GRAS Notice (GRN) No. 496
http://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/default.htm

ORIGINAL SUBMISSION

SPHERIX consulting ChromaDex

GRN 000496

December 18, 2013

RECEIVED
DEC 19
Division of Biotechnology and GRAS Notice Review

Office of Food Additive Safety
HFS-255
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740

To Whom It May Concern:

Enclosed please find three copies of the dossier entitled "Generally Recognized As Safe (GRAS) Notification for the Use of Aqueous Broccoli Seed Extract Powder in Food," accompanying appendices, and GRAS Panel Consensus Statement. We have prepared these documents for Brassica Protection Products LLC.

The data and information that serve as the basis for this GRAS determination is available for review and copying at reasonable times at the office of Claire L. Kruger, Ph.D., D.A.B.T., President, Spherix Consulting, Inc., 11900 Parklawn Drive, Suite 200, Rockville, MD 20852, Telephone: 301-897-0611; Facsimile: 301-897-2567; Email: clairek@chromadex.com, or will be sent to FDA upon request.

Should you have any questions or concerns, please contact me at the number listed above.

Sincerely,

(b) (6)

Claire L, Kruger, Ph.D., D.A.B.T.
President

Enclosures:

Three copies of the dossier entitled "Generally Recognized As Safe (GRAS) Notification for the Use of Aqueous Broccoli Seed Extract Powder in Food" and accompanying Appendices

Three copies of the GRAS Panel Consensus Statement for the above-referenced GRAS Notification

11900 Parklawn Drive, Suite 200
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Generally Recognized As Safe (GRAS) Notification for the Use of Aqueous Broccoli Seed Extract Powder in Food

Prepared for:

Brassica Protection Products LLC
250 S. President Street, Suite 2000
Baltimore, MD 21202

Prepared by:

Spherix Consulting, Inc.
A Division of ChromaDex, Inc.
11900 Parklawn Drive, Suite 200
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July 10, 2013

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I. GRAS EXEMPTION CLAIM

A. Name and Address of Sponsor

Brassica Protection Products LLC
250 South President Street, Suite 2000
Baltimore, Maryland 21202 USA
Contact: Antony Talalay
atalalay@brassica.com
(410) 732-1200

B. Common or Usual Name of GRAS Substance

The substance that is the subject of this Generally Recognized As Safe (GRAS) determination is the concentrated, spray-dried aqueous extract of *Brassica oleracea* var. *italica* Plenck (Brassicaceae) seeds.

C. Intended Use

The spray-dried, concentrated aqueous extract of *Brassica oleracea* var. *italica* Plenck (Brassicaceae) seeds from Brassica Protection Products (hereinafter, "aqueous broccoli seed extract powder") is intended to be used as an ingredient in teas, energy drinks, thirst quenching drinks, and non-carbonated flavored waters.

D. Basis for GRAS Determination

This GRAS determination for the use of aqueous broccoli seed extract powder as an ingredient in selected foods and beverages as described in Section C of this chapter is based upon scientific procedures as described under 21 CFR §170.30(b). The intake of aqueous broccoli seed extract powder from the intended uses specified above, has been shown to be safe and GRAS, using scientific procedures, under the Federal Food, Drug, and Cosmetic Act (FFDCA), Section 201(s). To demonstrate that aqueous broccoli seed extract powder is safe, and GRAS, under the intended conditions of use, the safety of the intake of aqueous broccoli seed extract powder has been determined to be GRAS by demonstrating that the safety of this level of intake is generally recognized by experts qualified by both scientific training and experience to evaluate the safety of substances directly added to food, and is based on generally available and accepted information.

The proposed use of aqueous broccoli seed extract powder as an ingredient in foods has been determined to be safe through scientific procedures set forth under 21 CFR §170.30(b) based on the following:

- The aqueous broccoli seed extract powder is produced from non-genetically modified broccoli seed, complies with food-grade product specifications, and is standardized to a glucoraphanin content of 13-20%, w/w.
- The Class 1 glucosinolates glucoraphanin and glucoerucin account for 99.6 % of the glucosinolate fraction of five commercial lots of the aqueous broccoli seed extract powder. The Class 2 (sinigrin, gluconapin, epigoitrin and progoitrin), Class 3 (gluconasturtiin), and Class 4 (glucobrassicin) glucosinolates comprise the remaining 0.4%.
- Analyses of multiple production lots of aqueous broccoli seed extract powder document appropriate process control of contaminants (microbes, pesticides, and heavy metals).
- The proposed level of addition of aqueous broccoli seed extract powder to teas, energy drinks, thirst quenching drinks and non-carbonated flavored waters is 115 mg/serving. Using the National Center for Health Statistics' 2009-2010 National Health and Nutrition Examination Surveys data, 36.5% of the total U.S. population was identified as consumers of these beverages. An Estimated Daily Intake (EDI) calculation of the aqueous broccoli seed extract powder from its proposed uses indicates that the mean intake of the extract is 283 mg/person per day or 4.5 mg/kg body weight per day. The heavy consumer (90th percentile, all-user) intake is 554 mg/person per day or 8.8 mg/kg body weight per day.
- At a typical content of 13 – 22% Class 1 glucosinolates (13-20% glucoraphanin, plus up to 2% glucoerucin) in the commercial aqueous broccoli seed extract powder, the mean intake of these glucosinolates would range between 37 – 62 mg/day (approximately 1 serving cooked broccoli) and the 90th percentile glucosinolate intake would range between 72 – 122 mg/day (approximately 3 servings cooked broccoli).
- Estimates of intake for broccoli and other *Brassica* vegetables were derived using numerous food consumption surveys. Analyses indicate that the average and heavy broccoli consumers ingest 16.4 and 50.9 g/day, respectively. For reference, one serving of raw broccoli is 36 g and one serving of cooked broccoli is 78 g.
- Raw broccoli is estimated to contain 85 mg total glucosinolates/100 g and cooked broccoli is estimated to contain 51 mg total glucosinolates/100 g. Therefore, usual

glucosinolate intake based on broccoli and/or other *Brassica* vegetable consumption is in the range of 40–50 mg glucosinolates/day.

- Regulatory guidance from various authorities in the U.S. and abroad recommends the consumption of 5-10 servings of vegetables per day.
- The proposed use of aqueous broccoli seed extract powder results in mean consumer intake of glucosinolates at a level that is consistent with background consumption from broccoli. Heavy beverage consumers would ingest the amount of glucosinolates present in three servings of cooked broccoli per day. Therefore, these amounts of glucosinolates from the proposed uses are determined to be safe because they are equivalent to or less than exposure from normal diet and consistent with global authoritative dietary recommendations.
- The corroboration of safety of these levels of intake is provided by a critical review of human and animal studies documenting the metabolic fate, physiologic activity and lack of adverse health effects associated with ingestion of levels of glucosinolates exceeding the proposed levels of ingestion.

The GRAS status of aqueous broccoli seed extract powder (compliant with the established food grade specifications for aqueous broccoli seed extract powder), under the intended conditions of use proposed by Brassica Protection Products, LLC, has been made through the deliberations of Thomas E. Sox, Ph.D., J.D.; Andrew Wallace Hayes, Ph.D., D.A.B.T.; and Roger Clemens, Dr.PH. These individuals are qualified by scientific training and experience to evaluate the safety of food and food ingredients. These experts have carefully reviewed and evaluated the publicly available information summarized in this document, including the safety of aqueous broccoli seed extract powder and the potential human exposure to broccoli seed extract resulting from its intended use as an ingredient in teas, energy drinks, thirst quenching drinks, and non-carbonated flavored waters, and have concluded:

> *There is no evidence in the available information on aqueous broccoli seed extract powder that demonstrates, or suggests reasonable grounds to suspect, a hazard to the public when aqueous broccoli seed extract powder is used at the intended use levels in the proposed applications. Aqueous broccoli seed extract powder is GRAS for use in selected foods as proposed by Brassica Protection Products LLC.*

Therefore, aqueous broccoli seed extract powder is safe and is GRAS at the proposed levels of ingestion. Aqueous broccoli seed extract powder is, therefore, excluded from the definition of a food additive, and may be used in the U.S. without the promulgation of a food additive regulation by the FDA under 21 CFR.

E. Availability of Information

The data and information that serve as the basis for this GRAS determination will be available for review and copying at reasonable times at the office of Claire L. Kruger, Ph.D., D.A.B.T., President, Spherix Consulting, A Division of ChromaDex, Inc., at 11900 Parklawn Drive, Suite 200, Rockville, MD 20852. Telephone: 240-565-5501; Facsimile: 240- 621-7549; Email: clairek@chromadex.com, or be sent to FDA upon request.

F. Signature

Pursuant to the criteria provided in proposed 21 CFR 170.36, Brassica Protection Products LLC hereby determines that the use of aqueous broccoli seed extract powder in food under the intended conditions of use is exempt from the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act, because Brassica Protection Products LLC has determined that such use is Generally Recognized As Safe through scientific procedures.

(b) (6)

Signature
Antony Talalay, Authorized Representative of
Brassica Protection Products LLC

July 10, 2013
Date

000012

II. DESCRIPTION OF SUBSTANCE

A. Identity

1. Chemical Name and Identity

The substance that is the subject of this GRAS Determination is a hot water extract of *Brassica oleraceae* var. *italica* Plenck (Brassicaceae) seeds that is concentrated and spray dried with maltodextrin and silicon dioxide. The broccoli seeds are non-genetically-modified, are grown in the state of California, and are chemically selected for their specific glucosinolate content. The seeds are shipped to Canada in virgin totes for processing at a food-grade facility, where they are tested for gross microbiological content upon arrival and then stored in their original bulk sacks at ambient temperature until extraction. Seeds are subjected to hot water extraction and the resulting extract is concentrated and spray dried to a powder. The main constituents of the commercial aqueous broccoli seed extract powder are maltodextrin, glucoraphanin, protein, ash, moisture, silicon dioxide, glucoerucin, plus a small amount (≤ 0.2%, w/w) of fat, and traces of other glucosinolates.

Glucosinolates are a structural class of plant secondary metabolites that are characteristic of the members of the family Brassicaceae, whose members include cruciferous vegetables such as broccoli, cabbage, watercress, Brussels sprouts, cauliflower, turnip, mustard, radish, and horseradish. The structural subtypes and distribution of glucosinolates in plants, including broccoli, have been discussed in the literature (Fahey, et al. 2001 "The chemical diversity..."). Major classes of glucosinolates can be grouped into 4 categories according to their sidechain ("R-") group which consists of either an aliphatic group (Class 1), an allyl (unsaturated) group (Class 2), an aromatic (*e.g.*, phenylethyl) group (Class 3) or an indole group (Class 4) (See Figures 1 and 2 for general and specific molecular structures). The major glucosinolates in broccoli florets are glucoraphanin, glucobrassicin, gluconasturtiin and glucoiberin (Goodrich, et al. 1989; Song and Thornalley 2007), whereas the major glucosinolates in the aqueous extract of broccoli seed are glucoraphanin and glucoerucin. In the Goodrich and Song studies, glucoerucin was not analyzed for; therefore, it is likely that glucoerucin also occurs in broccoli florets.

Five lots of the aqueous broccoli seed extract powder that is the subject of the current GRAS determination were analyzed for glucosinolate content and it was demonstrated that the glucosinolate fraction was comprised of >99% Class 1 glucosinolates (including glucoraphanin and glucoerucin) with ≤ 0.4% of the glucosinolate content represented as Class 2, 3, and 4 glucosinolates (Table 1). Class 2 glucosinolates were detected at trace amounts that fell below their reporting limits, provided in parentheses: sinigrin (< 0.0056%, w/w), gluconapin (<

0.0061%, w/w), epiprogoitrin (< 0.0044%, w/w), progoitrin (< 0.0055%, w/w). Class 3 glucosinolates were present at very low levels: gluconasturtiin (0.02-0.04%, w/w). Class 4 glucosinolates were detected below their reporting limit, as well: glucobrassicin (< 0.0064%, w/w). See Table 2 for chemical structures of the major glucosinolate components of the aqueous broccoli seed extract powder.

The non-glucosinolate portion of the extract powder contains maltodextrin, ash, protein, moisture, silicon dioxide, and trace amounts of fiber and oil (see Tables 5 and 6). Based on a mass spectrometry-based screening analysis of the extract powder, other constituents determined to possibly be present, but which were not quantitated, include: small organic acids (*e.g.*, citric acid; malic acid; malonic acid; methylmalonic acid; pantothenic acid; mesaconic acid; *alpha*-hydroxyglutaric acid), low molecular weight phenolics (*e.g.*, 4-hydroxybenzoic acid/salicylic acid; gentisic acid/protocatechuic acid), simple sugars (*e.g.*, glucuronic acid; *D*-glucose), and substances derived from maltodextrin (*e.g.*, mannoheptulose; gluconic acid).

2. Common or Trade Names

The concentrated, spray-dried aqueous broccoli seed extract powder described in this GRAS determination is manufactured by Brassica Protection Products, LLC and sold world-wide under the name "SGS Broccoli Seed Extract Powder."

3. CAS Registry Number

Aqueous broccoli seed extract powder does not have a Chemical Abstracts Registry Number (CAS No.). Table 1 presents the CAS Nos., where available, for the principal characteristic components of SGS Broccoli Seed Extract Powder.

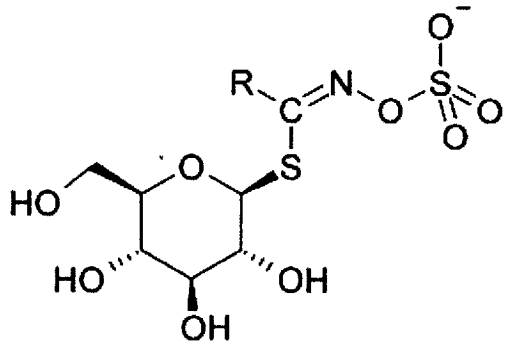

Figure 1. Generalized Structure for Glucosinolates

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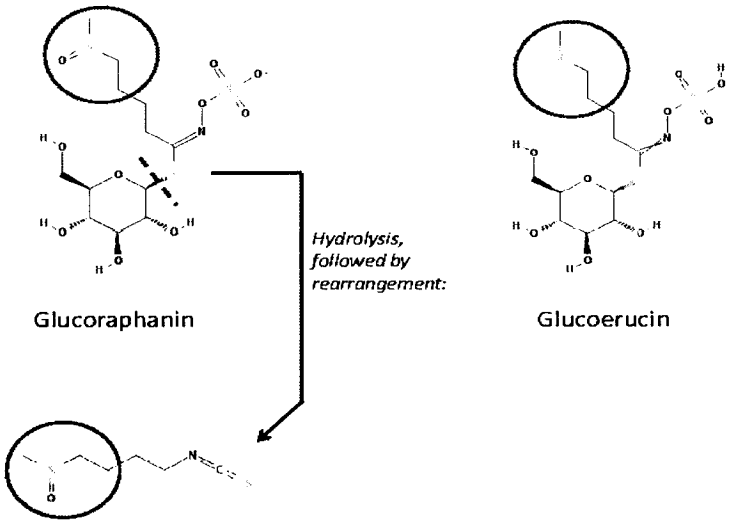
1. Glucosinolates which generate an aliphatic isothiocyanate <u>without</u> a terminal double bond

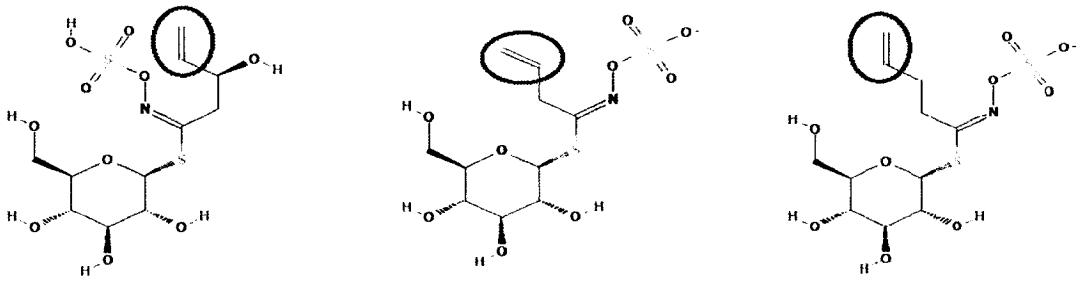


Glucoraphanin

Glucoerucin

Sulforaphane (isothiocyanate)

