The Solae Company™

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Company Confidential Information

September 9, 2006

RECEIVED 9/11/06 OOPLDS

Barbara O. Schneeman, Ph.D.
Office of Nutritional Products, Labeling and Dietary Supplements
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740

Re: Petition for Allergen Labeling Exemption of Solae Soy Lecithins When Used as Processing Aids

Dear Dr. Schneeman:

Pursuant to Section 403(i) 21 U.S.C. 343(i) of the Federal Food, Drug and Cosmetic Act ("Act"), as amended by Section 403(w) (6) of the Food Allergen Labeling and Consumer Protection Act [FALCPA (2004)], Solae, LLC (Solae) submits this Petition to exempt its proprietary soy lecithin products (SOLEC™E, SOLEC™F, SOLEC™3F-SB, SOLEC™64-CX, SOLEC™152, SOLEC™HR and SOLEC™8140), from declarative allergen labeling when used as processing aids.

Solae established that Solae soy lecithin products, used as processing aids in a variety of food processing applications, provide significantly less ***potential soy protein/allergen*** than the standard soy lecithin reference used in development of the FDA Guidance to Industry. We conclude that our products would qualify for exemption from labeling beyond the 18 month interim term (wherein inspectors are instructed to use discretionary inspection authority for allergen labeling soy lecithin products), as provided for in the Guidance, based on level of potential allergen in the product and use level of soy lecithins as processing aids.

The Petition contains background information covering the definition and characterization of Solae soy lecithin products, their potential for use as processing aids in a variety of food applications, and extrapolated potential exposure data from industry product use data documenting use as processing aids in processed food categories marketed in the United States.

The information provided contains some proprietary data that is protected by law from disclosure. Solae believes disclosure of such information could potentially aid any competitors in making similar attestments; as such the information has been shared internally on a need-to-know basis and should be held as confidential. In this Petition, Solae will provide scientific evidence to demonstrate that Solae soy lecithins, as characterized by the method(s) provided in this Petition, when used as processing aids, ***would not be expected to cause an allergic response that poses a risk to human health.***

Please feel free to contact me directly if you have any specific questions regarding the information provided in the Petition.

Sincerely,

Janet E. Collins/db

Janet E. Collins, Ph.D., R.D.
Director, Global Regulatory Affairs
Solae, LLC
Telephone: 202-728-3622
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cc: Paul M. Kuznesof, Ph.D. [(CFSAN) paul.kuznesof@fda.hhs.gov.]

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Pursuant to Section 403(i) 21 U.S.C. 343(i) of the Food, Drug and Cosmetic Act, as amended by Section 403(w) (6) of the Food Allergen Labeling and Consumer Protection Act (FALCPA, 2004)[1] Solae, LLC submits this Petition to exempt its proprietary soy lecithin products (SOLEC®E, SOLEC®F, SOLEC®3F-SB, SOLEC®64-CX, SOLEC®152, SOLEC®HR and SOLEC®8140) from declarative allergen labeling when used as processing aids. Based upon the FDA/CFSAN "Guidance for Industry,"[2] Solae established that Solae soy lecithin products provide significantly less potential soy protein/allergen than the standard soy lecithin reference used in development of the Guidance.[3]

FALCPA 203(w)(6) provides that any person may petition the Secretary of the U.S. Department of Health and Human Services (Secretary) to exempt a food ingredient described in section 201(qq)(2) from the allergen labeling requirements. The petitioner must demonstrate that such food ingredient, as derived by the method specified in the Petition, does not cause an allergic response that poses a risk to human health.[4] FALCPA establishes labeling requirements for the "eight major foods or food groups accounting for some 90% of food allergies," including milk, eggs, fish, crustacean shellfish, tree nuts, peanuts, wheat and soybeans. Any non-commodity packaged food containing one of the eight major allergens must be labeled with the word, "contains," followed by the name of the food source from which the major food allergen is derived (such as, soy lecithin), or the common or usual name of the major food allergen (soy) must be provided in the list of ingredients.

In its Guidance, FDA stated that protein levels, as characterized by hexane insoluble (HI) matter, must meet the Food Chemicals Codex (FCC)[5] standard for HI. We agree that the FCC standard can be a useful benchmark minimum by which to characterize soy lecithin products in commerce in the US and internationally. In this case, a maximum level of 0.3% HI is acceptable for a product to fit the FCC standard for soy lecithin- and according to the Guidance, this level of HI, when present in a processing aid, would not require labeling as presumably, when used as a processing aid at this level, would not reasonably be expected to pose harm to human health.

In this Petition, the HI levels present in Solae soy lecithins are documented to be significantly below the level referenced from the Food Chemicals Codex[6] and also well below that level documented in scientific literature as "allergenic" to soy-allergic subjects. The objective of the Petition is to gain exemption of Solae soy lecithin products, derived from soybean processing, from specific designation on the ingredient statement when used as processing aids.

