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The Report of the Expert Panel on the Generally Recognized as Safe Determination of the Proposed Uses of Soy Leghemoglobin Protein Derived from *Pichia pastoris* as a Food Ingredient

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Introduction

Impossible Foods Inc. (Impossible Foods) convened a panel of independent scientists (Expert Panel), qualified by their scientific training and relevant national and international experience in the evaluation of the safety of food ingredients, to conduct an independent, critical and comprehensive evaluation of the available safety information on soy leghemoglobin protein preparation (LegH Prep), and to determine if the proposed uses as a protein component in ground beef replacement (analogue) products would be Generally Recognized as Safe (GRAS) based on scientific procedures. The Expert Panel consisted of Professor Joseph F. Borzelleca, Ph.D. (Virginia Commonwealth University School of Medicine), Professor Michael W. Pariza, Ph.D. (University of Wisconsin-Madison), and Professor Stephen L. Taylor Ph.D. (University of Nebraska-Lincoln).

An initial review was conducted and a summary of the results was made available to the Expert Panel as part of the "GRAS NOTIFICATION FOR SOYBEAN LEGHEMOGLOBIN PROTEIN DERIVED FROM *PICHIA PASTORIS*" (not dated) and a "TECHNICAL SUMMARY OF SOYBEAN LEGHEMOGLOBIN PROTEIN DERIVED FROM *PICHIA PASTORIS* " (dated May 30, 2014). Impossible Foods conducted further studies to confirm the safety and GRAS status of the proposed uses of LegH Prep and made this information and data available to the Expert Panel. A comprehensive search of the scientific literature on plant and animal hemoglobins and related products was conducted by Impossible Foods as part of the preparation of the new GRAS notice that is the subject of this report, as well as during the preparation of the supportive Technical Summary. Impossible Foods reported to the Expert Panel that their search failed to identify anything further on the safety of LegH Prep. The Expert Panel, independently and collectively, critically evaluated the new information and data and re-evaluated the original information and data, and other information deemed appropriate or necessary and information pertaining to the method of manufacture, product specifications, batch analyses, intended levels of use, exposure estimates, and the safety of LegH Prep.

Following its independent, critical evaluation of the available information, the Expert Panel convened by teleconference and email correspondence, and unanimously concluded that the intended use in ground beef analogue products of soy leghemoglobin protein derived from *Pichia pastoris*, manufactured consistent with current Good Manufacturing Practice (cGMP) and meeting appropriate food-grade specifications, is GRAS based on scientific procedures. A summary of the basis for this conclusion appears below.

Impossible Foods proposes to market the soy leghemoglobin protein produced in the yeast *Pichia pastoris* in the United States for use as a protein component to create a flavor impact in ground beef analogue products.

Hemoglobin proteins are found in most organisms, including bacteria, protozoa, fungi, plants and animals (Hardison, 1998). Hemeproteins are classified as globin/non-globin and symbiotic/non-symbiotic. Hemoglobin, myoglobin, and leghemoglobin are examples of globin proteins. Cytochrome oxidases, hemocyanins, and methemalbumin are examples of non-globin hemeproteins (Everse, 2004) (Jokipii-Lukkari, Frey, Kallio, & Haggman, 2009). Plant hemoglobins are classified according to function as symbiotic or non-symbiotic (Gupta, Hebelstrup, Mur, & Igamberdiev, 2011). Symbiotic hemoglobins are found predominantly in leguminous plant species. The most studied symbiotic hemoglobins are the leghemoglobins of nitrogen fixing legumes where they facilitate oxygen diffusion within root tissues. Non-symbiotic hemoglobins have been identified in a wide range of legume and non-legume plants. The highest expression levels for non-symbiotic plant hemoglobin are observed in metabolically active or stressed tissue (Anderson, Jensen, Leewellyn, Dennis, & and Peacock, 1996).

