ORIGINAL SUBMISSION

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high performance ingredients

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SENT *VIA* FEDEX

August --, 2012

Mary Ditto, 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

GRAS Notice for Potato Protein Isolates

Dear Dr. Ditto:

In accordance with 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 (17 April 1997)], I am submitting in triplicate, as the Notifier [Solanic, P.O. Box 15, 9640 AA Veendam, The Netherlands], a Notice of the determination, on the basis of scientific procedures, that potato protein isolates manufactured by Solanic, as defined in the enclosed documents, is GRAS under specific conditions of use as an ingredient in multiple food categories, and therefore, is exempt from the premarket approval requirements of the *Federal, Food, Drug and Cosmetic Act*. Information setting forth the basis for the GRAS determination, includes a comprehensive summary of the data available that has been reviewed by an independent panel of experts (the Expert Panel) qualified by scientific training and experience to evaluate the safety of potato protein isolates in traditional food products.

Should you have any questions or concerns regarding this GRAS Notice, please do not hesitate to contact me at any point during the review process so that we may provide a response in a timely manner.

Sincerely,

Marco Giuseppin
Chief Technology Officer
Solanic

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OCT 18 2012
Division of
Biotechnology and
GRAS Notice Review

GRAS Exemption Claim for Potato Protein Isolates

Submitted to:

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

Submitted by:

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August 10, 2012

GRAS Exemption Claim for Potato Protein Isolates

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I GRAS EXEMPTION CLAIM

I.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)]

Solanic B.V., an AVEBE group Company, hereby claim that the use of potato protein isolates, as described in Section I.D below, is exempt from the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act because we have determined that such use are Generally Recognized as Safe (GRAS).

Signed,

Date

Marco Giuseppin
Chief Technology Officer
Solanic
Marco.Giuseppin@solanic.eu

I.B Name and Address of Notifier

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9640 AA Veendam
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I.C Common Name of the Notified Substance

Potato protein isolates

I.D Conditions of Intended Use in Food

1. Foods in which the Substance is to be used

Solanic, an AVEBE group company, intends to market their minimally processed potato protein isolates as ingredients in multiple food and beverage categories in the United States (U.S.). Specifically, Solanic intends to market 2 distinct potato protein isolates, a high molecular fraction (>35 kDa) and a low molecular fraction (between 35 and 4 kDa). Where necessary, the high molecular fraction will be abbreviated as HMW and the low molecular fraction as LMW. These abbreviations do not constitute commercial names of the products. LMW and/or HMW potato protein isolates are proposed for technological use in the following categories: alcoholic beverages; baked foods and baking mixes; cheeses; dairy product analogs; egg products; fats and oils; frozen dairy desserts and mixes; fruit and water ices; gelatins, puddings, and fillings;

meat products; milk products; nuts and nut products; plant protein products; poultry products; and as an amino acid nutrient during fermentation. Solanic's LMW and HMW potato protein isolates also are intended for nutritive uses in sports drinks and protein sports bars at use levels of between 1 to 10%.

The individual proposed food-uses and use-levels for potato protein isolates employed in the current intake analysis are summarized in Table I.D-1.

Table I.D-1 Summary of the Individual Proposed Uses and Use Levels for Potato Protein Isolates in the United States (2003-2008 NHANES data)

Food Category	Proposed Food-Uses	Potato Protein Use Level (%)	
		LMW	HMW
Technological Uses			
Alcoholic Beverages	Wine (processing-aid)	0.01 to 0.025[a]	
Baked Goods and Baking Mixes	Gluten-free Bread[b]	0.5 to 6	
	Gluten-free Cakes[b]	0.5 to 6	
Cheeses	Natural Cheese	NA	0.01
Dairy Product Analogs	Non-dairy Coffee Whiteners	0.2 to 4	
	Non-dairy Whipped Topping	0.2 to 1	
Egg Products	Meringues	0.2 to 4	NA
Fats and Oils	Creamy Salad Dressings (egg-based)	0.2 to 1	NA
	Mayonnaise	0.2 to 1	NA
Frozen Dairy Desserts and Mixes	Frozen Yogurt	0.2 to 1	
	Ice Cream	0.2 to 1	
Fruit and Water Ices	Sorbet	0.2 to 1	NA
Gelatins, Puddings, and Fillings	Mousses	0.2 to 4	NA
Meat Products	Beef Patties	0.2 to 3	
	Deli Meats	0.2 to 3	
	Injected Ham (fresh)	0.2 to 3	
	Pate	0.2 to 3	
	Sausages and Hot Dogs	0.2 to 3	
Milk Products	Yogurt	0.5	NA
Nuts and Nut Products	Coated Peanuts	1 to 15	
Plant Protein Products	Vegetable-based Meat Analogs	0.5 to 6	
Poultry Products	Injected Chicken and Turkey (Fresh)	0.2 to 3	
Fermentation-aid[c]	Processing-aid	NA	
Nutritive Uses[d]			
Beverages and Beverage Bases	Adult Sports Drinks (Recovery Drinks)[e]	1 to 10	
Grain Products and Pastas	Adult Protein, Granola, and Health Bars	1 to 10	

HMW = high molecular weight; LMW = low molecular weight; NA = not applicable
[a] Approximately 90% of the added potato protein will be removed using standard filtration methods employed by the wine industry.
[b] There were limited food codes for gluten-free products in the NHANES 2003-2008 database; thus, surrogate codes were selected.
[c] Potato protein isolates are intended for use as an amino acid nutrient during fermentation.
[d] Low glycoalkaloid preparations will be used for nutritive food uses.
[e] There were no food codes available for recovery-type sport drinks in the NHANES 2003-2008 database; thus, surrogate codes for meal replacement beverages (milk- and non-milk-based drinks) and energy drinks were selected.

2. Purpose for Which Substance is Used

Solanic's minimally processed potato proteins display excellent water binding, fat binding and foaming properties. Technical food use include uses as foaming aids (*e.g.*, gluten free bread, and egg-free mousse), softening-aids (*e.g.*, gluten-free breads), emulsifiers (*e.g.*, dressings, ice-creams, and whipping cream) emulsification and gelling in meat products, fining agents in wine, and as fermentation aid. Flavoring uses also are proposed (*e.g.*, cheeses).

Potato protein isolates also are intended for use in nutritional products including sports bars and sports drinks targeted to mature individuals seeking foods to supplement the diet with a balanced source of non-animal protein.

3. Description of the Population Expected to Consume the Substance

Potato protein isolates intended for technical food uses (Table I.D-1) are expected to be consumed by members of the general U.S. population who may be reasonably be expected to consume at least one food within the aforementioned food categories.

Food products to which potato protein isolates are added to foods for nutritive uses (sports bars and sports beverages) will be marketed to mature individuals seeking a balanced, and/or, vegetarian sources of protein in the diet. Nutritive food uses of Solanic's potato protein isolates are not proposed for use as a source of dietary protein in food products targeted to children or infants (*e.g.*, baby food, children's meal supplement beverages).

I.E. Basis for the GRAS Determination

Pursuant to Title 21, Section 170.30 of the *Code of Federal Regulations* (CFR) (U.S. FDA, 2012), potato protein isolates have been determined to be GRAS on the basis of scientific procedures.

I.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:

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Fax.: +31- (0)598-66 42 72

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

II. DETAILED INFORMATION ABOUT THE IDENTITY OF THE NOTIFIED SUBSTANCE

II.A. Identity

Solanic's potato protein isolates are a homogeneous off-white, free flowing powder free from objectionable flavor or odor and is characterized as comprising greater than 90% protein and less than 10% moisture by weight.

1. Common or Usual Name

Potato Protein Isolates; High Molecular Weight (HMW) potato protein isolate; Low Molecular Weight (LMW) potato protein isolate.

2. Trade Name

No trade names have been designated for these ingredients at this time.

II.B. Method of Manufacture

Solanic's potato protein ingredients are obtained from potato tuber juice, which originates from the AVEBE starch production process. AVEBE's production uses various cultivars of commercially available, non-genetically modified potatoes (*Solanum tuberosum* subsp. *tuberosum*) that are inspected for quality prior to their use. All potatoes are accepted and processed at the Gasselternijveen location, The Netherlands, in accordance with AVEBE ISO 9001, and Hazard Analysis Critical Control Point (HACCP) plans.

Solanic's potato protein isolates are manufactured at the Solanic manufacturing facility using mild processing techniques according to cGMP and HACCP measures. The manufacturing process also has received food safety certification from the British Retail Consortium (BRC). The raw potatoes are washed and rasped to produce a potato juice, which is separated from the potato solids using mechanical separation techniques such as rasps, centrisieves, and decanters. Sodium bisulphite is added during rasping to prevent browning. The potato juice is then subjected to a second physical separation step (centrisieving) to remove fibers and any remaining undesirable fine particulates. The potato proteins are isolated from the juice as fractions using chromatographic materials[1] similar to those currently used for isolation of milk proteins from skim milk and whey. Citric and formic acid are used for elution of the HMW and LMW fractions, which are subsequently concentrated by ultrafiltration and conditioned by mild temperature treatment (temperature ranges between 20 to 25°C) and addition of pH regulators (hydrochloric acid and sodium hydroxide). Chromatography regeneration solvents include

[1]The chromatography resin used for isolation of AVBE's potato protein isolates is the subject of an effective Food Contact Substance Notification (FCN No. 528) (U.S. FDA, 2005). Residues of the food contact material are not detectable in the finished product.

formic acid and sodium hydroxide. The conditioning also includes a polishing step using activated carbon to remove excess triglycoalkaloids (TGA) from the protein concentrates. The use of a secondary ion-exchange (polystyrene/divinylbenzene) processing step in accordance with 21 CFR §173.25 is applied for further reduction of the TGA content of the material for food nutritive food applications where higher concentrations of the potato protein isolates are intended for addition to a food (U.S. FDA, 2012). All processing-aids used during the manufacturing process described herein are permitted by U.S. regulation, determined to be GRAS, or the subject of an effective Food Contact Notification, for their respective uses in the manufacture of potato protein isolates.

The final concentrates are spray dried or, depending on the application, stored as stable concentrates of >20% dry matter (Figure II.B-1). Finally, UV treatment[2] of the potato protein isolates is conducted to control and reduce microbial counts in the finished product. In all process steps, the potato protein isolates are not subjected to processing conditions that would have detrimental effects on the native protein characteristics. Production temperatures are kept at room temperature of 20 to 25°C, and the pH values in all steps are strictly controlled and in the range of 4.8 to 6.

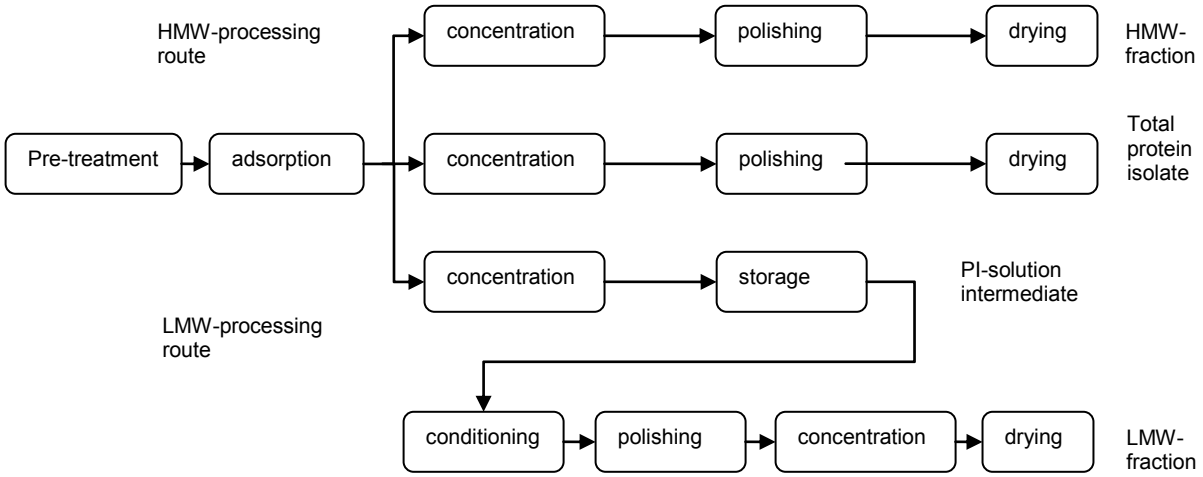

Figure II.B-1 Schematic Overview of the Manufacturing Process for Isolated Potato Protein (LMW and HMW Fractions)

II.C. Specifications for Food Grade Material

Food grade specifications have been developed for Solanic's potato protein isolates. Each batch of HMW and LMW product is specified to contain >90% potato protein, and contains limits

[2]The use of ultraviolet light during the manufacturing of AVEBE's protein isolates for control of surface microorganisms is used in accordance with 21 CFR §179.39 (U.S. FDA, 2012).

for heavy metal and microbial contamination. Each lot of material also is analyzed for the presence of glycoalkaloids, sulphite, and lysinoalanine. Product specifications for HMW and LMW potato protein isolates are presented below in Table II.C-1.

Table II.C-1 Product Specifications

Parameter	HMW	LMW	Method
Dry Matter	>910 g/kg	>910 g/kg	RA-05-014
Protein (amino acids)	>900 g/kg dry matter	>900 g/kg dry matter	DBGT/AZA/001, DBGT/AZA/002, DBGT/AZA/003
Ash	<100 g/kg dry matter	<100 g/kg dry matter	ISO 5984
Total glycoalkaloids	<312 mg/kg protein	<312 mg/kg protein	WP 001; Solanic method; based on AOAC 997.13
Sulphite	<100 mg/kg	<100 mg/kg	RA-09-011
Lysino alanine (free)	<100 mg/kg	<100 mg/kg	AAAFURZH.30N
Lysino alanine (total)	<500 mg/kg	<500 mg/kg	AAAFURZH.30N
pH (1% solution)	6.0 to 7.0	3.0 to 4.0	-
Solubility (5% solution)	>80% (pH 6.5)	>80% (pH 3.2)	-
Heavy Metals			
Mercury	<10 ppb	<10 ppb	KBBL: (Q1) ANAL-10222
Cadmium	<0.4 ppm	<0.1 ppm	OP-QC-FP-H3-08-006NL
Arsenic	<50 ppb	<10 ppb	KBBL: (Q1) ANAL-10222
Lead	<0.1 ppm	<0.1 ppm	OP-QC-FP-H3-08-006NL
Microbial			
Aerobic count	<100,000	<100,000	RA-06-021/ISO 4833
Enterobacteriaceae	<10	<10	RA-06-061/ISO 7402
Sulphite reducing *Clostridium*	<100	<100	RA-06-100
Salmonella spp	absent in 25 g	absent in 25 g	NEN-EN-ISO 6579
Listeria Monocytogenes	absent in 25 g	absent in 25 g	NEN-EN-ISO 11290-1
Escherichia coli	absent in 1 g	absent in 1 g	NEN-ISO 16649-2
Yeast and Moulds	<10	<10	RA-06-041/ISO 7954

Analysis of 3 non-consecutive lots of HMW and LMW potato protein isolates demonstrates that the manufacturing process as described in Section II.B produces a consistent product which is in compliance with the product specifications. A summary of the chemical analysis for the 3 lots of LMW and HMW potato proteins is presented in Table II.C-2. Batch analyses for 3 lots of LMW potato protein isolates subjected to an additional ion-exchange purification step for removal of glycoalkaloids also is presented (Table II.C-3).

Table II.C-2 Batch Analyses

(b) (4)

Parameter	HMW			LMW		
Dry Matter (% w/w)	94.6	95.1	94.3	93.6	93.7	93
Protein (amino acids) (% w/w)	85.3	85.1	85.7	88.6	88	87.3
Ash (% w/w)	3.60	3.13	5.38	0.78	1.65	2.2
Total glycoalkaloids (α-solanine + α-chaconine) (mg/kg)	102	271	241	281	278	249
Sulphite (SO_2) (ppm)	12	10	12	42	40	28
Lysinoalanine (total)	ND	ND	ND	ND	ND	ND
Heavy Metals						
Mercury (ppb)	<10	<10	<10	<10	<10	<10
Lead (ppm)	0.1	0.1	0.1	0.2*	0.2*	0.4*
Arsenic (ppb)	<50	<50	<50	94*	95*	113*
Cadmium (ppm)	0.4	0.5	0.4	<0.1	<0.1	<0.1
Microbial						
Total aerobic count	2.3E+04	1.4E+04	1.3E+03	1.0E+02	2.0E+02	8.0E+02
Enterobacteriaceae (cfu)	<10	<10	<10	<10	<10	<10
Sulphite reducing *Clostridium* (cfu)	<100	<100	<100	<100	<100	<100
Salmonella spp. (cfu/25 g)	absent	absent	absent	absent	absent	Absent
Listeria monocytogenes (cfu/25 g)	absent	absent	absent	absent	absent	absent
Escherichia coli (cfu/g)	absent	absent	absent	Absent	absent	Absent
Yeast and Moulds (cfu)	<10	<10	<10	<10	<10	<10

ND = Not Detected.

*Solanic notes that the levels of lead and arsenic are outside of the product specification; however, the levels remain low and these lots were prepared using a bentonite filtration step that is no longer used during manufacturing. Bentonite filtration has been replaced with activated carbon filtration to reduce the levels of trace minerals in the ingredient. As shown in table Table II.C-3, replacement of bentonite filtration with activated carbon produced an appreciable lowering of minerals in the ingredient.

Table II.C-3 Batch Analyses of LMW (Low Glycoalkaloid Content)

Parameters	LMW		
(b) (4) [redacted]			
Dry Matter (% w/w)	93.7	92.8	92.6
Protein (N*6.25) / Dry Weight (%)	98.5	99.4	97.6
Ash (% w/w)	ND	ND	ND
Total glycoalkaloids (α-solanine + α-chaconine) (mg/kg protein)	49	80	78
Heavy Metals			
Lead (ppm)	ND	<0.1	<0.1
Cadmium (ppm)	ND	<0.05	<0.05
Elution Solvent			
Formic acid (%w/w)	0.035%	<D.L.	<D.L.
Microbial			
Total aerobic count	<50000	<50000	<50000
Enterobacteriaceae (cfu)	<10	<10	<10
Sulphite reducing *Clostridium* (cfu)	<100	<100	<100
Salmonella spp. (cfu/25 g)	absent	absent	absent
Listeria monocytogenes (cfu/25 g)	absent	absent	absent
Escherichia coli (cfu/g)	absent	absent	absent
Yeast and Moulds (cfu)	<10	<10	<10

D.L. (Detection Limit) = 0.065% w/w

II.D Additional Compositional Analyses

1. Mass Balance Analyses

Analytical characterizing LMW and HMW potato protein isolates to 100% purity is presented in Table II.D-1. The ingredients are well characterized, and largely consist of nutrients that are common to the diet. The analytical data presented below across a number of analytical endpoints is consistent between each lot, which provides additional support that the manufacturing process is adequately controlled.

Table II.D-1 Mass Balance Analyses (in mass %)

Parameter	HMW			LMW		
(b) (4) [redacted]						
Dry matter (dm)	94.6	95.1	94.3	93.6	93.7	93
Moisture	5.4	4.94	5.7	6.4	6.3	7
Crude ash	3.6	3.13	5.38	0.78	1.65	2.2
Crude fat	0.19	0.15	0.12	0.18	0.03	0
Crude fiber	0.61	0.49	0.57	2.03	0.28	1.06
Protein content (Kjeldahl; N*6.25)	85.3	85.1	85.7	88.6	88	87.3

Table II.D-1 Mass Balance Analyses (in mass %)

Parameter	HMW			LMW		
(b) (4) [redacted]						
Glycoalkaloids[1]	0.01	0.03	0.02	0.03	0.03	0.02
Phosphorus (P)	0.145	0.22	0.17	0.002	0.002	0.003
Sodium (Na)	1.83	1.78	1.58	0.08	0.08	0.11
Potassium (K)	0.1887	0.1573	0.1249	0.038	0.0425	0.0135
Chloride (Cl)	0.16	0.03	0.03	1.49	1.8	1.782
Citric acid	4.24	4.27	3.85	ND	ND	ND
Formic acid	0.07	0.04	0.03	0.11	0.1	ND
Total	101.9	100.4	103.3	101.6	100.2	99.5

ND = not detected; dm = dry matter
[1]Total of α-solanine + α-chaconine

2. Protein and Amino Acid Content

Solanic's HMW and LMW potato protein isolates contain a balanced amino acid profile. Analytical data characterizing the protein composition and amino acid content of the HMW and LMW potato protein isolates are shown below in Table II.D-2.