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[1] Food Allergen Labeling and Consumer Protection Act of 2004. 21 USC 301. Public Law 108-282, August 2 2004.
[2] FDA/CFSAN Guidance for Industry: Guidance on the labeling of certain uses of lecithin derived from soy under section 403(w) of the Federal Food, Drug, and Cosmetic Act. 2006. USDHHS, FDA/CFSAN, College Park, MD.
[3] Food Chemicals Codex, 5th ed., FDA/CFSAN, Washington DC.
[4] FALCPA Section 203(w) (6) (A, C).
[5] Ibid.
[6] Ibid.

Background

Soy Protein Allergy Prevalence

Prevalence of soy protein allergy in the general population is not known.[7] In diagnostic tests, including double blind placebo controlled food challenges (DBPCFC), a relatively low rate of soy allergy is reported. Giampietro et al. (1992)[8] reported that 3% of 317 children tested positive to soy allergy in DBPCFC; Magnolfi et al. (1996)[9] reported similarly, that of 704 generally allergic children, only 1.1% were found to be soy allergic in a DBPCFC.

Bruno et al. (1997)[10] reported from a multi-center study of 505 allergic children some 1.2% children were soy positive, while Burks et al (1998)[11] reported that of 187 allergic children, only 1.8% were positive to soy challenge. Other studies that compiled food intolerance estimates report 0.3 to 4.4% soy "allergy" in adults and children (Bjornsson et al., 1996[12]; Young et al., 1994[13] ; and Sampson and McCaskill, 1985[14]). According to the FDA Guidance for Industry[15] "...definitive studies assessing the prevalence of soy allergy are lacking, it is currently estimated that 0.2% of children and adults in the US are allergic to soy. Based on this estimate, soy allergy appears to be less prevalent than allergies to other major food allergens." Taylor[16] surmises a similar prevalence (0.1-0.2% of the US population) based on calculations used to predict soy allergic response from various testing sources.

Allergenicity of Soy Protein

According to FDA Guidance for Industry[17] there is no consensus on the minimal dose of soy protein that will elicit an adverse effect (LOAEL). Further complicating the assessment is that soy protein does not appear to elicit the level of observed response as that from other food allergens, as reported in a review article by Cordle (2004)[18]

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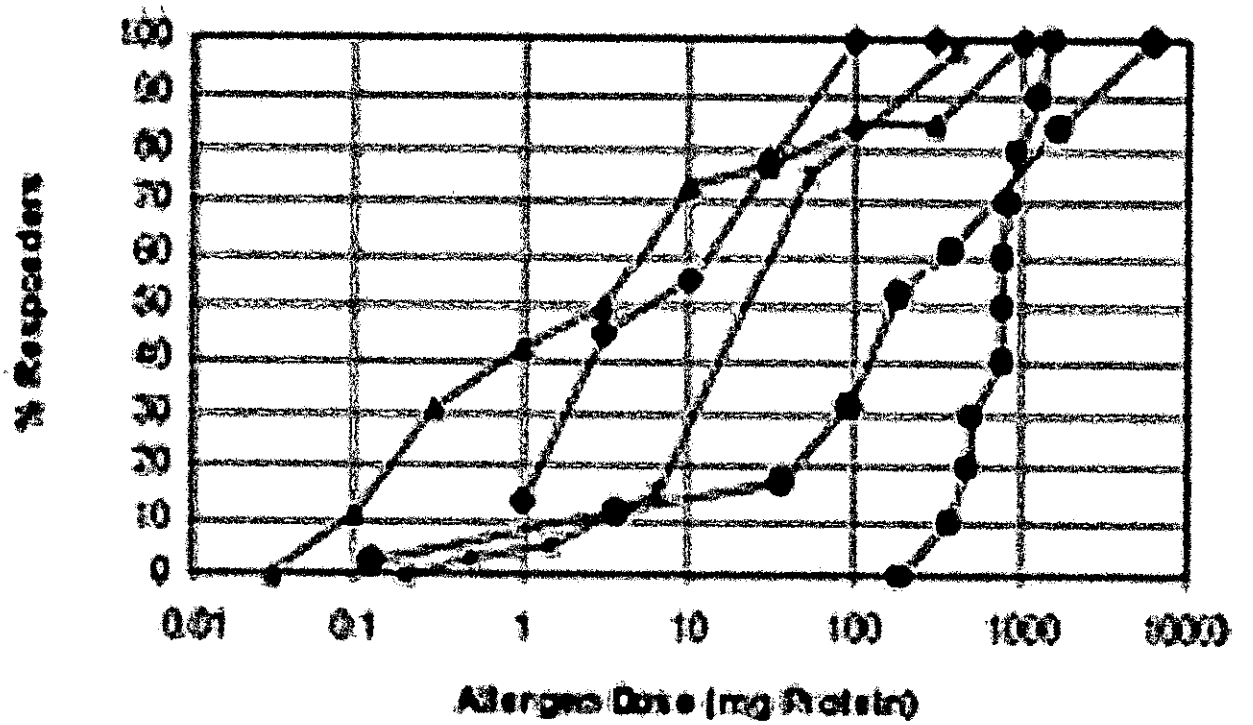
[7] Taylor, S. 2006. Estimating prevalence of soy protein allergy. The Soy Connection. Chesterfield, MO.
[8] Giampietro et al. 1992. Ann. Allergy 69: 143.
[9] Magnolfi et al. 1996. Ann. Allergy Asthma Immunol. 77: 197.
[10] Bruno et al. 1997. Pediatr. Allergy Immunol. 8: 190.
[11] Burks et al. 1998. J. Pediatr.
[12] Bjornsson et al. 1996. Ann. Allergy Asthma Immunol. 77: 327.
[13] Young,et al. 1998. Lancet. 334: 1127.
[14] Sampson, H.A. and McCaskill, C.C. 1985. J. Pediatri. 107: 669.
[15] FDA/CFSAN 2006. Guidance for Industry: Guidance on the Labeling of Certain Uses of Lecithin Derived from Soy Under Section 403(w) of the Federal Food, Drug, and Cosmetic Act. USDHHS/FDS/CFSAN, College Park MD>
[16] Ibid.
[17] Ibid.
[18] Cordle, C. 2004. J. Nutri. 134: 1213S.

