groups (11.75 g/day and 11.42 g/day), respectively.

Table 8. Mean *per capita* fatty acid intake from oil blend in target foods pre- and post- LSHOLLSO replacement, U.S. population.

Fatty Acids	g/day			% En		
	Pre replacement	Post-replacement	% CHANGE	Pre replacement	Post-replacement	% CHANGE
Palmitic 16:0	1.27	0.50	-60.6	0.61	0.23	-62.3
Stearic 18:0	0.50	0.45	-10.0	0.24	0.21	-12.5
Oleic 18:1	5.08	8.86	74.4	2.36	4.18	77.1
Linoleic 18:2	5.93	2.96	-50.1	2.82	1.39	-50.7
Linolenic 18:3	0.55	0.46	-16.4	0.27	0.22	-18.5
Total Fat Intake	14.02	14.02	0.0	6.61	6.61	0.0

Table 9. 90th percentile per user fatty acid intake from oil blend in target foods pre- and post-LSHOLLSO replacement, U.S. population.

Fatty Acids	g/day			% En		
	Pre replacement	Post-replacement	% CHANGE	Pre replacement	Post-replacement	% CHANGE
Palmitic 16:0	2.78	1.11	-60.1	1.17	0.47	-59.8
Stearic 18:0	1.09	0.96	-11.9	0.46	0.41	-10.9
Oleic 18:1	11.02	19.33	75.4	4.70	8.21	74.7
Linoleic 18:2	13.00	6.55	-49.6	5.51	2.77	-49.7
Linolenic 18:3	1.33	1.02	-23.3	0.58	0.43	-25.9
Total Fat Intake	30.05	30.05	0	12.94	12.94	0

Table 10. Mean per capita fatty acid intake from total diet pre- and post- LSHOLLSO replacement, U.S. population.

Fatty Acids	g/day			% En		
	Pre replacement	Post-replacement	% CHANGE	Pre replacement	Post-replacement	% CHANGE
Palmitic 16:0	14.38	12.84	-10.7	6.07	5.32	-12.3
Stearic 18:0	6.96	6.83	-1.8	2.91	2.84	-2.5
Oleic 18:1	27.34	30.92	13.1	11.49	13.24	15.2
Linoleic 18:2	14.75	13.45	-8.8	6.23	5.61	-10.0
Linolenic 18:3	1.42	1.38	-2.9	0.61	0.59	-3.6
Total Fat Intake	79.58	79.58	0.0	33.61	33.61	0.0

Table 11. 90th percentile per user fatty acid intake from total diet, pre- and post- LSHOLLSO replacement, U.S. population.

Fatty Acids	g/day			% En		
	Pre replacement	Post-replacement	% CHANGE	Pre replacement	Post-replacement	% CHANGE
Palmitic 16:0	23.62	20.24	-14.3	8	6.53	-18.4
Stearic 18:0	11.75	11.42	-2.8	3.94	3.81	-3.3
Oleic 18:1	45.20	53.34	18.0	15.16	18.60	22.7
Linoleic 18:2	25.66	22.63	-11.8	9.22	7.81	-15.3
Linolenic 18:3	2.49	2.35	-5.8	0.95	0.87	-8.2
Total Fat Intake	129.40	129.40	0.0	42.88	42.88	0.0

Summary of dietary intake

Replacement of hydrogenated soybean oil, liquid oils, and oil blends under the proposed food uses of LSHOLLSO does not change the total daily intake of fat in the U.S. diet. The fatty acid with the highest increase due to the replacement is oleic acid. For the U.S. population, there is an overall increase in the total daily intake of oleic acid from 27.34 g/day to 30.92 g/day on a mean per capita basis, and an increase from 45.2 g/day to 53.34 g/day at the 90th percentile per user; these increases correspond to 13.1% and 18.0%, respectively. These post-replacement increases are expected, since the oleic acid fraction in the oils targeted for replacement is much lower, ranging from 15-22% in cottonseed oil to 51-70% in canola oil, compared to the 71.7% oleic acid observed in LSHOLLSO. Palmitic acid is the fatty acid with the second greatest dietary impact following replacement with LSHOLLSO. A reduction from 14.38 g/day to 12.84 g/day in the mean *per capita* group and from 23.62 g/day to 20.24 g/day for the 90th percentile per user group was determined; these reductions correspond to 10.7% and 14.3%, respectively. The total reduction in saturated fat intake (palmitic and stearic combined) was 12.5% for the mean *per capita* group, and 17.1% for the 90th percentile per user group. These dietary changes – elevation of oleic acid and decrease in saturated fat – are consistent with current dietary recommendations which encourage reductions in *trans* fats and saturated fats, and further urge that most dietary fats be comprised of polyunsaturated and monounsaturated fats such as oleic acid (Lichtenstein et al., 2006; USDA, 2005; WHO, 2003). While a slight decrease was noted for linoleic acid post-substitution, the slightly lower level still satisfies the AI for individual subgroups established by the IOM (IOM, 2002). Linolenic acid intake was essentially unchanged before and after LSHOLLSO substitution, and meets the AI for all individual subgroups (IOM, 2002). Thus replacement with LSHOLLSO has no nutritional consequence on linolenic acid intake in the U.S. population. Dietary intakes of linolenic and

linoleic acids for all age and gender sub populations pre- and post- replacement can be found in Appendix D.

In summary, replacement of hydrogenated oils, liquid oils and oil blends with LSHOLLSO under the proposed food uses results in changes in the fatty acid composition in the U.S. diet that lead to higher oleic acid intake, and lower consumption of saturated fats (palmitic and stearic acids) and linoleic acid, with no impact on total fat intake. Given that this assessment assumes that all of the targeted oil components of the foods proposed for replacement at all eating occasions in the U.S. are replaced with the new soybean oil, the results presented in this report represent a theoretical maximal effect of LSHOLLSO on fatty acid composition of the diet. Further, the nutritional impact of LSHOLLSO under the intended conditions of use is estimated to result in changes in fatty acid consumption that are in line with current dietary guidelines for fatty acid intake (Lichtenstein et al., 2006; USDA, 2005; WHO, 2003).

Current Regulatory Status and Background Dietary Intakes

Stearic, linoleic, oleic and palmitic acids are permitted for use as ingredients in food in the U.S. (21 CFR 172.860; 21 CFR 184.1065; 21 CFR 184.1090; 21 CFR 184.1329; 21 CFR 184.1505). There are currently no specific regulations in the U.S. governing the use of α-linolenic acid as a food ingredient.

α-Linolenic acid (ALA) is a normal component of soybean oil occurring at levels ranging from 4.5 to 11% in commercial soybean oil (CODEX, 2005). It is the parent compound of the n-3 polyunsaturated fatty acid (PUFA) family of essential fatty acids, and therefore, an important component in the diet (Beare-Rogers et al., 2001). ALA is present at high levels in vegetable fats and oils, especially, linseed and flaxseed oil, and present in lower levels in many different seed oils (Innis and Elias, 2003; Ratnayake et al., 1992). Additional rich sources of ALA include currant oil from the seeds of Ribes, perilla oil (*Perilla frutescens*), and dragonhead oil (*Dracocephalum moldavica*) (Stuchlik and Zak, 2002; Vecera et al., 2003). DeFilippis and Sperling (2006) reported the ALA content of flaxseeds, butternuts, canola oil, walnuts, fish (catfish, mackerel, salmon, tuna) and flaxseed oil to be 18.1, 8.7, 9.3, 9.1, 0.2, and 53.3 g per 100 g food item, respectively.

Oleic acid is a common component of vegetable oils, and constitutes as much as 90% of the fatty acid content of high oleic sunflower oil (CODEX, 2005). Other high oleic oils that are commonly consumed include olive oil (55 – 83% oleic acid), canola oil (51 – 70 % oleic acid), and safflower oil (70 – 83% oleic acid) (CODEX, 2005). Currently, there is no recommended minimum intake for oleic acid in the U.S., as the IOM has not set a recommended daily allowance for this fatty acid (IOM, 2002). However, the Adult Treatment Panel-III (ATP-III) recommends that monounsaturated fats such as oleic acid comprise up to 20% of total energy intake for optimal dietary control of cholesterolemia (NCEP, 2001). For comparison, the average consumption of monounsaturated fat in Italy, where a 'Mediterranean diet' is common, fluctuates between 18-20% of total dietary fatty acids (Poli et al., 2008). A separate study

conducted by Hulshof et al. (1999) reports that the mean consumption of oleic acid across Europe ranges between 12.4 g/d (Norwegian women) up to 36.9 g/d (Spanish men), whereas intake exceeds 55 g/d in the 90th percentile group of Greek men. The values for Greek and Spanish men exceed the mean *per capita* (30.92 g/d) and 90th percentile per user (53.34 g/d) intake estimated for U.S. populations post-replacement with LSHOLLSO (see Tables 10 and 11), and establish historical precedence for this level of oleic acid intake.

F. Animal studies

Delaney et al. (2008) conducted a 90-day rat feeding study using high oleic (70%) soybeans to demonstrate that there is no biologically relevant difference with respect to body weight/gain, food consumption/efficacy, mortality, clinical signs of toxicity, or ophthalmological observations compared to commercial soybeans[3]. Consequently, this study demonstrates high oleic (70%) soybean is as nutritious and wholesome as non-GM soybeans.

To demonstrate the safety of ethyl oleate, the ethyl ester of oleic acid, Bookstaff et al. (2004) conducted a 91-day feeding study in rats (20 males and 20 females per group), comparing the safety of ethyl oleate to high oleic safflower oil. In the feeding study, test rats consumed ethyl oleate at up to 10% of the diet (high-dose males and females consumed 5.5 and 6.1 g/kg/day, respectively), while control rats were fed 10% high oleic soybean oil, which contained 80% oleic acid (where total dietary oleic acid intake was 8%); both diets were supplemented with an additional 5.6% commodity soybean oil. Both diets were well tolerated throughout the study period, and there were no toxicologically significant findings in any of the measured parameters for either group. The following data were collected: clinical observations, body weight gain, appearance of the feces, ophthalmic examination, hematology, coagulation, clinical chemistry, urinalysis, organ weight, histopathology, male and female reproductive assessments (i.e., sperm viability and estrous cycle assessments). These data demonstrate that both ethyl oleate and high oleic soybean oil, at daily intake of up to 10% of the diet (equivalent to 66% of total oil intake), are safe with no observed adverse effects at these dietary levels.