Table II.D-2 Protein and Amino Acid Analyses

Parameter	HMW			LMW		
(b) (4) [redacted]						
Protein (% w/w)						
Kjeldhal N*6.25	85.3	85.1	85.7	88.6	88.0	87.3
Kjeldhal N*6.25 (dm)	90.2	89.5	90.9	94.7	93.9	93.9
Sprint Analyzer N*6.25	85.7	88.3	85.7	84.6	85.3	87.2
Sprint Analyzer N*6.25 (dm)	90.1	92.9	90.9	90.4	91.0	93.8
Amino Acids (g/kg as is)						
Aspartic acid + Asparigine	107.84	110.68	111.94	127.53	125.61	120.61
Threonine	65.53	65.15	66.04	47.16	45.94	43.88
Serine	50.62	52.32	53.27	50.64	50.35	47.56
Glutamic acid + glutamine	114.29	113.85	116.22	85.39	83.75	79.41
Proline	41.32	42.57	43.18	51.16	50.26	44.92
Glycine	39.21	41.08	41.57	53.54	52.00	48.21
Alanine	54.36	53.55	54.45	26.54	26.22	24.84
Valine	42.22	43.97	44.88	71.15	65.40	64.96
Isoleucine	37.95	37.65	39.41	50.23	45.92	45.47
Leucine	95.79	96.56	98.24	94.31	91.29	87.65

Table II.D-2 Protein and Amino Acid Analyses

Parameter	HMW			LMW		
(b) (4) [redacted]						
Tyrosine	55.13	54.16	55.31	56.24	51.11	51.86
Phenylalanine	54.40	54.97	55.72	64.97	60.87	59.49
Lysine	65.67	66.37	67.46	71.09	69.23	65.06
Histidine	17.48	17.22	17.58	15.49	14.72	14.04
Arginine	36.84	37.33	38.39	42.32	40.87	38.84
Cysteïne	6.41	7.35	7.42	23.59	23.68	23.30
Methionine	25.99	24.44	24.93	12.25	13.92	13.15
Tryptophan	9.35	9.88	10.13	12.23	11.82	13.75

dm = dry matter

3. Protease Inhibitor Content of Potato Protein Isolates

Potatoes contain several protease inhibitors that inhibit all of the major pancreatic digestive endo- and exopeptidases responsible for protein digestion in the gastrointestinal tract of higher animals (Pearce *et al.,* 1983), including inhibitors of serine (Suh *et al.*, 1991; Walsh and Twitchell, 1991), aspartic acid (Mares *et al.*, 1991; Walsh and Twitchell, 1991) and cysteine proteases (Brzin *et al.*, 1988; Rowan *et al.*, 1990; Walsh and Twitchell, 1991).

The Solanic laboratory has used the common ISO certified procedures optimized for animal feed for the analysis of proteolytic inhibition activity of food products. These methods are based on the use of chromogenic substrates like N_{α}-Benzoyl-L-arginine-p-nitroanilide (L-BAPA) or Nα-Benzoyl-L-arginine ethyl ester (BAEE) and require a completely solubilized, clear solution for accurate measurements. This method is sensitive to the sample matrix, and accuracy of the analysis is affected in non-aqueous matrices. This analysis method is therefore undesirable because Solanic's protein isolates are intended for applications in a variety of food products that vary in fat-, oil-, protein- and carbohydrate-content. To circumvent limitations of the chromogenic based assays, Ruseler-van Embden *et al*. (2004) developed an analysis method that incorporated azocasein as a protease substrate and assessed the loss of proteolytic activity upon incubation in the presence of a protease inhibitor rich emulsion. Solanic had recently developed and validated a procedure for the accurate and reproducible determination of trypsin inhibitory activity in a variety of complex systems which is based on the azocasein method (Spelbrink *et al*., 2011). This method allows Solanic to accurately measure the protease inhibitor activity of foods to which the company's potato proteins have been added. The protease inhibitor activity of Solanic's potato protein isolates against trypsin and chymotrypsin evaluated using the azocasein method is shown below in Table II.D-3. The Solanic laboratory has analyzed the protease inhibitor activity of multiple batches of its LMW isolates produced in 2009 through 2010. The protease inhibitor activity ranged between 230 to 320 mg/g powder, with a typical value of 275 mg/g powder.

Table II.D-3 Trypsin and Chymotrypsin Inhibitory Activity of HMW and LMW Isolates

Protease Activity[1]	HMW			LMW		
(b) (4) [redacted]						
Trypsin Inhibitor Activity	64/66	89/91	90/89	299/312	282/301	290/290
Chymotrypsin inhibitor activity	31/31	45/48	37/38	192/177	249/242	278/286

Data presented as duplicate analyses of samples from a single lot
[1] mg protease inhibited/g protein as-is, based on Azocasein method as described by Spelbrink *et al.* (2011).

II.E Stability

1. Bulk Stability

Two year accelerated shelf-life studies have been initiated for the HMW and LMW isolates. The study was based on the ICH harmonized tripartite guidelines for stability testing and evaluation of pharmaceuticals for human use (ICH, 2003a,b). The studies were conducted using a 15 kg bag of the HMW potato protein isolate (Lot No. Solanic 206P) and 10 kg bag of LMW potato protein isolate (Lot No. Solanic 306P). Samples were stored at ambient conditions (between 15 and 25°C) in the warehouses of Solanic until used for the stability study, and batches (both HMW and LMW) were produced in April 2010. The respective 15 and 10 kg samples were to third party for storage under accelerated conditions (40°C/75% Relative Humidity) for a period of up to 36 months. At time-points of 0, 1, 3, 6, 12 18, 24 and 36 months, ~600 g samples were obtained from the bulk products. The sampled materials were collected in sterile containers and analysed at Solanic. Storage conditions during the accelerated stability study were 40 ± 2˚C and 75 ± 5% Relative humidity. Analytical result for each time-point up to the current 18 month time-point are presented in Table II.E-1

Table II.E-1 Batch Analyses

Parameter	Specification	LMW Results	Specification	HMW Results
(b) (4) [redacted]				
Appearance				
0 month	White/Grey	White/Grey	White/Grey	White/Grey
1 month		White/Grey		White/Grey
3 month		White/Grey		White/Grey
6 month		White/Grey		White/Grey
12 month		White/Grey		White/Grey
18 month		White/Grey		White/Grey
pH (1% solution)				
0 month	2.8 - 4.5	3.3	6.0 – 7.5	6.6
1 month		3.3		6.7
3 month		3.2		6.7
6 month		3.2		6.6

Table II.E-1 Batch Analyses

Parameter	Specification	LMW Results	Specification	HMW Results
(b) (4) [redacted]				
12 month		3.3		6.5
18 month		3.2		6.6
Conductivity (1% solution)				
0 month	Max 6.0	0.74	Max 6.0	0.86
1 month		0.78		0.85
3 month		0.80		0.80
6 month		0.78		0.85
12 month		0.65		0.67
18 month		0.70		0.77
Clarity				
0 month	N.D.	145	N.D.	322
1 month		208		320
3 month		168		329
6 month		180		336
12 month		195		283
18 month		168		235
Loss on Drying (%)				
0 month	4.0 - 8.0	6.81	4.0 – 8.0	5.36
1 month		7.18		6.70
3 month		7.29		6.93
6 month		7.55		7.64
12 month		8.2		9.4
18 month		8.3		10.7
Protein (%d/w)				
0 month	<90% d/w	96.9	<90% d/w	87.9
1 month		96.5		88.2
3 month		96.6		84.8
6 month		95.1		83.2
12 month		95.6		85.2
18 month		N.D.		-
Solubility (%) (1% solution)				
0 month	≥80%	99.8	≤80%	95.8
1 month		100.0		88.5
3 month		100.0		88.4
6 month		100.0		91.9
12 month		100.0		92.0
18 month		100.0		92.6
Total aerobic count (CFU/g)				
0 month	≤ 50,000	200	≤50,000	450,000
1 month		2100		800
3 month		5900		100

Table II.E-1 Batch Analyses

Parameter	Specification	LMW Results	Specification	HMW Results
(b) (4) [redacted]				
6 month		<1000		1000
12 month		110		230
18 month		200		500
Yeast and Mould (CFU/g)				
0 month	<100	<10	≤100	<10
1 month		<10		<10
3 month		<10		<10
6 month		N.D.		N.D.
12 month		<10		<10
18 month		N.D.		N.D.
Aerobic spores (CFU/g)				
0 month	≤1000	100	≤1000	200
1 month		100		300
3 month		200		100
6 month		<100		<100
12 month		<100		<100
18 month		<100		<100
Anaerobic spores (CFU/g)				
0 month	<1000	100	<1000	500
1 month		<100		<100
3 month		<100		200
6 month		<100		<100
12 month		<100		<100
18 month		<100		<100

N.D. = Not Determined; % d/w = percent dry weight

2. Trypsin Inhibitor Stability during Thermal Treatments

The stability of the 3 classes of protease inhibitors during cooking has been investigated (Huang *et al*., 1981). The authors evaluated the effects of boiling, oven baking (375°C) and microwave cooking on the stability and activity of potato protein inhibitors I, and II, and carboxypeptidase inhibitors isolated form Russet Burbank potatoes. Potato protein Inhibitor II was the least stable and did not survive either preparation method. Inhibitor I was present after boiling, but not after microwave heating. The carboxypeptidase inhibitors were not affected by boiling, and were partially resistant to oven baking, with concentrations decreasing after 60 minutes of baking. Microwave cooking resulted in limited denaturing of the carboxypeptidases, with concentrations decreased by 25% after 7 minutes. The high stability of the carboxypeptidase inhibitors is consistent with their simple structure stabilization *via* disulfide bridging throughout the protein.

Similar observations were observed for stability of Solanic's potato protein isolates. The protease inhibitors present in Solanic's potato protein isolates are heat liable, and conditions

common to food processing (pasteurization, baking) were shown to inactivate the trypsin inhibitory proteases in the ingredients. The effect of pasteurization treatment at 80°C for 30 minutes on the protease inhibitor activity of Solanic's HMW and LMW isolates is shown in Table II.E-2. Over 90% of the trypsin inhibitory activity of the LMW isolate was inactivated under these treatment conditions. As expected, the chymotrypsin inhibitory activity of the isolates were more stable to thermal processing. As shown in Figure II.E-1, thermal inactivation of the protease inhibitors within the potato protein isolates is rapid, occurring within 5 minutes of treatment.

Table II.3-2 Effect of Heat Treatment on Trypsin and Chymotrypsin Inhibitory Activity of Solanic's Potato Protein Isolates (80°C during 30 min)

Potato Protein Isolate	Treatment	TIA (mg inhibited trypsin/mg DM)	CTIA (mg inhibited chymotrypsin/mg DM)
HMW	Native	0.030	0.072
	Heated	0.012	0.030
LMW	Native	0.794	0.296
	Heated	0.058	0.131

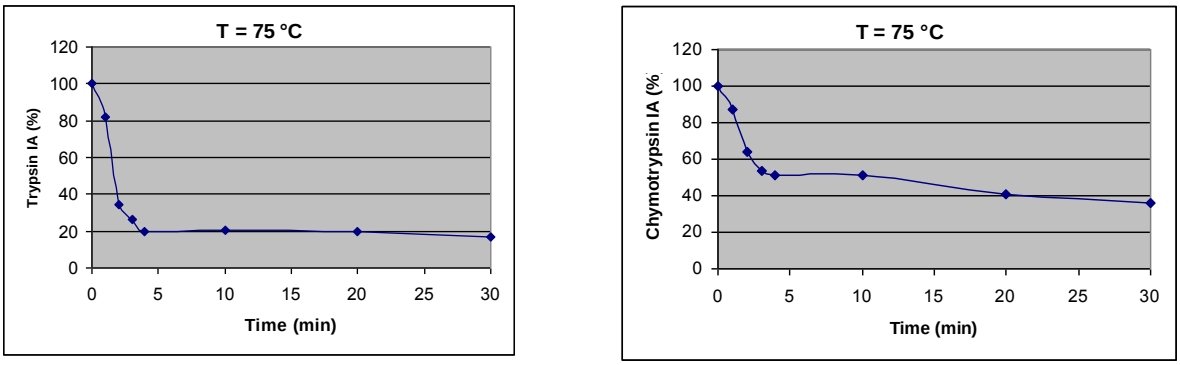

Figure II.E-1 Effect of Heat Treatment (75°C and pH 6) on Trypsin and Chymotrypsin Inhibitory Activity of Solanic's LMW Potato Protein Isolate

III. SELF-LIMITING LEVELS OF USE

Self-limiting use levels are not known.

IV. DETAILED SUMMARY OF THE BASIS FOR SOLANIC'S GRAS DETERMINATION

Potato protein is a natural constituent of potatoes, and contributes to the nutritional profile of the tuber. The manufacturing of Solanic's potato protein isolates is conducted using non-disruptive physical separation processes and does not involve any chemical steps that would intentionally alter the composition or structure of the isolated protein relative to that present in the intact potato. The majority of food uses to which potato protein isolates will be added are subjected to comparable thermal processing conditions (*e.g.*, baking, frying, boiling) that are typically applied to potatoes consumed in the U.S. Potatoes and their constituents, including proteins, have a long history of consumption by humans. The nutritional and toxicological considerations for Solanic's potato proteins isolates are therefore similar to those recognized for the agricultural commodity itself. Safety was based, in-part, on analytical data characterizing recognized toxicants and other anti-nutritional factors that are relevant to the potato. The Organisation for Economic Cooperation and Development (OECD) consensus document on potatoes (OECD, 2002) was used to identify key substances for compositional evaluation. Accordingly, exposures to glycoalkaloids (*i.e.*, α-solanine and α-chaconine) and protease inhibitors from the consumption of Solanic's potato proteins under their intended conditions of use were considered. The safety of exposure to potato glycoalkaloids and dietary protease inhibitors from soybean and potatoes has been extensively evaluated and reported in the literature. Generally available authoritative opinions and published studies evaluating the toxicity and anti-nutritional effects of dietary potato glycoalkaloids and protease inhibitors respectively were sufficient to assess the safety of exposure to these substances from the consumption of potato protein isolates under their intended conditions of use. Published data evaluating the toxicity of Solanic's potato protein isolates, administered at the highest permissible dietary concentration, were without adverse effects in male and female Wistar rats following subchronic administration. Rats are known to be highly sensitive to dietary trypsin inhibitors. The absence of adverse effects on pancreatic function in this animal model further supports the safety of the consumption of Solanic's potato protein isolates by humans. Nutritional considerations pertaining to the dietary quality of potato protein was reviewed by Solanic. Studies in pigs have demonstrated that cooked potato protein is a balanced source of dietary protein and is equivalent to other sources of high quality protein such as casein and animal protein. Although most food applications to which Solanic potato protein are added are expected to be thermally processed, the ability of animals to digest minimally processed potato proteins may reduced. However, based on the limited consumption of minimally processed potato proteins under the conditions of intended use, any reductions in the nutritional bioavailability were not of nutritional significance as such effects are offset by the background consumption of dietary protein in the diet. Potatoes are not known to contain substances that are of mutagenic potential. Accordingly studies evaluating mutagenicity/genotoxicity of potato protein isolates were not considered necessary for the safety assessment. Nevertheless, studies demonstrating that Solanic's potato protein isolates are not mutagenic or genotoxic in the Ames assay using

Salmonella and *Escherichia coli* tester strains in the presence and absence of metabolic activation have been conducted, which further corroborate the conclusion that potato protein isolates do not represent a mutagenic or genotoxic risk.

A summary of all data and information relevant to Solanic's scientific procedures GRAS evaluation of the company's potato protein isolates under the conditions of intended use is presented in Sections IV.A through IV.G below. Finally, 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 similarly concluded that the intended uses of Solanic's potato protein isolates are GRAS based on scientific procedures (see Section IV.H]).

IV.A Probable Consumption Estimates

1. Current uses of Potato Protein Ingredients in the U.S.

Protein isolates from the potato are not listed within the Code of Federal Regulations (CFR) as an approved food additive, or GRAS affirmed substance. In 2002 the FDA issued a letter of no objection in response to AVEBE's exemption claim for the GRAS self-determination of the use of coagulated potato protein, hydrolyzed potato protein, or clarified hydrolyzed potato protein as a water binder, foaming aid, or emulsifier at use-levels between 0.1 to 3.0% (U.S. FDA, 2002). Solanic's minimally processed potato protein isolates represent a second generation product with improved technical function and are intended to replace current GRAS uses of coagulated potato protein in the U.S. food supply.

2. Estimated Consumption of Potato Protein from Natural Occurrences in Food

Potatoes are ranked third (preceded only by rice and wheat) in terms of their importance as a food for human consumption, and in the USA are recognized as a staple of the normal human diet (Camire *et al*., 2009). The protein content of potatoes is reported to range from 1 to 1.5% of tuber fresh weight (Oritz-Medina, 2007). An online based database containing time-series and cross sectional data on food and agriculture for 200 countries has been developed by The Food and Agriculture Organization of the United Nations (FAO) (FAOSTAT, 2011). These consumption estimates are adjusted to account for production, trade, feed and seed uses, waste, other utilization not related to food use. The consumption estimates also include data on *per capita* daily protein supply quantity for each food. Using the FAOSTAT database, *per capita* estimates of potato protein consumption were determined for a number of potato consuming countries over a period between 1967 through 2007 (Figure IV.A-1). In the U.S., consumption of potatoes has remained relatively stable over several decades, with average potato consumption of 50 kg/person/year, or approximately 137 g/person/day. This estimate is similar to United States Department of Agriculture (USDA) estimates, where annual *per capita* intake of approximately 118 lbs of potatoes from all sources (canned, chips, dehydrated, fresh, and frozen), equivalent to approximately 147 g/day, was estimated (USDA-ERS, 2010). Higher intakes have been determined for European and South American countries. Although potato

consumption is declining in many European countries, historical data show that consumption of potatoes in 1967 was in excess of 200 kg/person/day resulting in daily intakes of potatoes and potato protein of 566 and 9.1 g/person/day respectively.

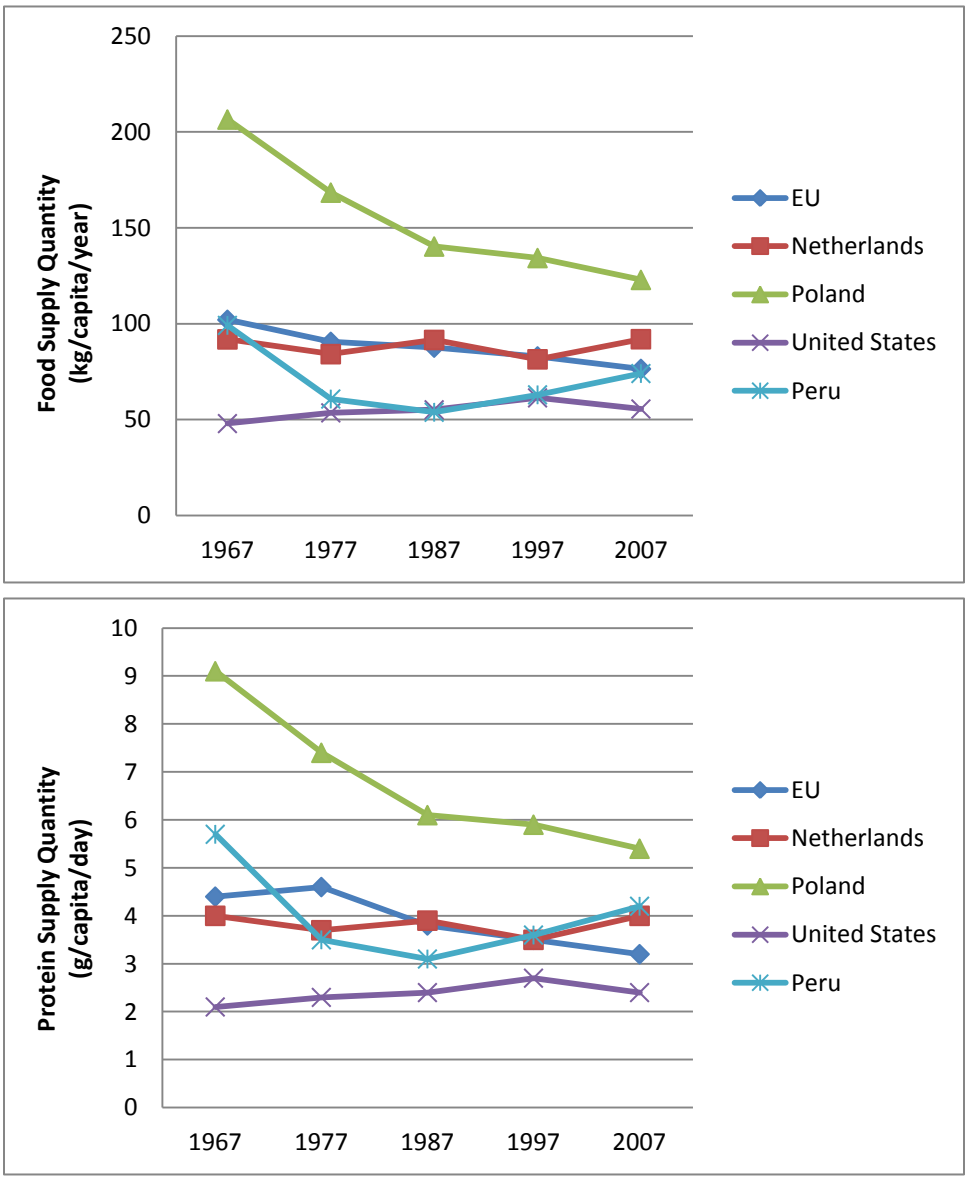

Figure IV.A-1 Food Balance Data for Potato Consumption in Various Countries

Data represents *per capita* estimates of potato consumption and potato protein consumption.
Source: FAOSTAT (2011).