2. Glucosinolates which generate an isothiocyanate containing a terminal double bond which is electrophilic



Progoitrin

Sinigrin

Gluconapin

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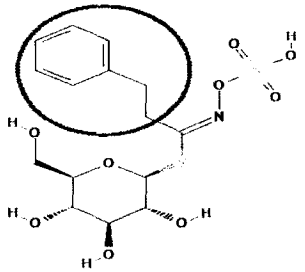
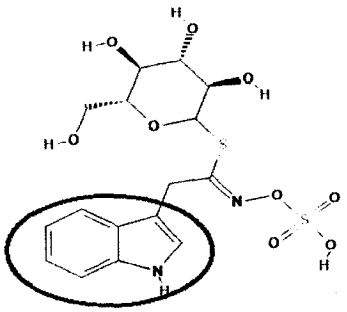
3. Glucosinolates which generate a phenylethyl isothiocyanate



Gluconasturtiin

4. Glucosinolates which generate an indole-containing isothiocyanate



Glucobrassicin

Figure 2. Molecular Structures for the Four Major Classes of Glucosinolates in Cruciferous Plants Used as Human Foods
Aliphatic or alkyl (Class 1), Allyl (Class 2), Phenylethyl (Class 3) and Indole (Class 4)

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Table 1. Characteristic Glucosinolate Components of Aqueous Broccoli Seed Extract Powder

Component	Chemical Abstracts Services Registry Number (CAS No.)	Percent in final product (%, w/w)[1]
Glucoraphanin	21414-41-5 (for the potassium salt)	11.14-14.10
Glucoerucin	21973-56-8 (for the potassium salt)	1.26-2.00
Glucoiberin	554-88-1 (for the potassium salt)	0.14-0.19
Gluconasturtiin	499-30-9 (for the potassium salt)	0.02-0.04
A hexyl glucosinolate (either *n*-hexyl-, 3-methylpentyl- or 4-methylpentylglucosinolate)	Not found	0.01-0.05

[1] Note that the method used to quantitate specific glucosinolates as reported here differs slightly from that used to report the glucoraphanin content which is reported on the Certificates of Analysis for the aqueous broccoli seed extract powder (Wade, et al. 2007). Regarding the results reported in this table, there may have been some minor analyte loss due to sample preparation clean up steps.

Table 2. Aqueous Broccoli Seed Extract Powder Principal Glucosinolate Components and Structures

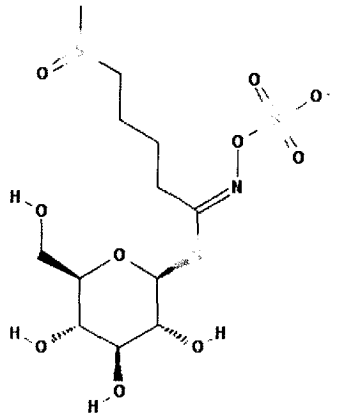
Chemical name	Structure
Glucoraphanin	

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Table 2. Aqueous Broccoli Seed Extract Powder Principal Glucosinolate Components and Structures

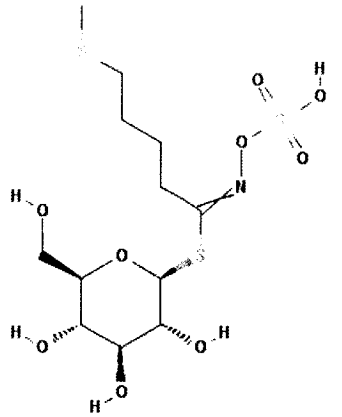
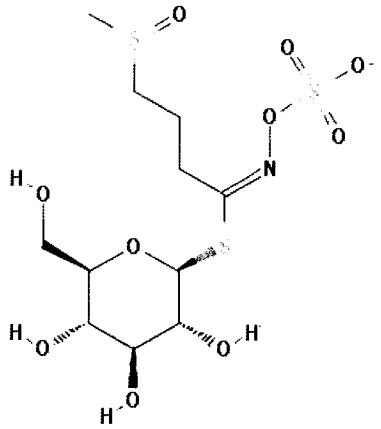
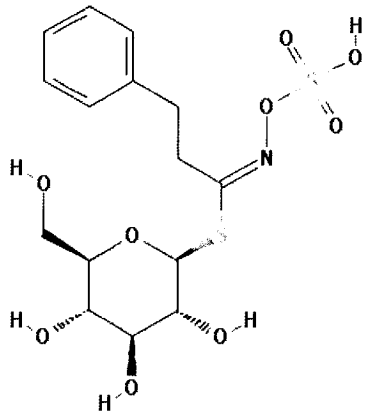
Chemical name	Structure
Glucoerucin	
Glucoiberin	
Gluconasturtiin	

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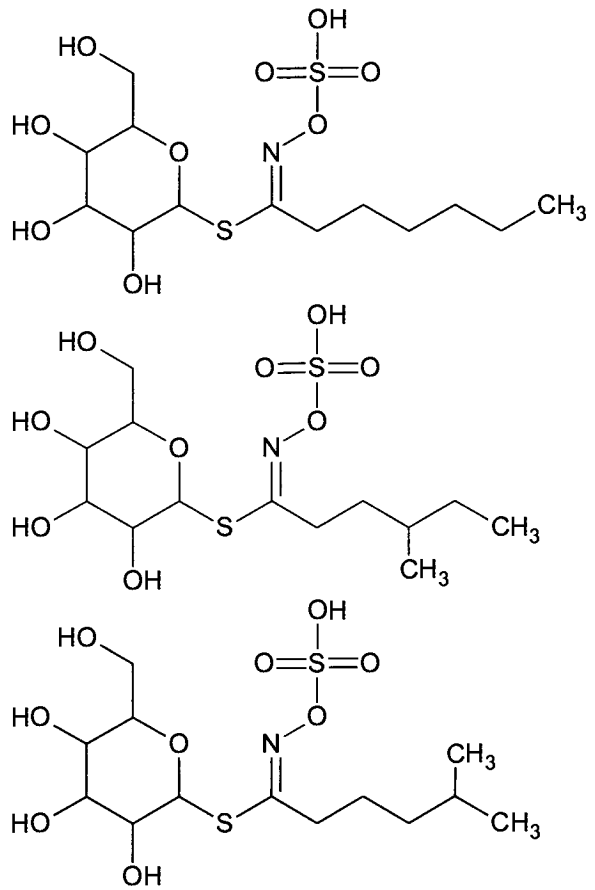
Table 2. Aqueous Broccoli Seed Extract Powder Principal Glucosinolate Components and Structures

Chemical name	Structure
Hexyl glucosinolate (either *n*-hexyl-, 3-methylpentyl- or 4-methylpentylglucosinolate)	

4. Composition

"SGS Broccoli Seed Extract Powder" is the trade name for the concentrated, spray dried aqueous extract of the seeds of *Brassica oleraceae* var. *italica* (Brassicaceae). The extract is standardized, for commercial purposes, to a minimum of 13% glucoraphanin content as reported on the Certificate of Analysis using the analytical method of Wade and co-workers (2007). In their analyses, ChromaDex (Table 5) also followed the Wade et al. method. In addition, they carried out specific sample clean-up steps that were intended to reduce the concentration of maltodextrin in the sample to be injected, in order to improve the sensitivity of the analysis for

non-maltodextrin components. However, these clean-up steps may also have reduced the glucoraphanin content slightly.

B. Physical and Chemical Properties

The physical and chemical properties of the aqueous broccoli seed extract powder are presented in Table 3.

Table 3. Aqueous Broccoli Seed Extract Powder Physical and Chemical Properties

Property or Characteristic	Value
Description	Powder extract from broccoli seeds
Latin binomial	*Brassica oleraceae* var. *italica* Plenck (Brassicaceae)
Country of origin	Seeds grown in the United States; processed in Canada
Particle size	98% through 40-mesh US
Appearance	Off-white to tan powder, with natural variations
Odor	Mild and somewhat characteristic of broccoli
Moisture content	Maximum 8%, w/w

C. Production Process

1. Raw Material Identity

Seed suppliers for the *Brassica oleraceae* var. *italica* Plenck (Brassicaceae) (broccoli) seed that is used in the production of the aqueous extract are located in California, USA. The broccoli seed is produced from cultivated plants of controlled stock that are selected for their specific glucosinolate content. The original seed stock was selected from commercially available seed which exhibited desired performance, composition and quality characteristics. The current year's field that is designated for seed stock provision for the following year is isolated from other *Brassica* crops to prevent cross-pollination. Fields are manually inspected and any off-type plants are physically removed during the growth period. Harvesting procedures prevent stock seed from being mixed with non-stock seed during harvest, transport, cleaning and packaging.

The broccoli seed has been independently tested for "% GMO in a 'nonGMO' sample" by Biogenetic Services, Inc.; however, the test method and endpoint measured was not stated. There are no known commercial sources of GMO broccoli at this time.

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Brassica Protection Products LLC states that the broccoli seeds used in the manufacture of the SGS-brand aqueous broccoli seed extract powder are neither irradiated nor treated with ethylene oxide.

2. Agricultural Practices

As stated above, the broccoli seeds are non-genetically-modified, are grown in the state of California, are open-pollenated, and are chemically selected for their specific glucosinolate content. The growers and processor of the broccoli seeds are required by Brassica Protection Products to conform to the standards and practices established by the California Department of Food and Agriculture (CDFA). The farming operations are monitored by the Inspection and Compliance branch of the CDFA, which insures that the crops produced conform to the applicable standards established by the state. These inspections include evaluation of the agricultural and handling practices, chemical analysis in support of food and environmental safety requirements and the assurance that any fertilizers used meet the quality assured by the fertilizer manufacturer. Brassica Protection Products selects its seed suppliers based on their compliance with applicable California Agricultural board practices. Proximity of broccoli plants to other *Brassica* species in adjacent fields is also a criterion for individual field selection.

Harvest time is determined based on the dryness of the majority of the seeds present in the field and also on the optimum yield of seed. During harvest, the fields are cut in swaths. The cut stems are allowed to sit for a few days to dry. Then the seeds are separated from the stems and placed into virgin totes. Totes are transported via truck to an area where seeds are separated out from stems, insects, and any other unwanted material. This is the conditioning step. Seeds are transferred to new virgin totes and are then shipped off to the manufacturer in Canada. The Canadian facility is food-grade and when the seeds arrive they are tested for gross microbiological content and are then stored in their original bulk sacks in a dry warehouse at ambient temperature until extraction.

3. Manufacturing Facility

The aqueous broccoli seed extract powder is produced by Innovation Place Bioprocessing Center, located at 107 Research Drive, Saskatoon, Saskatchewan, S7N 3R2, Canada. This facility holds site license number 30084 from Health Canada, is authorized for manufacturing and is in compliance with GMP requirements outlined in Part 3 of the Natural Health Products Regulations of Canada. The facility has Kosher certification for co-packing extracts for other companies (UKD-ID OUV2-2AD89C6; Pareve, OU).

4. Manufacturing Process

See Figure 3 for a flow diagram of the manufacturing process for the production of aqueous broccoli seed extract powder.

First, the broccoli seeds are fed into a food-grade tank containing municipal hot water, or the second wash (also using municipal water) resulting from the immediately preceding batch, at not less than 95°C. The mixture is then held at or above 95°C for an extended period. This critical step not only reduces microbial load, but also inactivates the endogenous myrosinase enzyme (Rungapamestry, et al. 2006; Van Eylen, et al. 2008) which converts glucosinolates to their respective isothiocyanates (*e.g.*, glucoraphanin to sulforaphane). After the hot water extraction, the mixture is cooled and then separated into liquid extract and wet seed by filtering through a food-grade stainless steel screen. The liquid is cooled to 70 ± 5 °C and this temperature is maintained until addition of silicon dioxide and maltodextrin at a later step, in preparation for spray-drying. This cooling step is a critical control point. The wet seed is then subjected to a second hot water wash. As mentioned above, this second wash may be recycled and used as the primary extractant of the immediately following batch of seed. At the end of the run, any remaining final wash is discarded.

The liquid is decolorized/deodorized using activated charcoal and then centrifuged and filtered through diatomaceous earth. The spent charcoal is discarded. The filtered extract is then concentrated. This is a critical control step. The concentrated broccoli seed aqueous extract is normalized for solids content and then silicon dioxide is added at not more than 2%, w/w, in addition to maltodextrin.

The pH of the concentrated extract with added silicon dioxide and maltodextrin is measured and recorded, and then the liquid is spray dried. Spray-drying is a critical control step. One hour after startup an oven moisture analysis is carried out to determine product moisture. The dryer parameters are adjusted to maintain product moisture within specifications. Every three hours an oven solids analysis is carried out to confirm the automatic moisture readings.

The final, formulated spray dried broccoli seed aqueous extract is handled by personnel wearing masks, clean sleeves and fresh gloves during the packing process. The product is packed into food-grade drums (double-lined with food-grade polyethylene bags) (see Appendices P7 and 8 which are labeled prior to filling. Inclusion of a dessicant pack is necessary due to the hygroscopic nature of the final product. The outer bag is sealed and the drum lid is sealed using tamper evident seals. All steps in the packaging and sealing process are documented.

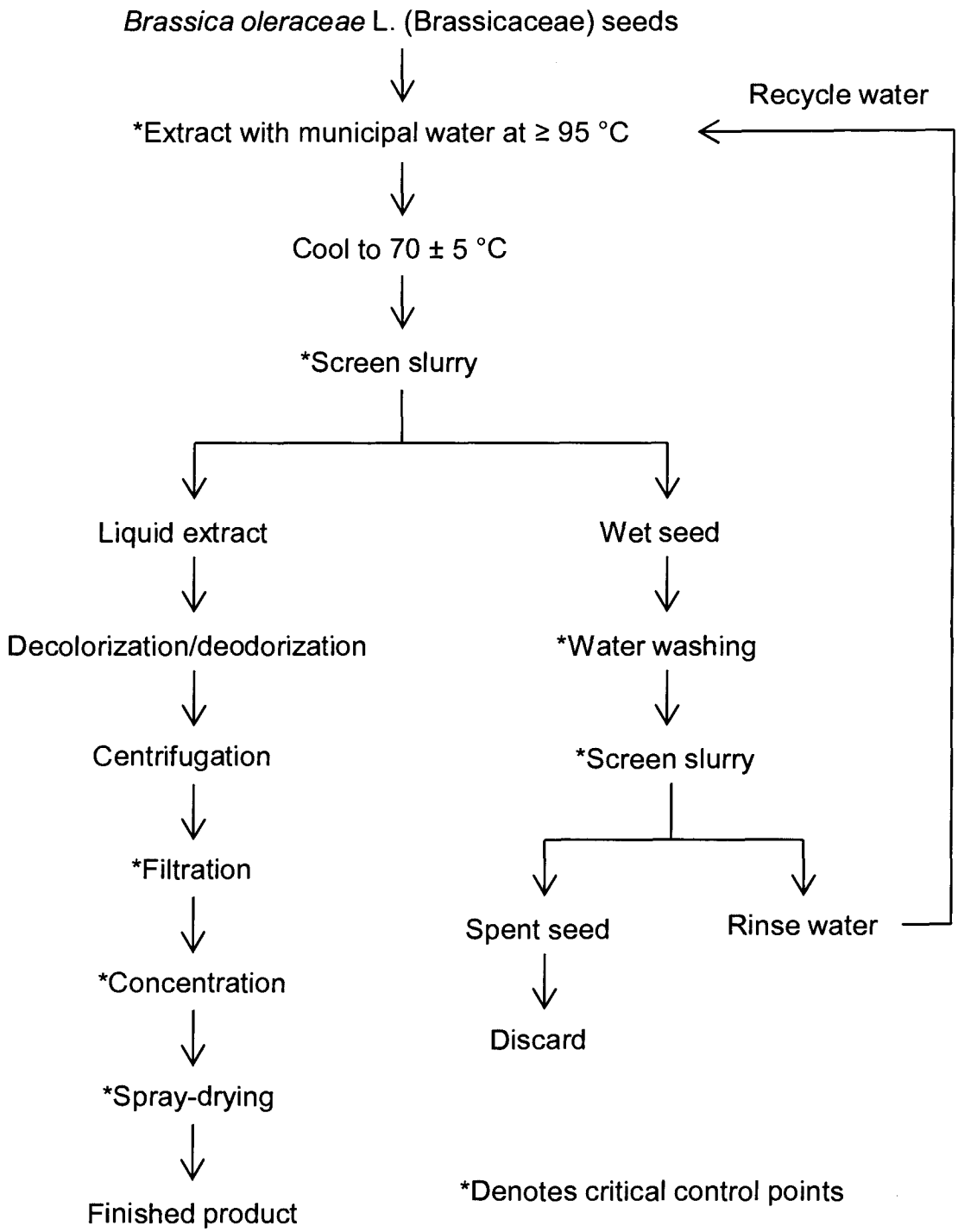

Figure 3. Process Flow Summary for the Production of the Aqueous Broccoli Seed Extract Powder

5. Hazard Analysis and Critical Control Points (HACCP) Plan

There is a HACCP plan in place.