G. Studies in Humans

The TRANSFAIR study provides a clear example of the history of safe chronic consumption of oleic acid, especially through the consumption of high-oleic oils such as olive oil (Hulshof et al., 1999). The authors used dietary consumption surveys derived from 14 European countries to

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[3] Soybean components were added to the diets in the following amounts: 20% meal, 1.5% hulls, 1.5-1.7% oil. The oil composition of HOSO (high oleic soybean oil) is: 6.44% palmitic acid; 5.89% stearic acid; 70.6 % oleic acid; 5.5% linoleic acid; 7.2% linolenic acid.

estimate total fat intake and total fatty acid intake within the respective populations of each country. For the estimation of individual fatty acid intake, food composition data was derived by measuring the fatty acid profiles of 100 food samples representing 95% of the total fat intake for each country. The lowest daily oleic acid intake was observed in German men (mean 17.6 g/d; 90th percentile 25.8 g/d) and Norwegian women (mean 12.4 g/d; 90%ile 19.0 g/d). On a percent energy (%en) basis, monounsaturated fatty acid (MUFA) consumption in men accounted for 9.0 and 11.5 %en at the mean and 90th percentile, respectively; in women these values were 8.7 and 10.7 %en at the mean and 90th percentile, respectively.

The highest intake of oleic acid, for both men and women, was observed in Greece[4]. The daily intake of oleic acid among Greek males (aged 23 to 64 years) was estimated to be 32.3 and 55.8 g/day in mean and 90th percentile consumers, respectively. MUFA consumption corresponded to 17.9 and 27.8 %en for the mean and 90th percentile respectively. In Greek females, oleic acid was consumed in comparable quantities as seen in Greek males, in mean and 90th percentile per capita groups (28.5 and 49.3 g per person per day, respectively). MUFA consumption in Greek women corresponded to 18.1 and 27.7 %en for the mean and 90th percentile, respectively. A separate study revealed that median total monounsaturated fat intake in Greek populations was 22.4 %en in females and 21.3 %en in males, or 46.5 g/day and 55.9 g/day, respectively (Trichopoulou et al., 2003). In Spain, the lowest quartile of olive oil consumers had a MUFA intake of ~15 %en, while those in the highest quartile consumed ~20 %en as monounsaturated fats (Serra-Majem et al., 2003). As shown in Figure 3, there are many European countries where the intake of oleic acid is comparable to, or exceeds, oleic acid intake following the introduction of LSHOLLSO to the U.S. diet. These quantitative data support the safe use of LSHOLLSO under the intended uses based on the long history of safe consumption of oleic acid, especially in countries such as Greece and Spain with high oleic acid consumption. The mean and 90th percentile intake of oleic acid intake among men and women in different countries is represented in Figure 3 to allow comparison to the levels of U.S. LSHOLLSO intake in the pre- and post-replacement analysis described earlier.

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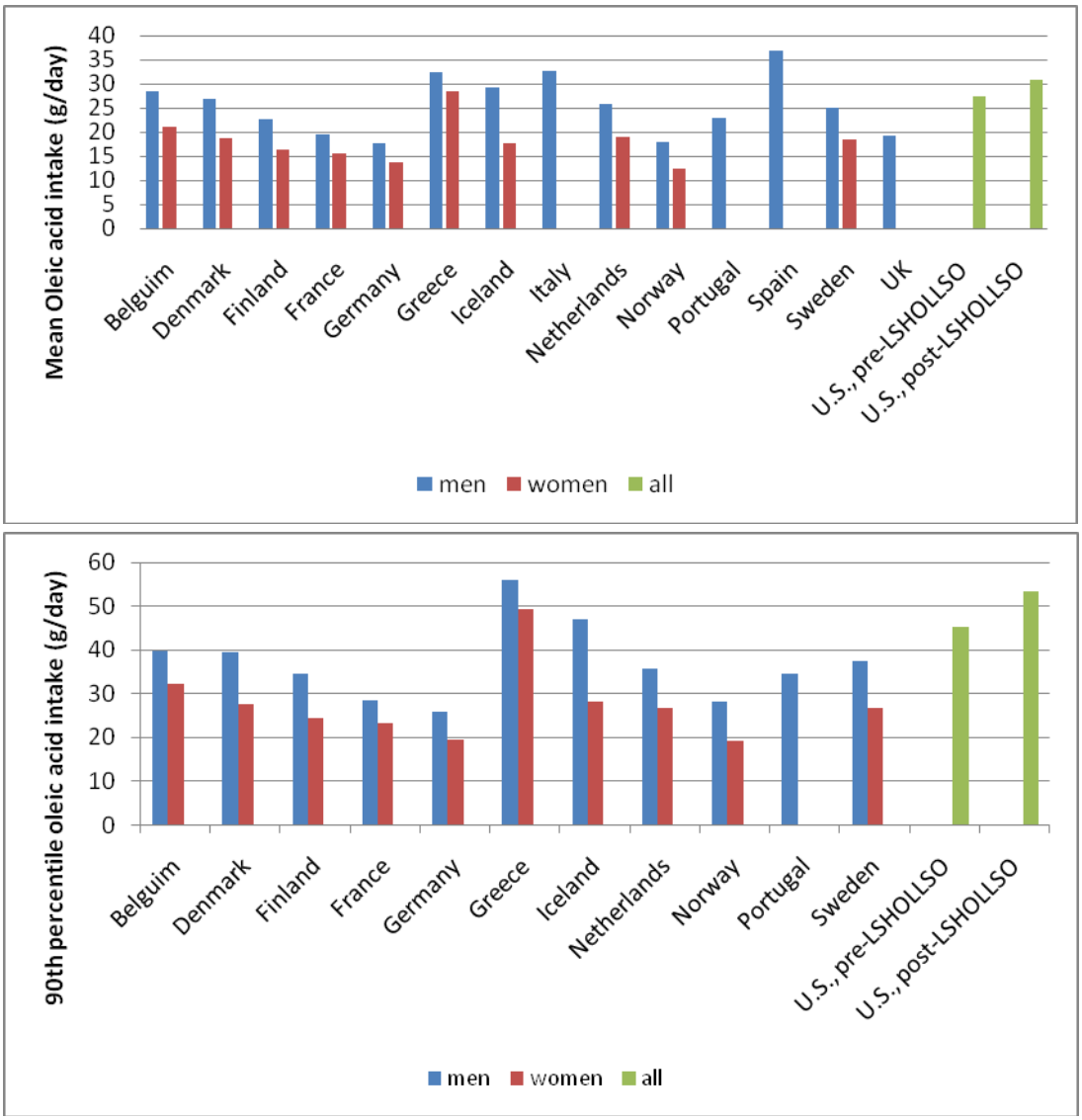
[4] In the study published by Hulshof et al. (1999), Spanish men had a mean intake of 36.9 g/day of oleic acid; however, no data were available for the 90th percentile or for women. Consequently, data for Greece were presented in the text.



Adapted from Hulshof et al., 1999.

Figure 3. Mean and 90th percentile oleic acid intake in European populations (g/day) compared to U.S. intake pre- and post- LSHOLLSO replacement.

Collectively, the scientific literature supports the conclusion that dietary oils rich in oleic acid are a suitable option to provide energy when replacing the use of oils high in saturated fats or *trans* fatty acids. A summary of the data available in the scientific literature that supports healthy oleic acid intake from epidemiological studies and meta-analyses is provided in Table 12. Importantly, no adverse effects due to consumption of high oleic acid diets have been observed in clinical trials (Baer et al., 2004), or are otherwise reported in the literature. Ranges of oleic acid that have been used in clinical interventions with successful cardiovascular outcomes are presented in Table 13.

Table 12. Studies assessing the safe intake of dietary oleic acid.

Epidemiological studies
Hulshof et al., (1999). In several Western European countries, a considerable proportion of energy is derived from saturated fatty acids. Reduced intake of cholesterol-raising fatty acids, saturated fatty acids and trans-fatty acids included, is recommended. Further, there is strong support for safe chronic consumption of dietary oleic acid (i.e. mean intakes of 32.3 g/day and 28.5 g/day in Greek men and women, respectively) at levels that exceed current U.S. intake.
Yoneyama et al., (2007). Based on data from over 3000 Japanese participants, increased intake of oleic acid, linoleic acid, and α-linolenic acid would reduce serum C-reactive proteins levels (a marker of inflammation), especially when there is moderate dietary intake of long chain PUFAs such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA).
Meta-analyses
Mensink et al., (2003). Meta-analysis of 60 clinical trials to estimate effects of specific fatty acids on cardiovascular heart risk factors concludes that these risk factors are reduced most effectively when dietary *trans* fatty acids and saturated fatty acids are replaced with *cis* unsaturated fatty acids.
Mente et al., (2009). The authors conducted a systematic review of the MEDLINE database, for all prospective cohort studies and randomized controlled trials published from 1950-2007 to examine the association between nutrient intake, dietary components and dietary patterns relative to coronary heart disease and related clinical outcomes. As a result, they identified protective dietary factors (vegetables, nuts, monounsaturated fats) and harmful factors (*trans* fatty acids, high glycemic index foods).

Table 13. Levels of dietary oleic acid/monounsaturated fatty acids (% energy) in clinical studies with positive cardiovascular outcomes.