Finally, the average daily consumption of potatoes in Ireland in the early 19th century was 4 to 6 kg, supplemented with milk, eggs and fish; corresponding potato protein intakes of between 60 to 90 g would been expected and would have provided a more than an adequate supply of dietary protein (Bourke, 1968). High consumption estimates also have been reported for rural areas of Peru, where the consumption of potatoes is reported to be as high as 200 to

800 g/person/day in infants and adult women respectively, which corresponds to intakes of 4 and 16 g of potato proteins/day respectively (Camire *et al.*, 2009).

3. Background Consumption of Trypsin Inhibitors and Comparison to Trypsin Inhibitor Activity of Foods Containing Solanic's Potato Protein Isolates

Solanic's isolated potato proteins are produced from starch potatoes. An experiment comparing the protease inhibitor activities of native and processed starch and consumption potatoes was conducted by Solanic. The varieties Seresta (the most commonly grown starch potato), Bildstar and Nicola (commonly grown consumption potato varieties) were used for this comparison. Processing conditions were similar to the normal potato boiling practice *i.e*. boiling during 20 min. in which the heart of the potato reaches a temperature of 75°C. The pH was measured but not adjusted. For all 3 varieties pH was approximately 6.0. In Table IV.A-1, the protease inhibitor activity of the 3 varieties is presented. Under the conditions of this experiment 85 to 92% of the trypsin inhibitory activity was inactivated during cooking. Similar to observations noted previously (Section II.E.2), chymotrypsin inhibitors present within the potato are more stable to heat treatment and between 55 to 77% of the inhibitory activity was reduced during cooking.

Table IV.A-1 Trypsin and Chymotrypsin Inhibitor Activity in Various Potato Varieties

Variety	Treatment	TI[1]	% inhibition	CTI[1]	% inhibition
Seresta	Native	5.9	85	4.0	55
	Processed	0.9		1.4	
Bildstar	Native	6.4	88	4.6	70
	Processed	0.8		1.4	
Nicola	Native	3.5	92	2.2	77
	Processed	0.3		0.5	

[1] mg protease inhibited per g potato fresh weight; CTI = chymotrypsin inhibition; TI = trypsin inhibition. Analytical method used: L-BAPA

Protease inhibitors are common in the diet. The background protease inhibitory activity of several commonly consumed foods was evaluated by Solanic (Spelbrink *et al*., 2011). Depending on the food product, trypsin inhibitor activity (TI) was shown to vary greatly from 0 (sausage with 1% caseinate) to 4.7 (meringue with egg protein) mg TI/g food. In soy infant formula, TI is reported to range from 0.3 to 2.7 mg TI/g formula (Garthoff *et al.*, 2002a). Based on average daily infant formula consumption, an infant consuming soy-based formula is estimated to consume 50 mg TI/day (~10 mg TI/kg body weight for 5 kg infant). Solanic also has noted that residual trypsin inhibitor consumption in the Polish population, which consumes a substantial amount of boiled potatoes, was estimated to be approximately 380 mg/day. For the British population, background consumption of trypsin inhibitors was estimated to be approximately 295 mg TI/person/day from the household diet alone (Doell *et al*., 1981). Moreover, although more recently, potato consumption in the Western diet has decreased to

approximately 100 g/day, in the past, intakes exceeding over 1,000 g/day were not unusual. Based on these past potato intake estimates, humans were expected to consume 800 mg TI/day from consumption of cooked potatoes alone.

Using the azocasein method, Solanic evaluated the trypsin inhibitory activity of several foods to which the LMW potato proteins are intended for use. As shown in Table IV.A-2, residual trypsin inhibitory activity was noted for most food applications in which LMW potato proteins were added. Highest residual activity was observed for the mayonnaise and meringue uses. Regardless of the food application and processing conditions, the trypsin inhibitory activity was comparable to that analyzed in the boiled potato (Table IV.A-1).

Table IV.A-2 Residual Trypsin Inhibited (TI) Activity of Various Food Applications in which Solanic's LMW Potato Proteins were Used (Azocasein method)

Application	LMW Use Level (% fw)	Treatment	TI[1]	Δ TI[2] (%)	TI inactivation (%)
Gluten free cake	3.0	Oven baking; 180°C; 20-25 min	0.72	ND	85
Mayonnaise	0.7	High shear mixing with local elevated temperatures	0.87	93	-
Mayonnaise low fat	0.7	High shear, pasteurization; 80°C; 30 min	0.24	71	72
Mayonnaise high fat	2.0	High shear mixing with local elevated temperatures	1.28	91	-
	1.0	High shear and pasteurization; 80°C; 30 min	0.49	78	62
Meringue	4.2	Oven baking; 135°C; 35-40 min oven drying; 85°C; 2 h	1.78	ND	91
Pâté	1.0	Pasteurize; 80°C; 20 min	0.74	100	53
Wine, red	0.25	Precipitation, no heat	0.13	15	-
Wine, white	0.25	Precipitation, no heat	0.05	20	-
Yoghurt	0.20	Unprocessed	1.48	ND	-
Yoghurt	0.20	UHT 113°C; 5 s	0.10	ND	93

% fw = percent fresh weight; ND = un-supplemented controls not determined; UHT = ultra high temperature
[1] TI/g = mg Trypsin inhibited/g product as-is
[2] Δ TI = % increase of TI activity compared to un-supplemented control food

The trypsin inhibitor activity of LMW potato protein containing food products and comparison to commercial and laboratory prepared non-potato protein counterparts was evaluated by Solanic Laboratories (Spelbrink *et al*., 2011). As shown in Table IV.A-3, the TI activity of egg protein powder is comparable the TI activity of Solanic's LMW potato protein isolate. In general, LMW containing applications like ice cream, lollypops and mayonnaise contain approximately twice the amount of TI as the same applications with a reference protein. The same holds true for pâté up to an inclusion level of 1% LMW. The commercial and egg protein meringues showed a considerable amount of TI. The Solanic LMW meringues showed a wide variation in TI probably depending on batch to batch variation and meringue size. Two salamis were tested, 1 with and

1 without LMW protein. Both salamis displayed low levels of trypsin inhibitory activity. Sausages prepared with Solanic LMW protein were significantly higher in TI activity than sausages prepared with caseinate. Increasing the amount of LMW protein resulted in an increase in TI activity. Soy products showed trypsin inhibitory activity ranging from 0.26 to 0.54 mg TI/g product, whereas yoghurts had very low TI activity.

Table IV.A-3 Trypsin Inhibitory Activity of Several Foodstuffs

Sample	% Protein	TI (mg TI/g finished product)
Solanic LMW protein powder	-	264
Egg protein powder	-	151
Ice cream 3% milk powder	3.0	2.1
Ice cream 1% Solanic LMW protein	1.0	4.2
Lollypop, egg protein	-	1.5
Lollypop, Solanic LMW protein	-	2.3- 4.9
Mayonnaise, commercial	-	0.07 -0.14
Mayonnaise, egg yolk	2.5% egg yolk	1.1
Mayonnaise, Palsgaard 5430	0.75% Palsgaard 5430	1.2
Mayonnaise, Solanic LMW protein	0.75% 306P	2.0
Meringue, commercial	-	3.3
Meringue, egg protein	3.5	4.7
Meringue (Solanic LMW)	3.5	1.78
Meringue (Solanic LMW)	3.5	0.83
Meringue (Solanic LMW)	3.5	13.2
Pate 0% Solanic LMW protein	0	0.5
Pate 0.5% Solanic LMW protein	0.5	0.8
Pate 1% Solanic LMW protein	1.0	1.1
Pate 1.5% Solanic LMW protein	1.5	1.8
Salami, Solanic LMW protein	-	0.1
Salami, Solanic LMW protein	-	0.0
Sausage, 1% caseinate (a)	1.0	0.0
Sausage, 0.3% Solanic LMW protein	0.3	0.6
Sausage, 0.5% Solanic LMW protein	0.5	2.2
Sausage, 1.0% Solanic LMW protein	1.0	2.8
Sausage, 1.0% Blend pea protein	1.0	0.8
Soy drink	-	0.26
Soy burger (inside, lyophilized)	-	0.46
Soy milk	-	0.48
Soy oil	-	0.54
Soy drink yoghurt	-	0.04-0.10
Yoghurt, commercial	-	0.02-0.06

4. Estimated Consumption of Potato Protein Isolates from Intended Food Uses

Estimates for the intake of potato protein isolates were based on the proposed food-uses and use-levels (Table I.D-1) in conjunction with food consumption data included in the National Center for Health Statistics' (NCHS) NHANES (CDC, 2006, 2009; USDA, 2009, 2010). The data from the 2003-2004, 2005-2006, and 2007-2008 cycles of the NHANES survey were combined to provide a larger population from which to estimate potato protein isolates consumption. Calculations for the mean and 90th percentile all-person and all-user intakes, and percent consuming were performed for each of the individual proposed food-uses of potato protein isolates. Similar calculations were used to determine the estimated total intake of potato protein isolates resulting from all proposed food-uses of potato protein isolates combined. In both cases, the per person and per kilogram body weight intakes were reported for the following population groups:

- infants, ages 0 to 2;
- children, ages 3 to 11;
- female teenagers, ages 12 to 19;
- male teenagers, ages 12 to 19;
- female adults, ages 20 and up;
- male adults, ages 20 and up; and
- total population (all age and gender groups combined).

It should be noted that this type of intake methodology is generally considered to be "worst case" as a result of several conservative assumptions made in the consumption estimates. For example, it is often assumed that all food products within a food category contain the ingredient at the maximum specified level of use. In addition, it is well established that the length of a dietary survey affects the estimated consumption of individual users. Short-term surveys, such as the typical 2- or 3-day dietary surveys, overestimate the consumption of food products that are consumed relatively infrequently.

As discussed, coagulated potato proteins and hydrolysates derived thereof are currently marketed in the U.S. for use in multiple food categories; exposure to potato protein from these food uses was estimated to be 1.9 g/day (U.S. FDA, 2002). Solanic's potato protein isolates are intended for use as alternative to coagulated potato proteins in food and therefore background exposure to coagulated potato proteins will not contribute, in an additive manner, to the exposure to potato proteins in the diet following introduction to the U.S. food supply from the proposed uses described in Table I.D-1.

4.1 Estimated Daily Intake of Low Molecular Weight Potato Protein Isolate from Proposed Technological Uses in the U.S.

The estimated total intake of LMW potato protein isolate on a per g/person/day and per kilogram body weight basis from the proposed technological uses in the U.S. by population group is summarized in Tables IV.A-4 and IV.A-5, respectively.

Approximately 90.3% of the total U.S. population were identified as potential consumers of LMW potato protein isolate from the proposed technological uses (21,362 actual users identified). Consumption of the proposed technological uses by the total U.S. population resulted in an estimated mean all-person and all-user intakes of LMW potato protein isolate of 2.1 g/person/day (34 mg/kg body weight/day) and 2.3 g/person/day (38 mg/kg body weight/day), respectively. The 90th percentile all-person and all-user intakes of LMW potato protein isolate from the proposed technological uses by the total U.S. population were 4.7 g/person/day (77 mg/kg body weight) and 4.8 g/person/day (80 mg/kg body weight), respectively.

On an individual population basis, the greatest mean all-person intake of LMW potato protein isolate on an absolute basis was determined to occur in male adults at 2.7 g/person/day (31 mg/kg body weight/day). The greatest mean all-user intake of LMW potato protein isolate on an absolute basis also was observed to occur in male adults 2.9 g/person/day (34 mg/kg body weight/day). Infants displayed the lowest mean all-person and all-user intakes of LMW potato protein isolate, on an absolute basis with a value of 0.7 and 1.1 g/person/day, respectively. On a body weight basis, the mean all-person and all-user intakes of LMW potato protein isolate were highest in children and infants, respectively, with an intakes of 61 and 90 mg/kg body weight/day, respectively. The lowest all-person and all-user mean intakes of LMW potato protein isolate on a per kilogram body weight basis were observed to occur in female adults at 27 and 29 mg/kg body weight/day, respectively.

Table IV.A-4 Summary of the Estimated Daily Intake of Low Molecular Weight Potato Protein Isolate from Proposed Technological Uses in the U.S. by Population Group (2003-2008 NHANES Data)

Population Group	Age Group (years)	% Users	Actual # of Users	All-Person (g/day)		All-User (g/day)	
				Mean	90th Percentile	Mean	90th Percentile
Infants	0 to 2	64.2	1,510	0.7	2.0	1.1	2.3
Children	3 to 11	89.5	3,689	1.6	3.6	1.8	3.7
Female Teenagers	12 to 19	87.7	2,182	1.6	3.6	1.9	3.7
Male Teenagers	12 to 19	87.0	2,154	2.5	5.3	2.8	5.6
Female Adults	20 and up	92.7	6,197	1.9	4.0	2.1	4.1
Male Adults	20 and up	92.4	5,630	2.7	5.8	2.9	6.0
Total Population	All Ages	90.3	21,362	2.1	4.7	2.3	4.8

When heavy consumers (90th percentile) were assessed, the estimate for the all-person and all-user intakes of LMW potato protein isolate from the proposed technological uses were determined to be greatest in male adults at 5.8 g/person/day (69 mg/kg body weight/day) and 6.0 g/person/day (72 mg/kg body weight/day). The lowest 90th percentile all-person and all-user intake estimates of LMW potato protein isolate were observed to occur in infants, with a value of 2.0 and 2.3 g/person/day, respectively, on an absolute basis. On a body weight basis, infants were determined to have the greatest all-person and all-user 90th percentile intakes of LMW potato protein isolate, with values of 158 and 187 mg/kg body weight/day, respectively. The lowest all-person and all-user 90th percentile intakes of LMW potato protein isolate on a body weight basis was observed to occur in female adults at 58 and 60 mg/kg body weight/day, respectively.

Table IV.A-5 Summary of the Estimated Daily Per Kilogram Body Weight Intake of Low Molecular Weight Potato Protein Isolate from Technological Uses in the U.S. by Population Group (2003-2008 NHANES Data)

Population Group	Age Group (years)	% Users	Actual # of Users	All-Person (mg/kg)		All-User (mg/kg)	
				Mean	90th Percentile	Mean	90th Percentile
Infants	0 to 2	64.2	1,510	58	158	90	187
Children	3 to 11	89.5	3,689	61	136	68	143
Female Teenagers	12 to 19	87.7	2,182	28	62	32	66
Male Teenagers	12 to 19	87.0	2,154	38	83	44	87
Female Adults	20 and up	92.7	6,197	27	58	29	60
Male Adults	20 and up	92.4	5,630	31	69	34	72
Total Population	All Ages	90.3	21,362	34	77	38	80

4.2 Estimated Daily Intake of High Molecular Weight Potato Protein Isolate from Proposed Technological Uses in the U.S.

The estimated total intake of HMW potato protein isolate on a per g/person/day and per kilogram body weight basis from the proposed technological uses in the U.S. by population group is summarized in Tables IV.A-6 and IV.A-7, respectively.

Approximately 89.2% of the total U.S. population were identified as potential consumers of HMW potato protein isolate from the proposed technological uses (21,181 actual users identified). Consumption of the proposed technological uses by the total U.S. population resulted in an estimated mean all-person and all-user intakes of HMW potato protein isolate of 2.0 g/person/day (32 mg/kg body weight/day) and 2.2 g/person/day (36 mg/kg body weight/day), respectively. The 90th percentile all-person and all-user intakes of HMW potato protein isolate from the proposed technological uses by the total population were 4.5 g/person/day (72 mg/kg body weight/day) and 4.7 g/person/day (77 mg/kg body weight/day), respectively.

On an individual population basis, the greatest mean all-person and all-user intakes of HMW potato protein isolate on an absolute basis were determined to occur in male adults at 2.6 and 2.8 g/person/day, respectively. Male teenagers also were determined to have a mean all-user intake of HMW of 2.8 g/person/day. Infants displayed the lowest mean all-person and all-user intakes of HMW potato protein isolate, on an absolute basis with a value of 0.6 and 1.0 g/person/day, respectively. On a body weight basis, the mean all-person and all-user intakes of HMW potato protein isolate were highest in children and infants, respectively, with an intake of 57 and 78 mg/kg body weight/day, respectively. The lowest all-person and all-user mean intakes of HMW potato protein isolate on a per kilogram body weight basis were observed to occur in female adults at 24 and 27 mg/kg body weight/day, respectively.

Table IV.A-6 Summary of the Estimated Daily Intake of High Molecular Weight Potato Protein Isolate from Proposed Technological Uses in the U.S. by Population Group (2003-2008 NHANES Data)

Population Group	Age Group (years)	% Users	Actual # of Users	All-Person (g/day)		All-User (g/day)	
				Mean	90th Percentile	Mean	90th Percentile
Infants	0 to 2	64.5	1,512	0.6	1.8	1.0	2.2
Children	3 to 11	90.2	3,717	1.5	3.5	1.7	3.6
Female Teenagers	12 to 19	87.4	2,164	1.5	3.4	1.8	3.6
Male Teenagers	12 to 19	84.2	2,116	2.3	5.2	2.8	5.5
Female Adults	20 and up	90.9	6,104	1.8	3.8	1.9	4.0
Male Adults	20 and up	91.4	5,568	2.6	5.6	2.8	5.9
Total Population	All Ages	89.2	21,181	2.0	4.5	2.2	4.7

When heavy consumers (90th percentile) were assessed, the estimate for the all-person and all-user intakes of HMW potato protein isolate from the proposed technological uses were determined to be greatest in male adults, at 5.6 and 5.9 g/person/day, respectively. The lowest 90th percentile all-person and all-user intake estimates of HMW potato protein isolate were observed to occur in infants, with values of 1.8 and 2.2 g/person/day, respectively, on an absolute basis. On a body weight basis, infants were determined to have the greatest 90th percentile all-person and all-user intakes of HMW potato protein isolate, with a value of 148 and 176 mg/kg body weight/day, respectively. The lowest all-person and all-user 90th percentile intakes of HMW potato protein isolate on a body weight basis were observed to occur in female adults, at 55 and 57 mg/kg body weight/day, respectively.

Table IV.A-7 Summary of the Estimated Daily Per Kilogram Body Weight Intake of High Molecular Weight Potato Protein Isolate from Proposed Technological Uses in the U.S. by Population Group (2003-2008 NHANES Data)

Population Group	Age Group (years)	% Users	Actual # of Users	All-Person (mg/kg)		All-User (mg/kg)	
				Mean	90th Percentile	Mean	90th Percentile
Infants	0 to 2	64.5	1,512	51	148	78	176
Children	3 to 11	90.2	3,717	57	128	63	138
Female Teenagers	12 to 19	87.4	2,164	26	58	30	62
Male Teenagers	12 to 19	84.2	2,116	36	79	43	87
Female Adults	20 and up	90.9	6,104	24	55	27	57
Male Adults	20 and up	91.4	5,568	30	67	32	68
Total Population	All Ages	89.2	21,181	32	72	36	77

4.3 Estimated Intake of Solanic's Potato Protein Isolates from Proposed Nutritive Food Uses of Solanic's LMW Potato Protein Isolate

Solanic's LMW and HMW potato proteins isolates are proposed for addition to adult sports drinks and protein health bars at use levels between 1 and 10%. The proposed nutritive uses and use levels for Solanic's LMW and HMW potato protein isolates are identical, therefore, the estimated daily intake of LMW and HMW potato protein isolates from the individual proposed food-uses are discussed together in the following sections and are referred to as potato protein isolates.

The estimated total intake of the potato protein isolates on a per g/person/day and per kilogram body weight basis from the proposed food-uses in the U.S. by population group is summarized in Tables IV.A-8 and IV.A-9, respectively.

Only 8.5% of the total U.S. population was identified as potential consumers of the potato protein isolates from the proposed nutritive food-uses (2,087 actual users identified). Consumption of the potato protein containing foods under the proposed foods by the total U.S. population resulted in an estimated mean all-person and all-user intakes of the 1.3 g/person/day (20 mg/kg body weight/day) and 11.2 g/person/day (165 mg/kg body weight/day), respectively. The 90th percentile all-person and all-user intakes of the potato protein isolates by the total population were 1.4 g/person/day (20 mg/kg body weight/day) and 30.0 g/person/day (429 mg/kg body weight/day), respectively.

On an individual population basis, the greatest mean all-person and all-user intakes of Solanic's potato protein isolate on an absolute basis were determined to occur in male adults at 1.9 and 15 g/person/day, respectively. Children displayed the lowest mean all-person and all-user intakes of the potato protein isolate on an absolute basis, with a value of 0.3 and 3.5 g/person/day, respectively. On a body weight basis, the mean all-person and all-user intakes of the

potato protein isolates were highest in male adults, with an intake of 24 and 186 mg/kg body weight/day, respectively. The lowest all-person and all-user mean intakes on a per kilogram body weight basis were observed to occur in female teenagers at 9 and 74 mg/kg body weight/day, respectively.