6. Definition of Batch and Lot

Numerous subunits of material (internally referred to as batches) comprise the entirety of a run's production. A single lot is typically comprised of 7-16 batches. Lots can be traced back to a specific field or fields.

7. Nonconforming Material

In the event a batch of aqueous broccoli seed extract powder is demonstrated to be nonconforming with its specifications or is atypical according to usual production data, a nonconformance report form shall be used. For example, when there is excessive product loss (*e.g.*, more than expected for regular processing), product is contaminated, product is produced that does not meet specification, or if there is a product complaint. If the nonconformance has produced off-specification product that has been packaged, the packaged material shall be segregated from acceptable product. The original nonconformance report shall be forwarded to the Quality Assurance department of the Bioprocessing Center and an investigative review of the rejected material will occur. Determinations that are made as a result of the review may be implemented. Reworking of out-of-specification material is handled on a case-by-case basis; when this occurs, the resultant material is intended for human consumption and final product must conform to the food-grade specifications that are presented in Table 4. Material that does not meet the specification for glucoraphanin content may be reworked by blending with material that is high in glucoraphanin *as long as the other line items on the specification are met in all batches being blended, and appropriate documentation of the process is carried out.*

8. Processing Aids and Water Quality

The processing aids used in the manufacture of the final spray dried aqueous broccoli seed extract powder are appropriate for food manufacturers and are either established as GRAS substances, or are commonly used in food production and/or conform to the Food and Chemical Codex (FCC) standards. In particular, diatomaceous earth is GRAS as per 21CFR §573.340 and GRN 87, and conforms to Food and Chemical Codex (FCC) monograph standards; silicon dioxide is GRAS as per 21 CFR §172.480 and GRN 321; activated charcoal conforms to FCC monograph standards; and maltodextrin is GRAS as per 21 CFR §184.1444 and conforms to FCC monograph standards.

The water used to produce the aqueous broccoli seed extract powder is municipal water that meets Canadian public drinking water standards.

9. Final Product Certification and Statements of Assurance

The SGS Broccoli Seed Extract (aqueous broccoli seed extract powder) has a letter of Kosher certification from the Orthodox Union of America.

The final aqueous broccoli seed extract powder contains up to 11% protein, w/w. Regarding content of potential allergenic proteins, broccoli is not one of the major eight allergens requiring labeling in the U.S. (soy, wheat, egg, crustacean shellfish, milk or other dairy, fish, peanuts and tree nuts); nor is there any contact with these eight specific substances, or any animal-source ingredients, during or after the manufacturing process. However, some reports have documented clinical hypersensitivity to various *Brassica* species (Blaiss, et al. 1987; Hernández, et al. 2005; Vovolis, et al. 2009), especially mustard (Caballero, et al. 2002; Figueroa, et al. 2005; Rance and Dutau 1997; Vidal, et al. 1991) and rape (canola) (Poikonen, et al. 2006; Poikonen, et al. 2008); in some cases, symptoms were due to agricultural-based exposures that occurred while harvesting the crop (Suh, et al. 1998). The incidence of mustard allergy in children in a France-based study is reported to be 1.1% (Morisset, et al. 2003). The incidence of canola seed hypersensitivity in a UK-based study is estimated at 2% (Trinidade, et al. 2010). Broccoli allergy is also reported, albeit less frequently, and population-based occurrence rates are unavailable (Dölle, et al. 2013; Liao, et al. 2013). The aqueous broccoli seed extract powder will be labeled in such a way that consumers are alerted to the fact that it is derived from broccoli seed.

D. Production Characterization

1. Product Specifications and Batch Analysis Results

Product specifications for the aqueous broccoli seed extract powder are presented in Table 4.

Batch analysis results on the specific glucosinolate and moisture content of commercial lots of aqueous broccoli seed extract powder are provided in Table 5. Results from proximate and other analyses are provided in Table 6. Some additional analyses on single lots are included for reference. Regarding composite samples that are reported on in Table 6, these samples were generated using the sampling plan methodology that is outlined in Appendix P10. Table 7 reports the summary content of the broccoli seed aqueous extract and includes the ranges of components reported in Tables 5 and 6.

It was noted that the glucoraphanin values reported on the Certificates of Analysis were ~10-23% higher than the values ChromaDex reported in Table 5. ChromaDex carried out the analyses for glucoraphanin and other glucosinolates using the Wade, et al. 2007 method conditions, but specified that they used sonication, precipitation and syringe filtering (PTFE) during sample preparation, which may have caused some loss of analyte(s). Wade, et al. 2007 did not specify how they prepared samples and, therefore, it is likely that other labs following this method may not necessarily have all used the same sample preparation protocols prior to analysis. Also, ChromaDex used an Agilant 1200 Series HPLC system, while Wade and co-workers used Waters instrumentation. Together, these differences may account for the variation that is reported from different labs for the glucoraphanin content of these five analyzed lots.

Erucic acid was a potential undesirable constituent of the aqueous broccoli seed extract powder. However, it was recognized that oils will be minimally soluble in an aqueous extract. Erucic acid was measured in the oil fraction of one batch of aqueous broccoli seed extract. The oil fraction comprises up to ~0.15%, w/w, of the finished product. Erucic acid was found to constitute only 0.74% of the oil fraction (0.15%), amounting to a total erucic acid content of 0.0011% in the final commercial product. This extremely low erucic acid content does not present any concern, considering that the product will be consumed at approximately 115 mg per serving in selected foods. This calculates to a theoretical exposure of 0.00128 mg erucic acid per serving, or between 1-2 μg/serving, which is a negligible amount.

See Table 7 for a summary of the content of the aqueous broccoli seed extract powder. Table 7 is derived from the range of results of components reported on in Tables 5 and 6.

Table 4. Product Specifications for Aqueous Broccoli Seed Extract Powder

Substance	Units of results	Limits	Methods
Glucoraphanin	%, w/w	13.0-20.0%	Wade, et al. 2007
Moisture	%, w/w	< 8.0%	Loss on drying
Heavy metals			
Lead	ppm	< 1.5 ppm	ICP-MS (Appendix C9)
Arsenic	ppm	< 2.0 ppm	ICP-MS (Appendix C9)
Cadmium	ppm	< 0.5 ppm	ICP-MS (Appendix C9)
Mercury	ppm	< 0.5 ppm	ICP-MS (Appendix C9)
Microbiology			
Total aerobic colony count	cfu/g	≤ 3,000 cfu/g	MFHPB-33[1] or MFHPB-18[2]
Yeast and mold	cfu/g	≤ 100 cfu/g	MFHPB-22[3]
Coliforms	cfu/10 g	< 5 cfu/10 g	MFHPB-34[4]
Escherichia coli	cfu/10 g	< 5 cfu/10 g	MFHPB-34[4]
Salmonella spp.	cfu/25 g	Negative/25 g	MFHPB-20[5]
Staphylococcus aureus	cfu/10 g	< 5 cfu/1 g	MFHPB-21[6]

cfu = colony-forming units

[1]MFHPB-33. Government of Canada, Health Products and Food Branch, Ottawa, "Enumeration of total aerobic bacteria in food products and food ingredients using 3M™ Petrifilm™ aerobic count plates," February 2001. http://www.hc-sc.gc.ca/fn-an/res-rech/analy-meth/microbio/volume2/mfhpb33-01-eng.php

[2] MFHPB-18. Government of Canada, Health Products and Food Branch, Ottawa, "Determination of the aerobic colony count in foods," October 2001.

[3] MFHPB-22. Government of Canada, Health Products and Food Branch, Ottawa, "Enumeration of yeasts and moulds in foods," January 2004. http://www.hc-sc.gc.ca/fn-an/res-rech/analy-meth/microbio/volume2/mfhpb22-eng.php

[4]MFHPB-34. Government of Canada, Health Products and Food Branch, Ottawa, "Enumeration of *Escherichia coli* and coliforms in food products and food ingredients using 3M Petrifilm™ *E. coli* count plates," September 2012. http://www.hc-sc.gc.ca/fn-an/res-rech/analy-meth/microbio/volume2/mfhpb34-01-eng.php

[5]MFHPB-20. Government of Canada, Health Products and Food Branch, Ottawa, "Isolation and identification of *Salmonella* from food and environmental samples," March 2009. http://www.hc-sc.gc.ca/fn-an/res-rech/analy-meth/microbio/volume2/mfhpb20-01-eng.php

[6]MFHPB-21. Government of Canada, Health Products and Food Branch, Ottawa, "Enumeration of *Staphylococcus aureus* in foods," September 2005. http://www.hc-sc.gc.ca/fn-an/res-rech/analy-meth/microbio/volume2/mfhpb21-01-eng.php

Table 5. Glucosinolate Analyses (%, w/w) on Various Lots of Aqueous Broccoli Seed Extract Powder

Component	Method	Lot Number						
		20121009	20120423	20120305	20111121	20110502	20110302	20090120
Glucoraphanin	[1]ChromaDex analysis[†] or [2]Other lab results, as reported on the Certificate of Analysis	[1]12.55 [2]14.7	[1]13.53 [2]14.9	[1]12.12 [2]14.7	[1]14.10 [2]16.5	[1]11.14 [2]13.71	[2]15.75	[2]13.3
Glucoerucin	[1]ChromaDex analysis	1.71	1.80	1.26	2.00	1.64	Not reported	Not reported
Gluconasturtiin		0.03	0.03	0.03	0.04	0.02	Not reported	Not reported
Glucoiberin		0.18	0.17	0.18	0.14	0.19	Not reported	Not reported
Unknown glucosinolate (either n-hexyl-, 3-methylpentyl- or 4-methylpentylglucosinolate)		0.01	0.05	0.02	0.03	0.02	Not reported	Not reported
Sinigrin		< 0.0054	< 0.0056	< 0.0054	< 0.0055	< 0.0054	Not reported	Not reported
Gluconapin		< 0.0059	< 0.0061	< 0.0059	< 0.0059	< 0.0059	Not reported	Not reported
Epiprogoitrin		< 0.0043	< 0.0044	< 0.0043	< 0.0044	< 0.0043	Not reported	Not reported
Progoitrin		< 0.0053	< 0.0055	< 0.0053	< 0.0054	< 0.0053	Not reported	Not reported
Glucobrassicin		< 0.0062	< 0.0064	< 0.0062	< 0.0062	< 0.0062	Not reported	Not reported
Total Glucosinolates[¥] [¥]Calculation uses the glucoraphanin value given in the ChromaDex report.		***14.51***	***15.61***	***13.64***	***16.34***	***13.04***	Not reported	Not reported

[†]ChromaDex carried out these analyses for glucoraphanin and other glucosinolates using the Wade, et al. 2007 method, and specified in the report (not provided) that they used sonication, precipitation and filtering (PTFE) during sample preparation, which may have caused some loss of sample. A different laboratory besides ChromaDex typically analyzes the extract powder for the glucoraphanin content that is reported on the Certificate of Analysis (CoA); this lab also uses the Wade, et al. 2007 method. However, the authors of this publication did not specify how to prepare samples and, therefore, it is likely that each laboratory following this method may not necessarily use the same sample preparation protocols prior to analysis. The values reported by ChromaDex for glucoraphanin content are approximately 10-23% lower than the values which are reported by the other laboratory for the Certificate of Analysis (CoA) of each lot, reflecting the interlaboratory variability in the measurement of glucoraphanin.

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Table 6. Proximate and Other Analyses for Both Single Lots and Composite Samples

Component	Method	Units	Lot Number or Composite						
			20110502	20090121	20090120	Composite of 12 lots (not specified)	Composite of lots 20110221, 20110223, 20110228, and 20110302	Composite of lots 20111121, 20111122, 20111123, 20111128, and 20111129	Composite of lots 20110221, 2011022, 20110228, and 20110302
Crude fiber	AOCS Ba 6-84	%, w/w	< 0.1 (insoluble) 0.75 (soluble)	0.01	<0.01	Not measured	Not measured	< 0.01	0.0
Oil	POS internal method based on Troëng 1955	%, w/w	0.13	0.02	0.02	< 0.5	Not measured	0.14	0.0
Erucic acid	AOAC 969.33 (prep) and AOAC 996.06 (quantification)	%, w/w; as fractional composition of the overall oil content	0.74% of oil; (=0.001% erucic acid in the current batch having 0.13% oil content)	Not measured	Not measured	Not measured	Not measured	Not measured	Not measured
Alpha carotene	AOAC 2005.07	IU/100 g	Not measured	Not measured	Not measured	Not measured	< 0.5 IU/100 g	Not measured	Not measured
Trans-beta carotene			Not measured	Not measured	Not measured	Not measured	< 0.5 IU/100 g	Not measured	Not measured
Cis-beta carotene			Not measured	Not measured	Not measured	Not measured	< 0.5 IU/100 g	Not measured	Not measured
Protein	AOCS Ba 4e-93	%, w/w	10.1	9.54	9.34	9.64	Not measured	9.46	10.7
Ash	AOCS Bc 5-49	%, w/w	12.4	13.8	13.0	12.95	Not measured	11.6	13.9
Calcium	AOAC 990.08C	%, w/w	0.310	Not measured	Not measured	Not measured	Not measured	Not measured	Not measured
Iron	AOAC 990.08C	%, w/w	0.0046	Not measured	Not measured	Not measured	Not measured	Not measured	Not measured
Vitamin C	HL 7018	%, w/w	0.00348	Not measured	Not measured	Not measured	Not measured	Not measured	Not measured
Moisture	AOCS Ba 2a-38	%, w/w	7.04	6.99	6.55	2.86	Not measured	5.79	4.95
Maltodextrin	Addition by weight	% w/w	≤ 35.0	≤ 35.0	≤ 35.0	≤ 35.0	≤ 35.0	≤ 35.0	≤ 35.0
Silicon dioxide	Addition by weight	% w/w	2.0	2.0	2.0	2.0	2.0	2.0	2.0
TOTAL		%, w/w	***67.09***	***67.36***	***65.92***	***62.95***	--	***64.00***	***66.55***

000029

Table 7. Summary of the Content of Aqueous Broccoli Seed Extract Powder	
Component	Weight percent (w/w)
Glucosinolates	13-17
Moisture	≤ 8.0
Maltodextrin	≤ 35.0
Silicon dioxide	≤ 2.0
Oil	≤0.15
Fiber	≤ 0.01
Protein	9-11
Ash	12-14
TOTAL	**79-87%**

2. Non-Glucosinolate Components

The non-glucosinolate portion of the extract powder contains significant amounts of maltodextrin, ash, protein, moisture, and silicon dioxide, plus trace amounts of fiber and oil (see Tables 5-7); although there will be natural variability present from lot-to-lot, these components are anticipated to account for a total of 66-70%, w/w, of the aqueous broccoli seed extract powder. As regards the remaining uncharacterized 13-21% of the extract, additional work involving a mass spectrometry-based screening of the extract indicated that various compound classes were present, such as: small organic acids (*e.g.*, citric acid; malic acid; malonic acid; methylmalonic acid; pantothenic acid; mesaconic acid; *alpha*-hydroxyglutaric acid), low molecular weight phenolics (*e.g.*, 4-hydroxybenzoic acid/salicylic acid; gentisic acid/protocatechuic acid), simple sugars (*e.g.*, glucuronic acid; *D*-glucose), and substances derived from maltodextrin (*e.g.*, mannoheptulose; gluconic acid). The presence of these compound classes are not considered to be of toxicological concern and are ubiquitous in the food supply.