Reference	Diet composition	Study duration	Study population
Lichtenstein et al. (2006)	17.8 % en as oleic acid	5 weeks	16 women, 14 men
Perez-Jimenez et al. (1995)	22 % en as MUFA	4 weeks	22 men
Jansen et al. (2000)	22 % en as MUFA	4 weeks	41 men
Abbey and Nestel (1994)	17.8 % en as oleic acid	3 weeks	10 women, 5 men
Baer et al. (2004)	17.5 % en as oleic acid	5 weeks	50 men
Allman-Farinelli et al. (2005)	20.3 % en as MUFA	5 weeks	27 men
Thijssen et al. (2005)	19.1% en as MUFA	5 weeks	27 women, 18 men
Mensink (2008)	18.9% en as MUFA	3 weeks	33 women, 11 men

The level of oleic acid in the human intervention studies (see Table 13) ranged between 17-22% of total energy intake. These levels are consistent with those observed in traditional 'Mediterranean' diets, where total monounsaturated fats contribute between 18-28% of total caloric intake in adult populations (Hulshof et al., 1999; Poli et al., 2008; Serra-Majem et al., 2003; Trichopoulou et al., 2003). Following substitution of dietary fats with LSHOLLSO, mean *per capita* oleic acid intake is estimated to be 13.2 %en (increased from 11.5 %en), and 18.6 %en (increased from 15.2 %en) in the 90th percentile group. These upward shifts in oleic acid intake post-substitution are consistent with the dietary recommendations of many health groups. For example, the Adult Treatment Panel-III (ATP-III) recommends that monounsaturated fatty

acids comprise up to 20% of total energy intake for optimal dietary control of cholesterolemia (NCEP, 2001).

Changes in fatty acid intake resulting from replacement with LSHOLLSO are consistent with American Heart Association diet and lifestyle recommendations for cardiovascular disease risk reduction, where a key recommendation is to limit intake of saturated fat to 7 %en, *trans* fat to 1 %en, and to minimize intakes of partially hydrogenated fats (Lichtenstein et al., 2006). Similarly, the USDA Dietary Guidelines for Americans (DHHS and USDA, 2005) offers the following recommendations:

1. Consume less than 10 percent of calories from saturated fatty acids.
2. Keep total fat intake between 20 to 35 percent of calories, with most fats coming from sources of polyunsaturated and monounsaturated fatty acids, such as fish, nuts, and vegetable oil.
3. Limit intake of fats and oils high in saturated and/or *trans* fatty acids, and choose products low in such fats and oils.

Small reductions in polyunsaturated fatty acid intake in the replacement assessment, such as observed for linoleic acid (mean *per capita* 14.8 g/d pre-replacement versus 13.4 g/d post-replacement), are expected to be nutritionally insignificant, particularly in light of the fact that this reduction is compensated by increases in dietary oleic acid intake, and a modest decrease in saturated fat intake.

H. Other Data Pertaining to the Safety of LSHOLLSO

Potential Allergenicity

The inverted repeat RNA involved in the gene suppression of *FAD2* and *FATB* is not anticipated to encode for the production of a protein so no additional assessment of potential allergenicity or toxicity is needed.

The only introduced protein in MON 87705 is the 5-enolpyruvylshikimate-3-phosphate synthase protein from *Agrobacterium* sp. strain CP4 (CP4 EPSPS) that confers glyphosate tolerance to Roundup® herbicide. The EPA has previously reviewed and established a tolerance exemption for CP4 EPSPS[5], and the genetic material necessary for the production of this protein in or on all raw agricultural commodities (40 CFR §180.1174). This exemption was based on the following safety information:

[5] CP4 EPSPS (5-enolpyruvylshikimate-3-phosphate synthase) is the protein which provides tolerance to glyphosate (Roundup®) herbicide in this and other Roundup Ready soybean varieties.

1. The donor organism, *Agrobacterium* sp. strain CP4, is not a known human or animal pathogen and is not known to induce allergenic responses in humans.

2. A history of safe use of CP4 EPSPS protein has been demonstrated, based on the similarity of the CP4 EPSPS protein in MON 87705 to EPSPS proteins naturally present in food crops (e.g., soybean and corn) and in microbial food sources such as bakers yeast (*Saccharomyces cerevisiae*). The CP4 EPSPS protein is functionally equivalent to native plant EPSPS proteins except for the lack of affinity for glyphosate. In addition, there is a long history of safe use regarding the CP4 EPSPS protein since the introduction of Roundup Ready® crops in 1996, which include Roundup Ready soybean, Roundup Ready Flex cotton, and Roundup Ready Corn 2.

3. No biologically relevant structural similarities were observed between the CP4 EPSPS protein and allergens, toxins, and pharmacologically active proteins, which support the conclusion that CP4 EPSPS is not likely to pose a human health concern. This conclusion is also supported by the rapid degradation of CP4 EPSPS protein *in vitro* (Harrison et al., 1996) and in simulated digestive fluids using an industry standardized protocol (Thomas et al., 2004)

Several studies have evaluated the allergenicity of refined soybean oil in allergenic individuals, and it is generally accepted that refined soybean oils do not represent allergenic risks (Awazuhara et al., 1998; Hidalgo and Zamora, 2006; Paschke et al., 2001). Multiple studies have been performed using sera from soybean-sensitive individuals, and demonstrate that there is no significant difference in the allergenic potency of Roundup Ready soybeans containing CP4 EPSPS compared to conventional soybeans (Batista et al., 2007; Batista et al., 2005; Kim et al., 2006; Sten et al., 2004).

To conclude, LSHOLLSO is not expected to create an increased allergenic risk, based on the following: 1) The inverted repeat RNA involved in the gene suppression of *FAD2* and *FATB* is not anticipated to encode for the production of a protein. 2) Several studies have evaluated the allergenicity of refined soybean oil in allergenic individuals, and based on the results of these studies it is generally accepted that refined soybean oils do not represent allergy risks. 3) The CP4 EPSPS protein has been well investigated for allergenic potential and has a long history of safe use indicating that there is no evidence that this protein poses an allergenic risk.

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V. SUMMARY AND BASIS FOR GRAS CONCLUSION.

LSHOLLSO contains the same major fatty acids as commodity soybean oil, although not at the same levels. The major fatty acids in LSHOLLSO, just as in commodity soybean oil, are palmitic, stearic, oleic, linoleic and linolenic acids. The fatty acid profile of LSHOLLSO is similar to many other commercial oils currently available, including other high oleic vegetable oils (high oleic canola, high oleic safflower, high oleic sunflower), traditional oils such as olive oil that has a long history of consumption in the diet, and canola oil that has obtained GRAS status by the U.S. FDA. All of these comparator oils are suitable for use in deep-frying or spraying applications, and have a history of safe consumption in the diet. Storage stability data demonstrate that LSHOLLSO has excellent stability that is comparable to other commercial oils with high oxidative and heat stability, such as high oleic, low linolenic canola oil. LSHOLLSO is manufactured in accordance with current Good Manufacturing Practice (cGMP) and meets appropriate food-grade specifications.

Replacement of hydrogenated soybean oil liquid oils and oil blends under the proposed food uses of LSHOLLSO does not change the total daily intake of fat in the U.S. diet. The fatty acid with the highest increase due to the replacement is oleic acid. For the U.S. population, there is an overall increase in the total daily intake of oleic acid from 27.3 g/day to 30.9 g/day on a mean *per capita* basis, and an increase from 45.2 g/day to 53.3 g/day at the 90th percentile per user. Dietary intake of palmitic acid is reduced from 14.4 g/day to 12.8 g/day daily intake in the mean *per capita* group and from 23.6 g/day to 20.2 g/day for the 90th percentile per user group following replacement with LSHOLLSO. These changes – elevation of oleic acid and reduction in saturated fat – are consistent with current dietary recommendations which encourage limiting intake of *trans* fats and saturated fats, and suggest that most dietary fats be comprised of polyunsaturated and monounsaturated fats such as oleic acid (Lichtenstein et al., 2006; USDA, 2005; WHO, 2003).

Monsanto has determined that the scientific evidence presented above does not indicate any potential for adverse effects in humans following the consumption of LSHOLLSO under the conditions of intended use in foods. Following a critical evaluation of the scientific data available in the public domain that pertain to the safety of LSHOLLSO, a panel of experts, qualified by scientific training and experience to evaluate the safety of LSHOLLSO as a component of food, unanimously concluded that the proposed uses of LSHOLLSO are safe and suitable and are GRAS based on scientific procedures. Therefore, Monsanto has concluded that LSHOLLSO is GRAS under the intended conditions of use on the basis of scientific procedures.

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Table of CFR Sections Referenced (Title 21—Food and Drugs)		
Part	**Section §**	**Section Title**
101—Food labeling	101.12	Reference amounts customarily consumed per eating occasion
170—Food additives	170.3	Definitions
	170.30	Eligibility for classification as generally recognized as safe (GRAS)
172—Food additives permitted for direct addition to food for human consumption	172.185	TBHQ
	172.340	Fish protein isolate
	172.860	Fatty acids
173—Secondary direct food additives permitted in food for human consumption	173.270	Hexane
177—Indirect food additives: Polymers	177.2250	Filters, microporous polymeric
182—Substances generally recognized as safe	182.1073	Phosphoric acid
184—Direct food substances affirmed as generally recognized as safe	184.1033	Citric acid
	184.1065	Linoleic acid
	184.1090	Stearic acid
	184.1329	Glyceryl palmitostearate
	184.1505	Mono- and diglycerides
	184.1555	Rapeseed oil (canola oil)
	184.1666	Propylene glycol
	184.1763	Sodium hydroxide
186—Indirect food substances affirmed as generally recognized as safe	186.1256	Clay (kaolin)
Table of CFR Sections Referenced (Title 40—Protection of Environment)		
180 – Tolerances and exemptions from tolerances for pesticide chemicals in food	180.1174	CP4 Enolpyruvylshikimate-3-phosphate synthase (CP4 EPSPS) and the genetic material necessary for its production in all plants

Appendix A. Expert Panel Consensus Statement regarding the Generally Recognized as Safe (GRAS) status of Monsanto's Low Saturate, High Oleic, Low Linolenic acid Soybean Oil (LSHOLLSO) for use in foods.