Table IV.A-8 Summary of the Estimated Daily Intake of Solanic's Potato Proteins Isolates from Proposed Nutritive Food-Uses in the U.S. by Population Group (2003-2008 NHANES Data)

Population Group	Age Group (years)	% Users	Actual # of Users	All-Person (g/day)		All-User (g/day)	
				Mean	90th Percentile	Mean	90th Percentile
Children	3 to 11	6.9	283	0.3	na	3.5	5.1
Female Teenagers	12 to 19	8.3	205	0.6	1.4	4.5	15.0
Male Teenagers	12 to 19	8.0	196	1.2	1.2	11.6	28.0
Female Adults	20 and up	10.4	697	1.4	1.7	10.9	29.2
Male Adults	20 and up	10.0	612	1.9	1.9	15.0	38.4
Total Population	All Ages	8.5	2,087	1.3	1.4	11.2	30.0

na = not available

When heavy consumers (90th percentile) were assessed, the estimate for the all-person and all-user intakes of potato protein isolate from the proposed nutritive uses were determined to be greatest in male adults at 1.9 and 38.4 g/person/day, respectively. The lowest 90th percentile all-person and all-user intake estimates were observed to occur in male teenagers and children, respectively, with values of 1.2 and 5.1 g/person/day, respectively, on an absolute basis. On a body weight basis, female adults and male adults were determined to have the greatest all-person and all-user 90th percentile intakes of potato protein isolate, respectively, with a value of 24 and 467 mg/kg body weight/day, respectively. The lowest all-person and all-user 90th percentile intakes of potato protein isolate on a body weight basis was observed to occur in male teenagers and female teenagers, respectively, at 16 and 177 mg/kg body weight/day, respectively.

Table IV.A-9 Summary of the Estimated Daily Per Kilogram Body Weight Intake of Solanic's Potato Proteins Isolates from Proposed Nutritive Food-Uses in the U.S. by Population Group (2003-2008 NHANES Data)

Population Group	Age Group (years)	% Users	Actual # of Users	All-Person (mg/kg)		All-User (mg/kg)	
				Mean	90th Percentile	Mean	90th Percentile
Children	3 to 11	6.9	283	13	na	141	244
Female Teenagers	12 to 19	8.3	205	9	22	74	177
Male Teenagers	12 to 19	8.0	196	19	16	183	459
Female Adults	20 and up	10.4	697	19	24	153	413
Male Adults	20 and up	10.0	612	24	21	186	467
Total Population	All Ages	8.5	2,087	20	20	165	429

na = not available

IV.B Absorption, Distribution, Metabolism and Excretion

Studies characterizing the absorption, distribution, metabolism and kinetics of minimally processed potato proteins were not identified within the public domain. Potato proteins have a long-history of consumption in the food supply, and Solanic's LMW and HMW potato protein isolates are expected to be digested and metabolized in a similar manner to other commonly consumed dietary protein sources. Studies evaluating the bioavailability and nutritional quality of potato protein are presented in Section IV.E.

IV.C Toxicity of LMW and HMW Potato Proteins

1. Subchronic Toxicity Study

In a study provided by Solanic, groups of male and female Wistar Crl:(WI) BR rats (10/group/sex) were provided 0 (control), 15% HMW, 7.5% LMW, or 15% total potato protein isolates (TP: LMW + HMW), in the diet for a period of 90 days (Lynch *et al.*, 2012)[3]. The potato protein test ingredients were heat treated through a pasteurization procedure (30 minutes at 80°C) prior to addition to feed, and composition of the test diets, including analyses of TIA and glycoalkaloid content is presented in Table IV.C-1 below. As discussed in Section IV.E.2 below, dietary protease inhibitors are toxic to rodents. Consumption of diets supplemented with protease inhibitors from soybean or potato produce pancreatic hypertrophy in rodents. Secondary effects of reduced growth and feed consumption also are observed in these animals.

[3] The study was conducted at NOTOX B.V. ('s-Hertogenbosch, The Netherlands) in compliance with standards as described under: OECD (1998). OECD Principles of Good Laboratory Practice (as Revised in 1997). (OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring, no 1 - ENV/MC/CHEM(98)17 http://www.oecd.org/officialdocuments/displaydocumentpdf/?cote=env/mc/chem(98)17&doclanguage=en); OECD (1998), Repeated dose 90-day oral toxicity study in rodents. In: OECD Guidelines for the Testing of Chemicals - http://www.oecd.org/env/chemicalsafetyandbiosafety/testingofchemicals/oecdguidelinesforthetestingofchemicals.htm (OECD Guideline no 408).

Since it is recognized that the pancreatic response in rodents is not relevant to humans (Section IV.E.2), the test articles were pasteurized to partially inactivate the proteases in the ingredients ensuring that any adverse findings attributed to the test articles would be differentiated from secondary effects that may occur from pancreatic toxicity. Use of pasteurization reduced the TI activity of the LMW isolate by 80%, and the TI activity of LMW diet was determined to be 2-fold above the baseline level measured in the casein base chow diet. Although this dietary concentration was insufficient to induce pancreatic effects under the conditions of this study, in an earlier non-GLP (good laboratory practice) dose-ranging study, Solanic did identify one animal with an increase in pancreatic weights administered LMW potato protein isolate at dietary concentration of 15% (Van Otterdijk, 2007). The apparent increase in pancreatic weights in rats administered the pasteurized material at this dose prompted the use of the low dietary use level of 7.5% in the subchronic study.

Table IV.C-1 Compositions of the HMW, LMW, and TP Protein Diets

Parameter	Control diet	Dose group		
		15% HMW	7.5% LMW	15% TP
Proximates and Macronutrients				
Dry matter (%)	96.8	97.6	97.2	97.7
Crude protein, N x 6.25 (%)	18.0	18.0	18.0	18.0
Crude fat (%)	7.1	7.1	7.1	7.1
Crude fiber (%)	6.0	6.0	6.0	6.0
Crude ash (%)	5.6	5.1	5.4	5.3
Casein (%)	20.2	5.0	12.3	4.0
Starch (%)	37.0	37.1	37.2	37.1
Sucrose and maltodextrin (%)	21.1	22.3	21.9	22.8
N-free extracts	60.1	61.4	60.7	61.3
Minerals				
Calcium (%)	0.89	0.90	0.89	0.89
Phosphorus (%)	0.62	0.61	0.61	0.61
Sodium (%)	0.25	0.26	0.25	0.25
Magnesium (%)	0.21	0.21	0.21	0.21
Amino Acids				
Lysine (%)	1.500	1.500	1.500	1.500
Methionine (%)	0.628	0.625	0.625	0.628
Cysteine (%)	0.406	0.406	0.410	0.409
Threonine (%)	1.074	1.083	1.071	1.073
Tryptophan (%)	0.269	0.264	0.265	0.266
Lysine : methionine and cysteine (ratio)	0.690	0.687	0.690	0.691
Lysine : threonine (ratio)	0.716	0.721	0.714	0.715
Lysine : tryptophan (ratio)	0.179	0.176	0.176	0.177
Anti-nutrients and Toxins				
Trypsin inhibitor activity (TI)[a] (mg trypsin inhibited/g diet)	-	1.2	4.5	2.7

Table IV.C-1 Compositions of the HMW, LMW, and TP Protein Diets

Parameter	Control diet	Dose group		
		15% HMW	7.5% LMW	15% TP
Glycoalkaloids (α-cachonine + α-solanine)[a] (mg/kg diet)	ND	14	12	26
Energy				
Gross energy (MJ/kg)	18.9	19.1	19.0	19.1
Metabolizable energy (pig) (MJ/kg)	14.8	14.9	14.9	14.9
Metabolizable energy (Atwater) (MJ/kg)				
protein (%)	15.8	15.9	15.9	15.9
fat (%)	19	19	19	19
carbohydrates (%)	17	17	17	17
	64	64	64	64

ND = Not determined
[a] Theoretical concentration based on assayed (azocasein method) TI of the LMW (52.9±7.4), HMW (3.9±1.6), and TP (10.7±1.4) isolates on an as-is basis expressed as mg Trypsin inhibited/g.

A standard series of traditional toxicological parameters was evaluated including development of any clinical signs of toxicity, body weights and food consumption, and organ weights. Clinical pathology, macroscopy, and histopathology, as well as the functional observation test also were performed. Over the course of the study, the achieved intakes were calculated to be 8,571, 4,224, and 8,414 mg/kg body weight/day for the 15% HMW, 7.5% LMW, and 15% TP protein group males, respectively. The corresponding values in females were 9,698, 4,912, and 9,768 mg/kg body weight/day, respectively. The estimated activity of TI in the 7.5% LMW diet was 4.5 mg TI/g diet, resulting in the consumption of 189 mg TI/kg body weight/day.

No mortality or clinical signs of toxicity were observed in any of the potato protein-treated animals. Ophthalmological examinations did not reveal any adverse effects of treatment and functional tests on hearing ability, papillary reflex, static righting reflex, or grip strength were normal in all treated groups. During the overnight activity test, a statistically significant reduction in motor activity was scored in females treated with either 7.5% LMW or 15% HMW potato protein relative to controls. These effects were not considered treatment related as they were not observed in any of the male groups or in females consuming the TP diet. Food consumption, body and organ weights were not adversely affected by treatment.

Slight variations in hematological and clinical chemistry parameters were reported; these however were typically observed in one sex only and/or in only one of the treatment groups (LMW or HMW protein, or TP). It was further noted that the changes were not associated with any morphological alterations and were therefore considered not to represent adverse effects related to the dietary administration of the potato proteins. Males of both the LMW and HMW test groups were reported to exhibit slight increases in the incidence of vacuolation in the zona fasciculata of the adrenal cortex compared to the control group (*i.e.*, 7 and 5 affected animals in LMW and HMW protein test males, respectively, *versus* 3 animals in the control group). Although the severity of the vacuolation was reported to be only slight and it was noted that it

may have been a normal background finding typical of this rat strain and age, the authors could not exclude the possibility of a relationship to treatment. However, it was concluded that based on the low severity and incidence, the vacuolation did not adversely affect adrenal function and therefore was of no toxicological relevance. Furthermore, similar variations were not observed in any of the treated females of any of the test groups and while slightly increased vacuolation of the adrenals was noted in males of the LMW and HMW groups, the incidence of this finding in the TP males was lower than in the control group.

Rodents are highly sensitive to dietary trypsin inhibitors. The absence of reduced weight gain, pancreatic toxicity, or other evidence of toxicity/adverse effects in rodents consuming Solanic's potato protein isolates at the highest permissible dietary concentrations support the safety Solanic's potato protein isolates under the intended conditions of use in food and are consistent with the fact that potatoes have a long-history of wide-spread and safe consumption.

IV.D Genotoxicity

1. Ames

The mutagenicity of LMW and HMW potato protein isolates were investigated in 4 GLP-compliant studies using the *in vitro* bacterial reverse mutation assay (*i.e.*, Ames test) (van den Wijngaard, 2011a-d) (Table IV.D-1). The studies were conducted in accordance with OECD guidelines for toxicity testing of chemicals (Test 471[4]) and the FDA Center for Food Safety and Applied Nutrition, Redbook 2000 Toxicological Principles for the safety of Food Ingredients (IV.C.1a[5]).

LMW and HMW potato protein isolates (concentrations of 0 to 5,000 µg/plate) were tested using the Ames test in the histidine auxotroph strains of *Salmonella typhimurium* TA98, TA100, TA1535, and TA1537 and the tryptophan auxotroph strain of *Escherichia coli* WP2 *uvrA* (van den Wijngaard, 2011a- d). Negative (spontaneous reversion rate) and positive controls (*i.e.*, sodium azide, 9-aminoacridine, 2-nitrofluorene, N-ethyl-N-nitrosourea, 2-aminoanthracene, and benzo(a)pyrene) were included in the experiment. In 3 of the 4 studies, treatment with LMW or HMW potato protein isolates did not increase the number of revertant colonies above the negative control treatment in all strains tested in the absence or presence of metabolic activation [*i.e.*, Aroclor 1254-induced rat liver homogenate fraction (S9)]. In one of the AMES studies conducted with LMW potato protein, a slight increase in the mean number of revertant colonies was observed at 1,000 and 2,000 µg/plate in strain TA1535 in the absence of metabolic activation when compared to the negative control; however, these results were not

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[4] OECD Guidelines for the Testing of Chemicals. http://www.oecd.org/env/chemicalsafetyandbiosafety/testingofchemicals/oecdguidelinesforthetestingofchemicals.htm
[5] Redbook 2000 Toxicological Principles for the safety of Food Ingredients. http://www.fda.gov/Food/GuidanceComplianceRegulatoryInformation/GuidanceDocuments/FoodIngredientsandPackaging/Redbook/default.htm

reproducible when repeated (van den Wijngaard, 2011a). As a result, the authors concluded that the effect observed was "an artefact and was not biologically relevant". In all 4 studies, the authors concluded that LMW and HMW potato protein isolates were non-mutagenic in *S.* Typhimurium strains TA98, TA100, TA1535, and TA1537 and E. coli WP2 *uvrA,* both in the presence and absence of metabolic activation S9.

In addition, both LMW and HMW potato protein isolates were not toxic to any strain tested in the mutagenicity studies, as neither a decrease in the mean number of revertants nor a clearing of the background lawn of bacterial growth compared to the negative controls was observed among all 4 studies.

Table IV.D-1 Mutagenicity and Genotoxicity of LMW and HMW Potato Protein Isolates

Assay	Treatment	Results
LMW potato protein		
van den Wijngaard, 2011a *Salmonella* (TA98, TA100, TA1535, and TA1537) and *Escherichia coli* WP2 *uvrA*	First test (plate incorporation test): with and without S9 activation; 0, 62, 185, 556, 1,667, and 5,000 µg/plate Second test (plate incorporation test): with and without S9 activation; 0, 125, 250, 500, 1,000, and 2,000 µg/plate[1]	Negative in all strains with and without activation[2]
van den Wijngaard, 2011b *Salmonella* (TA98, TA100, TA1535, and TA1537) and *E. coli* WP2 *uvrA*	First test (plate incorporation test): with and without S9 activation; 0, 62, 185, 556, 1,667, and 5,000 µg/plate Second test (plate incorporation test): with and without S9 activation; 0, 63, 125, 250, 500, 1,000, and 2,000 µg/plate[1]	Negative in all strains with and without activation
HMW potato protein		
van den Wijngaard, 2011c *Salmonella* (TA98, TA100, TA1535, and TA1537) and *E. coli* WP2 *uvrA*	First test (plate incorporation test): with and without S9 activation; 0, 62, 185, 556, 1,667, and 5,000 µg/plate Second test (pre-incubation test): with and without S9 activation; 0, 22, 65, 194, 583, and 1,750 µg/plate	Negative in all strains with and without activation
van den Wijngaard, 2011d *Salmonella* (TA98, TA100, TA1535, and TA1537) and *E. coli* WP2 *uvrA*	First test (plate incorporation test): with and without S9 activation; 0, 62, 185, 556, 1,667, and 5,000 µg/plate Second test (pre-incubation test): with and without S9 activation; 0, 22, 65, 194, 583, and 1,750 µg/plate	Negative in all strains with and without activation

HMW = high molecular weight; LMW = low molecular weight

[1] As precipitation was observed in the first test at 1,667 µg/plate, a lower maximum concentration was used in the second test.

[2] In the second test, a slight increase in the mean number of revertant colonies compared to the spontaneous reversion of the negative control was observed at 1,000 and 2,000 µg/plate in strain TA1535 in the absence of metabolic activation. As a result, a third test was conducted only with strain TA1535; however, no increase was observed. Therefore, the authors concluded that the slight increase observed in the second test was an "artefact and was not biologically relevant", as the results were not reproduced in the first or third test conducted.

Based on the negative findings observed in the bacteria reversion assays in the presence or absence of metabolic activation Solanic's LMW and HMW potato protein isolates were concluded non genotoxicity/mutagenic under the conditions of these assays. Due to the wide-spread and long history and safe consumption potatoes in the food supply, additional characterization of the mutagenicity/genotoxicity of the ingredients using *in vivo* rodent bioassays, or *in vitro* mammalian cell based assays were not considered necessary.

IV.E. Nutritional Considerations

1. Nutritional Value

Dietary protein obtained through consumption of potatoes is of high nutritional value, and its utilizable protein as a percentage of its energy content is as high as that of wheat (FAO, 1990). As shown in Table IV.E-1 potato protein is a balanced source of essential amino acids, and the potato nitrogen content is remarkably close to animal protein. This contrasts to common vegetable sources of protein (corn/soybean/wheat) that are deficient in lysine and the sulfur containing amino acids. Potato protein therefore represents a balanced amino acid option for vegetarians. Isolated potato proteins have a similar amino acid composition, and as a percentage of total nitrogen, essential amino acids are typically present in larger quantities in potato protein isolates than in fresh potatoes. This is caused by the relative abundance of aspartic and glutamic acids, and lower content of essential amino acids in the non-protein nitrogen (free amino acids and peptides), which are not retained during chromatographic isolation.

Table IV.E-1 Typical Amino Acid Composition of Potato Proteins

Amino acid	Content (mg/g crude protein)							
	Potato Fresh[1]	Potato Coagulated[2]	HMW	LMW	Egg[3]	Beef[4]	Soy[5]	Wheat[6]
Isoleucine	39	49	39	45	55	43	49	33
Leucine	61	92	97	88	91	80	81	77
Lysine	56	76	67	65	77	84	67	24
Methionine (Met)	18	28	25	13	33	23	14	17
Cysteine (Cys)	18	13	7	23	24	10	16	58
Phenylalanine (Phe)	42	62	55	59	58	38	52	35
Tyrosine (Tyr)	36	54	55	52	43	31	38	26
Threonine	43	52	66	44	48	42	43	12
Tryptophan	12	14	10	14	17	12	50	37
Valine	52	66	44	65	69	49	27	24
Histidine	21	23	18	14	26	32	49	33

[1] Average of 58 references (Woolfe, 1987)
[2] Data based on Lichtenbelt (2001), Nestares *et al.* (1993) and Šimová *et al.* (1982)
[3] Egg, whole, raw – food code 125 (CNF, 2010)
[4] Beef, ground, raw – food code 2786 (CNF, 2010)
[5] Soy meal, defatted, raw – food code 3326 (CNF, 2010)
[6] Grains, wheat flour, white, all purpose, bleached – food code 4501 (CNF, 2010)

The total content of nitrogenous substances can be divided in protein nitrogen and non-protein nitrogen. Protein nitrogen is usually defined as those nitrogenous substances that may be coagulated by heat treatment. All remaining nitrogen containing products are non-protein nitrogen, mainly consisting of free amino acids and low molecular weight peptides. The ratio of protein to non-protein nitrogen is usually in the order of 1:1, however, this ratio can vary between cultivars within the species (Woolfe, 1987). The amino acid composition of the globular protein is genetically determined and the influence of environmental factors on protein composition is therefore limited. Data on the essential amino acid composition of varieties grown in various parts of the world show a variation of 10 to 15% in the content of essential amino acids in total nitrogen (Woolfe, 1987).

Limited studies have evaluated the nutritional quality of protein derived from potatoes using human subjects. Kofrányi *et al*. (1970) are frequently cited as having determined that potato protein is better suited to maintain the nitrogen balance of a human being than the protein of beef, tuna, wheat flour, soybean, rice, corn, or beans. The human requirement of potato nitrogen for this purpose would be 0.545 g/kg body weight. As reviewed by Woolfe (1987), humans have been maintained in good health by supplying the major part (>95%) of the nitrogen in their diet as potato nitrogen at a rate of approximately 0.52 g/kg body weight.

A limitation in the assessment of the nutritional value of a given protein from its amino acid composition is the fact that the efficiency of digestion of the protein and uptake of the fragments produced by digestion has a large influence on the amount and ratio of essential amino acids actually supplied to the body by the protein. This concern may be particularly relevant to Solanic's minimally processed potato proteins, which contain relatively large quantities of protease inhibitors that are known to inhibit digestive enzymes.

1.1 Nutritional Value of Processed Potato Protein in Animals

For assessing the digestibility of potato proteins, a number of digestibility values or tests are in use to rate digestibility, including protein efficiency ratio (PER), net protein utilization (NPU), or biological value (BV).

The matter of digestibility may be incorporated by determination of the PER value, which is the amount of weight gain per gram of protein consumed using rats from a single strain, fed isonitrogenous diets of the protein to be examined or casein for 28 days. The PER of casein is commonly set to 2.5 and is used as a reference value.

Experimental PER values reported in the literature for potato (total nitrogen or isolated processed protein) range from 1.8 to 2.3, and were reported to be comparable to the PERs derived for casein (Woolfe, 1987; Nestares *et al.*, 1993). The PER also can be computed from the essential amino acid composition, which produces a PER of 2.3 (Jewell *et al.*, 1980). Both

the experimentally derived PER and calculated PER values demonstrate that digestibility of processed potato protein in rats is good.

The nutritional value of a protein can also be reported as NPU, and/or BV. This is defined as the amount of nitrogen absorbed as a percentage of that consumed (in the case of biological value: intake corrected for fecal nitrogen loss). Nestares *et al.* (1993) reported an NPU of potato protein of 74% (BV 80%), compared to casein 82% and wheat gluten 44%. Šimová *et al.* (1982) reported NPU values for processed potato protein concentrates in the range of 73 to 77% (BV 79 to 83%). Overall, the evidence indicates that processed potato protein isolates would be readily digestible and comparable to whole potatoes.