Impossible Foods analyzed structures of plant non-symbiotic hemoglobins and symbiotic leghemoglobins and animal myoglobins including rice, soy, corn, barley, lupine, horse, tuna, and pig. Animal myoglobins, plant leghemoglobins and plant hemoglobins adopt the same globin fold and are structurally very similar. All globin proteins described above bind the chemically identical heme B prosthetic group involved in binding and/or transport of oxygen. The globin protein family is large, present in a wide range of organisms, and is well studied.

Identity and Characterization of Soy Leghemoglobin Protein

The chemical name of the characterizing component of LegH Prep is soy leghemoglobin protein. The source of the protein is the soybean plant *Glycine max* gene *LGB2*. Soy leghemoglobin protein is derived from the root nodules of the soy plant.

There is no Chemical Abstracts Number for soy leghemoglobin.

The proposed common or usual name of LegH Prep is "leghemoglobin (soy)."

Production of LegH Prep

The method of production involves four stages: construction of the production strain of *Pichia pastoris*, expression of soy leghemoglobin protein in submerged fermentation, enrichment, and stabilization of the expressed soy leghemoglobin protein.

All materials used in the production of LegH Prep are food grade and GRAS or high-quality chemical or pharmaceutical grades (USP, NF, or ACS grades) from approved suppliers and processing conditions are appropriate for food production and consistent with cGMP.

Preparation of the Production Strain for Fermentation

Production strain *Pichia pastoris* MXY0291 was constructed from recipient strain Bg11 (MXY0051) using a series of transformations with different expression constructs, in order to express soy leghemoglobin protein. In addition to the protein coding sequence for soy leghemoglobin, MXY0291 contains extra copies of native *Pichia pastoris* heme biosynthetic enzymes and modified *Pichia pastoris* transcription factor Mxr1, all expressed under the strong native *Pichia pastoris* alcohol oxidase promoter (*pAOX1*). This promoter has been demonstrated to produce high levels of recombinant proteins after producing biomass on glycerol and inducing *pAOX1* with methanol (Cereghino & Cregg, 2000). The genome of MXY0291 is fully sequenced and well characterized.

The production strain parent, *Pichia pastoris* Bg11, was derived from the well-characterized strain Y-11430, which is deposited in the collection at the Northern Regional Research Laboratories (NRRL). The lineage of *P. pastoris* strain NRRL Y-11430 was previously included in GRN 204, reviewed by the Agency in 2006.

There are no indications that *P. pastoris* has been associated with animal or human illness. The first *P. pastoris* strains were isolated from an oak tree and a chestnut tree and were deposited in the collection at the Northern Regional Research Laboratories (NRRL) (www.biogrammatics.com). Yeast strains screened by Phillips Petroleum for growth on methanol included two *P. pastoris* strains, designated NRRL Y-1603 (ATCC accession 28485) (ATCC, 2006b) and NRRL YB-4290 (NCAUR, 2006). Phillips Petroleum identified a *P. pastoris* strain with improved growth characteristics. The strain was designated 21-1 and deposited at NRRL, as NRRL Y-11430 . This strain is now available from ATCC as 76273. No records are available confirming that NRRL Y-1603 or NRRL YB-4290 is the progenitor of NRRL Y-11430, but it seems likely that one of them is the progenitor strain. NRRL Y-11430 was the progenitor strain for GS115, a histidine auxotrophic mutant *(his4-)*, a common *Pichia* strain provided in commercial kits by Invitrogen Corporation, and widely used as the parental strain of many biotechnology products, including FDA approved proteins such as Kalibitor® (ecallantide, for the treatment of acute attacks of hereditary angioedema, 2009). Additionally, the GS115 derived strain SMD1168 is used for the GRAS approved production of BD16449 Phospholipase C (Food and Drug Administration, 2006). Like GS115, the BioGrammatics, Inc. strain, Bg11 is also a derivative of NRRL Y-11430, and genomic sequencing data performed by BioGrammatics Inc. confirm the similarity of NRRL Y-11430, Bg11 and GS115. Additional taxonomic history of these strains is available in a 2009 manuscript by C. Kurtzman and on the Biogrammatics webpage (biogrammatics.com).