EXPERT PANEL CONSENSUS STATEMENT REGARDING THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF MONSANTO'S LOW SATURATE, HIGH OLEIC, LOW LINOLENIC ACID SOYBEAN OIL (LSHOLLSO) FOR USE IN FOODS

August 17, 2009

INTRODUCTION

Monsanto Company (Monsanto) convened an Expert Panel (the "Panel") of independent scientists, qualified by their relevant national and international experience and scientific training to evaluate the safety of food ingredients, to conduct a critical and comprehensive evaluation of the available pertinent data and information, and determine whether the intended uses of Low Saturated, High Oleic, Low Linolenic soybean oil (LSHOLLSO) as a food ingredient are safe and suitable and would be Generally Recognized as Safe (GRAS) based on scientific procedures. The Panel consisted of: Dr. Joseph F. Borzelleca, (Virginia Commonwealth University School of Medicine), Dr. Robert J. Nicolosi (University of Massachusetts, Lowell) and Dr. John A. Thomas (University of Indiana School of Medicine). Curricula vitae evidencing the Panel members' qualifications for evaluating the safety of food ingredients are provided in Attachment 1.

The Panel, independently and collectively, critically examined a comprehensive package of scientific information and data on LSHOLLSO compiled from the scientific literature and other published sources through June 2009 assembled by Monsanto and Cantox Health Sciences International (Cantox). In addition, the Panel evaluated other information deemed appropriate or necessary, including data and information provided by Monsanto. The information evaluated by the Panel included details pertaining to the method of manufacture and product specifications, supporting analytical data from several batches of LSHOLLSO, storage stability data, intended use-levels in specified food products, consumption estimates for all intended uses, and a comprehensive assessment of the available scientific literature pertaining to the safety of LSHOLLSO.

The Panel unanimously concluded that the intended uses in traditional foods described herein for Monsanto's LSHOLLSO, meeting appropriate food-grade specifications as described in the supporting dossier [Documentation Supporting the Evaluation of Monsanto's LSHOLLSO as Generally Recognized as Safe] and manufactured and used consistent with current Good

Manufacturing Practice (cGMP), are safe and suitable and GRAS based on scientific procedures. A summary of the basis for the Panel's conclusion is provided below.

DESCRIPTION OF LOW SATURATED HIGH OLEIC LOW LINOLENIC SOYBEAN OIL (LSHOLLSO)

Background Information

Monsanto Company has developed biotechnology-derived soybean, MON 87705, to generate nutritionally-improved soybean oil with decreased levels of saturated fats (16:0 palmitic acid and 18:0 stearic acid) and increased levels of oleic acid (18:1), with an associated decrease in linoleic acid (18:2) through suppression of FAD2 and FATB, two key enzymes in the fatty acid biosynthetic pathway. Gene suppression is attained in MON 87705 by the introduction of tandem endogenous soybean *FAD2* and *FATB* gene segments, resulting in production of an inverted repeat or double stranded (ds) RNA. Double stranded RNAs are commonly used by eukaryotes, including plants, for endogenous gene suppression and pose no novel risks from a food or feed perspective. Nucleic acids, such as RNA, have a long history of safe consumption and are considered GRAS by the U.S. Food and Drug Administration. The inverted repeat RNA is not anticipated to encode for the production of a protein and consequently no additional assessment of potential allergenicity or toxicity is needed. The only introduced protein in MON 87705 is the 5-enolpyruvylshikimate-3-phosphate synthase protein from *Agrobacterium* sp. strain CP4 (CP4 EPSPS) that confers glyphosate tolerance to Roundup® herbicide. LSHOLLSO is derived from soybean plants produced through a breeding cross of genetically modified high-oleic soybean variety (MON 87705) with a low linolenic acid soybean line (Vistive™). Vistive™ was developed through conventional breeding, and has naturally occurring mutations in the Fad3-1c and Fad3-1b genes.

The fatty acid profile of LSHOLL soybean has been purposefully altered to improve performance in frying applications, and consequently the composition of oil produced from LSHOLL soybeans is substantially different to that produced from conventional soybean cultivars. LSHOLLSO has significant increases in oleic acid, and corresponding decreases in saturated fatty acids (palmitic, stearic), and polyunsaturated fatty acids (linoleic and linolenic). Consequently, the fatty acid specification ranges established for LSHOLLSO are outside those for conventional soybean oil. Based on the analysis of 8 lots of processed LSHOLLSO, the fatty acid specification for the five major fatty acids are: palmitic acid (<4.0%); stearic acid (<4.0%); oleic acid (55 to 85%); linoleic acid (8 to 30%); α-linolenic acid (<4.0%). The composition of the remaining fatty acids are consistently within the range expected for soybean oil as defined under Food Chemical Codex (FCC, 2008). In addition, the levels of oleic acid in LSHOLLSO are comparable to oleic acid levels reported in the CODEX standards for several vegetable oils (canola, olive oil, high oleic safflower oil, and high oleic sunflower seed oil) which range

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between 55.0% (canola oil) and 90.7% (high oleic sunflower seed oil) of total fatty acids. These food oils are widely consumed in the diet under uses that are comparable to the intended uses of LSHOLLSO.

Manufacturing Process

The same manufacturing process used to extract oil from commodity soybeans is also used for LSHOLL soybeans. No new interventions or processes are introduced. Because traditional soybean oil manufacturing processes are used to produce LSHOLLSO, the altered fatty acid profile of LSHOLLSO is a reflection of the LSHOLL soybean variety, and not due to, or impacted by, the manufacturing process. All reagents and processing aids used in the manufacture of LSHOLLSO are approved for use in food production in the U.S.

Product Specifications

The product specifications and methods of analysis for LSHOLLSO are presented in Tables 1 and 2. Food Chemical Codex (FCC) specifications for conventional soybean oil also are included for comparative purposes.

Table 1 Specifications and analytical methods for soybean oil (FCC) and LSHOLLSO (Monsanto)

Parameter	Soybean Oil Specification (FCC)	LSHOLLSO Specification	Analysis Method
Fatty acid composition	See Table 2	See Table 2	AOCS Ch 2a-94, Ce 1f-96
Lovibond color (Yellow)	NMT 20	NMT 20	AOCS Cc 13j-97 (Lovibond)
Lovibond color (Red)	NMT 2.0	NMT 2.0	AOCS Cc 13j-97 (Lovibond)
Free fatty acids	NMT 0.1%	NMT 0.1%	AOCS Ca 5a-40
Iodine value	120 - 143	90 - 100	AOCS Cd 1c-85
Lead	NMT 0.1 mg/kg	NMT 0.1 mg/kg	AOC Ca 17-01
Peroxide value	NMT 10 meq/kg	NMT 10 meq/kg	AOCS Cd 8-53
Stability, (AOM)	NLT 7 h	NLT 30 h	AOCS Cd 12b-57
Unsaponifiable matter	NMT 1.5 %	NMT 1.5%	AOCS Ca 6a-40
Water	NMT 0.1%	NMT 0.1%	AOCS Ca 2c-25

NLT = not less than; NMT = not more than; FCC = Food Chemical Codex. AOM – Active oxygen method

Table 2 Specifications for fatty acid composition of soybean oil (FCC) and LSHOLLSO (Monsanto)

Fatty acid	FCC Soybean Oil Specification (% weight)	LSHOLL Soybean Oil Specification (% weight)
14:0 (myristic)	<0.5	<0.5
16:0 (palmitic)	7.0 – 12	< 4.0
16:1 (palmitoleic)	<0.5	<0.5
18:0 (stearic)	2.0 – 5.5	< 4.0
18:1 (oleic)	19 – 30	55 – 85
18:2 (linoleic)	48 – 65	8 – 30
18:3 (α-linolenic)	5 – 10	< 4.0
20:0 (arachidic)	<1.0	<1.0
20:1 (eicosenoic)	<1.0	<1.0
22:0 (behenic)	<0.5	<0.5
24:0 (lignoceric)	<0.3	<0.3

Monsanto provided batch analyses data for several lots of LSHOLLSO produced from soybeans grown at various U.S. locations. These data demonstrate that the manufacturing process produces a consistent product in terms of its chemical composition.

Stability

The storage stability of LSHOLLSO was evaluated at ambient (25°C) and elevated (40°C) temperatures for 30 days. At the end of the 30-day study period, peroxide values, iodine values and free fatty acid content were all well within the limits identified by FCC and the CODEX standard for soybean oils. Peroxide values (which reflect lipid oxidation levels[6]) of LSHOLLSO are lower compared to conventional soybean oil, and are comparable to the values observed for high oleic low linolenic canola oil, which has very good stability in commercial applications. These data indicate that LSHOLLSO exhibits excellent storage stability, and its stability under these conditions is comparable to high oleic low linolenic canola oil.

[6] Oxidation results in the production of compounds that give undesirable odor and flavor, leading to rancidity.

INTENDED USES AND EXPOSURE ESTIMATES

Intended Uses, exposure estimates and dietary impact

LSHOLL soybean oil is intended to replace hydrogenated soybean oil, liquid oil and oil blends (soybean, corn, canola, cottonseed, sunflower, palm, and rice bran oils) currently used to prepare various foods including meat, poultry and fish dishes (commercial and restaurant), fried eggs (commercial and restaurant), French fries, potato chips and puffs, creamy salad dressings, salty snacks, and grain mixture dishes. The consumption of LSHOLLSO from all proposed food uses was estimated by Exponent, Inc., using the proposed food uses and use levels in conjunction with food consumption data included in the National Health and Nutrition Examination Surveys (NHANES 2003-2006) (CDC, 2007). The current intake of total fat and five major fatty acids in the diet pre-substitution and post-substitution with LSHOLLSO was calculated, assuming 100% replacement with LSHOLLSO in all identified food applications.