When looking at the digestibility figures of processed potato protein isolates and after comparison them with digestibility figures of proteins from other sources, it may, be concluded that potato protein is an excellent source of essential amino acids.

There are no data available to date on the digestibility of amino acids and biological value of isolated and minimally processed potato proteins. Digestibility studies conducted with Solanic's coagulated potato protein have been conducted and are described below. The coagulated potato protein used in this study is a heat coagulated potato protein with low TGA content and is currently marketed for use in animal feed. In Table IV.E-2, the nutritional values as found in studies with rats are outlined.

Table IV.E-2 Nutritional Values of Potato Protein in Rat Studies (%)

	Nestares *et al.* (1993)		Boisen (1999)	
	Mean	**SD**	**Mean**	**SD**
Apparent digestibility	87.1	0.7	ND	ND
True digestibility	93.3	0.6	97.3	1.8
Biological value	79.5	1.1	81.5	2.7
Net protein utilization	74.2	1.1	79.3	3.3

ND = not determined; SD = standard deviation

The data reported by Nestares *et al.* (1993) were obtained from using common (French) potato protein, whereas Boisen used Protastar (an AVEBE coagulated potato protein product). Data show that the nutritional value of Protastar is somewhat higher in rats than common potato protein (Boisen, 1999).

Ileal amino acid digestibility studies with coagulated potato protein were carried out with young piglets (Beelen and Tolman, 1996), growing-finishing pigs (Beelen *et al.*, 1999) and veal calves (Verdonk *et al.*, 1999). Ileal amino acid digestibility of potato protein reported by these authors (Table IV.E-3) show that coagulated potato protein has a high amino acid digestibility.

Table IV.E-3 Apparent Ileal Amino Acid Digestibility of Potato Protein (%)

Item	Beelen and Tolman (1996) (young piglets)	Beelen *et al.* (1999) (growing finishing pigs)	Verdonk *et al.* (1999) (veal calves)
Crude protein (Nx6.25)	79	89	79
Isoleucine	86	93	89
Leucine	89	94	90
Lysine	86	93	88
Methionine	89	91	90
Cysteine	56	72	57
Phenylalanine	87	94	80
Tyrosine	87	95	89
Threonine	83	91	82
Tryptophan	77	87	77
Valine	86	93	86
Arginine	89	97	88
Histidine	86	94	80
Alanine	82	90	84
Aspartic acid	77	91	77
Glutamic acid	86	92	79
Glycine	76	90	78
Proline	83	97	83
Serine	82	91	82
Sum amino acids	84	92	83

1.2 Nutritional Value of Un-Processed Potato Protein

The majority of studies summarized in Section 1.1 above evaluated the digestibility of potato proteins that have been denatured, resulting in significant inactivation of the protease inhibitor fraction of the protein, which comprises approximately 50% of the total protein present within the vegetable. Several proposed applications of Solanic's LMW potato isolates are not subject to food processing conditions that would result in significant denaturing of the protein; therefore, these food applications are expected to result in dietary consumption of active potato proteases. Studies conducted in pigs administered feed supplemented with various raw potato formats have reported that the nitrogen content of raw potatoes is not effectively utilized (Whittemore *et al*., 1975; Livingstone *et al.*, 1979). Whether these effects are due to the inhibitory effects of potato proteases on digestive enzymes, or secondary matrix effects related to the non-digestibility of raw potato starch is unclear. The apparent amino acid ileal digestibility of minimally processed potato protein was evaluated by Jin *et al.* (2008). In this experiment weanling finishing pigs were administered potato proteins extracted from *S. tuberosum* L. cv. Gogu valley potatoes. The potato proteins were extracted from the potatoes using mechanical crushing, followed by the use of a protein extraction buffer, centrifugation and spray-drying. Although this extraction process is expected to largely retain the protease activity of the protein isolates, the use of β-mercaptoethanol (0.1%) may have partially denatured the proteases,

which are known to be rich in disulfide bonds. The potato proteins were administered to the diets of 280 Landrace x Yorkshire x Duroc weanling pigs (6.42 ± 0.74 kg; 23 ± 3 days of age), at concentrations of 0, 0.25, 0.5, or 0.75% for a period of 28 days. No significant differences in growth or feed conversion efficiency were observed between pigs randomized to either the potato protein groups or the potato-protein free control; similarly no statistically significant differences in apparent ileal digestibility were observed between these groups. The authors concluded that the potato protein containing diets "…improved the performance of weanling pigs."

The effect of un-processed potato protein and potato fiber on nutritional, physiological, and biochemical parameters in young pigs was recently evaluated by Tuśnio *et al*. (2011). The authors performed 2 experiments using young barrows (15 kg; synthetic line 990). In the first experiment, pigs (n=6 per group) were randomized to 1 of 3 groups shown below in Table IV.E-4. Animals were surgically fitted with post-valvular T-caecum cannula and housed in metabolic cages and administered their respective diets for 7 days. Digesta were collected during the last 3 days for measurement of apparent ileal digestibility of the diets. Experiment 2 was conducted using 4 groups of 6 pigs administered 1 of 4 test diets for a period of 21 days. Composition of the test diets is shown in Table IV.E-5. At the end of the experiment the animals were euthanized and the digestive tracts of the animals were dissected. Stomach, small intestine, liver and pancreas were weighed and samples of duodenum, mid jejunum and ileum were taken and preserved for histological examination.

Table IV.E-4 Composition of Diets, Experiment 1

Ingredient	**1**	**2**	**3**
Casein (g/kg)	225.0	112.5	112.5
Potato Protein Concentrate (g/kg)	-	123.5	123.5
Calculated Analyses			
ME (MJ/kg)	15.7	15.5	15.5
Crude Protein (g/kg diet)	177.5	177.5	180.0
Glycoalkaloids (mg/kg diet)	-	83.6	83.6
Trypsin Inhibitor Activity (mgTI/g diet)	-	0.48	0.58

mgTI = mg trypsin inhibited

Table IV.E-5 Composition of Diets, Experiment 2

Ingredient	Casein		Potato Protein Concentrate	
	Cellulose	Potato Fiber	Cellulose	Potato Fiber
Casein (g/kg diet)	430.0	430.0	430.0	430.0
Potato Protein Concentrate (g/kg diet)	127.5	127.5	-	-
Cellulose (g/kg diet)	-	-	175.0	175.0
Potato Fiber	60.0	-	60.0	-
Calculated Analyses				
ME (MJ/kg diet)	13.8	13.8	13.8	13.8
Crude Protein (mg/kg diet)	186.0	191.0	220.0	215.0
Glycoalkaloids (mg/kg diet)	-	-	118.5	118.5
Trypsin Inhibitor Activity(mgTI/g diet)	-	-	0.68	0.78

mgTI = mg trypsin inhibited

Based on the experimental findings, the authors reported that apparent ileal digestibility was significantly ($P<0.05$) reduced in the animals administered the potato protein diets relative to the animals consuming the casein controls. However, as shown in experiment 2, these apparent reductions in protein digestibility were not nutritionally significant. Consumption of potato protein diets did not adversely affect overall growth, body weight gain, or food to growth ratio. Potato protein did not affect the relative weight of the stomach and jejunum. Various effects on the morphometry of the small intestine were reported by the authors; however, "*these effects were inconsistent and differed among the segments*", and the authors concluded that "*The absence of apparent negative impact of PPC (potato protein concentrate) fed with CEL (cellulose) shows that solanidine glycoalkaloids at the dietary level of 118.5 mg/kg do not damage the structure of intestinal epithelium in pigs.*"

2. Digestive Protease Inhibition (Trypsin Inhibitors)

2.1 Animal Studies

Solanic's LMW isolate is expected to contain a heterogeneous mixture of potato proteins groups with protease inhibitor activity against the major digestive proteases secreted by the pancreas. The majority of the protease inhibitor activity of the LMW isolate is attributed to the serine protease PI-2. This protease inhibits the digestive enzymes trypsin and chymotrypsin *via* a mechanism that involves the formation of an irreversible protein complex that results in the inactivation of the protease *via* hydrolysis of a peptide bond within the inhibitor. The inhibitor is not released, however, because the fragments are held together by disulfide bridges on either side of the cleavage site. The reaction of mammals to the ingestion of serine protease inhibitors, also commonly termed trypsin inhibitors, is an increase in the production of digestive proteases to compensate for the loss of proteases to the protease-inhibitor complexes (Lee *et al.*, 1985). Protease inhibitors have been considered to be anti-nutrient factors due to observations in animals administered large dietary concentrations of proteases in the diet

(consumption of raw soybeans or potatoes), where impaired protein utilization, and adverse pancreatic effects have been reported in multiple animal species. An overview of the effects of consumption of protease inhibitors in animals is presented herein, and available data from human studies evaluating the consumption of potato proteins is discussed in Section 2.2., below. A discussion of species difference in the regulation of pancreatic function and associated differences in the pancreatic response of animals to dietary trypsin inhibitors also is presented (Section 2.3).

In mice, rats, and chickens, chronic consumption of diets supplemented with functional protease inhibitors (*e.g*., protein isolates from soybeans and potatoes), results in pancreatic hypertrophy (Bajko *et al.*, 1979, 1980; Pearce *et al.*, 1983; Hathcock, 1991; Grant *et al.*, 1993). During chronic consumption, pancreatic hypertrophy progresses to adenomas, tumors formation and a significant increase in mortality in rodents (Gumbmann *et al.*, 1989; Hathcock, 1991; Grant *et al.*, 1993).

The adverse effect of protease inhibitors in rodents may require protease inhibition of both trypsin and chymostrypsin as the chronic administration of a soybean bowman-birk inhibitor concentrate[6], did not result in pathological effects in any organ, nor were adverse effects on pancreatic weights reported in multiple animal species (Kennedy *et al*., 1993).

When incorporated as the predominant protein source in the diet, dietary proteases present in raw soybean or potato preparations also result in impaired nitrogen utilization rates, increased loss of dietary nitrogen in the urine and feces, and a corresponding diminished growth in the animals (Whittemore *et al.*, 1973, 1975; Livingstone *et al.*, 1979; Gumbmann *et al.*, 1989).

Direct comparisons of the effects of dietary proteases derived from soybean and potato have been evaluated by multiple authors (Lee *et al.*, 1985; Gumbmann *et al.,* 1989). Gumbmann *et al.* (1989) evaluated the pancreatic response of soy and potato protein protease inhibitors in rats and mice during short-term (28) and long-term (95 weeks) studies. During the short term study, the authors administered soybean or potato protein trypsin inhibitors in the diets of Sprague-Dawley rats at dietary concentrations of 0, 100, 200, or 400 mg TI/100 g. Trypsin inhibitor activity quantitation methods used by the authors were not described in the study. Effects of the diets on pancreatic weight are shown in Table IV.E-6. Statistically significant increases in pancreatic weights were observed for all treatment groups in the potato protein group.

[6]Although Soybean Bowman-Birk inhibitors are potent inhibitors of chymotrypsin, they are less effective inhibitors of trypsin

Table IV.E-6 Effect of Feeding Diets Containing Soybean and Potato TI Concentrates for 28-Days on Pancreatic Weights in Sprague-Dawley Rats

TI in Diet	Protein from Casein	Protein Efficiency Ratio	Pancreatic Weights
Soybean TI Concentrate			
100	9.8	3.29^{abc}	0.40^{cd}
200	9.5	3.30^{abc}	0.48^{b}
400	9.1	3.13^{bc}	0.53^{b}
Potato TI Concentrate			
100	9.7	3.06^{c}	$0.47^{b,c}$
200	9.3	2.62^{d}	0.49^{b}
400	8.7	2.40^{d}	0.63^{q}
0	10.0	3.42^{a}	0.366^{d}
0	8.7	3.33^{ab}	0.37^{d}

Means not sharing a common superscript letter are significantly different (P<0.05); Data adapted from Gumbmann *et al*., 1989

The authors observed that mice were less sensitive to the effects of TI in the diet, as the administration of soybean or potato protein isolates to the diets of weanling male Swiss Webster outbred mice at a dose of 200 mg TI/100 g diet did not have adverse effects on protein had marginal effects on pancreatic weight that were only statistically significant in the soybean group, which was increased by 15% (P<0.05) relative to controls. No effects on protein efficiency ratio or weight gain were observed in the mice fed the potato protein TI concentrates (Table IV.E-7).

Table IV.E-7 Protein Efficiency Ratio, Body Weight Gain and Pancreatic Weight in Swiss-Webster Mice Fed Soybean or Potato Protein TI Concentrates for 28 Days

Treatment	Protein Efficiency Ratio	Weight Gain (g)	Pancreatic Weight (% body weight)
Soybean	0.15^{b}	4.0^{b}	0.86^{a}
Potato	0.24^{a}	6.5^{a}	0.81^{ab}
None	0.24^{a}	4.9^{a}	0.75^{b}

Diets contained 20% protein from casein. Means not sharing a common superscript letter are significantly different (P<0.05%). Dietary TI was 200 mg TI/100 g diet. Adapted from Gumbmann *et al*. (1989).

The authors also reported that dietary protein was capable of offsetting the adverse effects of TI on PER and weight gain. Short-term administration of soybean TI concentrates at dietary concentrations of between 200 to 800 mg TI/100 g protein were reported to be severely growth depressing in rats when total dietary protein from casein was limited to 10%; however, when casein concentrations were increased to 20%, no adverse effects on protein efficiency or growth were noted at any of the TI dose levels. However, dietary casein was unable to offset the increase in pancreatic weights which was observed in all treatment groups. Long-term administration of soybean or potato protein TI concentrates was evaluated by the authors using 21-day-old male outbred albino Wistar rats and 21-day-old $B6C3F_1$ CrlBR hybrid mice

administered protease inhibitors in the dietary concentrations of 0, 100, or 200 mg TI/100 g diet for 95 weeks. Adaptation of the pancreas to the diets was observed in this study (Table IV.E-8). Slight yet statistically significant increases in pancreatic weight was only observed in the potato protein TI group at week 95 in the animals administered TI concentrates at the highest dietary concentration (200 mg TI/100 g diet). Although increases in nodular hyperplasia were noted in all the TI groups, statistical significance was only achieved in the animals randomized to the high-dose TI diets. Unlike rats, mice were able to fully adapt to the TI diets, with no adverse pancreatic effects (weight or histopathology) noted in these animals.

Table IV.E-8 Pancreatic Weights in Sprague-Dawley Rats and Swiss Webster Mice After Long-Term Feeding of TI Sources

TI dietary Conc. (mg TI/100 g diet)	Soybean TI Concentrate	Potato TI Concentrate	Control
Rats week 52			
0	-	-	0.27[b]
100	0.29[ab]	0.32[ab]	-
200	0.31[ab]	0.33[ab]	-
Rats week 95			
0			0.32[c]
100	0.36[abc]	0.33[c]	
200	0.39[ab]	0.39[ab]	
Mice week 95			
0			1.04[abc]
100	1.03[abc]	0.99[bc]	
200	1.14[a]	0.97[c]	

Diets contained 20% protein from casein. Means not sharing a common superscript letter are significantly different (P<0.05%). Adapted from Gumbmann *et al*. (1989)

A number of conclusions can be drawn from the studies by Gumbmann and colleagues (1989). First, the adverse effects of TI on pancreatic protein efficiency ratio and growth are determined by the dietary protein concentration, and adverse effects of proteases at these dietary concentrations could be completely offset by adequate concentrations of dietary protein. Secondly, although modest adaptation to dietary proteases can occur in rodents, particularly mice, the adverse pancreatic effects in rats are not ameliorated by dietary protein, and an apparent dietary threshold for development of nodular hyperplasia and adenoma in rats is 200 mg/100 g diet. And finally, the authors concluded that "*potato TI (trypsin inhibitor) produced changes in the pancreas of the rat that could not be distinguished from those associated with soy TI*" Based on this conclusion, observations obtained from studies evaluating effects of soybean trypsin inhibitors on animals were considered applicable to potato proteases.

Similar observations were reported by Lee *et al*. (1985), who evaluated the comparative effects of protease inhibitor enriched potato protein concentration and soy flour using male weanling

Wistar rats (40 to 50 g). The rats were administered casein based diets supplemented with potato protein concentrate at a level providing trypsin and chymotrypsin inhibitor activities comparable to that present in diets supplemented with protein derived from raw soy flour. Contents of the diets are shown below in Table IV.E-9.

Table IV.E-9 Composition of Diets (%) Containing Raw or Heated Protein Concentrate or Soy Flour

Ingredient	Potato Protein		Soy Flour	
	Raw	Heated	Raw	Heated
Casein	8.6	8.6	0	0
Potato Protein	2.7	2.7	0	0
Soy Flour	0	0	19.1	19.1
DL-Methionine	0	0	0.35	0.35
Protein Content[1]	10.2	10.2	10.3	10.3
TIx10^3 U/100 g diet	4230	419	4221	401
CI x10^3U/100 g diet	500	76	497	76

Adapted from Lee *et al*. (1985)
TI = Trypsin inhibition (U/100 g diet); CI = Chymotrypsin inhibition
[1] Based on N content of mixed diet (Nx6.25)

Rats (n=8 per groups) were administered their respective diets for 5 weeks and the following measurements were obtained: Growth, protein efficiency ratio, pancreas weight, protein digestibility, peptide test for measurement of pancreatic function and enzyme activities of trypsin, chymotrypsin, amylase, and lipase in the pancreas and small intestine. The effects of effects of the experimental diets on these parameters are shown below in Table IV.E-10. The authors reported that potato protein concentrate "*was found to produce the same general pattern of effects as raw soy flour, namely, an inhibition of growth, a decrease in PER and protein digestibility, an increase in the size of the pancreas, an increase in the recovery of p-aminobenzoic acid in the peptide test, and a depletion of enzyme in the pancreas with concomitant increased in the intestine (except for trypsin)*"

Table IV.E-10 Effect of Raw and Heated Potato Protein Concentrate and Soy Flour on Growth, Protein Digestibility, and Pancreas Size and Secretory Activity in Male Weanling Wistar Rats

Ingredient	Potato Protein		Soy Flour	
	Raw	Heated	Raw	Heated
Gain in Weight	103±15[c]	146±16[a]	72±12[c]	119±3[b]
Food Consumption, g	369±27[b]	419±27[a]	316±43[c]	425±28[a]
PER	2.16±0.23b	2.71±0.17a	2.21±0.18b	2.74±0.16a
Protein Digestibility[1] (Apparent %)	76.7±2.9[b]	84.5±1.6[a]	63.4±4.7[c]	76.7±3.1[b]
Size of Pancreas (% of body weight)	0.47±0.03[a]	0.34±0.04[b]	0.450.04[a]	0.35±0.04[b]
PABA recovery	41.5±7.0[a]	30.5±7.3[b]	42.1±7.9[a]	30.6±9.7[b]

Adapted from Lee *et al.* (1985)
[a-c] Mean values (± S.E) of 8 animals during an experimental period of 5 weeks. Mean values with unlike superscripts in the same row were significantly (P<0.05) different
[1] Protein digestibility (apparent) = N intake – N feces/Nitrogen intake X 100

2.1.1 USDA Studies Conducted in Pigs Administered Soybean Proteases

In contrast to the effects of dietary protease inhibitors in rodents, adverse effects on growth and pancreatic hypertrophy have not been reported following dietary studies in which high concentrations of dietary trypsin inhibitors were administered to pigs and monkeys on a repeat basis (Struthers *et al*., 1983; Harwood *et al*., 1986; Garthoff *et al*., 2002a).

The most recent and comprehensive studies investigating the nutritional effects of trypsin inhibitor were conducted by the USDA using swine, an animal model chosen for its close similarity to the human digestive system (Garthoff *et al.*, 2002a,b). Dietary soy TI extracted from raw soy flour was administered in the diet to 43 male neonatal (from post-natal day 3) miniature swine (600 to 950 g) for 39 weeks (Garthoff *et al.*, 2002a,b). This work was reported as 2 separate publications: The first study describing details of the study design, results of body weight, feed consumption and animal behavior; the second report detailing results of organ weight, gross and histopathological analyses, detailed biochemical investigations of the pancreas, and clinical chemistry, hematology, plasma cholecystokinin, and serum amylase activity (Garthoff *et al.*, 2002b). Details of the study are described below.

The animals were divided into 3 groups: sow control group (SC) (n=6), Autosow control group (ASC) (n=18), and Autosow TI (ASTI) group (n=19). Animals fed liquid diets by the Autosow were administered the food in 24 equal portions over the course of a day to mimic a typical suckling pattern. After acclimatization to the Autosow feeder (end of week 1), TI was added to the liquid diet of the ASTI group for weeks 1 to 6, providing a TI activity of 511 mg/100 g dietary dry matter. From weeks 6 to 39, ASC and ASTI animals were fed a liquid diet containing TI (506 mg TI/100 g dietary dry matter) or control article, immediately followed by standard chow based on body weight. The SC animals were fed standard chow ad libitum from week 6 and on.