BioGrammatics, Inc. further developed the NRRL-Y-11430 strain to remove the native *P. pastoris* plasmids using PCR primers unique to the plasmids to screen multiple single-colony isolates for the presence of the plasmids. One isolate without plasmids was selected to become the wild-type (wt) BioGrammatics strain, Bg10. Genomic sequence from Bg10 indicates the plasmids are no longer present, and, benchmarks the similarity of Bg10 with NRRL-Y11430, as well as with GS115. Biogrammatics, Inc. deleted the gene encoding for Aox1 from Bg10 using homologous recombination to generate Bg11, a strain that grows more slowly on methanol-containing induction media. Like NRRL Y-11430 and GS115, Bg11 does not contain antibiotic resistance genes.

Expression of Soy Leghemoglobin Protein in Submerged Fermentation, Enrichment and Stabilization

Soy leghemoglobin protein is obtained by fed-batch fermentation using the *P. pastoris* production strain MXY0291 described above. All media components are FCC approved or food-grade ingredients. The *P. pastoris* cells in the fermentation broth are lysed by bead mill mechanical shearing. Insoluble material within the lysate is removed by centrifugation and microfiltration. Ultrafiltration is used to concentrate soy leghemoglobin protein to at least 60 g/l. The resulting concentrated sample is stabilized with sodium chloride and sodium ascorbate and stored as a frozen liquid.

Specifications for Soybean Leghemoglobin Protein Product

LegH Prep is standardized to contain at least 60 grams per liter (g/l) soy leghemoglobin protein. Sodium chloride and sodium ascorbate are used to stabilize the product. All stabilizing agents are food grade. The product specifications, and batch analysis results, are presented below.

Table 1. LegH Prep specifications and batch analyses from five independent production runs.

	Specifications	PP-PGM2-16-015-101	PP-PGM2-16-088-101	PP-PGM2-16-102-101	PP-PGM2-16-144-101	PP-PGM2-16-200-101
Soy Leghemoglobin Protein (w/w)[1]	6 – 9%	6.74%	6.39%	6.28%	6.74%	6.95%
Soy Leghemolglobin Protein Purity (w/w)	≥65%	82%	71%	85%	77%	86%
Fat (w/w)	≤2%	0.05%	<0.01%	<0.01%	0.03%	0.08%
Carbohydrates (w/w)	≤4%	1.72%	0.99%	1.67%	2.01%	2.73%
Ash (w/w)	≤4%	1.87%	0.67%	2.63%	2.62%	2.74%
pH	6.5 – 8.5	7.19	7.19	7.38	7.01	6.77
Lead (ppm)	<0.4	<0.01	<0.01	<0.01	<0.01	<0.01
Arsenic (ppm)	<0.05	0.01	<0.01	<0.01	0.01	<0.01
Mercury (ppm)	<0.05	<0.005	<0.005	<0.005	<0.005	<0.005
Cadmium (ppm)	<0.2	<0.001	<0.001	0.001	0.003	0.001
Aerobic plate count (CFU/g)[2]	<10^4	<10	<10	<10	<10	<10
E. coli 0157:H7[3]	Absent by test	Absent by test	Absent by test	Absent by test	Absent by test	Absent by test
Salmonella spp.[4]	Absent by test	Absent by test	Absent by test	Absent by test	Absent by test	Absent by test
Listeria monocytogenes[5]	Absent by test	Absent by test	Absent by test	Absent by test	Absent by test	Absent by test

Stability of Soy Leghemoglobin

LegH Prep has been stored at -20 °C as a frozen liquid for at least 12 months with no observable change in soy leghemoglobin protein stability or performance in ground beef analogue products.