Replacement of hydrogenated soybean oil, liquid oils and oil blends under the proposed food uses of LSHOLLSO does not change the total daily intake of fat in the U.S. diet. The fatty acid with the highest increase due to the replacement with LSHOLLSO is oleic acid. For the U.S. population, there is an overall increase in the total daily intake of oleic acid from 27.34 g/day to 30.92 g/day on a mean *per capita* basis, and an increase from 45.2 g/day to 53.34 g/day at the 90th percentile per user, corresponding to increases of 13.1% and 18.0%, respectively. These post-replacement increases are expected, since the oleic acid fraction in the oils targeted for replacement is much lower, ranging from 15 to 22% in cottonseed oil to 51 to 70% in canola oil, compared to the 71.7% oleic acid observed in LSHOLLSO. Palmitic acid is the fatty acid with the second greatest dietary impact following replacement with LSHOLLSO. A reduction from 14.38 g/day to 12.84 g/day in the mean *per capita* group and from 23.62 g/day to 20.24 g/day for the 90th percentile per user group was determined, corresponding to decreases of 10.7% and 14.3%, respectively. LSHOLLSO replacement reduced saturated fat intake (palmitic and stearic combined) between 12.5 and 17.1%. These changes – elevation of oleic acid and decrease in saturated fat – are consistent with current dietary recommendations which encourage reductions in saturated fats, and suggest that most dietary fats be comprised of polyunsaturated and monounsaturated fats such as oleic acid (Lichtenstein et al., 2006; USDA, 2005; WHO, 2003). A slight decrease was noted for linoleic acid post-substitution; however, this intake level still satisfies the AI for individual subgroups established by the IOM (IOM, 2002). Linolenic acid was essentially unchanged before and after LSHOLLSO substitution, and thus replacement with LSHOLLSO has no nutritional consequence on linolenic acid intake in the U.S. population.

In summary, replacement of hydrogenated oils, liquid oils and oil blends under the proposed food uses results in modest changes in the fatty acid composition in the U.S. diet, with the primary consequence of higher oleic acid intake and lower consumption of saturated fats (palmitic and stearic acids) and linoleic acid. Replacement with LSHOLLSO does not impact

total fat intake or linolenic acid, and allows low *trans*-fat intake to be maintained. Given that this assessment assumes that all of the targeted oil components of the foods proposed for replacement at all eating occasions in the US are replaced with the new soybean oil, the results presented in this report represent a theoretical maximal effect of LSHOLLSO on fatty acid composition of the diet. The nutritional impact of exposure to LSHOLLSO under the intended conditions of use is estimated to result in changes in fatty acid consumption that are in line with current dietary guidelines for fatty acid intake (Lichtenstein et al., 2006; USDA, 2005; WHO, 2003).

SAFETY OF LSHOLLSO

Substantial equivalence of LSHOLLSO to other vegetable food oils.

The safety of LSHOLLSO is based on the long-standing history of safe consumption of the levels and types of fatty acids contained in commercial vegetable oils with a fatty acid profile similar to LSHOLLSO. The major fatty acids in LSHOLLSO (palmitic, stearic, oleic, linoleic and linolenic acids) are present at similar levels in several other commonly consumed vegetable oils. including other commercial high oleic vegetable oils (high oleic canola, high oleic safflower, high oleic sunflower), traditional oils such as olive oil that has a long-history of consumption in the diet, and canola oil that has obtained GRAS status by the U.S. Food and Drug Administration (FDA). LSHOLLSO does not contain any new fatty acids that are not presently found in vegetable food oils.

The levels of oleic acid in LSHOLLSO are comparable to oleic acid levels reported in the CODEX standards for several vegetable oils that are widely consumed (Table 3), as well as commercial high oleic low linolenic canola oil (HOLLCO). Since these food oils are widely available, and consumed in the diet under uses that are comparable to the intended uses of LSHOLL soybean oil, adverse toxicological or nutritional effects are not expected following the introduction of LSHOLL soybean oil to the U.S. marketplace.

The fatty acid profile of LSHOLL soybean is similar to another biotechnology-derived high oleic soybean, event DP 305423, which recently completed consultation at the FDA (FDA, 2009). The same event was approved as a Novel Food by Health Canada in May 2009 (http://www.hc-sc.gc.ca/fn-an/gmf-agm/appro/index-eng.php).

Table 3 Comparison of LSHOLLSO with ranges of fatty acids reported in the Codex Alimentarius Standards of Identity for Vegetable Oils.

Fatty acid	Specification						
	LSHOLLSO	Olive oil	Canola oil	HOSAFO[1]	Soybean oil	Sunflower oil	HOSFO[2]
C14:0	<0.5	<0.1	ND-0.2	ND-0.2	ND-0.2	ND-0.2	ND-0.1
C16:0	<4.0	7.5-20	2.5-7.0	3.6-6.0	8.0-13.5	5.0-7.6	2.6-5.0
C16:1	<0.5	0.3-3.5	ND-0.6	ND-0.2	ND-0.2	ND-0.3	ND-0.1
C18:0	<4.0	0.5-5.0	0.8-3.0	1.5-2.4	2.0-5.4	2.7-6.5	2.9-6.2
C18:1	60 -85	55-83	51.0-70.0	70.0-83.7	17-30	14.0-39.4	75-90.7
C18:2	8 – 25	3.5 -21	15.0-30.0	9.0-19.9	48.0-59	48.3-74.0	2.1-17
C18:3	<4.0	<1.5	5.0-14.0	ND-1.2	4.5-11.0	ND-0.3	ND-0.3
C20:0	<1.0	<0.8	0.2-1.2	0.3-0.6	0.1-0.6	0.1-0.5	0.2-0.5
C20:1	<1.0	NR	0.1-4.3	0.1-0.5	ND-0.5	ND-0.3	0.1-0.5
C22:0	<0.5	<0.3	ND-0.6	ND-0.4	ND-0.7	0.3-1.5	0.5-1.6
C24:0	<0.3	<1.0	ND-0.3	ND-0.3	ND-0.5	ND-0.5	ND-0.5

[1]HOSAFO = High Oleic Safflower Oil
[2]HOSFO = High Oleic Sunflower Oil.
ND: Not detected. NR: not reported

The concentration of tocopherols and phytosterols in LSHOLL soybean oil is consistent with levels found in conventional soybean oil. The composition and level of tocopherols in LSHOLLSO (mean 882 mg/kg) is comparable to that reported in the USDA food composition database for soybean oil (818 mg/kg), and is within the range reported for soybean in the CODEX standard (600 to 3370 mg/kg). Likewise, the level of total sterols in LSHOLLSO (3132 mg/kg) is similar to established standards used by CODEX (1800 to 4500 mg/kg) (CODEX, 2005) and the range reported in the literature (1860 to 3100 mg/kg) for conventional soybean oil (Johansson and Hoffmann, 1979).

Animal studies on oleic acid intake.

Delaney et al (2008) conducted a 90-day rat feeding study to demonstrate that there is no biologically relevant difference with respect to body weight/gain, food consumption/efficacy, mortality, clinical signs of toxicity, or ophthalmological observations in rats that are fed high oleic soybean-based diets compared to conventional soybean-based diets[7]. Consequently, this study demonstrates high oleic (70%) soybean is as nutritious and wholesome as non-GM soybeans.

[7] Soybean components were added to the diets in the following amounts: 20% meal, 1.5% hulls, 1.5-1.7% oil. The oil composition of HOSO is: 6.44% palmitic acid; 5.89% stearic acid; 70.6 % oleic acid; 5.5% linoleic acid; 7.2% linolenic acid.

To demonstrate the safety of ethyl oleate, the ethyl ester of oleic acid, Bookstaff et al. (2004) conducted a 91-day feeding study in rats (20 males and 20 females per group), comparing the safety of ethyl oleate to high oleic safflower oil. In the feeding study, test rats consumed ethyl oleate at up to 10% of the diet (high-dose males and females consumed 5.5 and 6.1 g/kg/day, respectively), while control rats were fed 10% high oleic soybean oil, which contained 80% oleic acid; both diets were supplemented with an additional 5.6% soybean oil. Both diets were well tolerated throughout the study period, and there were no toxicologically significant findings in any of the measured parameters for either group. The following data were collected: clinical observations, body weight gain, appearance of the feces, ophthalmic examination, hematology, coagulation, clinical chemistry, urinalysis, organ weight, histopathology, male and female reproductive assessments (i.e. sperm viability and estrous cycle assessments). These data demonstrate that both ethyl oleate and high oleic safflower oil, at daily intake of up to 10% of the diet (equivalent to 66% of total oil intake), are safe with no observed adverse effects at these dietary levels.

Human studies on oleic acid intake

Collectively, the scientific literature supports the conclusion that dietary oils rich in oleic acid are a suitable option to provide energy when replacing the use of oils high in saturated fats or *trans* fatty acids. In controlled clinical trials no adverse effects due to consumption of high oleic acid diets have been observed or reported (Baer et al., 2004). A summary of the data available in the scientific literature that supports healthy oleic acid intake from epidemiological studies and meta-analyses is provided in Table 4. Ranges of oleic acid that have been used in clinical interventions are presented in Table 5.

Table 4. Studies assessing the benefits of dietary oleic acid intake.
Epidemiological studies
Hulshof et al. (1999). In several Western European countries, a considerable proportion of energy is derived from SFA. Reduced intake of cholesterol-raising fatty acids, SFA and TFA included, is recommended. Further, there is strong support for safe chronic consumption of dietary oleic acid (i.e. mean intakes of 32.3 g/day and 28.5 g/day in Greek men and women, respectively) at levels that exceed US intake.
Yoneyama et al. (2007). Based on data from over 3000 Japanese participants, increased intake of oleic acid, linoleic acid, and ALA would reduce serum CRP levels (a marker of inflammation), especially when there is moderate dietary intake of long chain PUFAs such as DHA and EPA.
Meta-analyses
Mensink et al. (2003). Meta-analysis of 60 clinical trials to estimate effects of specific fatty acids on CVD risk factors concludes that these risk factors are reduced most effectively when dietary *trans* fatty acids and saturated fatty acids are replaced with *cis* unsaturated fatty acids.
Mente et al. (2009). The authors conducted a systematic review of the MEDLINE database, for all prospective cohort studies and RCTs published from 1950-2007 to examine the association between nutrient intake, dietary components and dietary patterns relative to CHD and related clinical outcomes. As a result, they identified positive dietary factors (vegetables, nuts, monounsaturated fats) and harmful factors (*trans* fatty acids, high glycemic index foods).

Table 5. Levels of dietary oleic acid/monounsaturated fatty acids (% energy) in clinical studies with no reported adverse effects.