3. Residual Impurities

The aqueous broccoli seed extract powder is tested routinely (on a per lot basis) for lead, cadmium, mercury, total aerobic plate count, yeasts and molds, Coliforms, *Escherichia coli*, *Salmonella* spp., and *Staphylococcus aureus*. Representative results for other impurities are presented in Table 8, and indicate that the production process for the manufacture of aqueous

broccoli seed extract powder reproducibly yields product that meets or exceeds the food-grade specifications for contaminants and impurities that are summarized here.

Regarding pesticides, the broccoli seed is required to come from plants that are farmed under conditions that meet or exceed the standards of the U.S. Department of Agriculture and the California Department of Food & Agriculture. As of August 1, 2011, a pesticide monitoring system was put in place by Brassica Protection Products that annually tests growers' compliance. Two representative lots of aqueous broccoli seed extract powder were subjected to comprehensive pesticide screening for 498 and 474 pesticide residues (20110302 and 20100726), respectively; results are provided in Appendices C12 and C13. All results were negative at the specific Limit of Detection for each pesticide.

Table 8. Contaminant Analyses Results for Aqueous Broccoli Seed Extract Powder

Component	Method	Units	Lot Number or Composite									
			20121009	20120423	20120312	20120305	A composite of 20110207, 20110208, 20110209	20111121	20110502	20110302	20090121	20090120
Lead Limit: < 1.5 ppm	AOAC 999.10 or ICP-MS (internal method; see Appendix C9)	ppm	0.02	0.03	Not measured	0.04	< 1.0	0.01	0.02	0.03	< 0.20	< 0.20
Arsenic Limit: < 2.0 ppm	AOAC 986.15 or ICP-MS (internal method; see Appendix C9)	ppm	0.44	1.0	Not measured	1.7	< 0.5	0.49	0.76	0.23	Not measured	Not measured
Cadmium Limit: < 0.5 ppm	AOAC 999.10 or ICP-MS (internal method; see Appendix C9)	ppm	0.02	0.03	Not measured	0.06	0.30	0.04	0.06	0.05	< 0.03	< 0.03
Mercury Limit: < 0.5 ppm	AOAC 977.15 or ICP-MS (internal method; see Appendix C9)	ppm	<0.005	< 0.005	Not measured	< 0.005	< 0.1	< 0.005	< 0.005	< 0.005	< 0.20	< 0.20
Aflatoxin B1	AOAC 2005.08	ppb	Not measured	Not measured	< 1.0	Not measured	< 0.2	Not measured	Not measured	Not measured	Not measured	Not measured
Aflatoxin B2		ppb	Not measured	Not measured	< 1.0	Not measured	< 0.1	Not measured	Not measured	Not measured	Not measured	Not measured
Aflatoxin G1		ppb	Not measured	Not measured	< 1.0	Not measured	< 0.2	Not measured	Not measured	Not measured	Not measured	Not measured
Aflatoxin G2		ppb	Not measured	Not measured	< 1.0	Not measured	< 0.1	Not measured	Not measured	Not measured	Not measured	Not measured
Total aerobic plate count Limit: ≤ 3000 cfu/g	MFHPB-33 or MFHPB-18	cfu/g	420	280	Not measured	190	Not measured	160	500	970	170	710
Yeast and mold count Limit: ≤ 100 cfu/g	MFHPB-22	cfu/g	Not detected	Not detected	Not measured	Not detected	Not measured	10	< 5	10	10	< 5
Coliforms Limit: < 5 cfu/10 g	MFHPB-34	cfu/10 g	Not detected	Not detected	Not measured	Not detected	Not measured	Not detected	Not detected	Not detected	< 5	< 5

000032

Table 8. Contaminant Analyses Results for Aqueous Broccoli Seed Extract Powder

Component	Method	Units	Lot Number or Composite									
			20121009	20120423	20120312	20120305	A composite of 20110207, 20110208, 20110209	20111121	20110502	20110302	20090121	20090120
Escherichia coli Limit: < 5 cfu/10 g	MFHPB-34	cfu/10 g	Not detected	Not detected	Not measured	Not detected	Not measured	Not detected	Not detected	Not detected	< 5	< 5
Salmonella spp. Limit: Negative/25 g	MFHPB-20	cfu/25 g	Not detected	Not detected	Not measured	Not detected	Not measured	Not detected	Not detected	Not detected	Not detected	--
Staphylococcus aureus Limit: < 5 cfu/10 g	MFHPB-21	cfu/10 g	Not detected	Not detected+	Not measured	Not detected	Not measured	Not detected	Not detected	Not detected	--	--

000033

4. Stability and Shelf Life

The stability of the aqueous broccoli seed extract powder, measured by glucoraphanin content over time, has been established under experimental conditions. The analytical method used to measure glucoraphanin content of samples has been published (Wade, et al. 2007) and samples were analyzed in the same laboratory across the entire study. Material was stored in standard containers used for the shipment and storage of the aqueous broccoli seed extract powder and the containers were held in a warehouse with an ambient temperature ranging from 59-86 °F (15-30 °C). Relative humidity was kept below 30% at all times and there was no direct exposure of the product to sunlight. Based on the results shown below in Table 9, the shelf life of the aqueous broccoli seed extract powder was determined to be 36 months from date of manufacture when stored in the original, sealed container.

Table 9. Stability of Glucoraphanin Content Over Time Across Various Lots of Aqueous Broccoli Seed Extract Powder (% Glucoraphanin, w/w)

	Lot number			
Time (months)	20090112	20090120	20100726	20100727
0	15.8	13.5	13.8	14.3
14	14.7	13.4	14.7	13.4
24	14.2	13.2	11.7	14.7
30	--	--	12.2	15
32	13.9	13.1	--	--
44	12.2	12.2	--	--
48	12.2	12.7	--	--

E. Method of Analysis in Foods

The method utilized to analyze glucoraphanin content is adapted from that published by Wade, et al. 2007.

III. HISTORY OF USE

Historical exposure is documented using an estimate of the background intake of broccoli from the diet. From this intake and literature reports of glucosinolate content in broccoli, a daily exposure to glucosinolates is calculated. A second calculation provides an estimate of the daily intake of aqueous broccoli seed extract powder with the corresponding glucosinolate intake, based on intended uses and use levels. These two calculations provide a comparison of the exposure to glucosinolates via consumption of aqueous broccoli seed extract powder versus exposure to glucosinolates via dietary intake of broccoli. The intended uses and use levels results in an estimated daily intake of aqueous broccoli seed extract powder that provides exposure to glucosinolates at or around the exposure to glucosinolates via broccoli intake. It is worth noting that most of the dietary exposure to broccoli is via usual consumption of *cooked* broccoli; the cooking process inactivates the plant enzyme myrosinase which converts glucosinolates into isothiocyanates. The manufacturing process of aqueous broccoli seed extract powder involves a hot water extraction which also inactivates myrosinase in the broccoli seed prior to extraction of the glucosinolates.

A. Historical Exposure

As broccoli is a common food in the diet, in this section we have provided a calculated estimated daily intake of broccoli and total glucosinolates.

1. Introduction

Estimates of intake for broccoli, brassica vegetables and the glucosinolates from consumption of these vegetables were derived from food consumption survey data, USDA vegetable utilization data and publicly available studies from the US and abroad. Analyses suggest that the average and heavy broccoli consumer ingests 16.4 and 50.9 grams broccoli/day, respectively. By comparison, one serving of raw broccoli is 36 grams and one serving of cooked broccoli is 78 grams. Regulatory guidance from the U.S. and abroad recommend consumption of 5-10 servings of vegetables per day. There are no specific recommendations for only *Brassica* vegetables; however, authoritative guidance and epidemiologic studies evaluating the association of *Brassica* and other vegetable consumption with health endpoints would suggest that an increase in broccoli intake would be both safe and healthy.

There are many factors that can affect estimates of glucosinolate intake from *Brassica* vegetable consumption. The number and types of *Brassica* vegetables included in the analysis, cultural food preferences, the analytical method used to estimate the glucosinolate content, the

processing and preparation methods employed which may result in loss of glucosinolates can all contribute to differing estimates of glucosinolate intake. However, current levels of glucosinolate consumption based on usual broccoli and/or *Brassica* intake are in the range of 40–50 mg/day (see Tables 10 and 11).

2. Food Consumption Survey Data

a. Introduction

Spherix Consulting has completed a dietary intake estimate of broccoli (raw or cooked combined), hereafter "broccoli R/C", by children and adults in the United States. Estimates for the intake of broccoli R/C were based on the food consumption data included in the National Center for Health Statistics' (NCHS) 2009-2010 National Health and Nutrition Examination Surveys (NHANES) (CDC, 2006; USDA, 2012; Bodner-Montville et al, 2006). Calculations for the mean and 90th percentile intakes were performed. The intakes were reported for the following population groups:

- children, ages 2 to 11 years (males, females, and all),
- adolescents, ages 12 to 18 years (males, females, and all),
- adults, ages 19 to 30 years (males, females, and all),
- adults, ages 31 to 50 years (males, females, and all),
- adults, ages 51 to 70 years (males, females, and all),
- adults, ages 71 years and older (males, females, and all),
- adults, ages 19 years and older (males, females, and all).

b. Survey Description

The most recent National Health and Nutrition Examination Surveys (NHANES) for the years 2009-2010 are available for public use. NHANES are conducted as a continuous, annual survey, and are released in 2-year cycles. In 2009-2010, approximately 10,000 people across the U.S. completed the health examination component of the survey. Any combination of consecutive years of data collection is a nationally representative sample of the U.S. population. It is well established that the length of a dietary survey affects the estimated consumption of individual users and that short-term surveys, such as the typical 1-day dietary survey, overestimate consumption over longer time periods (Gregory et al, 1995). Because two 24-hour dietary recalls administered on 2 non-consecutive days (Day 1 and Day 2) are available from the NHANES 2009-2010 survey, these data were used to generate estimates for the current intake analysis.

The NHANES provide the most appropriate data for evaluating food-use and food-consumption patterns in the United States, containing 2 years of data on individuals selected *via* stratified multistage probability sample of civilian non-institutionalized population of the U.S. NHANES 2009-2010 survey data were collected from individuals and households *via* 24-hour dietary recalls administered on 2 non-consecutive days (Day 1 and Day 2) throughout all 4 seasons of the year. Day 1 data were collected in-person in the Mobile Examination Center (MEC), and Day 2 data were collected by telephone in the following 3 to 10 days, on different days of the week, to achieve the desired degree of statistical independence. The data were collected by first selecting Primary Sampling Units (PSUs), which were counties throughout the U.S. Small counties were combined to attain a minimum population size. These PSUs were segmented and households were chosen within each segment. One or more participants within a household were interviewed. Fifteen PSUs are visited each year. For the 2009-2010 NHANES, there were 13,272 persons selected; of these 10,253 were considered respondents to the MEC examination and data collection. 9754 of the MEC respondents provided complete dietary intakes for Day 1 and of those providing the Day 1 data, 8,405 provided complete dietary intakes for Day 2.

In addition to collecting information on the types and quantities of foods being consumed, NHANES 2009-2010 collected socioeconomic, physiological, and demographic information from individual participants in the survey, such as sex, age, height and weight, and other variables useful in characterizing consumption. The inclusion of this information allows for further assessment of food intake based on consumption by specific population groups of interest within the total population. Among those who completed food intake survey on both Day 1 and Day 2, 8301 respondents also provided physiological information including age, sex and weight; of these 7738 were 2 years and older.

Sample weights were incorporated with NHANES 2009-2010 to compensate for the potential under-representation of intakes from specific population groups as a result of sample variability due to survey design, differential non-response rates, or other factors, such as deficiencies in the sampling frame (CDC, 2006; USDA, 2012).[1]

c. *Statistical Methods*

Consumption data from individual dietary records, detailing food items ingested by each survey participant, were collated by computer and used to generate estimates for the intake of

[1] A sample weight is assigned to each sample person. It is a measure of the number of people in the population represented by that sample person in NHANES, reflecting the unequal probability of selection, non-response adjustment, and adjustment to independent population controls.

broccoli R/C by the U.S. population. Estimates for the daily intake of broccoli R/C represent projected 2-day averages for each individual from Day 1 and Day 2 of NHANES 2009-2010 data; these average amounts comprised the distribution from which mean and percentile intake estimates were produced. Mean and percentile estimates were generated incorporating sample weights in order to provide representative intakes for the entire U.S. population. "All-person" intake refers to the estimated intake of broccoli R/C averaged over all individuals surveyed, regardless of whether they consumed food products containing broccoli R/C, and therefore includes "zero" consumers (those who reported no intake of food products containing broccoli R/C during the 2 survey days). "All-user" intake refers to the estimated intake of broccoli R/C by those individuals consuming food products containing broccoli R/C, hence the "all-user" designation. Individuals were considered users if they consumed 1 or more food products containing broccoli R/C on either Day 1 or Day 2 of the survey.

3. Foods Included in the Analyses

The individual foods that contain broccoli R/C and the broccoli contents in foods employed in the current intake analysis are summarized in Appendix I1. Food codes were chosen from the Food and Nutrition Database for Dietary Studies (FNDDS). In FNDDS, the primary (usually generic) description of a given food is assigned a unique 8-digit food code (CDC, 2006; USDA, 2012). Broccoli R/C contents in foods were generated with predefined recipes for the foods, which are available in NHANES 2009-2010 database.

4. Food Survey Results

Only 19.7% of the U.S. population are considered users of foods containing broccoli R/C (see "adults 19 years and older" in Table 10). To avoid underestimating the broccoli consumption by those broccoli-eaters, therefore, instead of "all-person", the "all-user" total intakes of broccoli R/C by the U.S. by population group are calculated and the results are summarized in Table 10.

For broccoli-eaters, defined as all-users in this report, on average, people eat 16.4 g/day and, at the 90th percentile, people eat 50.9 g/day of broccoli (see "adults 19 years and older" in Table 10). Children (2-11 years old) eat much less of broccoli (9.5 g/day on average and 34.3 g/day at the 90th percentile). The key intake in the calculation is cooked broccoli since few people consume the raw vegetable and the small numbers are not statistically reliable. In addition, most broccoli-containing foods are cooked (Appendix I1).

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Table 10. Estimated "All-user" Daily Intake (EDI) of Broccoli, Raw or Cooked (Broccoli R/C), by Population Group (2009-2010 NHANES Data)

Population Group	N_u/N_p [A]	% users [B]	Average Intake (g/day)	90th Percentile Intake (g/day)
Children 2-11 years old				
Males	135/855	14.3	9.3	38.8
Females	110/814	13.7	9.7	30.4
All	245/1669	14.0	9.5	34.3
Adolescents 12-18 years old				
Males	50/486	10.2	13.6	53.8
Females	58/453	11.5	20.4	72.1
All	108/939	10.9	17.4	53.8
Adults 19-30 years old				
Males	71/480	19.2	10.7	29.9
Females	85/548	18.6	12.2	50.9
All	156/1028	18.9	11.4	36.5
Adults 31-50 years old				
Males	114/798	15.3	17.5	46.8
Females	200/938	22.3	15.7	38.9
All	314/1736	19.0	16.4	43.7
Adults 51-70 years old				
Males	125/789	18.3	16.4	43.7
Females	160/766	24.6	24.0	77.5
All	285/1555	21.5	20.8	67.9
Adults 71 years and older				
Males	71/374	19.1	15.2	61.5
Females	82/437	19.2	10.5	34.3
All	153/811	19.1	12.3	38.8
Adults 19 years and older				
Males	381/2441	17.5	15.3	43.6
Females	527/2689	21.8	17.2	51.1
All	908/5130	19.7	16.4	50.9

[A] Number of people consuming one or more foods containing broccoli, raw or cooked on either Day 1 or Day 2 of the survey over the number of people surveyed.
[B] Percent of user when sample weights are considered.