The TI activity was analyzed for the ASC and ASTI groups and determined to be 0.022 and 0.92 mg TI/g sample, while the TI activity of the sow colostrum and milk was not determined. The presence of the low TI activity in the control liquid and standard USDA chow diets was reported to be likely related to the non-fat dry milk, white grease, casein and whey in the liquid diet, and soy meal and ground corn in chow diet, all of which have minimal TI activity. Blood samples monitoring effects of the diets on clinical chemistry were drawn at weeks 6, 18, 30, and 39.

From birth to 6 weeks of age, both ASC and ASTI groups grew exponentially, while the SC group grew at a linear rate. At week 1, the SC group was heavier than the other 2 groups due to slower food intake of pigs acclimatizing to Autosow feeder on days 3 to 7; however, by week 6, SC and ASTI animals had lower body weights than the ASC group ($P < 0.002$ for ASC *vs*. ASTI). Between weeks 2 and 6, the ASTI had 20% lower body weight gains ($p < 0.05$) than the ASC group however; the gains were equal by week 6. Feed intake increased exponentially in both Autosow groups, however, the ASTI group had 86 to 96% the feed intake of the ASC group ($P = 0.001$). The feed to weight gain ratio increased steadily in both groups and ranged from 0.74 to 1.08 kg dry matter intake/kg body weight gain in the ASC group, compared to 0.91 to 1.28 kg dry matter intake/kg body weight gain in the ASTI group. Although no statistical analysis was conducted on the feed efficiency between groups for this time period, the ASC diet appeared more efficient than the ASTI diet at each time-point evaluated (weekly). Although animals in both groups were active and ate well, 3 of 18 ASC piglets had loose stools compared to 14 out of 19 piglets in the ASTI group ($P=0.0009$) during the first 6 weeks of feeding.

From weeks 6 to approximately 19, the SC group continued linear rate of growth (approximately 500 g/week), while both Autosow groups had a large decrease in weight gain during week 7 (from 1,081 g during week 6 to 218 g in week 7 in the ASC group and from 1,157 g in week 6 to 118 g during week 7). Growth rate returned to pre-week 7 rates during week 8. Minor significant differences in body weights during weeks 7 to 9 were reported, however, no other significant effects were observed for the remainder of the study. Consumption of food was complete and consistent from weeks 8 to 22 in all 3 groups, with a slight increase in feed refusal in all 3 groups in the final 17 weeks of the study (no comparisons were conducted). Aflatoxin was detected at a level of 40.5 and 16.4 µg/kg in 2 batches of standard chow used in feeding between weeks 6 and 14; however, the authors reported that no biological consequences were observed. No evidence of infectious diseases or intestinal pathology was observed over the course of the study.

Statistically significant differences in blood chemistry parameters were observed in swine at 6 weeks of age. At this stage, cholesterol, total protein, globulin, albumin/globulin ratios, and creatinine were all significantly decreased in both Autosow groups compared to the SC group; while glucose was significantly higher. These parameters did not significantly differ between groups by the end of the study. Blood urea nitrogen (BUN), alkaline phosphatase (AP) and

serum-glutamic-oxalacetic transaminase were significantly elevated in Autosow groups at week 6 compared to the SC group. At week 39, BUN was still significantly elevated compared to SC, while AP became significantly decreased in both Autosow groups at weeks 18 and 30, remaining lower but not significantly so at week 39, compared to SC. No statistically significant differences were observed in white or red blood cell parameters and no statistically significant differences in the above mentioned parameters were observed between Autosow groups.

Relative liver weights were significantly increased in ASTI animals compared to the ASC group ($P < 0.05$) at 6 weeks of age (at 39-weeks of age, liver weights were elevated but not significantly). No significant effects on the weights of the pancreas, brain, thyroid, spleen, pituitary, kidneys, testis or lungs were observed. Total pancreas protein and pancreas amylase activity were significantly elevated in ASTI group *vs*. ASC group at 6 weeks ($P < 0.05$) but not at week 39. Plasma cholecystokinin (CCK) levels were not significantly different between any of the groups at any time-point of the study, however all 3 groups had a highly significant increase in CCK from week 6 to week 18 ($P = 0.0001$), followed by a significant decrease from weeks 18 to 30 ($P < 0.03$). No statistically significant differences in pancreas trypsin or chymotrypsin activity or pancreatic ornithine decarboxylase activity, RNA, DNA or extracted protein levels were observed between any of the groups. Necropsy revealed that all organ histopathology was within normal limits. No evidence for pancreatic increased mitotic activity, hyperplasia or hypertrophy was observed; however, extramedullary hematopoiesis of the spleen was observed with higher incidence in the ASTI group compared to ASC group at 6 weeks of age. Pancreatic acinar cell nuclear density was elevated but not significantly at week 39 in ASTI animals *vs*. control groups. Based on the results of their study and those of others, the authors concluded that “Overall, these results are consistent with the negative neoplastic effect of TI observed in the pancreas of all species tested except the rat…and provides added confidence that humans are not at increased risk for pancreatic neoplasia from foods containing natural TI activity.”

2.2 Human Studies

Potato proteinase inhibitor extracts are marketed in the United States as dietary supplements. Human studies evaluating the effects of dietary potato protease inhibitors on various parameters related to energy intakes in healthy subjects, as well as regulation of gastric emptying and maintenance of glucose and insulin status in individuals with Type 2 diabetes, have been reported (Hill *et al*., 1990; Schwartz *et al*., 1994; Spreadbury *et al*., 2003). In one study, participants were provided 1.5 g of proteinase inhibitors from potato (no further details about the extract were provided) in a high-protein soup, followed by a lunchtime test meal (Hill *et al*., 1990). In comparison to the control group, consumption of potato protein prior to the test meal was associated with a reduction in energy intake, which was postulated to be related to the effects of proteinase inhibitors on CCK release. Two further studies were identified which examined the potential effects of proteinase inhibitor from potatoes on parameters related to glucose control in healthy (Spreadbury *et al*., 2003) and diabetic subjects (Schwartz *et al*., 1994). In the study conducted by Schwartz *et al*. (1994) study participants with Type 2 diabetes

were provided a combination glucose-protein solution with or without 1.5 g of proteinase inhibitors. The proteinase inhibitor test article was obtained as an extract from raw potatoes, and comprised 70% proteinase inhibitors and 30% carbohydrate. Following consumption of the liquid proteinase inhibitor meal, significant reductions in serum insulin and plasma glucose levels, and plasma gastric inhibitory polypeptide value were observed. The rate of gastric emptying for 2 hours after the meal was decreased relative to rates observed following consumption of the same meal without added proteinase inhibitor. Postprandial plasma CCK levels were significantly increased for 15 minutes after ingesting the meal with proteinase inhibitor II.

Peters *et al.* (2011) evaluated the effect of potato protease inhibitor 2 (PI2) on appetite, food intake and plasma CCK levels in healthy humans. The study was conducted using three separate studies each using a two-way, placebo-controlled, balanced-order, cross-over design. Subjects (n=23 or 24 per study; mean age 41.3 years; BMI = 25 kg/m^2) were administered a minidrink containing 30 mg of PI2 or placebo 120 or 30 minutes before ad libitum lunch or 30 minutes before a fixed lunch. Endpoints measured during the study were self-reported satiety, ad libitum meal intake and plasma CCK and blood glucose. Confirmation of the protease inhibitor activity was conducted under simulated gut conditions. The authors reported that PI2 "*did not differ from control for any study parameters, in any substudy, despite confirmation of the inhibitory activity of PI2*".

No side-effects, including reports of adverse gastrointestinal effects, related to the consumption of the proteinase inhibitor II extract were reported in any of the studies (Hill *et al*., 1990; Schwartz *et al*., 1994; Peters *et al*., 2011).

2.3 Mechanism of Protease-Induced Pancreatic Hypertrophy and Relevance to Humans

Although the temporal sequence of events by which trypsin inhibition leads to pancreatic hypertrophy in rodents has not been definitively established, there is strong evidence to implicate that the effects occur *via* disruption of the negative feedback inhibition of trypsin on CCK production (Struthers *et al*., 1983; Sato *et al*., 2002; Komarnytsky *et al*., 2011; Nakajima *et al*., 2011). Cholecystokinin is an important gastrointestinal hormone with multiple down-stream effects on food digestion and absorption, and is the most important mediator of post-prandial production and secretion of pancreatic digestive enzymes (Wang and Cui, 2007). Cholecystokinin is secreted by I cells present throughout the intestinal mucosa, and stimulation of CCK secretion of I cells is mediated by degradation products of dietary protein (peptides, amino acids), and fat (fatty acids) (Wang and Cui, 2007). Several intestinal releasing factors [*e.g*., luminal CCK releasing factor (LCRF), diazepam-binding inhibitor, and monitor peptide] also have been identified, which function to stimulate CCK release by I cells. These releasing factors are sensitive to digestive proteases, and in rodents are constitutively expressed; thus, consumption of protease inhibitors results in increased plasma concentration of CCK. In

rodents, CCK secreted by I cells is transported to the systemic circulation and is transported to the pancreas directly stimulating CCK receptors (CCKR) on the pancreas resulting in pancreatic synthesis and release of digestive enzymes; chronic overstimulation of the pancreas by this hormone leads to hypertrophy and hyperplasia of the pancreatic acinar cells that is observed with chronic consumption of dietary protease inhibitors in these animals (Göke, 1990). Although comprehensive studies evaluating the temporal relationship between trypsin inhibitor induced CCK release and pancreatic hypertrophy in rodents are incomplete, species difference in the hormonal regulation of pancreatic function by CCK are evident. In contrast to findings in rodents where consumption of protease inhibitors is associated with large and statistically significant increases in plasma concentrations of CCK, studies conducted in pigs and humans have not substantiated that digestive proteases regulate CCK synthesis in a similar manner (Holm *et al.*, 1992; Reseland *et al.*, 1996; Garthoff *et al.,* 2002b). Similarly, clinical studies that have evaluated the effect of potato trypsin inhibitor consumption on plasma CCK levels in humans also demonstrate that dietary proteases do not influence circulating levels of CCK (Schwartz *et al.*, 1994; Peters *et al.*, 2011).

Further evidence that negative feedback regulation of pancreatic function by digestive proteases differs between humans and rodent are apparent from the observed differences in the *in vivo* responses of humans and rodents to camostate, a synthetic small molecule protease inhibitor. In rodents the administration of camostate in the diet at a dose 0.1% for a period of 1 week resulted in statistically significant increases in basal CCK concentrations (2-fold above baseline), marked increases in pancreatic protein synthesis and DNA content, and a corresponding 245% increase in pancreatic volume (Crozier *et al.*, 2008). Further studies by other investigators demonstrated that the pronounced growth of the exocrine pancreas in response to camostate was abolished by pharmacologic treatments with the CCKR antagonist L 364,718 (Göke, 1990). In humans, the consumption or intraduodenal administration of camostate at doses sufficient to abolish both trypsin and chymotrypsin activity have resulted in adaptive changes in pancreatic secretion activity, and a transient increase in pancreatic volume (Göke, 1990; Friess *et al.*, 1998); however the marked increases in basal concentrations of CCK observed in rodents administered camostate have not been reported in humans (Adler *et al.*, 1988a,b; Friess *et al.*, 1998). In addition, the administration of high affinity CCKR antagonist loxiglumide did not affect camostate induced pancreatic secretion (Göke, 1990). These findings indicate that unlike rodents, where digestive proteases play a major regulatory role in CCK induced pancreatic function, the regulation of pancreatic secretion in humans is mediated by multiple redundant pathways. For example, in rodents pancreatic function is regulated by both endocrine and neuroendocrine pathways, whereas the human pancreas does not contain functional CCKRs, and direct regulation of pancreatic acinar cell function *via* circulating CCK does not occur. In humans, CCK regulation of pancreatic function occurs *via* intestinal sensory nerve fibers within the lumen of the gastrointestinal tract, which excite both vagal cholinergic and enteropacreatic reflexes (Wang and Cui, 2007). Therefore, in humans, CCK regulation of pancreatic exocrine function is entirely under neural control, and CCKR activation only serves to

modulate pancreatic activity in this regard (Wang and Cui, 2007). Finally, species differences in the secretion of CCK releasing factors may further account for species differences in response to dietary trypsin inhibitors. In rodents, the CCK releasing factor LCRF appears to be constitutively expressed, and is controlled by feedback inhibition by digestive proteases. However, in the presence of dietary proteases, the negative feedback regulation of CCK releasing factors does not occur, resulting continual stimulation of CCK secretion. A similar feedback mechanism for regulation of LCRF does not been identified in humans; spontaneous release of LCRF into the intestinal lumen does not occur and secretion of LCRF must be stimulated by luminal amino acids and fatty acids (Wang and Cui, 2007).

Mechanistic studies in rodents and humans indicate that the feedback regulation of pancreatic function by dietary proteases differs between humans and rodents. Differences in the feedback control of CCK secretion and differences in the control of pancreatic function by CCK in humans and rodents account for the species differences that have been reported between rodents, pigs and human and non-human primates administered dietary trypsin inhibitors; these species differences further support the conclusion that the adverse effects of dietary trypsin inhibitors in rodents are not relevant to humans.

2.4 Carboxypeptidase Inhibition

In addition to proteases that inhibit trypsin and chymotrypsin, potato protein isolates, including Solanic's LMW isolate, contain inhibitors of the digestive exopeptidases carboxypeptidase A and B. The effects of diets containing potato derived proteases on the growth of newly hatched chicks was evaluated by Pearce and colleagues (1983). Approximately 100 newly born chicks were randomized to 1 of 6 groups administered a basal diet supplement (50% wt/wt) with control (glucose), autoclaved potato, raw potato, potato derived carboxypeptidase inhibitor, potato derived serine protease inhibitor, or soybean trypsin inhibitor. Serine protease and carboxypeptidase diets were administered at concentrations equivalent to that present in the raw potato diets. Diets were consumed by the animals for 10 days. As shown in Table IV.E-11 although slight increases in fecal protein were observed in the chick consuming carboxypeptidase inhibitors, the anti-nutrient effects of this diet were insufficient to affect weight gain or feed efficiency of the animals. Consumption of carboxypeptidases also was not associated with increases in pancreatic growth in these animals, and was in marked contrast to the pancreatic effects observed in the animals administered diets supplemented with raw potatoes, or trypsin inhibitor isolates.

Table IV.E-11 Summary of Effects of Various Diets Containing Protease Inhibitors on the Weight Gain, Feed Efficiency Fecal Protein, Mortality and Pancreas Growth in Newly Hatched Chicks

Diet	Weight gain (g/pen)	Feed efficiency	Fecal protein (mg/g)	Mortality (%)	Pancreas wt (g/100 g bw)
Control	675	1.46	65	0	0.501
Autoclaved potato	572	1.46	64	0	0.529
Raw potato	197*	4.82*	153*	20	0.829*
Carboxypeptidase inhibitor	595	1.55	80*	0	0.479
Potato protein trypsin inhibitor	494*	1.82*	135*	0	0.604*
Soybean trypsin inhibitor	514	1.80	135	0	0.653

*Significant difference (P<0.05) from control values. No statistical analyses conducted on soybean groups

3. Glycoalkaloids

Glycoalkaloids are a group of known toxins specific to the *Solanaceae* family of plants, including potatoes. The triglycoalkaloids (TGA) α-solanine and α-chaconine are the principal glycoalkaloids identified in potatoes, and since glycoalkaloids occur naturally in potatoes, exposure to these toxins *via* the normal background diet occurs in humans. Water soluble nortropane alkaloids, named calystegines, are known to be present in a number of plant species, including *Solanaceae*, specifically in eggplant *(S. melongena) an*d potatoes. Friedman *et al.* (2003) analyzed tubers of 8 potato varieties and found calystegine levels between 5 and 68 ppm in whole potato fresh weight (FW) (Friedman *et al*., 2003). However, the biological significance of this group of alkaloids in humans is not yet understood (OECD, 2002).

Glycoalkaloids are quite stable to heat at neutral pH, and are not destroyed during boiling of potatoes or during heat coagulation of potato protein in potato fruit juice.

Solanic has included a specification limit for TGA of not greater than 312 mg TGA/kg protein in both the LMW and HMW protein isolate ingredients. As reported in Table II.C-2, concentrations of total TGA in Solanic's potato protein isolates are typically below 300 mg/kg, and levels are further reduced for nutritive applications of the company's potato proteins using additional chromatography filtration methods to concentrations of below a detection limit of 100 ppm.

In the Novel Food decision (2002/150/EC) for AVEBE's coagulated potato proteins and hydrolysates thereof, it was decided that for *quantum satis* use of these ingredients, the level of TGA should not exceed 150 mg TGA/kg (w/w) product as such (EC, 2002).

Based on the *per capita* potato intakes reported by the USDA of approximately 147 g/day (Section IV.A.2), exposure to glycoalkaloids from potato consumption is estimated to be approximately 12 mg/day. The safety of the glycoalkaloids, α-solanine and α-chaconine, was considered by the Joint FAO/WHO Committee for Food Additives (JECFA) in 1992 (JECFA, 1993). Although the Committee could not establish a safe level of human consumption at the

time of the evaluation, it did conclude that consumption of potatoes, “frequently on a daily basis”, containing glycoalkaloids levels of 20 to 100 mg/kg, was of no safety concern.

Based on intake estimates for potato protein consumption from technical food uses as described in section IV.A, consumption of LMW potato protein by heavy consumers (90th percentile all-users) was estimated to be 5.4 and 3.4 g per person for male adults and children respectively. Corresponding consumption of TGA, assuming a conservative upper limit of 312 mg TGA/kg potato protein, would result in daily intakes of 1.68 and 1.06 mg for adults and children respectively. Among users of nutritive products containing Solanic’s potato protein isolates, high consumers (male adults) were estimated to consume up to 38.4 g of potato protein per day. Based on analytical data for the glycoalkaloid reduced LMW potato protein isolate (Table II.C-3), which contain below 100 ppm potato TGA’s, maximum exposure to TGA from nutritive food uses among heavy consumers would be potato proteins resulting in daily intakes of 3.8 mg TGA per person per day. These exposures are considered conservative estimates of exposure to TGA from introduction of Solanic’s potato protein isolates to the U.S. food supply under the proposed food uses, and would not appreciably alter total dietary intake of potato protein glycoalkaloids above background levels.

4. Lectins

Lectins, or hema-agglutinins, are carbohydrate-binding cell-agglutinatory proteins and they occur widely in both plants and animals. The potato tuber also contains a lectin, which agglutinates human, as well as a number of animal, erythrocytes (Woolfe, 1987; Safarikova and Safarik, 2000; Van Damme *et al*., 2004). Its function in the potato tuber is uncertain, and there is no knowledge about possible toxicity of this lectin. They are, however, heat labile and are not likely to survive the heat processing in the final applications. A comparison of lectins from a number of bean varieties revealed that their toxicity was highly dependent on the variety, but even the most toxic ones were easily deactivated by heat treatment (Liener, 1976). In view of the amount of potatoes eaten by man there is no evidence for any toxic effect of lectins in boiled potatoes. The lectin present in potatoes has a MW of approximately 100 kDa. Solanic analyzed overloaded gels of HMW and LMW isolated potato proteins and found no material at the 100 kDa band.

5. Allergenicity

5.1 Potato Allergens

Sol t1 (patatin) is a major storage protein of potato tubers and was the first identified potato allergen (Seppälä *et al.*, 1999, 2001; Tucke *et al.*, 1999; Astwood *et al.*, 2000; Majamaa *et al.*, 2001). In addition to patatin, four additional IgE-binding potato proteins with molecular masses ranging from 16 to 25 kDa were purified and identified by Seppälä *et al.* (2001). The proteins were identified as cathepsin D-, cysteine-, and aspartic protease inhibitors of potato and which belong to the family of soybean trypsin inhibitors (Kunitz type). This group of protease inhibitors

is relatively stable to digestive proteases and heating and shows binding with IgE from sera of atopic infants and children with suspected food allergy. Patatin, cathepsin D-, cysteine-, and aspartic protease inhibitors were designated PAT1_SOLTU, Sol t2, Sol t3.0101, Sol t3.0102, and Sol t4 and are included in the in the SwissProt databank.

Sol t1 is considered to be the most important potato allergen. Heat denaturation of Sol t1 occurs at approximately 55°C, and is not associated with a complete unfolding of the protein (Pots *et al.*, 1998).

Sol t2 is a glycoprotein identified as an allergen and is an aspartic protease inhibitor (cathepsin D protease inhibitor, PDI) (API11_SOLTU) with a MW of approximately 25 kDa (SwissProt). According to Seppälä *et al.* (2001) the MW is approximately 20 kDa and according to Pouvreau (2004) approximately 22 kDa. Differences in observed MW can be the result in using either reducing or non-reducing SDS media with Western blotting techniques.