From this review (Figure 1), it is apparent that the dose to elicit allergic response is orders of magnitude lower with soy than with other known allergens.

Figure 1: Food Allergen Reaction Thresholds*



Cordell, 2004[19]
Ingested allergen dose (mg protein) vs. % allergic responses in challenged patients.
Allergens in (number) patients: peanut (31), hazelnut (32), egg (33), milk (27), soy (27).

Soy is more similar in its allergenic profile to milk and eggs than to the severe allergens, peanuts, tree nuts and shellfish.

- Allergic reactions to soy occur predominately in children ≤ 3 years of age (Bock, 1987)[20]
- Most children in the US who have reactions to soy outgrow the allergy by age four.[21]
- The most common symptoms of soy allergy are similar to those of egg or milk allergies: stomach upset, rashes and hives (Sicherer et al., 2000).[22]
- It is appropriate to exercise good manufacturing practices with soy lecithin, as those employed when milk- and/or egg-containing foods or ingredients are handled.

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[19] Ibid.
[20] Bock, S.A. 1987. Pediatrics, 79:638.
[21] Ibid.; and Host, A. 1990. Allergy. 45:587.
[22] Sicherer, S.H. 2000. Allergy. 55:515.

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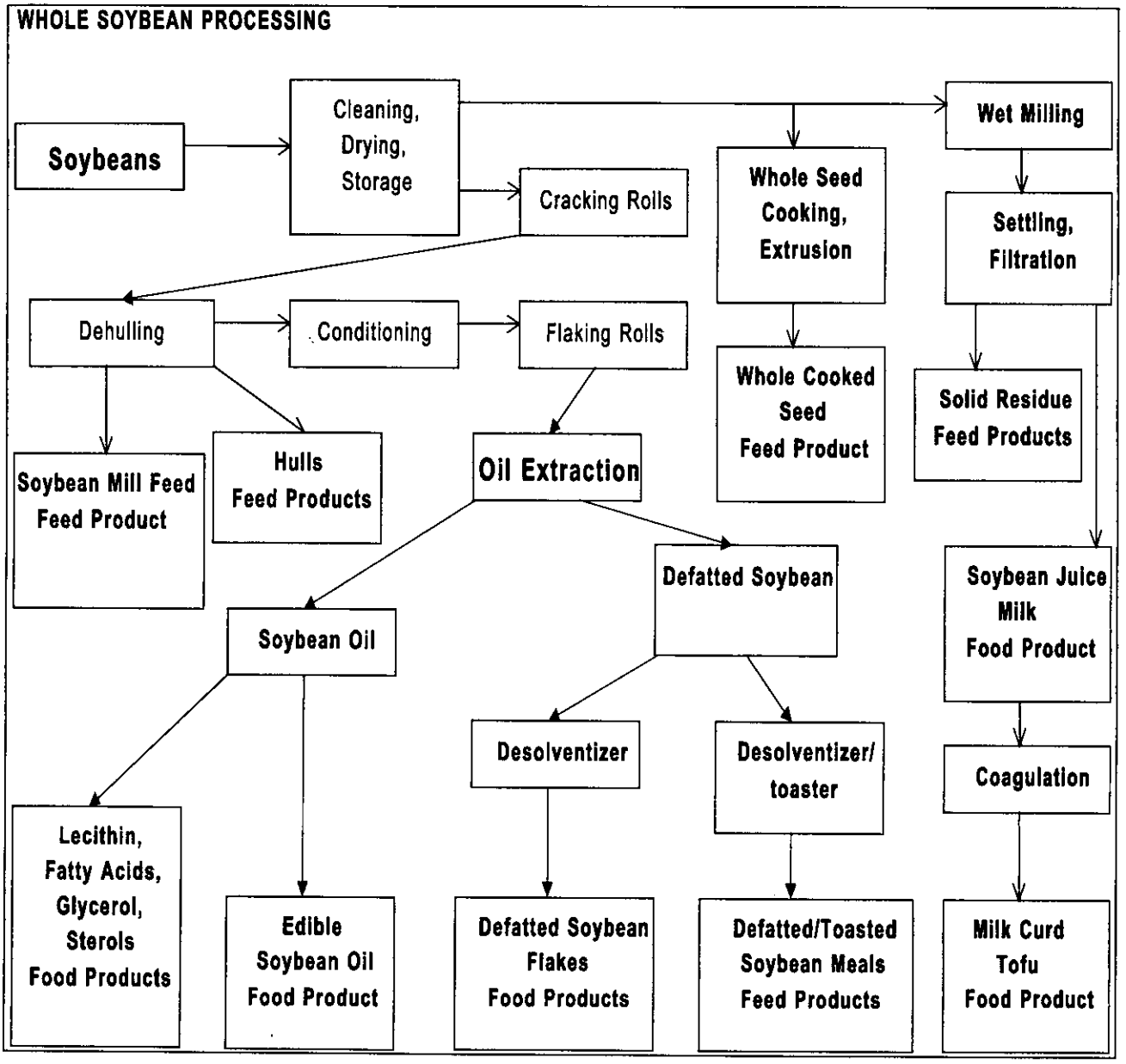
Soy Lecithin Characterization and Use