5. Other Available Estimates of Broccoli Intake

Vegetable utilization figures from USDA that provide per capita disappearance do not account for waste and thus may overestimate consumption; the raw broccoli per capita calculation is particularly deceiving because NHANES user data indicates that very little of the raw broccoli sold is actually consumed. Nevertheless, processed broccoli data from the USDA are more likely to reflect actual consumption. Data from the USDA utilization report show[2]:

Processed Broccoli Domestic Utilization: 828,100,000 lbs/year in Year 2011

Per capita use: 2.7 lbs/year (1.2247 kg/year), or 3.4 g/day

Calculation of user consumption from this number can be estimated by using the assumption that 19.7% of the population is broccoli eaters (NHANES 2009-2010 data); thus the average consumption is calculated to be 17.3 g/day per user. This number corroborates the data (16.4 g/day on average for the broccoli eaters in "adults 19 years and older") generated from the NHANES 2009-2010 analysis.

The estimated daily intakes of broccoli R/C by the U.S. population with NHANES 2009-2010 data can be compared with an unpublished report (Amann and Douglass 1999), which calculated intakes using the 1994-96 USDA Continuing Survey of Food Intakes by Individuals (CSFII) data (Table 2 in the report). The current calculation found that there are 132 foods that contain broccoli R/C in comparison with 81 foods reported in 1999. Unlike the current calculation, in which broccoli R/C contents in foods were generated from predefined recipes for the foods available in the NHANES 2009-2010 database; Amann and Douglass (1999) used their own "recipe file" with broccoli R/C contents in the foods, which were not documented in the report.

The unpublished report using 2009-2010 survey data (Amann and Douglass 1999) provided an estimate of mean and 90th percentile broccoli consumption of 47.8 and 109.4 g/day. The report, however, did not give a clear definition for "all-user"; in the Spherix report, individuals were considered a user if they consumed 1 or more food products containing broccoli R/C on either Day 1 or Day 2 of the survey or on both Day 1 and Day 2 of the survey. Assuming that the "all-user" definition in the 1999 report is the same as that defined in the current report[3], a comparison shows that, on average, the mean and 90th percentile broccoli R/C (based 2009-2010 survey data) consumption by US population ("adults 19 years and older") are now only 34.3% and 46.5%, respectively, of the intakes calculated 15 years ago (based on 1994-1996 CSFII

[2] Table 40 in Vegetables and Pulses Yearbook 2012. Available online at http://usda.mannlib.cornell.edu/MannUsda/viewDocumentInfo.do?documentID=1858

[3] Individuals were considered users if they consumed 1 or more food products containing broccoli R/C on either Day 1 or Day 2 of the survey.

survey data). Importantly, the assessment of total daily *Brassica* vegetable intake compared with broccoli intake alone from the 1999 internal report shows that the intake estimate is not increased with the addition of other vegetables in this category (Amann and Douglass 1999). Total vegetable intake for adults at the mean and 90th percentile were reported to be 47.8 (broccoli) and 46.3 (*Brassica* vegetables); 109.4(broccoli) and 110.9 (*Brassica* vegetables) g/day, respectively. Therefore, consumption of broccoli intake from the most current USDA survey can provide a good estimate of total glucosinolate intake.

6. Recommended Vegetable Intake

Epidemiologic evidence suggests that a high intake of cruciferous vegetables, which are rich in glucosinolates, are associated with lower risk of various cancers such as lung and colorectal cancer and there is some modest support for a role of increased intake in reducing risk for prostate and breast cancer (Higdon et al. 2007; Liu and Lv 2013; Liu et al. 2012, 2013; Wu et al. 2012). Prospective studies suggest that there may be health benefits associated with at least 5 weekly servings of cruciferous vegetables (Michaud et al. 1999; Feskanich et al. 2000; Giovannucci et al. 2003).

Regulatory and authoritative guidance from governments and associations around the world recommend intake of vegetables that exceed current consumption patterns. A recent CDC survey of U.S. fruit and vegetable daily consumption frequency among adults and adolescents indicates that 27.4 and 13.2% of adults and adolescents consume ≥ 3 servings of vegetables per day (http://www.cdc.gov/nutrition/downloads/StateIndicatorReport2009.pdf). This frequency should be compared against nutritional guidance which recommends 5-10 servings of vegetables per day. There are no specific recommendations for only brassica intake, however, the current intake assessment conducted by Spherix, indicated that the mean and 90th percentile consumption of 16.4 and 50.9 g/day, respectively, is less than 1 serving of vegetables (1 serving raw broccoli is 36 g; 1 serving cooked broccoli is 78 g). Therefore, authoritative guidance for vegetable consumption would suggest that an increase in broccoli intake would be both safe and healthy. Guidance from various countries is provided below:

a. United States

USDA Dietary Guidelines for Americans: The Executive Summary of Guidelines recommends consumption of a sufficient amount of fruits and vegetables while staying within energy needs. Two cups of fruit and 2 ½ cups of vegetables per day are recommended for a reference 2,000-calorie intake, with higher or lower amounts depending on the calorie level. (USDA Dietary Guidelines for Americans 2005, http://www.health.gov/dietaryguidelines/dga2005/document/).

The report of the Dietary Guidelines Advisory Committee (DGAC) on the Dietary Guidelines for Americans (2010) stresses that efforts are warranted to promote increased intakes of vegetables (especially dark-green vegetables, red-orange vegetables, and cooked dry beans and peas), fruits, whole grains, and fat-free or low-fat fluid milk and milk products (including calcium and vitamin D fortified soymilk) among all ages. (http://www.cnpp.usda.gov/dgas2010-dgacreport.htm)

The Dietary Approaches to Stop Hypertension (DASH) Eating Plan (HHS, NIH, National Heart, Lung and Blood Institute) recommends 4-5 daily servings of vegetables and 4-5 daily servings of fruits.(USDA Dietary Guidelines for Americans 2005, (http://www.health.gov/dietaryguidelines/dga2005/document/; http://www.nhlbi.nih.gov/health/public/heart/hbp/dash/new_dash.pdf.)

A collaboration of the American Cancer Society, American Diabetes Association and American Heart Association: In the *Make Healthy Choices* recommendations, suggests that people eat at least 5 servings of vegetables and fruits each day with a focus on fruits and vegetables that have the most color, as they are considered the most nutritious. (http://www.everydaychoices.org/).

b. Canada

In Canada's Food and Nutrition Guide "How much food do you need every day" (Health Canada 2007) guidance is provided for desirable dietary intakes of different types of food http://www.hc-sc.gc.ca/fn-an/food-guide-aliment/basics-base/quantit-eng.php

For adults over the age of 18, the Canada Food Guide recommends 7-10 servings of vegetables and fruit per day.

c. Australia

The Australian Guide to Healthy Eating recommends 5 servings per day from the vegetables and legumes group. (http://www.eatforhealth.gov.au/guidelines/australian-dietary-guidelines-1-5; current as of 15 Dec 2013).

d. European Union

Healthy Eating Guidance (Eurodiet 2000) provides population goals for nutrients and lifestyle that are consistent with the prevention of major public health problems in Europe. The guidance recommends eating > 400 g fruits and vegetables per day. (http://ec.europa.eu/health/ph_determinants/life_style/nutrition/report01_en.pdf)

e. France

General Healthy Eating Guidance France (Poster from PNNS program 2010) recommends at least 5 servings of fruits and vegetables per day. http://www.mangerbouger.fr/

f. Mexico

The principal guidance from the government in Mexico on nutrition, diet and food choices comes from the IMSS (2005) (Mexican Institute of Social Security) which recommends 3-5 servings of vegetables per day. (IMSS (Mexican Institute of Social Security). 2005. "GCS MUJERES2005 Respaldo" Global Credit Solutions Nutritional Support for Women 2005).

g. United Kingdom

Healthy Eating Guidance (also for prevention/ maintenance) described in the Eatwell Plate from the UK Foods Standards Agency recommends a least 5 servings of fruits and vegetables per day. (http://www.food.gov.uk/)

7. Estimates of Total Glucosinolate Intake From Broccoli and Other Cruciferous Vegetable Consumption

There are many factors that can affect estimates of glucosinolate intake and thus make comparison of studies difficult. The number and types of brassica vegetables included in the analysis, cultural food preferences, the analytical method used to estimate the glucosinolate content, specific processing and preparation methods that may result in loss of glucosinolates (McNaughton and Marks 2003; Goodrich et al. 1989), can all contribute to differing estimates. The glucosinolate content of *Brassica* vegetables reported in the literature is summarized in Table 11. Studies reported a mean and/or range of total glucosinolates (mg/100g fresh weight) content per vegetable. The mean glucosinolate content in raw broccoli (calculated from reported means and not from reported ranges) calculated from values reported in these studies is 84.9 mg/100g fresh broccoli. Similarly, the mean content in cooked broccoli was calculated to be 16.7 mg/100g, however, there were only four data points to consider. Importantly, cooking technique (time, use of water or steam) can contribute to variability; estimates of glucosinolate loss from researchers comparing fresh and cooked broccoli in the same study suggest that 40% loss is a reasonable estimate (Sones et al. 1984; Goodrich et al. 1989).

For broccoli-eaters, the NHANES analysis completed by Spherix indicates that, on average, broccoli users eat about 16.4 g/day and, at the 90th percentile, they consume about 50.9 g/day of broccoli. Raw broccoli is assumed to contain about 85 mg glucosinolates/100 g and cooked broccoli is assumed to contain 51 mg glucosinolates/100 g (assuming 40% loss during cooking). These consumption data result in a mean total glucosinolates exposure ranging from 8-

14 mg/day (assuming consumption of only cooked or only fresh broccoli); 90th percentile intake would range from 26-43 mg/day.

This background intake can also be compared to intakes in a serving of the vegetable. One serving of fresh broccoli is 36 g which would provide 30 mg total glucosinolates. One serving of cooked broccoli is 78 g which would provide 40 mg glucosinolates.

Although consumption of different *Brassica* species may provide slightly different proportions of glucosinolates, glucoraphanin is the predominate glucosinolate in *Brassica* vegetables (Table 11). Amann and Douglass (1999) reported a total glucosinolate consumption for all individuals 19 years and older of 20.2 mg/day based on the weighted mean glucosinolate content reported in *Brassica* vegetables. The intake based on maximum glucosinolate concentrations reported in these vegetables was 57.5 mg/day.

Studies reported in the literature provide estimates of background glucosinolate intake from broccoli and other cruciferous vegetables that corroborate the estimates given above. A study of 34 women over the age of 45 in the U.S. provided self-reported *Brassica* intake taken at two baseline timepoints. The minimum value, 25th percentile, median, 75th percentile and maximum values at the two baselines, respectively, were: (0,0); (0,0); (3.9,0); (20.3,10.4); (52.6,75.6) g/day (Fowke et al. 2001). A study of 33 women with an average age of 62 years had a mean 24 hour cruciferous vegetable consumption of 14.5 g (Fowke et al. 2002). Utilizing glucosinolate content estimates given above, the maximum glucosinolate ingestion would be close to 40 mg/day.

A database food intake data from 2,121 participants of the EPIC-Heidelberg cohort study in Germany provided a calculation of the mean total glucosinolate of 14.2 and 14.8 mg/day for men and women, respectively (Steinbrecher and Linseisen 2009). Other researchers have reported estimates of glucosinolate intake using glucosinolate data obtained from their own analyses of food. A mean intake of total glucosinolates of 4.7 mg/day was based on 5 different *Brassica* vegetables and the aggregated Czech food consumption data from 1993 (Hrncirik and Velisek 1997, cited in Steinbrecher and Lineisen 2009). Sones et al. (1984) reported a mean daily glucosinolate intake in the UK of from Brussels sprouts, cabbage, cauliflower, and turnip-swede (from fresh material) of 46.1 mg/person/day and from cooked material of 29.4 mg/day using the food intake data of the 1980 National Food Survey. A German study used per capita consumption from 1989 to report a mean intake of total glucosinolates of 43.1 mg/day and another estimate of mean intake of 41.1 mg/day from a representative population sample from the Potsdam region for 1992/1993 (Lange et al. 1992, 1994, cited in Steinbrecher and Lineisen 2009).

Table 11. Glucosinolate Content of *Brassica* Vegetables Based on Literature Reports

Vegetable	Preparation	Total Glucosinolates Mean (range)	Glucoraphanin	Glucoerucin	Gluconasturtiin	Glucobrassicin	Progoitrin/ Epiprogoitrin	Sinigrin	Gluconapin	Sulforaphane
		Mean (range), mg/100 g fresh weight								
Broccoli	Fresh	11.6[4] 11.90-88.56[15] 14.28-134.75[15] 19.28[14] 20.5-450[12] 23.0-64.6[15] 40.80 (19.3-127.5)[5] 50.7[1] (frozen) 61.1[1] 61 (2.86-169.51)[13] 68.6 (40.4-98.5)[3] 72.05 (43.8-144.1)[10] 72.37-282.36[15] 83.75 (45.5-116.9)[10] 89.99-119.99[15] 110.54 (67.7-199.6)[10] 127.49[14] 312[7]	1.40-58.85[15] 1.73-21.95[15] 3.74-101.35[15] 4.45[4] 6.07-38.77[15] 10.48-80.75[15] 11.6-34.0[15] 15.1 (2.4-27.0)[3] 19.15-69.59[15] 20.55-133.11[18] 21.02-133.11[15] 27.89 (12.6-38.5)[10] 42.56 (23.6-83.0)[10] 71-179[9] 218[7] 0-382[12]		0.81[6] 1.8[6] 1.81[13]	4.26[6] 4.96[4] 5.3[13] 11.04-52.79[18] 18.9[3] 26.64 (18.9-32.2)[10] 25.12 (10.2-45.3)[10] 50.9[7] 51.3-160[9]	0.06[4] 0.25[6] 2.4 (0-8.2)[3] 4.2[13] 3.3-67.1[9] 0-46.67[18] 0-182[12] ---------- 0[13] 0.32[6]	0.05[4] 0.16[6] 0.38[13] 0.6 (0-0.9)[3] 0-355.9[12]	0[4] 0-5.6[6] 3.98[13] 0-94.0[12]	188.5-370.3[11]
Broccoli	Seeds	735.08 (seeds)[21]	364.2 (seeds)[20] 181.51 (seeds)[21]	46.61 (seeds)[21]	<0.5 (seeds)[21] 3.89 (seeds)[21]	14.63 (seeds)[21]	<0.5 (seeds)[21] ---------- <0.1 (seeds)[21]	4.89 (seeds)[21]	5.82 (seeds)[21]	23-332 (seeds)[20]
Broccoli	Sprouts	107.69-249.65 (sprouts)[15] 117.45 (12d sprouts)[21] 129.94-363.57 (sprouts)[15] 141.48 (8d sprouts)[21] 209.32 (4d sprouts)[21] 1590[8] (3d old sprouts)	42.39 (12d sprouts)[21] 48.45-125.28 (sprouts)[15] 48.83 (8d sprouts)[21] 75.95-216.07 (sprouts)[15] 80.05 (4d sprouts)[21] 89.4 (2d sprouts)[20] 101.70-295.07 (sprouts)[15] 163.69 (sprouts)[16] 193.7 (3d sprouts)[20]	18.39 (8d sprouts)[21] 19.94 (4d sprouts) 21.10 (12 d sprouts)[21] 74.82 (sprouts)[16]	<0.5 (4d sprouts)[21] <0.5 (8d sprouts)[21] <0.5 (12d sprouts)[21]	7.96 (12d sprouts)[21] 8.67 (8d sprouts)[21] 11.43 (4d sprouts)[21]	<0.5 (4d sprouts)[21] <0.5 (8d sprouts)[21] <0.5 (12d sprouts)[21] ---------- <0.1 (4d sprouts)[21] <0.1 (8d sprouts)[21] <0.1 (12d sprouts)[21]	<0.5 (4d sprouts)[21] <0.5 (8d sprouts)[21] <0.5 (12d sprouts)[21]	0.97 (12d sprouts)[21] 1.07 (8d sprouts)[21] 1.17 (4d sprouts)[21]	104.3 (2d sprouts)[20]
Broccoli	Cooked	2.0[4], 7.0[4] (boiling, steaming) 20.7[1] (frozen) 37.2[1]	0.91[4]		0.20[6]	0.6[4] 1.34[6]	0.09[4] 0.25[6]	0[4]	0[4]	