Intended Uses in Food

LegH Prep is proposed to be used as a plant-based protein component in non-animal derived food products with the texture, nutrition, flavor and appearance of traditional animal derived foods. LegH Prep will impart a unique flavor impact to meat analogue products.

The high bioavailability of the heme iron component of soy leghemoglobin makes it suitable to enhance the dietary profile of many processed foods (Carpenter & Mahoney, 1992).

LegH Prep may be one of several Food and Drug Administration ("FDA" or "Agency") recognized plant proteins that will comprise ground beef analogue products. Other proteins may include, but are not limited to, commercially available proteins from soy, pea, mung bean, lentil, corn, potato, and wheat. Soy leghemoglobin will function to contribute to the flavour and nutritional quality of ground beef analogue products. A typical ground beef analogue product may contain:

Component	Meat Analogue
Protein	10%-25%
Plant Oils	0%-25%
Miscellaneous+	2%
Water	50%-75%

+Miscellaneous ingredients may include salt, flavors, vitamins, essential amino acids, etc.

LegH Prep will be added to the ground beef analogue products to deliver not more than 0.8% soy leghemoglobin protein.

Self-limitation of the Use of Soybean Leghemoglobin Protein Product

The use of LegH Prep in ground beef analogue products above the specified use-levels is largely self-limiting based on unacceptable organoleptic properties.

Estimated Dietary Intake

The vast majority of hemeproteins consumed in the diet are as myoglobin through consumption of meat and poultry products. Hemeprotein consumption was estimated using data from the "Retail Commodity Intakes: Mean Amounts of Retail Commodities per Individual, 2007-08. (Bowman, Martin, Clemens, Lin, & Moshfegh, 2013). For the US population, per capita mean consumption of meat and poultry products is 154 g/day and the 90th percentile intake is 308 g/day. Assuming an average myoglobin concentration for meat and poultry products of 0.5% (Yip & Dallman, 1996), the average per capita myoglobin consumption would be 0.77 g/day and the 90th percentile intake would be 1.54 g/day.

LegH Prep will be marketed for use in ground beef analogue products that provide consumers a flavorful and nutritious alternative to meat containing products. Impossible Foods has estimated daily intakes of soy leghemoglobin protein by assuming consumers will substitute the meat analogue product for the traditional meat product on a 1-for-1 basis.

Impossible Foods has assumed it will capture 100% of the total ground beef market with soy leghemoglobin protein-containing ground beef analogue products. 100% of the total ground beef market represents approximately 500 times the volume of the current meat analogue market size based on sales estimates[1]. The Estimated Daily Intake (EDI) of soy leghemoglobin in the target ground beef analogue applications was established using the Retail Commodity Intakes: Mean Amounts of Retail Commodities per Individual, 2007-08 (Bowman, Martin, Clemens, Lin, & Moshfegh, 2013). The results of that analysis are presented below. The estimates were calculated as follows:

For beef, the mean daily consumption is 59 grams. For ground beef, the mean consumption is 25 grams (59 grams x 42%). As the highest usage case, Impossible Foods assumes capturing 100% of this market with a ground beef analogue product consisting of not more than 0.8% soy leghemoglobin. This equates to a highest intake case of 200 mg/person/day of soy leghemoglobin (25 ground beef grams/person/day x 100% market x 0.8% soy leghemoglobin).

The estimated average daily intake of soy leghemoglobin in the intended applications will be 150 mg/person/day (0.6% soy leghemoglobin) and the maximum intake will be 200 mg/person/day (0.8% soy leghemoglobin). As noted above, this base case represents capturing 500 times the existing meat and poultry analogue market.

[1] Datamonitor estimates the US meat analogue volume was 53M kg in 2009. USDA-FAS Livestock and Poultry Report, April 2014 estimates 2014 US consumption of 11B kg beef, 8.5B kg pork, and 14B kg broilers. Therefore, the current meat analogue market is less than 0.2% of the overall meat market and capturing 1% of the meat market represents 5 times the current meat analogue market in the US.