Commercial sources of lecithin are predominantly vegetable oil seeds, with soybean being the largest contributor to commercial lecithin in the United States. Lecithins are derived from oil manufacturing whereby crude lecithins are separated from oils according to the process illustrated in Figure 2[23].

Figure 2: Soybean Processing Steps and Outputs (OECD 2001)



[23] OECD 2001. Consensus Document on Compositional Considerations for New Varieties of Soybean: Key Food and Feed Nutrients and Anti-Nutrients, Organization for the Economic and Cooperative Development, Paris, FR.

Commercial lecithins consist of phospholipids, glycolipids and fatty acids, and some residual protein.[24] During manufacture of soy lecithin most of the protein is removed. Lecithin is affirmed as generally recognized as safe (GRAS) with no limitation other than good manufacturing practice.[25] The GRAS affirmation specifies that soy lecithin must meet the specifications of the Food Chemicals Codex which stipulates that food grade lecithin may not contain more than 0.3% hexane-insolubles (300mg HI/100g lecithin).

Lecithins are used in a variety of commercial processed food products for several functional effects. When used as ingredients for functional reasons, lecithins serve largely as emulsifiers and stabilizers. The use of lecithins as a processing aid such as releasing agents for baked products, chewing gum, ham nets and other "non-stick" food applications (such as extrusion aids) also is important. Documents developed for customer and internal use by The Solae Company[26] state that when used as a processing aid, defined as, "an ingredient that aids in the food making process and does not have a technical or functional effect in the finished product," finished foods may include between 0.1 - 1.0% soy lecithin by weight.

Table 1: Soy Lecithin Use and Food Categories [Solae® Customers (2006)][27]

Product	**Processing Aid Function**	**Typical *inclusion* level, % by weight**
Ham Net Release Agent	Release of ham netting from smoked hams	**5%-10% lecithin in water/lecithin solution** that ham net is dipped in (no transference data exist)
Chewing gum	Multiple, e.g., viscosity modifier of gum base	**0.2-0.6%**
Sliced processed cheese	Anti-sticking / release aid (slices do not stick to one another)	**<0.25%** (sprayed onto cheese during processing)
Dry-Blended Beverages	Instantizing powders for anti-dust	**<1.0% of dry powder**
Every application requiring food-contact surface (or mold) release	Aerosol Spray Release Agent (pan release)	**5%-10% of aerosol release product** (no transference data exist)

[24] Martin-Hernandez et al. 2005. Characterization and quantification of proteins in lecithins. J. Ag. And Food Chem. 53: 8607.
[25] GRAS. 21 CFR 184.1400. Lecithin is Generally Recognized as Safe.
[26] The Solae Company. 2005. Solae Soy Lecithin- Solae business confidential.
[27] The Solae Company 2006. St. Louis, MO.

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Soy Lecithin Chemical Analysis

Lecithin is a complex mixture of fat soluble and insoluble components; its characteristic function is to serve as an emulsifier and it also serves as a processing aid (anti-sticking agent) in a variety of processes). The FDA standard of identity for soy lecithin characterizes soy chemistry by relating its identity to quantity of hexane insoluble materials (21CFR 184.1063, 21 CFR 172.814, 21 CFR 184.1400). By standard of identity, soy lecithin can not exceed 0.3% or 300mg/100g (3000ppm) lecithin of hexane insoluble matter (HI). Analytical methods reported in the literature and used historically, [as recently as 2005 (Martin-Hernandez et al., 2005),[28]] used separation methods designed to extract the "protein" fraction, believed to be precipitated in the hexane fraction, and quantify proteins using a variety of methods. According to the FDA Guidance to Industry[29] soy allergens would be present in the protein fraction of the ingredient and as such in the HI portion of the analysis, so that the specification would limit the amount of protein in food grade lecithin to 0.3% or 300mg/100g lecithin. Solae developed a fact sheet (2004)[30] to inform its customers about the protein content of soy lecithin and provided product specification information for two Solae lecithin products (CENTROPHASE®HR for pan release, and CENTROLEX®F as an extrusion aid). The HI specification for each (Attachment A) is a maximum of 0.02% HI. Additional Solae product data presented in Table 2 further demonstrate the low level of HI in existing Solae soy lecithin products used by our customers and will be used later to demonstrate potential contribution of protein from soy lecithin, when used as a processing aid, in finished food products.