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Table 11. Glucosinolate Content of *Brassica* Vegetables Based on Literature Reports

Vegetable	Preparation	Total Glucosinolates Mean (range)	Glucoraphanin	Glucoerucin	Gluconasturtiin	Glucobrassicin	Progoitrin/ Epiprogoitrin	Sinigrin	Gluconapin	Sulforaphane
		Mean (range), mg/100 g fresh weight								
Brussels sprouts	Fresh	29.70[4] 60-390[17] 73.0-91.4[15] 80.12[14] 90.5[1] (frozen) 94.07-221.17[15] 126.61[14] 156.4 (103.42-229.89)[13] 163.4 (147.0-180.9)[10] 200.20[14] 205.6 (61.7-400.8)[10] 220.3 (141.7-383.6)[10] 226.2 (145.5-393.9)[1] 246.1 (207.2-267.3)[10] 247.00[14] 252.72[14] 292.00[14] 445.50 (80.1-445.5)[14]	2.37[4] 3.58 (0.17-9.86)[10] 6.1[13]		2.96[13]	17.81[4] 20.1[13]	2.94[4] 3.23 (0.39-9.89)[10] 13.1[13] 26.44 (36.5-90.3)[10] 43.57 (11.4-118.2)[10] ---------- 0[12]	1.58[4] 3.83 (1.4-8.1)[10] 17.56-22.58[15] 22.0-25.3[15] 23.08-45.66[15] 40.2 (1.4-100.6)[10] 44.5 (1.6-111.4)[1] 44.66[13] 48.74 (9.9-140.8)[10]	1.56 (0.2-4.5)[10] 13.59 (2.7-45.3)[10] 22.83 (2.3-82.4)[10] 25.2 (2.5-90.9)[1] 35.97[13]	
Brussels sprouts	Cooked	27.67[4] 61.3[1] (frozen) 123.7 (59.7-254.2)[1] 148.00[14]	2.83[4]			15.49[4]	2.89[4]	1.51[4] 26.4 (1.2-76.5)[1]	14.8 (1.8-74.6)[1]	
Chinese cabbage	Fresh	8.9[5] 9.7-33.7[15] 20.47-20.97[15]					0.9-8.0[15]			
Cabbage—red	Fresh	18.79[14] 19.67[15] 26.50[14] 30.1-98.3[15] 64.2[5] 66.9[1] 68.8 (34.4-98.9)[10] 72.71 (39.3-104.3)[10] 73.12-124.02[15] 76.62 (26.5-76.6)[14] 90.91 (67.0-114.9)[10] 163.4 (88.2-234.4)[10] 204.3 (150.5-258.1)[10]	4.0-18.2[15] 22.83 (13.8-35.8)[10] 24.79 (20.4-29.2)[10]				3.23 (1.7-2.1)[10] 4.75 (3.9-5.6)[10]	3.0-16.7[15] 3.76 (0.5-9.2)[10] 4.52 (4.0-5.1)[10] 7.23[15]	3.69 (1.7-5.8)[10] 5.6 (5.2-6.1)[10]	
Cabbage—red	Seeds	1307.78 (seeds)[21]	< 0.5 (seeds)[21]	< 0.5 (seeds)[21]	18.08 (seeds)[21]	< 0.5 (seeds)[21]	< 0.5 (seeds)[21] 388.05 (seeds)[21]		114.66 (seeds)[21]	

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Table 11. Glucosinolate Content of *Brassica* Vegetables Based on Literature Reports

Vegetable	Preparation	Total Glucosinolates Mean (range)	Glucoraphanin	Glucoerucin	Gluconasturtiin	Glucobrassicin	Progoitrin/ Epiprogoitrin	Sinigrin	Gluconapin	Sulforaphane
		Mean (range), mg/100 g fresh weight								
Cabbage—red	Sprouts	246.81 (12d sprouts)[21] 516.42 (8d sprouts)[21] 907.82 (4d sprouts)[21]	< 0.5 (4d sprouts)[21] < 0.5 (8d sprouts)[21] < 0.5 (12d sprouts)[21]	< 0.5 (4d sprouts)[21] < 0.5 (8d sprouts)[21] < 0.5 (12d sprouts)[21]	< 0.5 (8d sprouts)[21] < 0.5 (12d sprouts)[21] 11.82 (4d sprouts)[21]	2.78 (8d sprouts)[21] 2.84 (12d sprouts)[21] 5.89 (4d sprouts)[21]	< 0.5 (4d sprouts)[21] < 0.5 (8d sprouts)[21] < 0.5 (12d sprouts)[21] 99.43 (12d sprouts)[21] 182.52 (8d sprouts)[21] 458.44 (4d sprouts)[21]		17.89 (12d sprouts)[21] 46.98 (8d sprouts)[21] 70.43 (4d sprouts)[21]	
Cabbage—red	Cooked	54.8[1]								
Cabbage	Fresh	30.62 (15.3-44.0)[10] 37.8[13] 42.66[14] 58.90 (42.7-108.9)[14]	0.3[13]		0.99[13]	3.1[13]	0.6[13] ---------- - 0[13]	19.59[19] 21.86[13] 26.3 (7.4-64.6)[1]	1.8 (0-5.3)[1] 2.04[13]	
Cabbage	Shredded	42.2[1] (coleslaw)								
Cabbage—savoy	Fresh	27.63[15] 59.47[14] 61.4-72.2[15] 73.20 (44.7-117.9)[10] 76.99 (59.5-209.0)[14] 108.9 (36.0-275.4)[1] 122.64[10] (single measurement) 205.28 (118.7-290.8)[10] 209.00[14]				55.17 (31.5-89.6)[10]	0.19 (0-0.5)[10] 0.62[10] (single measurement) 5.37 (2.2-11.5)[10]	4.84[15] 5.09 (0-14.2)[10] 12.8[10] 15.5-18.6[15] 33.40 (11.3-58.3)[10]		
Cabbage—savoy	Cooked	78.6 (31.5-165.1)[1]								

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Table 11. Glucosinolate Content of *Brassica* Vegetables Based on Literature Reports

Vegetable	Preparation	Total Glucosinolates Mean (range)	Glucoraphanin	Glucoerucin	Gluconasturtiin	Glucobrassicin	Progoitrin/ Epiprogoitrin	Sinigrin	Gluconapin	Sulforaphane
		Mean (range), mg/100 g fresh weight								
Cauliflower	Fresh	11.7[5] 14.1[15] 19.5-42.6[15] 31.76-64.23[15] 36.50[14] 40.5[1] (frozen) 42.10 (18.3-71.5)[10] 46.73 (26.3-80.4)[10] 53.3[13] 60.39 (13.4-202.8)[10] 62.0 (13.8-208.3)[1] 72.05 (13.4-231.6)[10] 78.60[14] 79.30 (25.4-199.6)[10]	1.7[13] 27.85 (0.79-83.0)[10]		1.34[13]	4.6[13]	0.9[13] ---------- 0[13]	1.14-13.07[15] 1.4-5.9[15] 3.4[15] 3.58 (1.0-5.9)[10] 12.83 (0.5-56.6)[10] 13.55 (0.5-56.6)[10] 14.2 (0.5-62.7)[1] 26.43[13]	0.7 (0-2.8)[1] 0.89[13]	
Cauliflower	Cooked	27.9[1] (frozen) 42.0 (9.4-111.1)[1]						10.0 (0-46.0)[1]	0.3 (0-1.1)[1]	
Collards	Fresh	98.08 (28.7-136.5)[10]						7.42 (4.5-10.3)[10]		
Horseradish	Fresh	160.12[14]								
Kale	Fresh	6.67[14] 24.86[15] 39.06-90.20[15] 62.2[5] 65.4-151.1[15] 85.94-127.84[15] 89.4[1] 106.5[13] 195.40 (140.7-267.0)[10]	7.0[13]		2.70[13]	8.6[13]	3.7[13] 27.30 (6.5-50.7)[10] ---------- 0[13]	2.2-22.7[15] 3.43-8.01[15] 19.10[15] 34.76 (22.4-70.7)[10] 42.33-76.08[15] 59.49[13]	5.94[13] 7.93 (2.2-14.2)[10]	
Kale	Cooked	69.1[1]								
Mustard greens	Fresh	25.7-112.6[15] 118.09 (118.1-544.5)[14] 155.75-275.23[15] 281.5[5]						0-203.64[15] 0.4-4.3[15] 8.04-93.96[15] 23.5-102.7[15]		
Radish—Root	Fresh	12.5[1] 70.97[14] 87.6-332.8[15] 92.81[14] 172.4[5]	1.6-15.0[15]							

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Table 11. Glucosinolate Content of *Brassica* Vegetables Based on Literature Reports

Vegetable	Preparation	Total Glucosinolates Mean (range)	Glucoraphanin	Glucoerucin	Gluconasturtiin	Glucobrassicin	Progoitrin/ Epiprogoitrin	Sinigrin	Gluconapin	Sulforaphane
		Mean (range), mg/100 g fresh weight								
Radish—Root	Seeds	1350.76 (seeds)[21]				< 0.5 (seeds)[21]			<0.5 (seeds)[21]	
Radish—Root	Sprouts	168.48 (12d sprouts)[21] 296.77 (8d sprouts)[21] 566.14 (4d sprouts)[21]				< 0.5 (4d sprouts)[21] < 0.5 (8d sprouts)[21] < 0.5 (12d sprouts)[21]			<0.5 (4d sprouts)[21] 5.73 (12d sprouts)[21] 5.84 (8d sprouts)[21]	
Radish—Root	Cooked	ND[1]								
Rutabaga	Fresh	20.44[14] 56.0 (39.2-165.7)[1] 92.00[14] 93.00 (20.4-140.5)[14] 140.48[14]							4.2 (0-34.4)[1]	
Rutabaga	Seeds	2131.97 (seeds)[21]	< 0.5 (seeds)[21]		49.79 (seeds)[21]	10.99 (seeds)[21]	671.36 (seeds)[21]	< 0.5 (seeds)[21]	684.75 (seeds)[21]	
Rutabaga	Sprouts	276.74 (12d sprouts)[21] 386.84 (8d sprouts)[21] 951.88 (4d sprouts)[21]	< 0.5 (4d sprouts)[21] < 0.5 (8d sprouts)[21] < 0.5 (12d sprouts)[21]		23.76 (8d sprouts)[21] 25.88 (4d sprouts)[21] 29.93 (12d sprouts)[21]	2.44 (12d sprouts)[21] 2.64 (8d sprouts)[21] 5.85 (4d sprouts)[21]	98.49 (12d sprouts)[21] 129.34 (8d sprouts)[21] 281.04 (4d sprouts)[21]	< 0.5 (4d sprouts)[21] < 0.5 (8d sprouts)[21] < 0.5 (12d sprouts)[21]	52.37 (12d sprouts)[21] 89.57 (8d sprouts)[21] 186.64 (4d sprouts)[21]	
Rutabaga	Cooked	29.1 (20.5-94.4)[1]							2.4 (0-21.0)[1]	
Turnip—Roots	Fresh	53.3[15] 50.4-81.7[15] 112.15-289.43[15] 130.24-676.54[15]		0.08[2]	48.4[2]	0.10[2]	0.09[2]		17.1[2]	
Turnip—Roots	Seeds	1131.06 (seeds)[21]	< 0.5 (seeds)[21]		41.68 (seeds)[21]	< 0.5 (seeds)[21]	38.32 (seeds)[21]		565.92 (seeds)[21]	
Turnip—Roots	Sprouts	474.76 (12d sprouts)[21] 766.07 (8d sprouts)[21] 938.63 (4d sprouts)[21]	< 0.5 (4d sprouts)[21] < 0.5 (8d sprouts)[21] < 0.5 (12d sprouts)[21]		12.53 (12d sprouts)[21] 39.84 (8d sprouts)[21] 40.10 (4d sprouts)[21]	< 0.5 (4d sprouts)[21] < 0.5 (8d sprouts)[21] < 0.5 (12d sprouts)[21]	13.07 (12d sprouts)[21] 26.76 (8d sprouts)[21] 28.57 (4d sprouts)[21]		278.19 (12d sprouts)[21] 396.79 (8d sprouts)[21] 457.23 (4d sprouts)[21]	
Turnip—Tops	Fresh	82.77 (35.6-129.9)[10]		0[2]	5.6[2]	0.04[2]	0.03[2]		34.3[2] 38.36 (14.2-67.4)[10]	
Turnip—Tops	Seeds	1364.30 (seeds)[21]	< 0.5 (seeds)[21]		33.97 (seeds)[21]	2.73 (seeds)[21]	45.00 (seeds)[21]	45.00 (seeds)[21]	986.10 (seeds)[21]	

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Table 11. Glucosinolate Content of *Brassica* Vegetables Based on Literature Reports

Vegetable	Preparation	Total Glucosinolates Mean (range)	Glucoraphanin	Glucoerucin	Gluconasturtiin	Glucobrassicin	Progoitrin/ Epiprogoitrin	Sinigrin	Gluconapin	Sulforaphane
		Mean (range), mg/100 g fresh weight								
Turnip—Tops	Sprouts	119.44 (12d sprouts)[21] 164.51 (8d sprouts)[21] 260.77[10] 736.66 (4d sprouts)[21]	< 0.5 (4d sprouts)[21] < 0.5 (8d sprouts)[21] < 0.5 (12d sprouts)[21]		<0.5 (8d sprouts)[21] <0.5 (12d sprouts)[21] 10.39 (4d sprouts)[21]	<0.5 (4d sprouts)[21] <0.5 (8d sprouts)[21] <0.5 (12d sprouts)[21]	10.46 (12d sprouts)[21] 13.08 (8d sprouts)[21] 24.42 (4d sprouts)[21]	10.46 (12d sprouts)[21] 13.08 (8d sprouts)[21] 24.42 (4d sprouts)[21]	99.64 (12d sprouts)[21] 128.01 (8d sprouts)[21] 542.39 (4d sprouts)[21]	
Watercress	Fresh	95.0[1] 658.20[14]								
Watercress	Cooked	ND[1]								