Reference	Diet composition	Study duration	Study population
Lichtenstein et al. (2006)	17.8 % en as oleic acid	5 weeks	16 women, 14 men
Perez-Jimenez et al. (1995)	22 % en as MUFA	4 weeks	22 men
Jansen et al. (2000)	22 % en as MUFA	4 weeks	41 men
Abbey and Nestel (1994)	17.8 % en as oleic acid	3 weeks	10 women, 5 men
Baer et al. (2004)	17.5 % en as oleic acid	5 weeks	50 men
Allman-Farinelli et al. (2005)	20.3 % en as MUFA	5 weeks	27 men
Thijssen et al. (2005)	19.1% en as MUFA	5 weeks	27 women, 18 men
Mensink (2008)	18.9% en as MUFA	3 weeks	33 women, 11 men

The level of oleic acid in the human intervention studies cited above ranged between 17 to 22% of total energy (en) intake. These levels are consistent with those observed in traditional 'Mediterranean' diets (Hulshof et al., 1999; Poli et al., 2008) where cardioprotection benefits are observed. Following substitution of dietary fats with LSHOLLSO, mean *per capita* oleic acid intake is estimated to be 13.2% en (increased from 11.5% en), and 18.6% (increased from 15.2% en) in the 90th percentile group. These upward shifts in oleic acid intake post-substitution are consistent with the advice of many health groups. For example, the Adult Treatment Panel-III (ATP-III) recommends that total energy contribution from monounsaturated fats comprise up to 20% dietary fat to control cholesterol (NCEP, 2001).

While the major fatty acid change in LSHOLLSO is an increase in oleic acid, other fatty acids – palmitic, linoleic and linolenic acids – are also altered in this oil, and their dietary impact following oil replacement were also considered. Dietary replacement with LSHOLLSO resulted in dietary changes in the mean *per capita* and 90th percentile groups, respectively, as follows: a modest decrease in palmitic (10.7%; 14.3%) and linoleic acid intakes (8.8%; 11.8%), and a slight decrease in stearic (1.8%; 2.8%) and linolenic acid intakes (2.9%; 5.8%). Both linolenic and linoleic acids are essential fatty acids, and adequate intakes have been established for each of these fatty acids (IOM, 2002). Importantly, post-LSHOLLSO replacement, intake of both linoleic and linolenic acids meet these adequate intake levels. In addition, the reduction observed in saturated fats, namely palmitic acid, is consistent with current dietary guidelines which urge reduced intake of saturated fatty acids to improve cardiovascular health (Lichtenstein et al., 2006; USDA, 2005; WHO, 2003).

Allergenicity

The inverted repeat RNA involved in the gene suppression of *FAD2* and *FATB* in MON 87705 is not anticipated to encode for the production of a protein, Thus, the only protein in MON 87705 requiring an assessment of allergenicity is the 5-enolpyruvylshikimate-3-phosphate synthase protein from *Agrobacterium* sp. strain CP4 (CP4 EPSPS) that confers glyphosate tolerance to Roundup® herbicide. LSHOLLSO is not expected to create an increased allergenic risk, based on the following: 1) Several studies have evaluated the allergenicity of refined soybean oil in allergenic individuals, and based on the results of these studies it is generally accepted that refined soybean oils do not represent allergy risks. 2) The level of CP4 EPSPS protein in LSHOLL soybean seed is low, 110 µg/g dry weight, which represents ~0.033% of total protein; since only trace amounts of protein are present in refined oils, the amount of CP4 EPSPS protein in the refined LSHOLLSO would be far less than this. 3) The CP4 EPSPS protein has been well investigated for allergenic potential and has a long history of safe use indicating that there is no evidence that this protein poses an allergenic risk. Based on this evidence, the EPA

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has reviewed and established a tolerance exemption for CP4 EPSPS[8], and the genetic material necessary for the production of this protein in or on all raw agricultural commodities (40 CFR §180.1174).

SUMMARY

The safety and GRAS status of the intended uses in frying and spraying food applications of Monsanto's LSHOLLSO is based on the following:

- LSHOLLSO does not contain any new fatty acids that are not presently found in conventional vegetable food oils. The major fatty acids in LSHOLLSO are palmitic, stearic, oleic, linoleic and linolenic acids.
- The fatty acid profile of LSHOLLSO is similar to many other commercial oils currently available. LSHOLLSO has a fatty acid profile that is comparable to other commercial high oleic vegetable oils (high oleic canola, high oleic safflower, high oleic sunflower), traditional oils such as olive oil that has a long-history of consumption in the diet, and canola oil that has obtained GRAS status by the U.S. FDA.
- Published studies indicate that high oleic acid vegetable oils with comparable fatty acid compositions to LSHOLLSO are suitable for use in deep-frying food applications.
- The increased content of oleic acid in LSHOLLSO is within the ranges outlined for several vegetable oils (olive oil, high oleic canola oil, and high oleic sunflower oil) based on CODEX standards of identity. All of these comparator oils are suitable for use in deep-frying or spraying applications, and a long-history of safe consumption in the diet (olive oil and canola oil specifically).
- The concentration ranges of the remaining fatty acids, total tocopherols and plant sterols were within the range for soybean oil or CODEX standards for other vegetable oils.
- Based on estimated intakes, no change in total dietary fat intake following the replacement of liquid soybean oil and liquid oil blends with LSHOLLSO is expected.
- LSHOLLSO is a refined vegetable oil manufactured using standard oil refining practices under cGMP.
- Published studies indicate that refined vegetable oils derived from common allergenic foods (soybeans, peanuts) are not an allergenicity risk to sensitive individuals.
- The nutritional impact of exposure to LSHOLLSO under the intended conditions of use is estimated to result in changes in the *per capita* and per user 90th percentile fatty acid consumption that are in-line with WHO, USDA and AHA dietary recommendations for heart health.
- Dietary intake assessment reveals that there is essentially no difference in linolenic acid intake pre- and post- replacement with LSHOLLSO (0.04 – 0.14 g/d decrease), and that intake of both linolenic and linoleic acid satisfy currently established adequate intake levels.

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[8] CP4 EPSPS (5-enolpyruvylshikimate-3-phosphate synthase) is the protein which provides tolerance to glyphosate (Roundup) herbicide in this product and in Roundup® Ready soybean varieties.

- The daily intake of oleic acid estimated for U.S. per capita intake using replacement modeling with LSHOLLSO does not exceed the daily intake in many European countries, and is within the range used in multiple clinical studies.

LSHOLLSO contains no fatty acids that are not presently found in conventional vegetable food oils. The major fatty acids in LSHOLLSO, just as in conventional soybean oil, are palmitic, stearic, oleic, linoleic and linolenic acids. The fatty acid profile of LSHOLLSO is similar to many other commercial oils currently available, including other high oleic vegetable oils (high oleic canola, high oleic safflower, high oleic sunflower), traditional oils such as olive oil that has a long-history of consumption in the diet, and canola oil that has obtained GRAS status by the U.S. FDA. All of these comparator oils are suitable for use in deep-frying or spraying applications, and have a long-history of safe consumption in the diet. The concentration ranges of the remaining fatty acids, total tocopherols and plant sterols were within the range for soybean oil, or the CODEX standards for other vegetable oils.

Replacement of hydrogenated soybean oil liquid oils and oil blends under the proposed food uses of LSHOLLSO does not change the total daily intake of fat in the U.S. diet. The fatty acid with the highest increase due to the replacement is oleic acid. For the U.S. population, there is an overall increase in the total daily intake of oleic acid from 27.3 g/day to 30.9 g/day on a mean *per capita* basis, and an increase from 45.2 g/day to 53.3 g/day at the 90th percentile per user. These increases post-replacement are expected, since the oleic acid fraction in the oils targeted for replacement is much lower, ranging from 15 to 22% in cottonseed oil to 51 to 70% in canola oil, compared to the 71.7% oleic acid observed in LSHOLLSO. Palmitic acid is the fatty acid with the second greatest dietary impact following replacement with LSHOLLSO. A reduction from 14.4 g/day to 12.8 g/day daily intake in the mean *per capita* group and from 23.6 g/day to 20.2 g/day for the 90th percentile per user group was calculated. These changes – elevation of oleic acid and decrease in saturated fat – are consistent with current dietary recommendations which encourage reductions in trans fats and saturated fats, and suggest that most dietary fats be comprised of polyunsaturated and monounsaturated fats such as oleic acid (Lichtenstein et al., 2006; USDA, 2005; WHO, 2003). While a slight decrease was noted for linoleic acid post-substitution, the slightly lower level still satisfies the adequate intake for individual subgroups established by the IOM (IOM, 2002). Linolenic acid was essentially unchanged before and after LSHOLLSO substitution, and thus replacement with LSHOLLSO has no nutritional consequence on linolenic acid intake in the U.S. population.

The inverted repeat RNA involved in the gene suppression of *FAD2* and *FATB* is not anticipated to encode for the production of a protein so no additional assessment of potential allergenicity or toxicity is needed. The only introduced protein in MON 87705 is the 5-enolpyruvylshikimate-3-phosphate synthase protein from *Agrobacterium* sp. strain CP4 (CP4 EPSPS) that confers

glyphosate tolerance to Roundup® herbicide. LSHOLLSO is not expected to create an increased allergenic risk, there is no evidence that CP4 EPSPS protein in LSHOLL soybeans is allergenic. Further, there is much published literature indicating that refined vegetable oils derived from common allergenic foods (soybeans, peanuts) pose no allergenic risk to sensitive individuals due to the trace levels of protein present.

Storage stability data demonstrate that LSHOLLSO has excellent storage stability that is comparable to other commercial oils with high oxidative and heat stability, such as high oleic, low linolenic canola oil. LSHOLLSO is manufactured in accordance with current Good Manufacturing Practice and meets appropriate food-grade specifications.

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CONCLUSION

We, the Expert Panel, have independently and collectively critically evaluated the data and information summarized above and conclude that the intended uses of Monsanto's Low Saturated, High Oleic, Low Linolenic (LSHOLL) soybean oil, produced and used consistent with current Good Manufacturing Practice (GMP) and meeting appropriate food-grade specifications presented in the supporting dossier [Documentation Supporting the Evaluation of Low Saturated High Oleic Low Linolenic Soybean Oil as Generally Recognized as Safe] are safe and suitable.