Table 2a. Summary of proposed uses of soy leghemoglobin protein in food applications based on Retail Food Commodity Intakes 2007-2008.

Food Category to be Replaced	Mean Consumption (g/day)[2]	Anticipated Market Share Replacement (%)	Anticipated Typical Use rate (%)	Soy Leghemoglobin Estimated Typical Daily Intake (mg/person/day)	Anticipated Maximum Use Rate (%)	Soy Leghemoglobin Estimated Maximum Daily Intake (mg/person/day)
Ground Beef	25	100	0.6	150	0.8	200

Table 2b. Summary of proposed uses of LegH Prep dry solids in food applications based on Retail Food Commodity Intakes 2007-2008.

Food Category to be Replaced	Mean Consumption (g/day)	Anticipated Market Share Replacement (%)	Anticipated Typical Use rate (%)	LegH Prep Dry Solids Estimated Typical Daily Intake (mg/person/day)	Anticipated Maximum Use Rate (%)	LegH Prep Dry Solids Estimated Maximum Daily Intake (mg/person/day)
Ground Beef	25	100	0.6	404	0.8	539

For the basis of safety testing, the 90th percentile consumption of soy leghemoglobin was calculated using 25 grams ground beef/person/day x 0.8% soy leghemoglobin/ground beef / 60 kg/person x 2. Therefore, the 90th percentile consumption equates to 6.67 mg/kg/day, which was used as the basis for safety testing.

Safety of Soy Leghemoglobin

Hemeproteins are found in bacteria, protozoa, fungi, plants and animals (Everse, 2004) (Hardison, 1998). Soy plants have been shown to express three hemoglobin proteins: symbiotic, non-symbiotic and truncated (Lee, Kim, & An, 2004). Symbiotic plant hemoglobins, which evolved from non-symbiotic hemoglobins (Gupta, Hebelstrup, Mur, & Igamberdiev, 2011), are commonly referred to as leghemoglobins. Symbiotic leghemoglobins, found predominately in legume root nodules, function in the nitrogen fixation process in concert with the bacterium *Rhizobium* where they facilitate oxygen diffusion within host root tissues. LegH Prep contains this symbiotic soy leghemoglobin derived from *Pichia pastoris.*

[2] Retail Food Commodity Intakes: Mean Amounts of Retail Commodities per Individual, 2007-08. U.S. Department of Agriculture, Agricultural Research Service, Beltsville, MD and US Department of Agriculture, Economic Research Service, Washington, D.C.
http://www.ncaur.usda.gov/SP2UserFiles/Place/12355000/pdf/ficrcd/FICRCD_Intake_Tables_2007_08.pdf

Anderson et al. demonstrated that the non-symbiotic hemoglobin in soybeans was expressed in various plant tissues including stems, shoots, cotyledon, leaves, and root hair (Anderson, Jensen, Leewellyn, Dennis, & and Peacock, 1996). These soybean tissues are commonly consumed in the diet in the form of bean sprouts. Commercial production of soybean sprouts is a six (6) day process from imbibition to packaging for retail sale (Lim, 2014). Sprouted barley, which is widely used in the beverage industry (malted barley) and in the baking industry (malted barley flour), has been reported to express hemoglobin one (1) day after imbibition (Duff, Guy, Xianzhou, Durnin, & Hill, 1998). Non-symbiotic hemoglobins are expressed in the rice embryo and in the coleoptiles and seminal root of sprouted rice, which are consumed as part of the diet (Lira-Ruan, Ruiz-Kubli, & Arredondo-Peter, 2011).