[1]Sones K, Heaney RK, Fenwick GR. J. Sci. Food Agric. 1984 35: 712-720.
[2]Carlson DG, Daxenbichler ME, Tookey HL. J. Amer. Soc. Hort. Sci. 1987 112: 179-183. Reported data were converted from μmol/100 g fresh weight.
[3]Lewis J and Fenwick GR. Food Chemistry 1987 25: 259-268.
[4]Goodrich RM, Anderson JL, Stoewsand GS. Journal of Food Processing and Preservation 1989 13: 275-280. Reported data were converted from mg/g fresh weight.
[5]Yen G-C and Wei Q-K. J. Sci. Food Agric. 1993 61: 471-475. Reported data were converted from mg/kg fresh weight.
[6]Betz JM and Fox WD. Chapter 14: High-performance liquid chromatographic determination of glucosinolates in *Brassica* vegetables. ACS Symposium Series 546: Food Phytochemicals for Cancer Prevention I: Fruits and Vegetables. Huang M-T, Osawa T, Ho C-T, Rosen RT, eds. American Chemical Society, Washington, D.C. 1994.
[7]Hansen M, Møller P, Sørensen H. J. Amer. Soc. Hort. Sci. 1995 120: 1069-1074. Reported data were converted from μmol/g dry weight based on a water content of 89.3% (w/w), per the USDA Nutrient Database (http://ndb.nal.usda.gov/ndb/search/list). Total glucosinolates were recalculated based on an estimated average MW of 445.
[8]Fahey JW, Zhang Y, Talalay P. Proc. Natl. Acad. Sci. USA 1997 94: 10367-10372. Reported data were converted from nmol/g dry weight based on a water content of 89.3% (w/w), per the USDA Nutrient Database (http://ndb.nal.usda.gov/ndb/search/list). Total glucosinolates were recalculated based on an estimated average MW of 445.
[9]Hansen M, Lausten AM, Olsen CE, Poll L, Sørensen H. Journal of Food Quality 1997 20: 441-459. Reported data were converted from μmol/g dry weight based on a water content of 89.3% (w/w), per the USDA Nutrient Database (http://ndb.nal.usda.gov/ndb/search/list).
[10]Rosa EAS, Heaney RK, Fenwick GR, Portas CAM. 3. Glucosinolates in Crop Plants. In: Horticultural Reviews, Vol. 19. Janick J, ed. John Wiley & Sons, Inc.: New York, 1997. Reported data were converted from μmol/100g fresh weight to mg/100g fresh weight. Total glucosinolates were recalculated based on an estimated molecular weight of 445.
[11]Chiang WCK, Pusateri DJ, Leitz REA. J. Agric. Food Chem. 1998 46: 1018-1021.
[12]Faulkner K, Mithen R, Williamson G. Carcinogenesis 1998 19: 605-609. Reported data were converted from μmol/g dry weight based on a water content of 89.3% (w/w), per the USDA Nutrient Database (http://ndb.nal.usda.gov/ndb/search/list).
[13]Kushad MM, Brown AF, Kurilich AC, Juvik JA, Klein BP, Wallig MA, Jeffery EH. J. Agric. Food Chem. 1999 47: 1541-1548. Reported data were converted from μmol/g dry weight based on the water content reported for fresh broccoli (89.3%, w/w), Brussels sprouts (86.0), cabbage (92.2), cauliflower (92.1), and kale

(84.0), per the USDA Nutrient Database (http://ndb.nal.usda.gov/ndb/search/list). Total glucosinolates were recalculated based on an estimated average MW of 445.

[14]McNaughton SA and Marks GC. British Journal of Nutrition 2003 90: 687-697.

[15]Verkerk R, Schreiner M, Krumbein A, Ciska E, Holst B, Rowland I, De Schrijver R, Hansen M, Gerhäuser C, Mithen R, Dekker M. Review. Glucosinolates in *Brassica* vegetables: The influence of the food supply chain on intake, bioavailability and human health. Mol. Nutr. Food Res. 2009 53: S219-S265. Reported data were converted from µmol/g dry weight or µmol/100g wet weight based on the water content reported for turnip (91.87%, w/w), radish (95.27), red cabbage (90.39), savoy cabbage (91.0), fresh broccoli (89.3), Brussels sprouts (86.0), Chinese cabbage (94.39), mustard greens (90.7), cauliflower (92.1), and kale (84.0), per the USDA Nutrient Database (http://ndb.nal.usda.gov/ndb/search/list). Broccoli sprouts were not found in the USDA Nutrient Database; water content was estimated at ~90%, w/w. Total glucosinolates were recalculated based on an estimated average MW of 445.

[16]Clarke JD, Hsu A, Riedl K, Bella D, Schwartz SJ, Stevens JF, Ho E. Bioavailability and inter-conversion of sulforaphane and erucin in human subjects in consuming broccoli sprouts or broccoli supplement in a cross-over study design. Pharmacological Research 2011 64: 456-463. Reported data were converted from µmol/g wet weight.

[17]Stoewsand GS. Bioactive organosulfur phytochemicals in *Brassica oleracea* vegetables—A review. Food and Chemical Toxicology 1995 33: 537-543. Reported data were converted from µg/g.

[18]Rosa EAS and Rodrigues AS. Total and individual glucosinolate content in 11 broccoli cultivars grown in early and late seasons. HortScience 2001 36: 56-59. Reported data were converted from mmol/kg dry weight based on the water content reported for fresh broccoli (89.3%, w/w), per the USDA Nutrient Database (http://ndb.nal.usda.gov/ndb/search/list).

[19]Rouzaud G, Young SA, Duncan AJ. Hydrolysis of glucosinolates to isothiocyanates after ingestion of raw or microwaved cabbage by human volunteers. Cancer Epidemiology, Biomarkers & Prevention 2004 13: 125-131. Reported data were converted from µmol/150 g.

[20]Gu Y, Guo Q, Zhang L, Chen Z, Han Y, Gu Z. Physiological and biochemical metabolism of germinating broccoli seeds and sprouts. Journal of Agricultural and Food Chemistry 2011 60: 209-213. Reported data were converted from mg/g dry weight to mg/100 g wet weight, based on the moisture content of broccoli seeds and sprouts reported in the paper (4.16% and 70.2%, w/w, respectively).

[21]Baenas N, Moreno DA, García-Viguera C. Selecting sprouts of Brassicaceae for optimum phytochemical composition. Journal of Agricultural and Food Chemistry 2012 60: 11409-11420.

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B. Estimated Daily Intake of Aqueous Broccoli Seed Extract Powder

In this section a calculation of the estimated daily intake (EDI) of aqueous broccoli seed extract powder by the U.S. population from all proposed uses in foods is provided.

1. Introduction

Brassica Protection Products intends to add aqueous broccoli seed extract powder to selected beverages in the U.S. food supply. The intended maximum use level is 115 mg aqueous broccoli seed extract powder per serving. A list of food products, default serving sizes and corresponding maximum use-level for aqueous broccoli seed extract powder are summarized in Appendix I2.

Spherix Consulting has completed an assessment of the consumption of aqueous broccoli seed extract powder by the U.S. population resulting from the proposed uses of aqueous broccoli seed extract powder. Estimates for the intake of aqueous broccoli seed extract powder were based on the proposed food uses and maximum use level in conjunction with food consumption data included in the National Center for Health Statistics' (NCHS) 2009-2010 National Health and Nutrition Examination Surveys (NHANES) (CDC, 2006; USDA, 2012; Bodner-Montville et al, 2006). Calculations for the mean and 90th percentile intakes were performed for all proposed food uses of aqueous broccoli seed extract powder combined. The intakes were reported for the following population groups:

- children, ages 2 to 5 years,
- children, ages 6 to 12 years,
- teenagers, ages 13-19 years,
- adults, ages 20 years and up,
- total population (all age groups combined, excluding infants of 0-2 years),
- total population (all age groups combined).

2. Food Consumption Survey Data

a. Survey Description

The most recent National Health and Nutrition Examination Surveys (NHANES) for the years 2009-2010 are available for public use. NHANES are conducted as a continuous, annual

survey, and are released in 2-year cycles. In 2009-2010, approximately 10,000 people across the U.S. completed the health examination component of the survey. Any combination of consecutive years of data collection is a nationally representative sample of the U.S. population. It is well established that the length of a dietary survey affects the estimated consumption of individual users and that short-term surveys, such as the typical 1-day dietary survey, overestimate consumption over longer time periods (Gregory et al, 1995). Because two 24-hour dietary recalls administered on 2 non-consecutive days (Day 1 and Day 2) are available from the NHANES 2009-2010 survey, these data were used to generate estimates for the current intake analysis.

The NHANES provides the most appropriate data for evaluating food-use and food-consumption patterns in the United States, containing 2 years of data on individuals selected *via* stratified multistage probability sample of civilian non-institutionalized population of the U.S. NHANES 2009-2010 survey data were collected from individuals and households *via* 24-hour dietary recalls administered on 2 non-consecutive days (Day 1 and Day 2) throughout all 4 seasons of the year. Day 1 data were collected in-person in the Mobile Examination Center (MEC), and Day 2 data were collected by telephone in the following 3 to 10 days, on different days of the week, to achieve the desired degree of statistical independence. The data were collected by first selecting Primary Sampling Units (PSUs), which were counties throughout the U.S. Small counties were combined to attain a minimum population size. These PSUs were segmented and households were chosen within each segment. One or more participants within a household were interviewed. Fifteen PSUs are visited each year. For the 2009-2010 NHANES, there were 13,272 persons selected; of these 10,253 were considered respondents to the MEC examination and data collection. 9754 of the MEC respondents provided complete dietary intakes for Day 1 and of those providing the Day 1 data, 8,405 provided complete dietary intakes for Day 2.

In addition to collecting information on the types and quantities of foods being consumed, NHANES 2009-2010 collected socioeconomic, physiological, and demographic information from individual participants in the survey, such as sex, age, height and weight, and other variables useful in characterizing consumption. The inclusion of this information allows for further assessment of food intake based on consumption by specific population groups of interest within the total population. Among those who completed food intake survey on both Day 1 and Day 2, 8301 respondents also provided physiological information including age, sex and weight; of these 7738 were 2 years and older.

Sample weights were incorporated with NHANES 2009-2010 to compensate for the potential under-representation of intakes from specific population groups as a result of sample

variability due to survey design, differential non-response rates, or other factors, such as deficiencies in the sampling frame (CDC, 2006; USDA, 2012).[4]

b. Statistical Methods

Consumption data from individual dietary records, detailing food items ingested by each survey participant, were collated by computer using R to read and edit the SAS files, and Matlab to generate estimates for the intake of aqueous broccoli seed extract powder by the U.S. population. Estimates for the daily intake of aqueous broccoli seed extract powder represent projected 2-day averages for each individual from Day 1 and Day 2 of NHANES 2009-2010 data; these average amounts comprised the distribution from which mean and percentile intake estimates were produced. Non-parametric statistical analyses were applied to the data. "All-person" intake refers to the estimated intake of aqueous broccoli seed extract powder averaged over all individuals surveyed, regardless of whether they consumed food products containing aqueous broccoli seed extract powder, and therefore includes "zero" consumers (those who reported no intake of food products containing aqueous broccoli seed extract powder during the 2 survey days). "All-user" intake refers to the estimated intake of aqueous broccoli seed extract powder by those individuals consuming food products containing aqueous broccoli seed extract powder, hence the "all-user" designation. Individuals were considered users if they consumed 1 or more food products containing aqueous broccoli seed extract powder on either Day 1 or Day 2 of the survey.

3. Food Usage Data

The individual proposed food uses for aqueous broccoli seed extract powder, default serving sizes and the corresponding maximum use-levels for aqueous broccoli seed extract powder (i.e., 115 mg per serving) employed in the current intake analysis are summarized in Appendix I2. Food codes representative of each proposed use were chosen from the Food and Nutrition Database for Dietary Studies (FNDDS). In FNDDS, the primary (usually generic) description of a given food is assigned a unique 8-digit food code (CDC, 2006; USDA, 2012).

4. Food Survey Results

The estimated "all-person" and "all-user" total intakes of aqueous broccoli seed extract powder from all proposed food uses of aqueous broccoli seed extract powder in the U.S. by population group is summarized in Table 12 and Table 13, respectively.

[4] A sample weight is assigned to each sample person. It is a measure of the number of people in the population represented by that sample person in NHANES, reflecting the unequal probability of selection, non-response adjustment, and adjustment to independent population controls.

Table 12. Estimated "All-person" Daily Intake (EDI) of Aqueous Broccoli Seed Extract Powder (ABSEP) from All Proposed Uses in the U.S. by Population Group (2009-2010 NHANES Data)

Population	N_u/N_p [A]	Percent users	Absolute EDI (mg ABSEP/day)		Weight-based EDI (mg ABSEP/kg/day)	
			Mean	90th Percentile	Mean	90th Percentile
Children, 2-5 years	100/764	13.1	17	28	1.1	1.7
Children, 6-12 years	242/1388	17.4	32	56	0.9	1.7
Teenagers, 13-19 years	355/1124	31.6	90	142	1.4	2.2
Adults, 20+ years	2224/5821	38.2	114	212	1.4	2.6
Total population (2+ years)	2921/9097 [B]	32.1	91	178	1.4	2.7
Total population	3564/9740 [B]	36.5	104	203	1.6	3.2

[A] Number of people consuming one or more foods containing aqueous broccoli seed extract powder on either Day 1 or Day 2 of the survey over the number of people surveyed.
[B]. 9704 respondents provided relevant dietary intakes for Day 1 and Day 2 and provided information including age; of these 9097 were 2 years and older, 235 were between the age of 1 and 2, and 408 were 0-1 year old.

Table 13. Estimated "All-user" Daily Intake (EDI) of Aqueous Broccoli Seed Extract Powder (ABSEP) from All Proposed Uses in the U.S. by Population Group (2009-2010 NHANES Data)

Population	N_u/N_p [A]	Percent users	Absolute EDI (mg ABSEP/day)		Weight-based EDI (mg ABSEP/kg/day)	
			Mean	90th Percentile	Mean	90th Percentile
Children, 2-5 years	100/764	13.1	128	210	8.1	13.3
Children, 6-12 years	242/1388	17.4	186	321	5.0	9.5
Teenagers, 13-19 years	355/1124	31.6	285	450	4.4	6.9
Adults, 20+ years	2224/5821	38.2	298	555	3.7	6.8
Total population (2+ years)	2921/9097 [B]	32.1	284	554	4.3	8.3
Total population	3564/9740 [B]	36.5	283	554	4.5	8.8

[A] Number of people consuming one or more foods containing aqueous broccoli seed extract powder on either Day 1 or Day 2 of the survey over the number of people surveyed.
[B]. 9704 respondents provided relevant dietary intakes for Day 1 and Day 2 and provided information including age; of these 9097 were 2 years and older, 235 were between the age of 1 and 2, and 408 were 0-1 year old.

5. Conclusions

In summary, 32.1% of the total U.S. population of 2+ years was identified as consumers of aqueous broccoli seed extract powder from the proposed food uses. The mean intakes of aqueous broccoli seed extract powder by the all aqueous broccoli seed extract powder consumers ("all-user") from all proposed food uses were estimated to be 284 mg/person per day or 4.3 mg/kg body weight per day. The heavy consumer (90th percentile all-user) intakes of aqueous broccoli seed extract powder from all proposed food-uses were estimated to be 554 mg/person/day or 8.3 mg/kg body weight per day. This results in a mean intake of 42.6-62.5 mg glucosinolates/day and a 90th percentile intake of 83.1-121.9 mg glucosinolates/day, from the aqueous broccoli seed extract powder.

In comparison, the mean and 90th percentile of broccoli intake is 16.4 g/person per day and 50.9 g/person per day. These levels of dietary broccoli intake result in an intake of 8-14 mg and 26-43 glucosinolates/day, respectively. One serving of fresh broccoli (36 g) provides 30 mg glucosinolates; one serving of cooked broccoli (78 g) provides 40 mg glucosinolates.

Therefore, mean intake of the aqueous broccoli seed extract power results in a glucosinolate intake similar to ingestion of 1-2 servings of cooked broccoli and 90th percentile intake approximates the glucosinolate intake one would receive from consuming 2-3 servings of cooked broccoli.

IV. INTENDED EFFECT

The intended effect of adding aqueous broccoli seed extract powder to food is to provide a dietary source of glucosinolates.