We further conclude that the intended uses of Monsanto's LSHOLL soybean oil, meeting appropriate food-grade specifications presented in the supporting dossier and produced and used consistent with current GMP, are Generally Recognized as Safe (GRAS) based on scientific procedures.

It is our opinion that other qualified experts would concur with these conclusions.

(b) (6) [redacted]

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ABBREVIATIONS

AI	Adequate intake
ALA	alpha-linolenic acid
AOCS	American Oil Chemists Society
Apo-A1	Apolipoprotein A-1
Apo-B1	Apolipoprotein B-1
CDC	Center for Disease Control
CETP	Cholesteryl ester transfer protein
cGMP	current Good Manufacturing Practices
CODEX	Codex Alimentarius
CP4 EPSPS	5-enolpyruvylshikimate-3-phosphate synthase
CRF	U.S. Code of Federal Regulations
CRP	C-reactive protein
CSO	Conventional soybean oil
CVD	Cardiovascular disease
DHA	Docosahexaenoic acid
En	energy
EPA	Eicosapentanoic acid
EPA	Environmental Protection Agency
FAD2	fatty acid desaturase
Fad3-1b	fatty acid desaturase
Fad3-1c	fatty acid desaturase
FATB	acyl-ACP-thioesterase
FCC	Food Chemical Codex
GM	Genetically modified
GRAS	Generally Recognized as Safe
HDL	High density lipoprotein
HOLLCO	High oleic low linolenic canola oil
HOSAFO	High oleic safflower oil
HOSFO	High oleic sunflower oil
IL-6	Interleukin 6
IOM	Institute of Medicine
LDL	Low density lipoprotein
LSHOLL	Low saturated, high oleic, low linolenic
LSHOLLSO	Low saturated, high oleic, low linolenic soybean oil
MCTs	Medium chain triacyglycerides
MON 87705	soybean variety with suppression of FAD2 and FATB genes
MUFA	Monounsaturated fatty acid
NCEP	National Cholesterol Education Program
NHANES	National Health and Nutrition Examination study
PHSBO	Partially hydrogenated soybean oil (also, PHSO)
PUFA	Polyunsaturated fatty acid
RBD	Refined, Bleached, Deodorized
SFA	Saturated Fatty Acids
TFA	Trans fatty acid
USDA	United States Department of Agriculture
VLDL	Very low density lipoprotein
WHO	World Health Organization

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Appendix B. Batch analyses of LSHOLLSO.

Table B-1. Batch analyses – chemical and physical characteristics of LSHOLLSO.

Fatty acid	LSHOLLSO specification	Lot ID						
		1121 8223	1121 8226	1121 8228	1121 8231	1121 8233	1121 8229 *	1122 2519*
Cold test	Passes test	Passed test	Passed test	Passed test	Passed test	Passed test	Passed test	Passed test
Color (Yellow/Red)	NMT (20Y/2.0R)	8.4Y 1.8R	1.7Y 0.1R	2.0Y 0.2R	2.8Y 0.3R	2.1Y 0.2R	2.4Y 0.2R	1.5Y 0.2R
Free Fatty Acids (%)	NMT 0.05%	0.02	0.003	0.006	0.01	0.01	0.03	0.03
Iodine Value	90-100	92.8	96.6	99	98.6	97.6	99.5	98.1
Peroxide Value (meq/kg)	NMT 1.0	LOD	LOD	LOD	LOD	LOD	LOD	LOD
Stability[1] (AOM) (h)	NLT 30	57.9	42.2	39.0	33.9	35.9	33.4	38.3
Unsaponifiable Matter (%)	NMT 1.0%	0.33	0.45	0.37	0.26	0.33	0.35	0.37
Moisture (%)	NMT 0.1%	0.0104	0.0108	0.0115	0.015	0.0092	0.0123	<0.01
Lead (mg/kg)	NMT 0.1ppm	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

[1]Stability (AOM) calculated from Rancimat data using proprietary OSI software (Omnion, Inc. 2004).
NMT = Not more than. NLT = Not less than. LOD = <0.02
*Lots 11218229 and 11222519 are generated from the same seed sample; lot 11218229 was processed from 6 kg seed at bench scale, while lot 11222519 was processed from 3700 kg of seed at pilot scale.

Table B-2. Batch analyses – fatty acid composition (% total fatty acids) of LSHOLLSO.

Fatty acid	Lot ID									LSHOLLSO specification
	1121 8223	1121 8226	1121 8228	1121 8231	1121 8233	1121 8229	1122 2519	1122 4519	1122 4518	
C14:0	0.02	0.02	0.02	0.02	0.02	0.02	0.02	<0.05	<0.05	<0.5
C16:0	2.43	2.35	2.59	2.54	2.52	2.42	2.49	2.48	2.42	< 4.0
C16:1	0.15	0.14	0.15	0.15	0.15	0.15	0.16	0.10	0.10	<0.5
C18:0	3.47	2.99	2.99	3.14	3.33	3.39	3.41	3.00	2.87	< 4.0
C18:1n9	78.3	75.5	72.4	72.7	73.3	72.1	71.7	64.62	64.82	55 – 85
C18:2	10.5	13.8	16.4	15.7	15.2	16.3	16.4	23.43	23.59	8 – 30
C18:3n3	2.30	2.59	2.78	2.87	2.72	2.88	2.96	3.42	3.30	< 4.0
C20:0	0.39	0.33	0.32	0.33	0.35	0.36	0.34	0.24	0.23	<1.0
C20:1	0.35	0.33	0.31	0.30	0.30	0.30	0.30	0.26	0.26	<1.0
C22:0	0.34	0.30	0.29	0.30	0.31	0.32	0.32	0.27	0.27	<0.5
C24:0	0.12	0.12	0.11	0.11	0.11	0.11	0.11	0.09	0.09	<0.3

Mean values determined here are used for comparison to the FA profile of other oils throughout this text, and were also used to define the FA composition of LSHOLLSO used in the dietary intake assessment.

Table B-3. Batch analyses - tocopherol and sterol composition of LSHOLLSO.

Fatty acid	Lot ID						Mean	range	Codex specifications
	1121 8223	1121 8226	1121 8228	1121 8233	1121 8229	1122 2519			
Tocopherols (mg/kg)									
α-tocopherol	72.5	75.3	73.5	61.9	64.2	51.3	68	51.3 – 75.3	9 – 352
γ-tocopherol	557	612	595	551	587	581	593	551 – 612	89 – 2307
δ-tocopherol	169	214	234	197	198	265	221	169 – 265	154 – 932
Total	798.5	901	902.5	809.9	849.2	897.3	882	798.5 – 902.5	600 - 3370
Sterols (mg/kg)									
Campesterol	630	722	783	632	660	413	640	413 – 783	
Stigmasterol	684	690	740	623	663	372	629	372 – 740	
β-sitosterol	1240	1530	1520	1240	1400	984	1319	984 - 1530	
Others	530	624	591	483	558	481	545	481 – 624	
Total	3084	3566	3634	2978	3281	2250	3132	2250 - 3634	1800 - 4500

Appendix C. Storage stability data for LSHOLLSO.

Table C-1. Storage stability parameters of LSHOLLSO and comparator oils measured at day 0, day 15 and day 30 at ambient (25 °C) and elevated (40 °C) temperatures.

Sample	Ambient (25°C)			Elevated (40°C)		
	Day 0	Day 15	Day 30	Day 0	Day 15	Day 30
Peroxide Value (Meq/kg)						
HOLLCO	0.225	0.253	0.355	0.225	0.317	0.403
CSO	0.760	0.861	0.904	0.760	0.880	1.207
LSHOLLSO	0.130	0.244	0.297	0.130	0.310	0.814
Free Fatty Acids (as % of Oleic Acid)						
HOLLCO	0.022	0.022	0.022	0.022	0.023	0.023
CSO	0.026	0.026	0.026	0.026	0.027	0.024
LSHOLLSO	0.033	0.033	0.033	0.033	0.033	0.033
Iodine Values						
HOLLCO	96	96	97	96	97	96
CSO	129	129	130	129	129	129
LSHOLLSO	99	99	100	99	99	100

HOLLCO = High oleic low linolenic canola oil, CSO = conventional soybean oil, LSHOLLSO = low saturated high oleic low linolenic soybean oil. See Appendix 3 for complete stability study report including description of test methods.

Table C-2. Fatty acid stability of LSHOLLSO and comparator oils measured at day 0, day 15 and day 30 at ambient (25°C) and elevated (40°C) temperatures.

Sample	Ambient (25°C)			Elevated (40°C)		
	Day 0	Day 15	Day 30	Day 0	Day 15	Day 30
C18:1 (as % Oleic Acid)						
HOLLCO	72.7	72.7	72.7	72.7	72.8	72.7
CSO	24.0	24.0	24.0	24.0	24.0	24.1
LSHOLLSO	73.5	73.7	73.6	73.5	73.6	73.2
C18:2 (as % Linoleic Acid)						
HOLLCO	16.5	16.3	16.5	16.5	16.3	16.7
CSO	52.9	53.0	53.2	52.9	52.9	53.1
LSHOLLSO	16.2	16.1	16.4	16.2	16.0	16.9
C18:3 (as % α-Linolenic Acid)						
HOLLCO	1.6	1.6	1.6	1.6	1.6	1.7
CSO	6.4	6.4	6.4	6.4	6.3	6.3
LSHOLLSO	2.9	2.8	2.9	2.9	2.8	3.0

HOLLCO = High oleic low linolenic canola oil,
CSO = conventional soybean oil,
LSHOLLSO = low saturated high oleic low linolenic soybean oil.

Appendix D. Mean per capita and 90th percentile per user fatty acid intake from total diet, pre- and post- substitution with LSHOLLSO.