Table 2: Solae® Soy Lecithin Hexane Insolubles (HI)*

Date of Reporting	Lecithin Product	Sample Set	Test Method+	HI Average (%)
July	SOLEC E	54	HI (Millipore) ASTM 707	[redacted]
July	SOLEC F	172	HI- AOCS 705	[redacted]
July	SOLEC 64-CX	35	HI (Millipore) ASTM 707	[redacted]
July	SOLEC 152	44	HI (Millipore) ASTM 707	[redacted]
June	SOLEC 3F-SB	136	HI- AOCS 705	[redacted]
August	SOLEC HR	6	HI- AOCS 705	[redacted]
August	SOLEC 8140	6	HI- AOCS 705	[redacted]

*Data collected over 12 months (2005-2006).
+ Methods provided in Annex I.

[28] Ibid.
[29] FDA/CFSAN 2006. Guidance to Industry, Guidance on the labeling of certain uses of lecithin derived from soy under section 403(w) of the Federal Food, Drug and Cosmetic Act. USDHHS/FDA/CFSAN, College Park, MD.
[30] The Solae Company, 2004. Soy lecithin application examples from internal document.

Historically, it has been assumed that the protein component(s) of lecithin are contained in the HI fraction, and thus, hexane extraction has been used in methods designed to quantify protein in soy lecithin (the FDA standard of identify for soy lecithin relates protein content to HI for which a limit exists). As of 2005, Martin-Hernandez et al. (2005), reported ***no validated methods*** were available to quantify protein in soy lecithin, and further the first step in the analysis typically is extraction using aqueous/organic solvents with protein content determined using different assays, including Bio-Rad Protein Assay, ELISA, and electrophoretic tests (Bradford; Paschke et al.; Lowry; Awazuhara et al; Gu et al.; Porras et al.; and Muller et al.). According to Martin-Hernandez et al. (2005), extraction with hexane-2-propanol-water, followed by amino acid analysis is the most suitable method for isolation and quantification of proteins from lecithins. A detection limit of 15mg protein/kg lecithin and quantification limit of 50mg protein/kg was reported, with standard soy lecithin ranging between 232mg and 1338mg protein/kg soy lecithin (232-1338ppm protein). Hexane extraction is the standard method used for analysis of protein in lecithin.[31]

Using data provided in Tables 1 and 2, with use levels from Solae soy lecithin products as a processing aid in various applications, the total range of protein from Solae soy lecithins used as processing aids in formulation of processed food products is calculated (Table 3).

Table 3. Calculated Exposure Levels to Soy Protein (HI) from Solae Soy Lecithins Used in a Variety of Applications

Application	Lecithin	HI Content	Usage Level	Serving Size	HI Exposure	#servings to consume 100 mg HI
		(mg/kg)	(%)	g/serving	mg/serving	#
Chewing Gum	Solec F	[redacted]	0.2 0.6	3 g/stick	[redacted]	[redacted]
Processed Cheese	Solec 152	[redacted]	0.25	30 g/slice	[redacted]	[redacted]
Waffles	Solec F	[redacted]	0.5	85 g/waffle	[redacted]	[redacted]
Dry Blended Beverage	Solec 152	[redacted]	0.5	30g/200ml	[redacted]	[redacted]

It can be seen from the data provided in Table 3 that the protein contribution, reported as HI, to the final food using various Solae commercial lecithin products as processing aids, is very low, making the presence of soy protein in the finished foods non-detectable by current methods of detection.

[31] Smith, A.K. and Circle, S.J. editors. 1972. Soybeans: Chemistry and Technology. AVI Publishing Company, Inc. Westport, CT.

In the FDA Draft Report on thresholds for allergens in foods[32] the LOAEL for soy was listed as 88-522mg protein. According to Taylor and Hefle (2004),[33] in an expert opinion rendered to FDA, in a low-dose challenge trial of more than 50 soy-allergic individuals, the minimal provoking dose was 88mg of soy protein (220mg of soybean) to elicit a mild, objective symptom. Further, they report the clinical evidence indicates a majority of soy-allergic individuals have minimal eliciting doses *above 500 mg soybean*. Therefore, not only is the amount of soy protein (or its allergenic protein) a factor in consideration but also the effective (allergenic) dose- which for soybean protein is roughly 100 fold lower than for minimal dose response from allergenic protein in egg, peanut and cows' milk.

In conclusion, we submit that these data and calculated HI values for processed foods clearly demonstrate when Solae soy lecithins (SOLEC®E, SOLEC®F, SOLEC®3F-SB, SOLEC®64-CX, SOLEC®152, SOLEC®HR and SOLEC®8140) are used as processing aids for ham net release, chewing gum and processed cheese slice anti-stick, and dry beverage anti-dust as examples, the maximum exposure dose to an individual to any HI would be [redacted] mg per serving in a dry blend beverage. One would need to drink [redacted] 200 ml servings of reconstituted milk to be exposed to a dose of 100 mg HI. A soy allergic consumer, as reported by Taylor and Hefle, would have to eat [redacted] sticks of chewing gum, [redacted] slices of processed cheese or [redacted] waffles to elicit even a mild objective reaction. These data represent conservative estimates since there is no reasonable means by which to quantify exposure of a consumer to say, for example, the soy lecithin that might be contained in the portion of a ham net that touched the surface of a slice of ham when the lecithin was used to help release the net from the ham. In each case, we have attempted to provide a reasonable case for what level of protein from lecithin might be found on the finished product given its use.