Impossible Foods analyzed plant symbiotic leghemoglobins (soy, lupine), non-symbiotic plant hemoglobins (rice, corn, barley), and animal myoglobins (horse, tuna, pig) and confirmed the structural similarity (cf. Annex 1, GRASN). The three dimensional structure of soybean leghemoglobin is highly similar to the non-symbiotic hemoglobins of corn, rice, and barley as well as mammalian myoglobin.

Globin proteins bind the identical heme prosthetic group and are involved in binding or transporting oxygen. The oxygen binding mechanism of soy leghemoglobin is similar to that of animal muscle myoglobin.

History of Safe Use

The safety of soy protein is well established. Soybeans have been part of the human diet for more than 5000 years.

In the 2004/2005 marketing year, 229 million metric tons of soybeans were produced worldwide. Although the majority of the crop is used for animal feed, approximately 14% is used for human food in the form of traditional soyfoods, e.g. tofu, soymilk, natto, miso, bean sprouts, and as soy protein ingredients used to formulate food products as diverse as infant formula, dairy and meat alternatives, nutritional supplements and energy bars. (Golbitz & Jordan, 2006) Plant and animal hemoglobin proteins are widely consumed in the human diet where they represent a highly bioavailable source of dietary iron for human nutrition. Plant-derived hemoglobins are prevalent in our food system through malted grain products and sprouted beans (pulses).

Regulatory Status

The use of soy proteins is widely accepted in the United States. The US Food and Drug Administration has affirmed the safety of soy protein isolates for inclusion in many products and has approved a health claim for soy protein and the reduced risk of coronary heart disease (21 CFR 101.82). In 2000, the US Department of Agriculture issued a ruling allowing soy protein to completely replace animal protein in the National School Lunch Program (Messina, 2006). The safety of soy protein in human food has been clearly established and affirmed by the two major food regulatory agencies in the US.

Repeated Dose (28-Day) Toxicity Studies in CRL- Sprague-Dawley-CD® IGS Rats

Two studies were conducted by Product Safety Laboratories (Dayton, NJ) consistent with OECD GLP Guidelines and with OECD Guidelines 407 and FDA'S Red Book for the initial study (43166) and with OECD Guideline 421 for the follow-up study (44856). Experimental and environmental conditions were the same including doses of LegH Prep (0, 250, 500, 750 mg soy leghemoglobin protein/kg bw/day), mode of administration (dietary admixture) and species and strain of rats.

In the initial study, although there were no consistent, dose-dependent, statistically significant treatment-related adverse effects reported, a NOAEL was not determined since potential perturbations on estrous cyclicity were reported at the low and high dose but not the middle dose. This lack of a dose-response suggested these effects were not treatment-related. Upon the advice of its scientific consults and as part of its products stewardship program, Impossible Foods secured the services of Dr. Karen Regan, an internationally recognized expert in reproductive toxicology, to review the results of this study and to assist in the design and execution of a follow up study, if deemed advisable. It was determined that OECD Guidelines for evaluating estrous activity (OECD 421) were most appropriate. The study was successfully executed. Dr. Regan independently completed her critical evaluation of the data and then reviewed her findings with the study pathologist who examined the tissues from the initial study. Dr. Regan and Product Safety Labs concluded that there were no test-substance related effects on reproductive macroscopic and microscopic parameters/observations, reproductive organ weights or estrous cyclicity in either study. The NOAEL was determined to be the highest dose tested, 750 mg soy leghemoglobin protein/kg bw/day. The Expert Panel concurs with these conclusions, there are no LegH Prep-related adverse effects on rat estrous cyclicity and the NOAEL is 750 mg soy leghemoglobin protein/kg bw/day.