Table D-1. Mean *Per Capita* Fatty Acid Intake from Total Diet, Pre- and Post- LSHOLLSO Replacement, US Males

Fatty Acids	Males 1-8 (g/day)		Males 9-19 (g/day)		Males 20-49 (g/day)		Males 50+ (g/day)	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Palmitic 16:0	12.30	11.02	17.38	15.30	18.75	16.72	15.51	14.12
Stearic 18:0	5.91	5.80	8.51	8.33	9.08	8.90	7.52	7.40
Oleic 18:1	22.07	24.55	32.27	36.36	35.65	40.15	30.54	34.16
Linoleic 18:2	10.70	9.80	16.32	14.75	18.76	17.11	16.76	15.52
Linolenic 18:3	0.99	0.99	1.46	1.46	1.77	1.73	1.64	1.58
Total Fat Intake	64.76	64.76	93.44	93.44	102.90	102.90	87.93	87.93
Fatty Acids	**Males 1-8 (%Energy)**		**Males 9-19 (%Energy)**		**Males 20-49 (%Energy)**		**Males 50+ (%Energy)**	
	Pre	**Post**	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**
Palmitic 16:0	6.18	5.53	6.23	5.40	6.04	5.31	6.16	5.57
Stearic 18:0	2.94	2.89	3.04	2.97	2.91	2.84	2.98	2.92
Oleic 18:1	10.96	12.20	11.54	13.16	11.47	13.06	12.02	13.55
Linoleic 18:2	5.25	4.81	5.78	5.16	6.08	5.49	6.56	6.04
Linolenic 18:3	0.50	0.50	0.52	0.51	0.57	0.56	0.65	0.63
Total Fat Intake	32.30	32.30	33.39	33.39	33.18	33.18	34.75	34.75

Table D-2. Mean *Per Capita* Fatty Acid Intake from Total Diet, Pre- and Post- LSHOLLSO Replacement, US Females

Fatty Acids	Females 1-8 (g/day)		Females 9-19 (g/day)		Females 20-49 (g/day)		Females 50+ (g/day)	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Palmitic 16:0	11.59	10.35	13.42	11.62	13.16	11.62	11.01	9.98
Stearic 18:0	5.59	5.48	6.55	6.39	6.36	6.22	5.35	5.25
Oleic 18:1	20.75	23.32	24.83	28.67	25.08	28.83	21.53	24.44
Linoleic 18:2	10.04	9.16	13.44	11.97	14.10	12.73	12.53	11.54
Linolenic 18:3	0.97	0.95	1.24	1.21	1.39	1.34	1.31	1.25
Total Fat Intake	60.85	60.85	72.86	72.86	73.64	73.64	63.29	63.29
	Females 1-8 (%Energy)		**Females 9-19 (%Energy)**		**Females 20-49 (%Energy)**		**Females 50+ (%Energy)**	
Fatty Acids	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**
Palmitic 16:0	6.29	5.61	6.15	5.24	6.01	5.25	5.94	5.15
Stearic 18:0	3.01	2.95	3.00	2.92	2.89	2.82	2.88	2.80
Oleic 18:1	11.13	12.49	11.35	13.30	11.43	13.27	11.49	14.00
Linoleic 18:2	5.35	4.88	6.10	5.36	6.49	5.81	6.69	5.92
Linolenic 18:3	0.53	0.52	0.56	0.55	0.65	0.62	0.71	0.65
Total Fat Intake	32.84	32.84	33.32	33.32	33.71	33.71	34.02	34.02

Table D-3. 90th Percentile *Per User* Fatty Acid Intake from Total Diet, Pre- and Post-LSHOLLSO Replacement, US Males

Fatty Acids	Males 1-8 (g/day)		Males 9-19 (g/day)		Males 20-49 (g/day)		Males 50+ (g/day)	
	Pre	Post[2]	Pre	Post	Pre	Post	Pre	Post
Palmitic 16:0	18.87	16.37	26.25	21.99	29.60	24.98	24.78	21.72
Stearic 18:0	9.30	9.13	13.07	12.66	14.30	13.96	12.13	11.82
Oleic 18:1	33.13	37.75	50.88	60.02	53.45	63.42	49.51	56.92
Linoleic 18:2	17.77	15.78	27.27	23.68	30.69	26.75	29.07	26.17
Linolenic 18:3	1.63	1.60	2.46	2.39	2.96	2.80	2.83	2.66
Total Fat Intake	100.50	100.50	142.10	142.10	155.60	155.60	139.80	139.80
Fatty Acids	**Males 1-8 (%Energy)**		**Males 9-19 (%Energy)**		**Males 20-49 (%Energy)**		**Males 50+ (%Energy)**	
	Pre	**Post**	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**
Palmitic 16:0	7.93	6.70	7.82	6.39	7.95	6.46	8.23	6.85
Stearic 18:0	3.80	3.69	3.92	3.79	3.92	3.76	4.08	3.96
Oleic 18:1	13.87	15.95	14.66	17.94	15.09	18.25	16.13	19.44
Linoleic 18:2	7.29	6.34	8.17	6.96	9.09	7.66	9.60	8.26
Linolenic 18:3	0.71	0.68	0.75	0.71	0.88	0.80	0.98	0.89
Total Fat Intake	39.66	39.66	41.06	41.06	42.13	42.13	44.19	44.19

Table D-4. 90th Percentile *Per User* Fatty Acid Intake from Total Diet, Pre- and Post-LSHOLLSO Replacement, US Females

Fatty Acids	Females 1-8 (g/day)		Females 9-19 (g/day)		Females 20-49 (g/day)		Females 50+ (g/day)	
	Pre	Post[2]	Pre	Post	Pre	Post	Pre	Post
Palmitic 16:0	17.49	14.82	20.61	17.21	20.59	17.27	17.65	15.34
Stearic 18:0	8.47	8.26	10.09	9.71	10.25	10.00	8.64	8.41
Oleic 18:1	31.64	37.00	38.13	45.62	40.32	49.49	34.71	41.69
Linoleic 18:2	16.95	14.85	22.55	19.42	23.36	20.20	21.81	19.38
Linolenic 18:3	1.54	1.49	2.07	1.95	2.35	2.16	2.29	2.11
Total Fat Intake	91.47	91.47	111.60	111.60	114.20	114.20	100.40	100.40
	Females 1-8 (%Energy)		**Females 9-19 (%Energy)**		**Females 20-49 (%Energy)**		**Females 50+ (%Energy)**	
Fatty Acids	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**
Palmitic 16:0	7.99	6.63	7.88	6.03	8.06	6.41	7.98	6.64
Stearic 18:0	3.90	3.80	3.96	3.79	3.98	3.80	3.94	3.80
Oleic 18:1	14.24	17.15	14.68	18.70	15.25	19.77	15.29	18.85
Linoleic 18:2	7.61	6.54	9.04	7.26	9.59	7.93	9.85	8.47
Linolenic 18:3	0.76	0.73	0.87	0.80	1.03	0.93	1.07	0.97
Total Fat Intake	40.29	40.29	41.74	41.74	44.12	44.12	43.74	43.74

Pages 000062-000143 of Curriculum Vitae removed in accordance with the Privacy Act of 1974.

SUBMISSION END

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DEC 1 8 2009
BY: (b)(6)

December 16, 2009

Susan Carlson, Ph.D.
General Health Scientist
U.S. Food and Drug Administration
Center for Food Safety and Applied Nutrition Office of Food Additive Safety Division of Biotechnology and GRAS Notice Review
5100 Paint Branch Parkway
College Park, MD 20740-3835

RE: GRN 306: Low Saturate, High Oleic, Low Linolenic Soybean Oil

Dear Dr. Carlson:

Thank you for your e-mail of November 24 regarding GRAS Notice 306, where you requested additional information as follows:

1. In the Method of Manufacture section (p. 5) please provide: (1) specifications for the "suitable adsorbent" (end of 2nd para on p. 5).
2. Where is Appendix 3 cited on p. 49 under Table C-1?
3. In the Basis for GRAS Determination section in sub-sections C, D, E, and summary of dietary intake: provide the mean and 90th percentile estimated daily intake values on a per user basis (not per capita) in each section.

We have revised our original GRAS Notice document to address the specific points you raised. Specifically, we have supplied additional information and clarification, which you can find attached in the document entitled: GRAS Notice for Low Saturated High Oleic Low Linolenic Soybean Oil (LSHOLLSO) (Revised December 16, 2009). These changes are as follows:

1. The "suitable adsorbent" used is TriSyl® S615 Silica, which is a glyceride oil purification adsorbent. A product specification sheet from our supplier, W.R. Grace & Co., is provided in Appendix E.
2. Our reference to Appendix 3 under Table C-1 was an error, and it should not be included in this notice. All appropriate data to assess the storage stability of LSHOLLSO is included in Table C-1 and Table C-2 on page 49 of the revised GRAS notice.
3. Sub-sections C, D and E in the section entitled 'Basis for GRAS Determination' have been revised to include the mean and 90th percentile estimated daily intake values on a

per user basis as requested. This data can be found in Tables 8-11, on pages 15-16 of the revised GRAS notice We would like to point out that, since all subjects used in this analysis (in this case NHANES participants) are food consumers when the total diet is evaluated, the per capita and per user total dietary fatty acid intakes (pre-replacement) are the same. In addition, since 97% of the NHANES subjects are 'users' of LSHOLLSO target foods, the fatty acid intakes from the total diet for the entire US population provides a reasonable approximation of the fatty acid intake from the total diet for users of target foods. These points are reiterated on page 13 of the revised GRAS notice in conjunction with the discussion of these data.

Should you have questions concerning the revisions to this GRAS Notice, please contact Dr. Russell Schneider, Regulatory Affairs Director, Washington D.C., (202-383-2866), me (314-694-6587), or Tracey Reynolds (314-694-8187)

Sincerely,

(b)(6)

Aaron Crawford
Pipeline Regulatory Manager
(314) 694-6587

cc: R. Schneider, Ph.D., Monsanto, Washington, DC
Regulatory Files

enc.: Two paper copies of the updated GRAS Notice for LSHOLLSO (Revised December 16, 2009)
CD containing the revised GRAS Notice for LSHOLLSO