The data provided clearly demonstrate that use of Solae brand soy lecithins, as processing aids over a range of food processing applications, does not pose a risk to public health. We request that Solae brand soy lecithins be exempted from allergen labeling when used as processing aids.

[32] FDA 2005. Draft Report- The US FDA Threshold Working Group (7); Approaches to establish thresholds for major food allergens and for gluten in food. Washington DC.
[33] Taylor and Hefle. 2004. Soy lecithin: An Expert Opinion (paper delivered to FDA).

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References Cited- in text order

Food Allergen Labeling and Consumer Protection Act of 2004. 21 USC 301. Public Law 108-282, August 2 2004.

FDA/CFSAN Guidance for Industry: Guidance on the labeling of certain uses of lecithin derived from soy under section 403(w) of the Federal Food, Drug, and Cosmetic Act. 2006. USDHHS, FDA/CFSAN, College Park, MD.

Food Chemicals Codex, 5th ed., FDA/CFSAN, Washington DC.

FALCPA Section 203(w) (6) (A, C).

Taylor, S. 2006. Estimating prevalence of soy protein allergy. The Soy Connection. Chesterfield, MO.

Giampietro et al. 1992. Ann. Allergy 69: 143.

Magnolfi et al. 1996. Ann. Allergy Asthma Immunol. 77: 197.

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Smith, A.K. and Circle, S.J. editors. 1972. Soybeans: Chemistry and Technology. AVI Publishing Company, Inc. Westport, CT.

FDA 2005. Draft Report- The US FDA Threshold Working Group (7); Approaches to establish thresholds for major food allergens and for gluten in food. Washington DC.

Taylor and Hefle. 2004. Soy lecithin: An Expert Opinion (paper delivered to FDA).

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Solae, LLC Petition for Allergen Labeling Exemption of Soy Lecithins when Used as Processing Aids

Annex I

Inspection Method: Hexane Insoluble Material in Lechithin, Code CM 705

Inpsection Method: Quantitative Hexane Insoluble Material by Millipore Filters, Code CM 707

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Inspection Method

Code: CM 705
Date: 09/07/2006
Issue No.: 3
Superceded Date: 08/02/2006
Superceded Number: 2
Title: Hexane Insoluble Material in Lecithin

Confidential

Statement of Intent

This method determines the substances insoluble in hexane under conditions of the test. A hexane solution of lecithin is filtered through a sintered glass crucible with a nominal pore size of 40 - 60 microns (µm). Retained material is dried, weighed, and reported as percent hexane insoluble material. The minimum reportable value is 0.01%

Safety Precautions

A. Review the MSDS for **ALL** materials listed in the Reagent section of this Test Procedure **BEFORE** performing the test.

B. Make sure that the appropriate Personal Protective Equipment (face shield/goggles, apron, chemical gloves, hood, respirator, etc.) are worn, based on a risk assessment of the potential safety hazards in this procedure. **Safety glasses with side shields must be worn in the lab at all times.**

C. Identify and be aware of any "high energy sources" (e.g., electrical, steam, compressed gas cylinders, or any liquid or gas line under pressure) involved with the Test Procedure or located in the immediate work area.

D. You must be trained to operate any laboratory equipment included in this Test Procedure **BEFORE** performing the test. This may include reading the operator's manual provided by the vendor and any site specific operation procedures. You must comply with the safety training program at your site.

E. Observe all prevailing laboratory safety operational procedures.

Equipment

1. Balance, analytical accurate to 0.1 mg (0.0001 g)

2. Erlenmeyer flasks, wide mouth, 250 ml

3. Filter funnel, Buchner, sintered glass, coarse porosity (Corning C), 30 ml (Clean in potassium dichromate - sulfuric acid cleaning solution before each use.

4. Filter flasks of convenient size. They should be fitted with 1-hole stoppers which will hold stem of filter funnel.

5. Cylinder, graduated, 100 ml

6. Oven, air or forced draft, at 105 ± 2°C

Equipment Preparation

Reagents
Hexane, A.C.S. grade

Caution: Hexane is flammable and a dangerous fire risk. The TLV is 50 ppm in air. A properly operating fume hood should be used when working with hexane.

Reagent Preparation and Disposal
Always refer to the individual chemical's MSDS for proper disposal. Refer to the site hazardous waste program for proper containment and disposal standards. EPA regulations define wastes that exhibit any of the following characteristics as hazardous:

Ignitability -	Ignitable wastes include liquids with a flash point of less than 140 degrees F solids which ignite spontaneously through absorption of moisture or friction; flammable gasses: and oxidizers (waste code D001)
Corrosivity -	Aqueous solutions with a pH less than or equal to 2, or greater than or equal to 12.5 (waste code D002)
Reactivity -	Substances that are unstable and readily undergo violent change: react violently with water: are capable of detonation when exposed to initiating source: or generate toxic fumes when exposed to water or corrosives (waste code D003)
Toxicity -	Refer to the MSDS for toxicity information. (waste codes D004-D043).