Allergenicity

Soybeans are acknowledged as a commonly allergenic food. Soybeans contain several allergenic proteins (Taylor, Panda, Goodman, & Baumert, 2014). Soy leghemoglobin is not identified among the known soybean allergens. Moreover, soy leghemoglobin is expressed in the root nodules of the soy plant rather than the bean. The potential allergenicity of soy leghemoglobin and the most abundant *Pichia pastoris* proteins (17 in total) present within LegH Prep can be assessed in the same manner as used for the novel proteins expressed in genetically engineered foods. The Codex Alimentarius Commission developed an assessment scheme for the analysis of potential allergenicity of proteins derived from biotechnology (Codex Alimentarius, 2003). This assessment is a multi-factorial approach which includes assessing the source of the protein for allergenicity, the sequence homology of the protein to known allergens, resistance to pepsin degradation and, if there is a high suspicion of allergenicity, specific serum screening. This analysis provides a likelihood of allergenic response by considering the totality of the evidence.

In its search of the biomedical literature, Impossible Foods did not find any publications implicating soy leghemoglobin or the 17 Pichia proteins in allergenicity or toxicity. Impossible Foods then enlisted Dr. Richard E. Goodman at the Food Allergy Resource and Research Program (FARRP) of the University of Nebraska to assess the potential allergenicity and toxicity of LegH Prep. Dr. Goodman conducted a comprehensive search of the biomedical literature to identify any published reports regarding possible allergenicity or toxicity associated with leghemoglobin and the Pichia proteins and any reports regarding health issues associated with human consumption. No negative reports were found.

Bioinformatics searches (amino acid sequence comparisons) were performed comparing the known sequence of soy leghemoglobin (GI:126241) and the 17 Pichai protein with known or putative allergens listed in the AllergenOnline.org, version 16 database using both FASTA full-length sequence alignments and search for 80 amino acid matches along the entire sequence looking for >35% identity. No significant alignments were found with soy leghemoglobin. Bioinformatics searches with the 17 most abundant residual *Pichia pastoris* proteins found in LegH Prep identified a few related protein sequences with sufficient similarity to exceed the Codex suggestion for potential cross reactivity (>35%). However, the sequence-related putative allergens identified in this search were not potent, common allergens, nor were any of them known to be allergenic when ingested. Moreover, comparison of the same *Pichia pastoris* proteins with all proteins in the NCBI Protein database identified far more significant matches to proteins found in commonly consumed fungi, including baker's yeast (*Saccharomyces* species). Thus, the bioinformatics searches did not reveal any similarities of concern between soy leghemoglobin and the 17 Pichia proteins and known allergens.

Dr. Goodman also tested the stability of LegH Prep in a model simulated gastric digestion study using the conditions recommended by Ofori-Anti et al. (Ofori-Anti, Ariyarathna, Chen, Lee, Pramod, & Goodman, 2008) to evaluate the pepsin stability of novel proteins in genetically modified crops. A positive correlation exists between the stability of abundant dietary proteins in this assay and the likelihood that they will be identified as food allergens. LegH Prep was very rapidly digested by pepsin (90% in less than 2 min). No stable protein fragments were detected either. On the basis of resistance to pepsin digestion, LegH Prep shows a low potential risk of allergenicity or toxicity.

Dr. Goodman stated "My conclusion from this "weight of evidence" approach to dietary protein safety is that the LegH Prep is very unlikely to present a risk of dietary allergy or toxicity to consumers."

Conclusions

We, members of the Expert Panel, have individually and collectively critically evaluated the information and data summarized above and other information deemed pertinent to the safety of the proposed uses of Soy Leghemoglobin Protein Preparation (LegH Prep). We unanimously conclude that the proposed uses as a protein component in ground beef replacement (analogue) products of LegH Prep, produced consistent with current Good Manufacturing Practice (cGMP) and meeting the appropriate food grade specifications presented above, are safe and suitable.

We unanimously conclude that the proposed uses as a protein component in ground beef replacement (analogue) products of LegH Prep, produced consistent with current Good Manufacturing Practice (cGMP) and meeting the food grade specifications presented above, are Generally Recognized As Safe (GRAS) based on scientific procedures.

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

(b) (6) [redacted]

09 August 2017
Date

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(b) (6) [redacted]

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