The proteins Sol t3.0101 and Sol t3.0102 belong to the potato cysteine protease inhibitors (PCPI). However, they represent a distinct group of proteins (Gruden *et al.*, 1997), as they show no homology to any other known cysteine protease inhibitor super families, but belong to a Kunitz-type Soy Trypsin Inhibitor (STI) family. PCPIs are in general single chain proteins with MW ranging from 20 to 25 kDa. Because of amino-acid substitutions on their surface further classification in subgroups was needed (Strukelj *et al.*, 1992; Krizaj *et al.*, 1993). Seppälä *et al.* (2001) classified Sol t3.0102 as potato cysteine protease inhibitor CP8.3_SOLTU, later renamed as CPI1_SOLTU. Sol t3.0101 is also a PCPI referred to as CPI10_SOLTU or PCPI-10. The MW of Sol t3.0102 and Sol t3.0101 are approximately 24 and 21 kDa, respectively.

Sol t4 was characterized by Seppälä as an aspartate protease inhibitor of 20 kDa. It is composed of a 16 kDa and a 4 kDa polypeptides joined by a disulfide bond. The exact physiologic functions of these aspartic protease inhibitors in potato tuber is still unclear (Strukelj *et al.*, 1992). In the SwissProt databank it was renamed to a serine protease inhibitor (SP17_SOLTU).

A potato protein with a MW of 12 to 14 kDa described Ebner *et al.* (1995) showed a cross-reactivity with recombinant Bet v2 (birch pollen, profilin) which was demonstrated by using antibodies to Bet v2. This cross-reactivity was also demonstrated with Bet v2 of apple, pear, celery and carrot. According to Ebner *et al.* (1995), no cross-reactivity of potato extract with Bet v1 (the major birch pollen allergen) occurred. In the SwissProt, no potato protein is included in the profilin family, whereas they are documented in the Bet v1 family. The potato proteins PRS1_SOLTU and PRS2_SOLTU, 2 proteins of approximately 17 kDa that are induced by injury, belong to this family. Also in studies of Calkhoven *et al.* (1987) cross-reactivity between birch pollen and potato extract was documented by a protein with a MW of 18 kDa. Due to the relation between PRS1, PRS2 and Bet v1 it can be concluded that the by Calkhoven *et al.*

(1987) observed cross-reactivity was not caused by protease inhibitors, also because the allergens Sol t 2 to 4 consists of 1 unit with a MW of approximately 20 kDa.

Beezhold *et al.* (1996) demonstrated cross-reactivity of the potato stress proteins WIN1_SOLTU and WIN2_SOLTU with latex by immunoblot inhibition analysis. The MW of the WIN proteins is unclear, but according to the SwissProt MW for the unprocessed WIN1 and WIN2 precursors they will be approximately 19 and 20 kDa, respectively. Beezhold *et al.* (1996) showed a homology of the 14 kDa C domain of hevein pre-protein (Hev b6) with these potato stress proteins. The SwissProt notifies a similarity in the WIN domains and the chitin-binding type-1 domain between the potato proteins WIN1, WIN2 and hevein. Heveins are important allergens in latex allergy (Wagner and Breiteneder, 2002) and 4 of them [Hev b2 (major allergen), Hev b6.02 (major allergen), Hev b7 (minor allergen) and Hev b8 (intermediate allergen, profilin)] have been associated with the latex-fruit syndrome. Another important latex allergen involved in the latex-fruit-syndrome is Hev b8 (intermediate allergen, profilin).

5.2 Human Data

Epidemiology of food allergy is mainly focused on 9 major allergens (*i.e.,* dairy products, soy, egg whites, wheat, peanuts, tree nuts, citrus fruits, shellfish and food additives). Case-reports of potato allergy are uncommon. Only one reference was found in which the epidemiology of potato is described. Weisnagel found in a retrospective study that 0.17% of the children (one child) in his medical practice in the period of 1992 to 2001 showed signs of allergy to potato.

The sensitizing potential of potato proteins in humans or rodents is unknown, and it is unclear if patients that show allergic reactions upon consuming raw or cooked potatoes have been sensitized by potato-proteins or that potato proteins mainly cross-react in patients allergic to other offending allergens.

Several atopic allergic reactions have been described with raw and cooked potato proteins (Pearson, 1966; Nater and Zwartz, 1967; Castells *et al.*, 1986; Quirce *et al.*, 1989; Wahl *et al.*, 1990; Seppälä *et al.*, 1999). In most human studies with potatoes (or potato-proteins) as the offending food (raw or cooked), the allergic patients used are either described as atopic individuals or as patients reactive to other foods and fruits (Wagner and Breiteneder, 2002), pollen [birch pollen Bet v1 and Bet v2, (Ebner *et al.*, 1995)]; olive tree pollen, (Barral *et al.*, 2004; Palomares *et al.*, 2005) or natural rubber latex (Beezhold *et al.*, 1996; Seppälä *et al.*, 2000; Reche *et al.*, 2001; Schmidt *et al.*, 2002; Tavadia *et al.*, 2002; Wagner and Breiteneder, 2002; Arif *et al.*, 2004; Palomares *et al.*, 2005). De Swert *et al.* (2002) evaluated the allergenicity of cooked white potatoes was in atopic children, selected on the basis of suspicion of allergy to cooked potatoes, using SPT and IgE immunoblots. The authors concluded that allergy to cooked potatoes may be a cause of severe allergic disease, with immediate reactions and eczema in some atopic infants and young children. However, the clinical significance and molecular specificity of hypersensitivity reactions to raw and cooked potatoes is still unclear

(Majamaa *et al.,* 2001). Positive oral and skin challenge responses to both raw and cooked potato in food allergic atopic infants were reported by Majamaa *et al*. (2001). Binding of IgE antibodies to patatin and positive SPTs and IgE antibody responses to Sol t1 were reported, suggesting that cooked and raw potato can be an allergenic food for infants suffering from atopic dermatitis.

In a case report of Gomez Torrijos *et al.* (2001) immediate urticaria and angiodema were described in a 19-year-old man upon contact with raw potato. Specific IgE binding to potato and positive responses in a skin prick test were observed. Beausoleil *et al*., (2001) reported a suspected case of anaphylaxis from consumption of a raw potato by a 4-year-old boy (Beausoleil *et al.*, 2001). The individual rapidly developed urticaria, angiodema, respiratory distress, vomiting and diarrhea following suspected consumption of a raw potato used for painting in pre-school. Skin prick tests to raw potato were positive however, the individual was not response to oral challenge with cooked potato. Oral challenge with raw potato was not performed, and the identity of the putative potato protein allergen was not determined.

Studies conducted with atopic individuals have suggested that raw and cooked potatoes can produce clinical reactions with symptoms similar to oral allergy syndrome. However, there is an absence of information characterizing the sensitizing potential of potatoes in humans. Sensitization in allergic patients to potato proteins can in many cases not be demonstrated (RAST and SPT with fresh potato).

5.3 Cross-Reactivity of Potato Proteins in Humans

Cross-reactivity to potato proteins (mainly raw potato proteins) has been shown in patients allergic to various foods and fruits, birch and olive tree pollen and natural rubber latex. Allergic patients with a so called para-birch syndrome are sensitized to birch pollen (major allergens Bet v1 and Bet v2) by the respiratory route and experience allergic symptoms not only to birch pollen, but also after contact with or ingestion of several fresh fruits and vegetables like apple, pear, celery, carrot and potato. Upon peeling potatoes or ingestion of raw or cooked potatoes local reactions of the mucosa of the upper aero-digestive tract (itching, wheezing, rhino conjunctivitis, eye irritation, inflammation, angiodema) or of the skin (eczema, contact urticaria) and systemic clinical symptoms (asthma, urticaria) might be experienced.

In birch pollen-induced (Bet v1) allergy no cross reactivity was observed with potato proteins by Ebner *et al.* (1995). However, cross-reactivity was shown of a potato protein with a MW of 12-14 kDa with recombinant Bet v2 (birch pollen allergen, profilin). However, the potato pathogenesis-related proteins PRS1_SOLTU and PRS2_SOLTU (both Bet v1 analogues) are still discussed in respect to cross-reactivity to Bet v1.

Cross-reactivity of Sol t1 (patatin) is primarily described with the latex allergen Hev b7 in patients allergic to natural rubber latex (NRL) in association with the so called latex-fruit-

syndrome (Wagner and Breiteneder, 2002). Moreover, Beezhold *et al.* (1996) demonstrated cross-reactivity of the potato stress proteins WIN1 and WIN2 with latex. This is especially the case in adults and less in children (Seppälä *et al.*, 2000; Schmidt *et al.*, 2002). However, the degree of homology of approximately 40% between these 2 proteins is low (Sowka *et al.*, 1998). These authors present evidence for the absence of cross-reactivity between Hev b7 isoforms and patatin, which is in contrast to the observations made by Beezhold *et al.* (1996). Hev b7 is considered to be a minor NRL allergen (Wagner and Breiteneder, 2002; Cullinan *et al.*, 2003) and other latex proteins (Hev b2, Hev b6.02 and Hev b8) are expected to play a more prominent role in the latex-fruit-syndrome (Wagner and Breiteneder, 2002; Barral *et al.*, 2004). The 46 kDa protein Hev b7 reacting with IgE from latex allergic patients was found to have both N-terminal (60%) and internal sequence homology with patatin (storage protein in various plant families *e.g.*, potato and tomato). This Hev b7 allergen in latex appears to represent a Hevea braziliensis homologue of patatin, which was identified as a major cross reactive protein in latex-associated potato allergy, especially in latex adults and less in children (Seppälä *et al.*, 2000; Schmidt *et al.*, 2002).

In a study of Reche *et al.* (2001) cross reactivity to tomato, latex and potato was observed using patients with a history of adverse reactions to tomato and IgE-mediated allergies. In a more recent study of Arif *et al.* (2004) a 43 kDa latex glycoprotein was isolated and purified with an IgE epitope on the carbohydrate moiety of the protein that is similar to a carbohydrate component on potato tuber patatin and which enables the latter protein to inhibited IgE binding to the Hev b13 protein.

In summary it can be concluded that cross-reactivity exist for various potato proteins in patients allergic to various foods and fruits (apple, pear, celery, carrot), pollen (birch, olive) or natural rubber.

5.4 Process and Digestive Stability

Studies measuring the processing (heat) and digestive (pH and enzymes) stability of potato proteins are limited. Although conflicting results are presented in several papers, the suspected allergenic potato proteins are considered to be rather unstable to heat and digestion. For instance in several patient-studies it was shown that raw potatoes gave rise to allergic reactions, whereas cooked potatoes resulted in a partial or total loss of its allergenic properties (Pearson, 1966; Castells *et al.,* 1986). In other studies patatin (Sol t1) and potato protease inhibitors (Sol t2-4) are reported to be relatively stable to digestive proteases and heating (Seppälä *et al.*, 1999, 2001; Majamaa *et al.*, 2001). Koppelman *et al.* (2002) and Pots *et al.* (1998) showed that as long as Sol t1 is not aggregated, denaturation of patatin on a secondary or tertiary folding level is reversible with only minor effects on IgE affinity. In studies of Astwood *et al.* (1996) the stability of various major, minor and non-allergenic food proteins was determined in the standard pepsin digestion assay. Potato acid phosphatase was easily digested (within 15

seconds) in the *in vitro* pepsin digestion assay under the conditions described for simulated gastric fluid in contrast to various strong allergens like Ara h2 from peanut.

5.5 Allergenicity Summary

Potato is not one of the major 8 food allergens. Based on available information within the literature, as reviewed herein, potatoes do not appear to be a significant allergenic food, especially when taking into account the scale of potato consumption around the world. Several proteins present in the potato protein fractions HMW and LMW are expected to present an allergic risk among sensitive consumers. Processing (heat) and digestion of these proteins may result in a partial or total loss of their allergenic properties. The suspected potato protein allergens are identified as Sol t1-4, PRS1-2 and WIN1-2; these proteins have shown cross-reactivity in patients allergic to various foods/fruits, pollen and latex. Clinical symptoms are in most cases restricted to the less severe local reactions of the mucosa and skin. Little is known of the sensitizing potential of these potato proteins. Although low-level occurrences of cross-reactivity may occur in individuals sensitive to birch-pollen and latex, allergenic responses in these individuals are not life-threatening, and these individuals can readily avoid consumption of such offending foods. Taken together, the available information suggests that the proposed food uses of Solanic's potato proteins will not present a greater allergic potential to that currently occurring from existing food uses of potatoes in the diet.

IV.H Expert Panel Evaluation

Solanic, an AVEBE group company, has determined that potato protein isolates, as described herein, are GRAS for food and beverage products as described in Table I.D-1, on the basis of scientific procedures. This GRAS determination is based on data generally available in the public domain pertaining to the safety of potato protein isolates, and on consensus among a panel of experts (the Expert Panel) who are qualified by scientific training and experience to evaluate the safety of the potato protein isolates as a component of food. The Expert Panel consisted of the following qualified scientific experts: Prof. Joseph F. Borzelleca, Ph.D. (Virginia Commonwealth University School of Medicine), Prof. Paul B. Pencharz. M.B., Ch.B., Ph.D., FRCP(C) (The Hospital for Sick Children, University of Toronto), and Prof. Stephen L. Taylor, Ph.D. (University of Nebraska).

The Expert Panel, convened by the Solanic, independently and critically evaluated all data and information presented herein, and also concluded that potato protein isolates were GRAS for food uses as described in Table I.D-1., based on scientific procedures. A summary of data and information reviewed by the Expert Panel, and evaluation of such data as it pertains to the proposed GRAS uses of potato protein isolates is presented in Appendix A.

IV.I Conclusion

Based on the above data and information presented herein, Solanic has concluded that the intended food uses of potato protein isolates, as described in Table I.D-1, are GRAS based on scientific procedures. General recognition of Solanic's GRAS determination is supported by the unanimous consensus rendered by an independent Panel of Experts, qualified by experience and scientific training, to evaluate the use of potato protein isolates in food, who similarly concluded that the intended uses of potato protein isolates as described herein are GRAS.

Food uses of Solanic's potato protein isolates as described herein may therefore be marketed and sold for its intended purpose in the U.S. without the promulgation of a food additive regulation under Title 21, Section 170.3 of the Code of Federal Regulations.

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CFR Sections Referenced (Title 21—Food and Drugs)		
Part	**Section §**	**Section Title**
170—Food additives	170.30	Eligibility for classification as generally recognized as safe (GRAS)
173—Secondary direct food additives permitted in food for human consumption	173.25	Ion-exchange resins
179—Irradiation in the production, processing and handling of food	179.39	Ultraviolet radiation for the processing and treatment of food

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Appendix A

Expert Panel Statement

Expert Panel Report On the Generally Recognized as Safe (GRAS) Use of Potato Protein Isolates as Direct and Secondary Direct Food Ingredients in Multiple Food and Beverage Products

August 10th, 2012

Solanic B.V. (Solanic), an AVEBE group company, intends to market 2 minimally processed potato protein isolates, derived from potato fruit juice of non-genetically modified (non-GMO) potatoes (*Solanum tuberosum* subsp. *tuberosum*), as secondary direct and direct ingredients in finished food and beverage products across multiple food and beverage categories in the United States (U.S.) (Table A-1). Specifically, Solanic intends to market 2 distinct potato protein isolates, a high molecular weight fraction (>35 kDa), a low molecular weight fraction (between 35 and 4 kDa). Where necessary, the high molecular weight fraction will be abbreviated as HMW, the low molecular weight fraction as LMW. These abbreviations do not constitute commercial names of the products.

Solanic convened a panel ("Expert Panel") of independent scientists, qualified by their scientific training and relevant national and international experience 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 proposed uses of Solanic's potato protein isolates are Generally Recognized as Safe (GRAS) based on scientific procedures. The Panel consisted of: Prof. Joseph F. Borzelleca, Ph.D. (Virginia Commonwealth University School of Medicine), Prof. Paul B. Pencharz. M.B., Ch.B., Ph.D., FRCP(C) (The Hospital for Sick Children, University of Toronto), and Prof. Stephen L. Taylor, Ph.D. (University of Nebraska).

The Panel, independently and collectively, critically evaluated a dossier, "**Documentation Supporting the Generally Recognized as Safe (GRAS) Determination of Potato Protein Isolates for Use as Ingredients in Multiple Food Categories,"** provided by Solanic which included a summary of the scientific data and information relevant to the safety of the proposed uses of minimally processed potato proteins. This information was prepared from a comprehensive search of the scientific literature through May 2012 and also included details pertaining to the method of manufacture and product specifications, supporting analytical data, intended conditions of use of the ingredients in food, estimated exposure under the proposed uses, and a comprehensive assessment of the available scientific literature pertaining to the safety of the material. In addition, the Expert Panel evaluated other information deemed appropriate or necessary.

Following its independent critical evaluation of such data and information, the Expert Panel convened *via* teleconference on Friday, March 9th, 2012, and unanimously concluded that the proposed uses described herein for Solanic's high molecular weight and low molecular weight potato protein isolates (HMW, and LMW isolates), meeting appropriate food-grade

specifications as described in the supporting dossier [**Documentation Supporting the Generally Recognized as Safe (GRAS) Determination of Potato Protein Isolates for Use as Ingredients in Multiple Food Categories**] and manufactured consistent with current Good Manufacturing Practice (cGMP), are GRAS based on scientific procedures. A summary of the basis for the Expert Panel's conclusion is provided below.

Solanic's potato protein isolates are produced from potato fruit juice obtained during the processing of potato starch. The ingredients are minimally processed using filtration, evaporative concentration, and column chromatography processing methods commonly used by food manufacturers. The manufacturing process is conducted in compliance with current Good Manufacturing Practice (cGMP), and in accordance with AVEBE ISO 9001, and Hazard Analysis of Critical Control Point (HACCP) plans. Briefly, commercial cultivars of non-GMO food grade potatoes (*Solanum tuberosum* subsp. *tuberosum*) are washed and rasped to produce a potato juice, which is separated from the potato solids using mechanical separation techniques such as rasps, centrisieves, and decanters. Sulfite is used as an antioxidant during rasping to prevent browning. The potato juice is then subjected to a second physical separation step (centrisieving) to remove fibers and any remaining undesirable fine particulates. The potato proteins are isolated from the juice using chromatographic methods involving food grade adsorption columns that fractionate the potato proteins on the basis of their isoelectric points. Citric and formic acids are used as pH adjusting agents for elution of the HMW and LMW protein fractions which are subsequently concentrated by ultrafiltration and conditioned by mild temperature treatment (temperature ranges between 20 to 25°C) and additions of pH regulators. The conditioning may also include a polishing step using activated carbon, and further processing with approved ion exchange columns to lower the concentrations of triglycoalkaloids (TGA) in the protein concentrates. In accordance with 21 CFR§179.39, ultraviolet (UV) light is used for treatment of the potato protein isolates to reduce microbial counts in the finished product (U.S. FDA, 2012). The final concentrates are spray dried or, depending on the application, stored as stable concentrates of >20% dry matter. The Panel understands that all processing-aids used during the manufacturing of the Solanic's potato protein isolates are used in accordance with applicable United States Federal Regulations, were determined to be GRAS, or the subject of an effective Food Contact Notification, for their respective manufacturing uses as described by Solanic.

The finished products are agglomerated white powders with a protein purity of >90% on a dry weight (dw) basis. The HMW isolate largely consists of patatin, small quantities of trypsin inhibitors, and amino acids. The LMW isolate contains a heterogeneous mixture of potato protease inhibitors and amino acids. Appropriate food grade specifications have been established for the ingredients, and contain suitable limits for heavy metals, residual sulphite, total glycoalkaloids, and microbial contaminants (Table B-1). Batch analyses of multiple non-consecutive lots of Solanic's LMW and HMW isolates demonstrate that the LMW and HMW isolates are consistently manufactured to meet the production specifications. Additional

analytical data was presented confirming the absence of pesticides, dioxins and PCBs, mycotoxins, lysinoalanine, 3-monochloropropanol, and acrylamide at levels of toxicological concern.

Solanic's potato protein isolates are proposed for a variety of technical food uses (*e.g.*, flavoring aid, foaming, and emulsification aids; fining agent in wine, and fermentation nutrient) at use levels of between 0.01 to 6.0%. Solanic's LMW and HMW isolates also are intended for addition to adult sports beverage products (*e.g.*, sports recovery type beverage), and protein health bars as a non-animal source of dietary protein within these products. Using information on the proposed food uses of HMW and LMW potato proteins in conjunction with NHANES (2003 through 2008) survey data, the estimated intakes of HMW and LMW potato proteins were calculated using Crème® computer software. Mean all-user total population consumption of LMW and HMW potato protein isolates from technical food uses was estimated to be 2.3 and 2.2 g/person/day respectively (38 and 36 mg/kg body weight). Among all-users, 90th percentile consumption of LMW and HMW potato protein isolates were 4.8 and 4.7 g (80 and 77 mg/kg body weight) per person respectively. Solanic's potato protein isolates also are proposed for nutritive food uses interchangeably in adult sports drinks, and protein health bars at a use level of between 1 and 10%. Mean and 90th percentile all-user total population consumption of Solanic's potato protein isolates from nutritive food uses was estimated to be 11.2 and 30.0 g/person/day respectively (165 and 429 mg/kg body weight respectively). Highest consumption was estimated for male adults aged 20 and up, where mean and 90th percentile consumption was calculated to be 15.0 and 38.4 g/person/day, corresponding to intakes of 186 and 467 mg/kg on a body weight basis.