Procedure

1. Heat all samples except deoiled lecithin to 60°C for a minimum amount of time in order to make them more fluid. Mix thoroughly to suspend any sediment and insure homogeneity.

2. Weigh approximately 10 g of sample to an accuracy of 0.01 g into a flask. Add 100 ml of hexane and shake until dissolved.

3. Filter under reduced pressure through a filter funnel which has been previously heated to 105°C for 1 hour, desiccated and weighed to an accuracy of 0.1 mg. Wash the flask twice with 25 ml portions of hexane and pour the washings through the funnel.

4. Place the funnel in an oven at 105 ± 2°C for 1 hour. Remove from oven, cool to room temperature, and weight to an accuracy of 0.1 mg.

Calculations (Results)
$$\text{Hexane Insoluble Matter (\%)} = \frac{(\text{g of filtered material})(100)}{(\text{g of Sample})}$$

where

g of filtered material = dried filter weight (step 4) - filter weight (step 3)

References
AOCS Official Method Ja 3-87

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Inspection Method

Code: CM 707
Date: 09/07/2006
Issue No.: 2
Superceded Date: 11/11/2004
Superceded Number: 1
Title: Quantitative Hexane Insoluble Material by Millipore Filters

Confidential

Statement of Intent

This method measures the particulate materials of lecithin by quantitatively measuring the hexane insoluble material retained on a Millipore 37 mm aerosol analysis monitor filter with matched weight membranes with a 0.8 µm pore size. The method is an adaptation of A.S.T.M. Method D2276-71 "Particulate Contaminant in Aviation Turbine Fuels". Results are reported in ppm.

Safety Precautions

1. Review the MSDS for **ALL** materials listed in the Reagent section of this Test Procedure **BEFORE** performing the test.

2. Make sure that the appropriate Personal Protective Equipment (face shield/goggles, apron, chemical gloves, hood, respirator, etc.) are worn, based on a risk assessment of the potential safety hazards in this procedure. **Safety glasses with side shields must be worn in the lab at all times.**

3. Identify and be aware of any "high energy sources" (e.g., electrical, steam, compressed gas cylinders, or any liquid or gas line under pressure) involved with the Test Procedure or located in the immediate work area.

4. You must be trained to operate any laboratory equipment included in this Test Procedure **BEFORE** performing the test. This may include reading the operator's manual provided by the vendor and any site specific operation procedures. You must comply with the safety training program at your site.

5. Observe all prevailing laboratory safety operational procedures.

Equipment

1. Balance, analytical accurate 0.0001 g (0.1 mg)
2. Beaker, 600 ml
3. Beaker, 250 ml
4. Class stirring rod
5. Petri dish
6. Watch glass
7. Millipore 37 mm aerosol analysis monitor filter; pore size: 0.8 µm; Type: Matched weight; Cat. No. MAWPO37AM; membrane weights matched to within 0.1 mg.; or equivalent; Some laboratories use MAWPO37PM. This version has a pad under the membranes making it easier to remove the membranes.

8. Filtration flask, 1 liter
9. Funnels (2), short stem, 65 mm. diameter
10. Tubing, Tygon, Two 2-inch pieces to fit funnel stem
11. Rubber stopper with glass tube
12. Ring stand with clamps and ring for funnel
13. Forceps, flat-bladed, non-pointed tips
14. Oven, static type (without fan assisted air circulation)
15. Vacuum system: Water aspiration; Mechanical pump (if used) should be explosion proof, corrosion resistant, and properly vented.
16. 100 or 50 mL volumetric pipet
17. Pipet bulb
18. Magnetic stirrer and stir bar or glass stirring rod to mix sample solutions.
19. Wash bottle for filter hexane.

Equipment Preparation

1. All glassware and containers in contact with the sample or solvent should be washed with detergent, rinsed with high purity water, and oven dried.

Reagents

1. Hexane (A.C.S. grade) that has been filtered through one of the Millipore contamination monitors. (One filter can be set aside and used only for this purpose.) All references to hexane in this method refer to such filtered hexane.
2. High purity, filtered water for rinsing cleaned glassware prior to drying.

Reagent Preparation and Disposal

Always refer to the individual chemical's MSDS for proper disposal. Refer to the site hazardous waste program for proper containment and disposal standards. EPA regulations define wastes that exhibit any of the following characteristics as **hazardous**:

Ignitability -	Ignitable wastes include liquids with a flash point of less than 140 degrees F solids which ignite spontaneously through absorption of moisture or friction; flammable gasses: and oxidizers (waste code D001)
Corrosivity -	Aqueous solutions with a pH less than or equal to 2, or greater than or equal to 12.5 (waste code D002)
Reactivity -	Substances that are unstable and readily undergo violent change: react violently with water: are capable of detonation when exposed to initiating source: or generate toxic fumes when exposed to water or corrosives (waste code D003)
Toxicity -	Refer to the MSDS for toxicity information. (waste codes D004-D043).

Precautions

1. Hexane is flammable and a dangerous fire risk. The TLV is 50 ppm in air. A properly operating fume hood must be used when working with hexane.

Procedure

1. SAMPLE PREPARATION

   A. Deoiled lecithin - Lecithin must not contain powdered flow aid or insoluble additives.

      1. Into a 600 mL beaker, weigh 100 g of deoiled lecithin to an accuracy of 0.01 g. Add 400 mL hexane to the deoiled lecithin and cover with a watch glass. Allow the deoiled lecithin to dissolve.

      2. When the sample is dissolved, mix with a glass stirring rod, and using a glass funnel, transfer quantitatively to a 500 mL volumetric flask. Make up to volume with additional hexane, rinsing the beaker with hexane in a wash bottle, quantitatively transferring the washings to the volumetric flask. After the flask is made to volume, insert a magnetic stirring bar and mix with a magnetic stirrer until the sample aliquot is removed so that all particles stay suspended.

   B. Fluid lecithin

      1. Warm fluid and plastic lecithin to 60°C, mixing well with a spatula before weighing the sample.

      2. Into a 250 mL beaker, weigh 10 grams of the test lecithin to an accuracy of 0.01 g. Add 100 mL of hexane, cover with a watch glass, stir until the lecithin is completely dissolved.

2. SAMPLE FILTRATION

   A. Preparation of the membrane filter:

      1. Connect a Millipore matched weight contamination analysis monitor, as shown in Figure I.

      2. Place the red and yellow plastic plugs into a Petri dish labeled for the sample.

      3. Apply vacuum and pass 10 -15 mL aliquots of hexane down the funnel to make certain all connections are tight.

   B. Sample Filtration

      1. Deoiled lecithin

         a. Pipet 100 mL of the sample solution from 500 mL volumetric flask into the funnel. Retain the initial solution until testing is completed in case sample must be rerun.

            NOTE: If the 100 gram deoiled lecithin sample made up to 500 mL in hexane is not optically clear, the sample aliquot should be reduced to 30 mL. Otherwise, clogging of the filter will probably result.

         b. Rinse the pipet with hexane from a wash bottle and then rinse the funnel with 100 mL of hexane. (See precaution 4 below.)

         c. Proceed to 3. – Membrane Processing

      2. Fluid lecithin

         a. When the lecithin is dissolved, mix with a glass stirring rod and quantitatively transfer to the monitor filter via the connected class funnel.

         b. Double rinse the beaker with hexane from a wash bottle, again transferring quantitatively (pouring down the stirring rod) the washings. If any fatty deposit exists on the outside of beaker lip, wash this with hexane onto the filter.

         c. After all the material has filtered, make a final rinse of the funnel-monitor with 100 ml of hexane.

         d. Proceed to 3. – Membrane Processing

3. MEMBRANE PROCESSING

   A. Release the vacuum, remove the Millipore monitor, and open the plastic case with the edge of a small spatula.

   B. Holding the monitor, push up gently through the bottom hole with a small lab spatula (or

equivalent).

C. Use forceps to grasp the matched weight monitor membrane filters at the very edge and transfer the membrane filters to the Petri dish labeled for the sample with the 2 plastic plugs. If a membrane is torn or chipped, the membranes should be discarded and the sample retested.

D. Separate the two membranes with a micro-spatula. Support one membrane over each plastic plug using the red to support the lower membrane (reference) and the yellow to support the top membrane (sample). Cover the Petri dish at once with a watch glass.

NOTE: Care must be exercised in handling the membranes with forceps. The membranes must be kept upright and should be separated while still damp with hexane. Otherwise, dry particles from the top membrane could be lost by any sharp movement. Petri dishes for holding the membranes should be kept covered with watch glasses to reduce pick up of any particulates during membrane drying.

E. Place the Petri dish containing the membranes into a 90 ± 5°C oven for 30 minutes.

F. Remove the Petri dish from the oven and allow to cool for 30 minutes.

G. Zero the analytical balance and weigh the bottom membrane (red plug). Record the weight to the nearest 0.0001 g.

H. Zero the analytical balance and weigh the top membrane (yellow plug). Record the weight to the nearest 0.0001 g.

NOTE: The membranes weigh about 0.0400 g (40 mg). It was found necessary to zero the analytical balance between each membrane weighing.

Calculations (Results)

1. Deoiled Lecithin

$$\text{HIM (ppm)} = \frac{(\text{Difference in Filter Weights (g)}) \times (500\text{ mL})}{(\text{Sample Weight (g)}) \times (\text{mL Filtered})} \times 1{,}000{,}000$$

2. Fluid Lecithin:

$$\text{HIM (ppm)} = \frac{\text{Difference in Filter Weights (g)}}{\text{Sample Weight (g)}} \times 1{,}000{,}000$$

Where

Difference in filter weights = top filter weight – bottom filter weight
Sample weight = g of sample weighed
500 = Final volume for deoiled Lecithin samples
mL Filtered = Actual volume of deoiled Lecithin solution filtered.
1,000,000 = Factor for converting g/g to ug/g (ppm)

References

A.S.T.M. D2276-71 "Particulate Contaminant in Aviation Turbine Fuels"