Solanic's potato protein isolates are minimally processed, and were considered substantially equivalent to potato proteins present in whole and processed potatoes, and potato containing foods; thus safety information obtained through history of safe use of potatoes in the human diet were considered relevant to the safety of Solanic's LMW and HMW potato protein ingredients. In the U.S., consumption of potatoes has remained relatively stable over several decades, with average potato consumption of 50 kg/person/year, or approximately 137 g/person/day (2 g potato protein/person/day) (FAOSTAT, 2011). Based on historical estimated *per capita* intakes of potatoes in the European Union (EU), daily intakes of up to 566 g of potatoes/person/day were estimated for Poland in 1967. Corresponding intake of potato protein by this population was reported to be 9.1 g/person/day (FAOSTAT, 2011). Higher consumption estimates were reported for Peru, where the consumption of potatoes is reported to be as high as 200 to 800 g/person/day in infant and adult women, which corresponds to intakes of 4 and 16 g of potato proteins per day respectively (Camire *et al.*, 2009). Historical references describing the consumption of potatoes in Ireland at the turn of the century have estimated that the consumption of between 4 to 6 kg of potatoes/person/day was common (Bourke, 1968); with corresponding potato protein intakes of between 60 to 90 g/person/day, potato protein would have been the principal source of dietary protein.

The toxicity of Solanic's LMW and HMW potato protein isolates was evaluated in a 13-week dosed feed study using Wistar rats (Lynch *et al*., 2012). The study was conducted in accordance with OECD 408 guidelines using current Good Laboratory Practice (cGLP). In this study, groups of 10 male and 10 female Wistar Crl:(WI) BR rats 5 weeks of age were provided chow diets supplemented with 0 (control), 7.5% LWM potato protein, or 15% HMW potato protein for a period of 13 weeks. A third experimental group was provided a mixture of HMW and LMW potato protein at 15% of the diet; this was designated total protein (TP). In their native forms, all three potato protein isolates were expected to contain active protease inhibitors. It is well established that rodents are highly sensitive to dietary trypsin inhibitors. Repeated consumption of trypsin inhibitors in the diet results in pancreatic hyperplasia and impaired weight gain in rats and mice (Gumbmann *et al*., 1986, 1989); however, these effects are not considered relevant to humans (see latter discussion below). Therefore to prevent the confounding effects of the trypsin inhibitors on pancreatic function and growth, which would complicate interpretation of the experimental data, the protease activity of the potato protein isolates was partially inactivated by mild pasteurization at 80°C for 30 minutes. Based on the weekly food consumption data, intakes of 4224 and 4917 mg/kg body weight/day of LMW protein and 8571 and 9698 mg/kg body weight/day of HMW protein were calculated for males and females, respectively. Male and female rats in the third group consumed 8414 and 9768 mg/kg body weight of TP respectively. The trypsin inhibitory activity of the test articles used in the study was measured using the azocasein colorimetric method[1] developed by Spelbrink *et al*. (2011). The trypsin inhibitory activities of the LMW, HMW, and TP test articles were 52.9±7.4, 3.9±1.6, and 10.7±1.4 mg trypsin inhibition (TI)/g protein respectively, corresponding to dietary concentrations of 4.5, 1.2, and 2.7 mg TI/g diet. The results of the study were largely unremarkable, and the HMW, LMW, and TP protein isolates were well-tolerated and without adverse effects. No-observed-adverse-effect levels (NOAEL) of 4224 mg/kg body weight and 8571 mg/kg body weight for male rodents, the highest doses tested, were determined for the LMW and HMW potato protein isolates respectively. A NOAEL of 8414 mg/kg body weight was established for the male rats in the TP group. Based on the TI activity of the LMW test article [59.8 mg Trypsin Inhibited (TI) per g LMW protein], male rats randomized to the LMW potato protein isolate group were estimated to consume 252 mg TI/kg body weight. Male rats in the HMW and TP groups were estimated to be exposed to trypsin inhibitors at doses of 71.1 and 149.8 mg/kg body weight respectively. Although The Expert Panel recognized limitations in the study design, notably the use of a single dietary concentration/dose of each test article, these levels represented the highest dietary concentrations that could be administered to the animals: Dietary concentrations of the HMW or TP potato protein in excess of 15% would have resulted in nutritional imbalances in the animals;

[1] The assay involves the use azocasein as a protease substrate and assesses the loss of proteolytic activity upon incubation in the presence of a PI-rich emulsion. This method contrasts with the traditional methods used for feed and food, which incorporate artificial chromogenic substrates such as Nα-Benzoyl-Larginine-p-nitroanilide (L-BAPA) or Nα-Benzoyl-L-arginine ethyl ester (BAEE) and therefore require a completely solubilized, clear solution for accurate measurements. The data are expressed as amount of protease inhibited per amount of sample material (mg Trypsin Inhibited/kg material).

and the dose of 7.5% LMW potato protein was selected on the basis of results from pilot studies which suggested that higher dietary concentrations were associated with hypertrophic effects on the pancreas. Limitations in the appropriateness of graded dosing in animal toxicity studies of macronutrients have been addressed (*e.g*., EFSA, 2011). The Expert Panel considered that information obtained from the animals within the Total Protein treatment group could serve as a suitable "low dose" comparator for use in the interpretation of any findings in either of the high dose treatment groups. The Expert Panel agreed that the authors' NOAEL determinations were appropriate.

Potato protein isolates have been the subject of a limited number of human studies. In studies in which potato proteinase inhibitor-containing extracts were provided to healthy and diabetic male and female adult subjects at single doses of up to 1.5 g, no adverse effects attributed to potato protein were reported (Hill *et al.,* 1990; Schwartz *et al*., 1994; Peters *et al*., 2011). Findings in these studies are conflicting. Schwartz *et al*., reported that consumption of 1.5 g of potato protein inhibitor 2 (PI2) within a liquid meal (50 g glucose + 20 g whey protein) resulted in a delay in gastric emptying, and corresponding delay of glucose absorption in individuals with type 2 diabetes. Placebo controlled cross-over studies conducted in healthy human subjects administered 30 mg of PI2 in a min-drink prior to consumption of ad-libitum or fixed meals did not produce functional effect on behaviors and physiological appetite and intake control measures (Peters *et al*., 2011). The Expert Panel considered the low quantities of potato proteins consumed and the short study durations to limit their value to the safety assessment of Solanic's potato protein isolates.

Potatoes are known to contain several natural toxins and anti-nutrient compounds, specifically protease inhibitors and glycoalkaloids (α-solanine and α-chaconine), which require assessment for safety under the proposed uses since exposure to these compounds from foods containing HMW and LMW potato proteins may differ from the historical exposure to these compounds through the consumption of potatoes in the diet. Based on the proposed specification of 312 mg of total glycoalkaloids/kg of isolate, and the proposed maximum use level of 10%, concentrations of glycoalkaloids in foods containing LMW and HMW potato proteins would be 31.2 mg/kg of food; these levels are comparable to typical background concentrations of glycoalkaloids that have been measured in modern potato varieties that are consumed in the diet (NTP, 1998). The safety of the glycoalkaloids, α-solanine and α-chaconine, was considered by the Joint World Health Organization (WHO)/Food Agriculture Organization of the United Nations (FAO) Expert Committee on Food Additives (JECFA) (JECFA, 1993). Although the committee did not establish an Acceptable Daily Intake (ADI), the expert committee did conclude that "… the large body of experience with the consumption of potatoes, frequently on a daily basis, indicated that normal glycoalkaloid levels (20-100 mg/kg) found in properly grown and handled tubers were not of concern." The U.S. Department of Agriculture has established a guideline of 200 mg/kg for total glycoalkaloid content of parents and offspring of potential potato varieties (NTP, 1998). The Expert Panel considered the background exposure to glycoalkaloids

in the diet from consumption of potatoes and potato containing foods, and concluded that the additional exposure to glycoalkaloids under the proposed food uses of Solanic's HMW and LMW potato protein ingredients would not pose a safety concern. The Expert Panel also understands that Solanic's LMW potato protein isolates intended for use for nutritive uses will be further processed to reduce the glycoalkaloid concentrations of the ingredient to below 100 mg/kg, which will further limit exposure to glycoalkaloids in the diet from the proposed food uses of the LMW isolate.

On a weight to weight basis, the HMW and LMW potato fractions contain between 13- and 200-fold greater concentrations of protease inhibitor activity respectively relative to that present in an equivalent quantity of raw potatoes. Stability studies show that the protease activity of these ingredients is expected to be reduced by 50 to >90% depending on the processing conditions (*e.g*., baking) of the intended food use. Solanic has further shown that protease inhibitors are common to the diet and that a number of commonly consumed foods (*e.g*., eggs, soy milk, infant formula) contain protease inhibitor activities at levels that are comparable to those occurring in foods to which Solanic's LMW potato protein isolates are added for technical purposes. The majority of technical food uses are expected to be subjected to food processing conditions that are comparable to those currently applied to whole and processed potatoes, therefore consumption of Solanic's potato protein isolates from proposed technical food uses would not appreciably add to total background intake of potato protein trypsin inhibitors in the diet. The Expert Panel recognized that nutritive uses in adult sports drinks and protein health bars may result in significant consumption of active trypsin inhibitors from the addition of the LMW isolate to these foods since these foods may not be subjected to processing conditions that would result in significant denaturation of the protein isolates. The mean and 90th percentile estimated exposure to the LMW isolate among heavy consumers (males aged >20 years) of sports drinks and protein health bars was 15.0 and 38.4 g per person per day (186 and 467 mg/kg body weight). The average trypsin inhibitor activity of Solanic's LMW potato protein isolate was 275 mg trypsin inhibition/g protein. Therefore exposure to trypsin inhibitors by the aforementioned heavy consumers would be 51.2 and 128 mg trypsin inhibition/kg body weight among mean and 90th percentile all-users respectively. Since Solanic's HMW isolate is proposed for use as an alternative protein source to the LMW isolate, and the protease inhibitor activity of the HMW isolate is 3 to 4 fold lower than that of the LMW isolate, nutritive uses of the HMW isolate will not increase total exposure to trypsin inhibitors in the diet beyond that provided by nutritive food uses of the LMW isolate. Additive exposures to typsin inhibitors in the diet from food uses of the HMW isolate were therefore not considered. Although consumption of proteinase inhibitors in the diet has an apparent history of safe consumption by humans, the long-term feeding of soybean and potato derived protease inhibitors to rodents and/or chickens results in pancreatic hypertrophy, which progresses to pancreatic adenomas and increased mortality (Gumbmann *et al*., 1986, 1989). Multiple studies comparing the qualitative and quantitative effects of potato and soybean protease inhibitors have shown that "*potato TI (trypsin inhibitor) produced changes in the pancreas of the rat that could not be distinguished*

from those associated with soy TI" (Lee *et al*., 1985; Gumbmann *et al*., 1989). The Expert Panel therefore considered studies conducted using soybean trypsin inhibitors to be relevant to the safety evaluation of potato protease inhibitors.

In contrast to findings in rodents, long-term consumption of dietary protease inhibitors at high concentrations does not affect pancreatic function in pigs or non-human primates (*e.g*., 2 to 4 year old male and female cebus monkeys, and adolescent female rhesus monkeys) (Harwood *et al*., 1986; Garthoff *et al*., 2002a,b). Specifically, no adverse effects on the pancreas were reported in weanling pigs following a 39-week treatment period with a partially purified soybean derived trypsin inhibitor preparation at doses of 140 to 276 mg trypsin inhibition/kg body weight/day (Garthoff *et al*., 2002a,b). The adverse effects of dietary proteases on pancreatic function observed in rodents are believed to be mediated by the loss of feedback inhibition of cholecystokinin (CCK) (Struthers *et al*., 1983; Sato *et al*., 2002; Komarnytsky *et al*., 2011; Nakajima *et al*., 2011). In rodents CCK is a major regulator of pancreatic secretory activity, and overstimulation of the pancreas by this hormone results in an initial adaptive hypertrophy, which progress to hyperplasia and adenoma formation during chronic exposure (Göke, 1990). In humans, digestive enzymes do not directly regulate CCK synthesis (Wang and Cui, 2007), accordingly, the loss of digestive enzyme activity from the consumption of synthetic protease inhibitors has not been reported to result in increases in basal levels of CCK (Adler *et al*., 1988a,b; Friess *et al*., 1998). Moreover, the rodent pancreas contains high affinity CCKR which are responsive to increased circulating concentrations of CCK in the plasma. The human pancreas does not express functional CCKR, and therefore is not responsive to circulating CCK (Wang and Cui, 2007). Control of pancreatic function in humans is mediated by luminal concentrations of CCK through neuroendocrine mechanisms (Wang and Cui, 2007). The significant species difference in the hormonal regulation of pancreatic function between humans and rodents, and the absence of adverse pancreatic effects of chronic protease consumption in pigs and non-human primates, support the conclusion that the effects of dietary proteases on pancreatic function observed in rodents is not relevant to humans.

Although studies in pigs and rodents have shown that coagulated potato protein is a well balanced source of dietary protein (Nestares *et al*., 1993; Beelen and Tolman, 1996; Beelen *et al*., 1999; Boisen, 1999), the digestibility of the protein may be reduced when consumed in their unprocessed form. Tuśnio *et al*. (2011) evaluated the effects of diets supplemented with potato protein in young barrows (21 kg; synthetic line 990). Barrows (n= 6/group) were administered diets containing casein alone as the sole protein source, or 11% casein supplemented with 12% potato protein concentrate. Diets were administered for 7 days and digesta collected over the last three days for measurement of apparent ileal digestibility. The glycoalkaloid and trypsin inhibitor concentration of the potato protein concentrate was 677 mg/kg and 3910 mg TI/kg (approximately 6.77 mg/kg and 3910 mg TI/kg feed). The authors reported that amino acid bioavailability was significantly reduced in the pigs consuming the potato protein supplemented diets relative to animals in the control group. However, the authors also reported that the effect

of potato protein concentrates was not nutritionally significant. Studies conducted in rodents, which are highly sensitive to the antinutrient activity of potato proteases, show that the anti-nutrient effects of dietary proteases are counteracted when sufficient protein from other food sources is present in the basal diets (Gumbmann *et al*., 1989). The results of dietary studies in chicks, an animal model used to evaluate the nutritional wholesomeness of feeds due to their rapid growth rates, demonstrated that native potato proteinase inhibitors are of lower nutritional value relative to their thermally denatured counterparts (Pearce *et al*., 1983). The Expert Panel concluded that there was insufficient experimental evidence to comment on the nutritional value of minimally processed potato proteins for use as an exclusive or primary source of protein in the diet. The Expert Panel noted that median protein intakes by male and females, aged 19 to 30, were estimated to be 70.4 and 107.1 g per person per day. Among heavy users of adult sports drinks and protein health bars, substantially higher background intakes of protein is expected as the recommended protein intake in these individuals is reported to range between 1.4 to 2.0 g/kg body weight, which would be in excess of 150 g of protein per day for a 90 kg athlete (Lemon, 1996). Therefore, background intakes of protein are expected to be sufficient to overcome any deficiencies in the protein quality of native potato proteases. Moreover, the physiological effects of dietary proteases would be transient, occurring immediately following consumption of the food, and would not influence protein digestion during subsequent meals throughout the day.

The Expert Panel reviewed generally available information characterizing the allergenic potential of potato proteins. Potatoes are a common food ingredient in the diet of infants in many countries, and in its cooked form, is one of the first solid foods introduced to infants usually at 4 to 6 months of age (Monti *et al*., 2011). Although potatoes are frequently consumed across multiple demographic groups and age ranges in many countries, food allergy to potatoes is uncommon, and to-date, case-reports of potato food allergenicity have only been reported for children (Monti *et al*., 2011). The identities of several potato allergens have been characterized, and include the storage protein patatin (Sol t1). Several protease inhibitors have been identified as potato allergens (Seppälä *et al*., 1999, 2001; Majamaa *et al*., 2001); these allergens are expected to be present in Solanic's potato protein isolates in their native forms. Information characterizing the thermal and digestive stability of potato allergens is limited. Pearson (1966) and Castells *et al*. (1986) reported that cooking of potatoes results in partial or total loss of its allergenic properties. In contrast, other studies have reported that both patatin, and potato protease inhibitor Sol t2-4 are relatively stable to digestive proteases and heating (Seppälä *et al*., 1999; Majamaa *et al*., 2001; Seppälä *et al*., 2001). The Expert Panel also evaluated anecdotal reports that cooked potatoes may be of lower allergenic potential in some individuals. For example, Beausoleil *et al*. (2001) reported a case of anaphylaxis to raw potato in a 4-year-old child. The individual developed urticaria, angioedema, respiratory distress, vomiting and diarrhea after biting into a raw potato used for painting in preschool. Skin prick tests to fresh potato were positive, however, the individual was not responsive to oral challenge with cooked potato. Since potatoes are typically cooked prior to consumption there is an absence of

historical references that can be used to determine the sensitization potential of native potato proteins in children. The Panel noted that the identity of the allergens involved in the few cases of reported allergic reactions to raw potatoes (*e.g.*, Beausoleil *et al.*, 2001) has not been determined, and thus it is unknown if these allergens are present in the Solanic protein isolates. Nevertheless, the possibility that wide-spread introduction of minimally processed potato proteins to finished food and beverage products within the U.S. food supply may result in increased incidences of sensitization in infants and children therefore could not be excluded. However, on the basis that the frequency of potato allergenicity is low, and the fact that the proposed food uses of Solanic's minimally processed potato proteins in adult sports drinks and protein bars are unlikely to be frequently consumed by infants or children, the risk of widespread exposure in this sensitive population group was considered very low. The allergenic potential of foods containing Solanic's potato proteins in processed foods is not expected to differ substantially from existing potato containing foods currently on the market.

Conclusion

We, the members of the Expert Panel, have independently and collectively critically evaluated the information summarized above and conclude that the proposed food uses described in Table A-1 in Attachment A of Solanic's potato protein isolates, meeting appropriate food-grade specifications and manufactured in accordance with current Good Manufacturing Practice, is Generally Recognized as Safe, based on scientific procedures.

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

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Prof. Stephen L. Taylor. Ph.D. Department of Food Science and Technology University of Nebraska	Date

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[redacted]

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ATTACHMENT A

Individual Proposed Food-Uses and Use-Levels for Potato Protein Isolates in the U.S.

Table A-1 Summary of the Individual Proposed Uses and Use Levels for Potato Protein Isolates in the United States (2003-2008 NHANES data)			
Food Category	**Proposed Food-Uses**	**Potato Protein Use Level (%)**	
		LMW	**HMW**
Technological Uses			
Alcoholic Beverages	Wine	0.01 to 0.025[a]	
Baked Foods and Baking Mixes	Gluten-free Bread[b]	0.5 to 6	
	Gluten-free Cakes[c]	0.5 to 6	
Cheeses	Natural Cheese	N/A	0.01
Dairy Product Analogs	Non-dairy Coffee Whiteners	0.2 to 4	
	Non-dairy Whipped Topping	0.2 to 1	
Egg Products	Meringues	0.2 to 4	N/A
Fats and Oils	Creamy Salad Dressings (egg-based)	0.2 to 1	N/A
	Mayonnaise	0.2 to 1	N/A
Frozen Dairy Desserts and Mixes	Frozen Yogurt	0.2 to 1	
	Ice Cream	0.2 to 1	
Fruit and Water Ices	Sorbet	0.2 to 1	N/A
Gelatins, Puddings, and Fillings	Mousses	0.2 to 4	N/A
Meat Products	Beef Patties	0.2 to 3	
	Deli Meats	0.2 to 3	
	Injected Ham (fresh)	0.2 to 3	
	Pate	0.2 to 3	
	Sausages and Hot Dogs	0.2 to 3	
Milk Products	Yogurt	0.5	N/A
Nuts and Nut Products	Coated Peanuts	1 to 15	
Plant Protein Products	Vegetable Meat Analogs	0.5 to 6	
Poultry Products	Injected Chicken and Turkey (Fresh)	0.2 to 3	
Fermentation aid[d]	Finning agent	N/A	
Nutritive Uses[e]			
Beverages and Beverage Bases	Adult Sports Drinks (Recovery Drinks)[f]	1 to 10	
Grain Products and Pastas	Adult Protein, Granola, and Health Bars	1 to 10	

HMW = high molecular weight; LMW = low molecular weight; NA = not applicable

[a] It should be noted that 90% of the added potato protein will be removed using standard filtration methods employed by the wine industry.

[b] There were no food codes available for gluten-free bread in the NHANES 2003-2008 database; thus, surrogate codes for low gluten bread were selected.

[c] There were no food codes available for gluten-free cakes in the NHANES 2003-2008 database; thus, surrogate codes for low gluten cakes were selected.

[d] Potato protein isolates are intended for use as an amino acid nutrient during fermentation. Intake exposures are expected to be negligible relative to proposed food-uses. Thus, exposures from this food use were not considered during calculation of intake estimates.

[e] Nutritive food uses will be limited to low glycoalkaloid preparations.

[f] There were no food codes available for recovery-type sport drinks in the NHANES 2003-2008 database; thus, surrogate codes for meal replacement beverages (milk- and non-milk-based drinks) and energy drinks were selected.

SUBMISSION END