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V. METABOLIC FATE, PHYSIOLOGICAL EFFECTS AND SAFETY OF GLUCOSINOLATES AND CORRESPONDING ISOTHIOCYANATE METABOLITES

Broccoli, like other crucifers, contains a structural class of compounds called glucosinolates that, when hydrolyzed by the enzyme myrosinase, form the pungent isothiocyanates that are responsible for the characteristic flavor of these vegetables. Myrosinase occurs in the cells of cruciferous vegetables such that it is sequestered away from the glucosinolates until the plant's structural integrity is compromised, via chewing or plant disease, for example. Once the myrosinase contacts the glucosinolates, hydrolysis occurs, releasing the isothiocyanates which deter predators or delight afficionados. Certain human gut flora microbes exhibit myrosinase-like activity. Therefore, hydrolysis of glucosinolates to isothiocyanates can occur in the plant itself, in the context of cutting or chewing, and in the colon during digestion. Isothiocyanates are readily absorbed in the gastrointestinal tract once they are liberated from their glucosinolate precursors and are distributed with the blood flow. The timecourse for absorption of isothiocyanates and their appearance in the blood depends on the form that is ingested: isothiocyanates versus glucosinolates; and whether myrosinase is present. For example, ingestion of glucosinolates without active myrosinase means that isothiocyanates are not released until the glucosinolates reach the colon, resulting in a slower appearance of isothiocyanates (and their metabolic products) in the blood and urine. Overall bioavailability of isothiocyanates varies widely but is greater from raw cruciferous vegetables (8.2-113% reported in one publication) versus cooked (1.8-43% from the same publication). Isothiocyanates are rapidly metabolized via conjugation with glutathione, further catabolized and then excreted in the urine as mercapturic acids (degraded glutathione conjugates). Isothiocyanates do not accumulate in tissues and are cleared from the body within 36 hr. The typical American diet does not include many cruciferous vegetables; therefore, controlling the exposure to glucosinolates via consumption of aqueous broccoli seed extract powder to levels approximating background dietary levels is a reasonable strategy to assure the safety of this product.

Immature broccoli florets contain up to four structural classes of glucosinolates, the aliphatic glucosinolates ("Class 1"), allyl glucosinolates ("Class 2"), aromatic glucosinolates ("Class 3"), and indole glucosinolates ("Class 4"), while the earlier developmental forms of this plant, namely broccoli seeds and sprouts, contain mainly Class 1 glucosinolates (specifically, glucoraphanin and glucoerucin), and only minor, if any, amounts of Class 2, 3, and 4 glucosinolates (Anon 2010; Melchini et al. 2010; Clarke et al. 2011a; Clarke et al. 2011b). The broccoli we typically consume (green florets) contains lower amounts of Class 1, 2, and 3 glucosinolates and higher amounts of Class 4 glucosinolates, in comparison with broccoli seeds and sprouts. Consistent with these findings, five lots of the Brassica Protection Products, LLC aqueous broccoli seed extract powder contained >99% of Class 1 glucosinolates (glucoraphanin and glucoerucin) and ≤0.05% of Class 2, 3, and 4 glucosinolates (Table 5). Class 1

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glucosinolates contain an aliphatic R-group, Class 2 glucosinolates contain an allyl R-group, Class 3 glucosinolates contain an aromatic R-group, and Class 4 glucosinolates contain an indole R-group. In vivo, all classes are hydrolyzed to isothiocyanate derivatives by myrosinases that are either endogenously present in glucosinolate-containing foods or produced by flora present in the gastrointestinal tract (Figure 4; Getahun and Chung 1999; Rouzaud et al. 2004; Wagner and Rimbach, 2009; Shapiro et al. 2001; Halkier and Gershenzon 2006; Holst and Williamson 2004). Class 1-, 2-, and 3-derived isothiocyanates are then subsequently conjugated to mercapturic acid and excreted in the urine, whereas Class 4-derived isothiocyanates can be further modified to indole-3-carbanol and then ascorbigen, which is excreted in urine as well (Hauder et al. 2011).

Because the aqueous broccoli seed extract from Brassica Protection Products, LLC contains the Class 1 glucosinolates glucoraphanin and glucoerucin as 99.6% of the total glucosinolate content of the extract, the following section reviews the metabolic fate, physiological effects, and safety of Class 1 glucosinolates and their isothiocyanate metabolites. The amount of Class 2 (sinigrin, gluconapin, epigoitrin and progoitrin), Class 3 (gluconasturtiin), and Class 4 (glucobrassicin) glucosinolates in the aqueous broccoli seed extract were at or below the detection limits of the assays. Because Class 2, 3, and 4 glucosinolates are present in other dietary sources such as mustard, horseradish, and flavoring agents (which can reach levels up to 0.13% (*e.g.*, as allyl isothiocyanates in foods)) at levels higher than in the aqueous broccoli seed extract powder produced by Brassica Protection Product, LLC, the safety issues surrounding Class 2, 3, and 4 glucosinolates (IARC 1985) are minimally relevant here and will not be discussed.

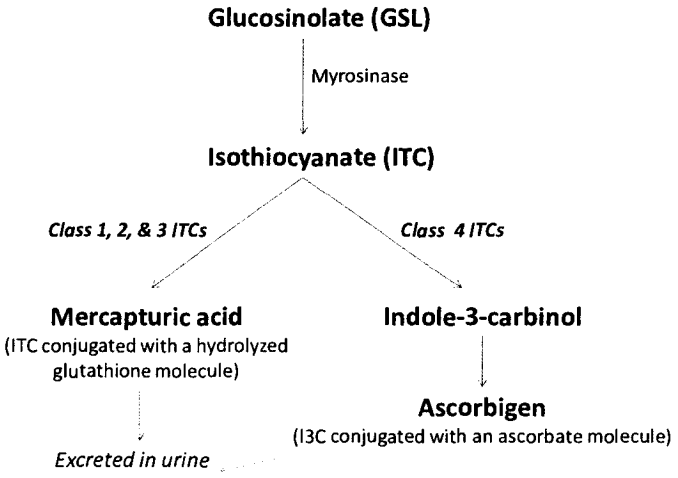

Figure 4. Generalized Scheme for Hydrolysis of Glucosinolates and Formation and Metabolism of Corresponding Isothiocyanates Ingested from Vegetables Consumed by Humans and Animals
Glucosinolates are initially converted to isothiocyanates by myrosinase. Class 1, 2, and 3 isothiocyanates are then conjugated with mercapturic acid (a specific dithiocarbamate), whereas Class 4 isothiocyanates can undergo additional metabolic steps (*Adapted from Shapiro et al. 2001*)

A. Pharmacokinetics of Glucosinolates and Isothiocyanates

1. Summary

Results from animals dosed with various glucosinolates show that absorption of isothiocyanates consumed in broccoli is rapid, occurring within 12 hr. However, appearance of isothiocyanate-conjugates found in the blood or urine is delayed following ingestion of cooked broccoli because of the absence of myrosinase which then requires hydrolysis of glucosinolates to isothiocyanates by gastrointestinal tract microbiota to liberate isothiocyanates for absorption/metabolism. Distribution in available animal studies shows that, after ingestion, isothiocyanate-conjugates are mainly present in the blood, gastrointestinal tract and excretory organs, indicating that there is no accumulation in the body.

Metabolism of hydrolyzed isothiocyanates in humans involves conjugation by glutathione in the liver shortly after absorption and sequential conversion to form mercapturic acid that is excreted in urine (see Figure 4). Excretion of isothiocyanate-conjugates is relatively rapid and essentially complete in humans with blood levels near baseline within 24-36 hr after ingestion of glucosinolates from foods.

2. Absorption of Glucosinolates and Isothiocyanates

The bioavailability of glucosinolates and their isothiocyanate and indole metabolites was assessed in 15 clinical studies (Table 15; Conaway et al. 2000; Getahun and Chung 1999; Cramer and Jeffery 2011; Cramer et al. 2012; Fahey et al. 2012; Clarke et al. 2011a; Clarke et al. 2011b; Egner et al. 2011; Vermuelen et al. 2008; Vermuelen et al. 2006; Rungapamestry et al. 2007; Petri et al. 2003; Ye et al. 2002; Shapiro et al. 2001; Shapiro et al. 1998) and two animal studies (Zhang et al. 2010; Bheemreddy and Jeffery 2007). Although quantitative studies on the direct absorption of glucosinolates are lacking, the bulk of the studies indicate that both glucosinolates and isothiocyanates are absorbed, however, isothiocyanates are more readily absorbed and exogenous sources of myrosinase increase glucosinolate-derived isothiocyanate bioavailability which thereby increases isothiocyanate excretion.

a. Human Studies

Conaway et al. (2000) evaluated the bioavailability of isothiocyanates from fresh and steamed broccoli with nearly identical amounts of glucosinolates (~1.0-1.1 μmol/g fresh wt.; ~0.45-0.49 mg) and reported that the relative absorption of isothiocyanates, quantified by 24-hr urinary excretion of mercapturic acid-conjugated metabolites of isothiocyanates in urine of 12 healthy men, was 32.3 ± 12.7% and 10.2 ± 5.9% of the amounts available from ingested

glucosinolates from fresh (+myrosinase) and steamed broccoli (-myrosinase), respectively. Total isothiocyanate-glutathione metabolites (isothiocyanate-metabolites) in plasma peaked between 0 and 8 hours and were approximately 3-4 fold higher with fresh compared to steamed broccoli.

Getahun and Chung (1999) evaluated the bioavailability of glucosinolate-derived isothiocyanates following ingestion of watercress, which are rich in the Class 3 phenylethyl glucosinolates (Rose et al. 2000). Nine human volunteers ingested cooked watercress that contained 475 μmol (~211 mg) glucosinolates. The absorption of isothiocyanates quantified by total recovery in 24 hr urine samples ranged from 5.6 to 34.8 μmol (~2.5-15.5 mg), corresponding to absorption of 1.2–7.3% the total amount of potential isothiocyanates from the amount of glucosinolates ingested. When the same volunteers were given a similar amount of glucosinolates from fresh watercress, urinary isothiocyanate metabolites representing amounts of absorption ranged from 17.2 to 77.7% of the total ingested isothiocyanates. When cooked watercress juice was incubated with fresh human feces under anaerobic conditions, approximately 18% of glucosinolates was hydrolyzed to isothiocyanates in 2 hr, suggesting that the gastrointestinal microbiota has the potential to hydrolyse glucosinolates to isothiocyanates.

Fahey et al. (2012) also assessed the efficiency of conversion of glucosinolates to isothiocyanates by gastrointestinal microbiota and subsequent absorption from the gastrointestinal tract by measuring total isothiocyanate metabolites in 12-hr urine samples in 45 healthy individuals given a broccoli sprout aqueous extract containing 200 μmol glucosinolates (~ 89 mg) (170 μmol glucoraphanin, 74 mg). The range of total 24-hour isothiocyanate metabolites excreted in urine following the administration of 200 μmol (~ 89 mg) glucosinolate (as an indicator of sulforaphane absorption) varied widely from 1.1%–40.7% with a mean 11.8%. The authors noted that although there were large differences in conversion and absorption efficiency of glucoraphanin between individuals as quantified by urinary isothiocyanate metabolites, the values within individuals was much more uniform suggesting significant differences in the intestinal microbiota and ability to convert glucosinolates to isothiocyanates in different individuals.

Cramer and Jeffery (2011) evaluated the bioavailability of the isothiocyanate metabolite sulforaphane in four healthy male subjects following the administration of a powder containing glucoraphanin with and without an air-dried broccoli sprout powder that contained myrosinase. The absorption of isothiocyanates, measured by levels of total isothiocyanate metabolites in 24 hr urine samples and levels in plasma over time, was 74%, 49%, and 19% of the glucoraphanin ingested from broccoli sprouts (~70 μmol, or 12.4 mg, sulforaphane), combination of sprouts + broccoli powder (- myrosinase) (131.7 μmol, or 23.4 mg total sulforaphane), and broccoli

powder (-myrosinase) (no sulforaphane detected, but upon addition of myrosinase, the powder released 61.7 μmol, or 10.9 mg, sulforaphane) diets, respectively. Combining the broccoli sprout powder that contained myrosinase with the powder that lacked myrosinase increased the levels of isothiocyanate absorption, but to levels that were lower than the amount seen in the subjects that were given the broccoli powder that contained myrosinase.

A similar follow-up study by Cramer et al. (2012) evaluated the absorption of sulforaphane from the glucoraphanin-rich broccoli powder (-myrosinase) used in Cramer and Jeffery (2011), but in combination with fresh broccoli sprouts, which contain active myrosinase. Four healthy volunteers each consumed meals containing one cup of dry cereal and a half cup of yogurt supplemented with or without broccoli sprouts (equivalent to 70 μmol sulforaphane, or 12.4 mg), glucoraphanin-containing powder (equivalent to 120 μmol sulforaphane, or 21.3 mg), or a combination of broccoli sprouts and glucoraphanin-containing powder (190 μmol sulforaphane, or 33.7 mg). Each phase of the experiment was separated by 1 week. The 24 hr urinary recoveries for sulforaphane metabolites, as a measure of absorption/conversion in the gastrointestinal tract, were 65, 60 and 24% of the dose ingested from the combination supplement, broccoli sprout and glucoraphanin powder (-myrosinase) supplements, respectively. Compared with the glucoraphanin powder (-myrosinase) or sprouts alone, combining broccoli sprouts that contained myrosinase with the glucoraphanin powder (-myrosinase) increased the amount of isothiocyanate absorption.

Clarke et al. (2011a) evaluated the bioavailability of sulforaphane and erucin in a crossover study in which 12 volunteers (19-50 yr) were given either broccoli powder supplements (6 tablets, devoid of myrosinase; ~3 g freeze-dried broccoli sprouts) or fresh broccoli sprouts (40 g). The supplements contained 121 and 40 μmol of glucoraphanin and glucoerucin, or 52.8 and 16.8 mg, respectively. The broccoli sprouts contained 150 and 71 μmol of glucoraphanin and glucoerucin, or 65.5 and 29.9 mg, respectively. The bioavailability of sulforaphane, erucin, and total isothiocyanates in the blood and urine was significantly lower with powdered broccoli supplements compared to fresh broccoli sprouts. The peaks in plasma concentrations and urinary excretion were also delayed when subjects consumed the broccoli supplement indicating a lag in the time for bioconversion to absorption/excretion, confirming the importance of myrosinase in fresh plant materials for optimizing of sulforaphane and other glucosinolates bioavailability. An additional report by Clarke et al. (2011b) reported that subjects who consumed a broccoli supplement without myrosinase (~3 g freeze-dried broccoli sprouts as 6 pills) had 5 times lower absorption, versus broccoli sprouts (68 g), as quantified by total 24-hr excretion of conjugated sulforaphane metabolite and, 8-fold lower absorption of erucin defined by metabolite excretion levels. The sprouts contained 218.4 μmol glucoraphanin (95.3 mg), 67.1

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μmol glucoerucin (28.2 mg), and 33.8 μmol glucoiberin (14.3 mg). The supplement contained 220.3 μmol glucoraphanin (96.2 mg), 75.9 μmol glucoerucin (31.9 mg), and 31.7 μmol glucoiberin (13.4 mg).

Egner et al (2011) quantified the bioavailability and absorption of 800 μmol glucoraphanin (349.2 mg) or 150 μmol sulforaphane (26.6 mg) from an aqueous broccoli sprout extract in a cross-over study involving 50 Chinese participants. Bioconversion and absorption was significantly greater ($p<0.001$; mean =70%) from the sulforaphane beverage than with the glucoraphanin beverage (mean = 5%) as measured by urinary excretion of total sulforaphane metabolites in 12 hr urine samples. Interindividual variability in excretion was also considerably lower with sulforaphane than with the glucoraphanin beverage that required hydrolysis by gastrointestinal tract bacteria to be available for absorption.

Vermeulen et al. (2008) reported similar effects on increased bioavailability of sulforaphane from broccoli glucosinolates in the presence of plant myrosinase. Eight men given 200 g of crushed fresh or cooked broccoli (-myrosinase) in an open cross-over study had higher amounts of sulforaphane metabolites in blood and urine in the fresh broccoli group versus the cooked broccoli group. The cooked broccoli contained 26 mg glucoraphanin, 2.3 mg glucoiberin, and 45.6 mg glucobrassicins; isothiocyanate content was not reported. The fresh broccoli 8.9 mg sulforaphane; glucosinolate content was not reported. Absorption of sulforaphane, measured by excretion of sulforaphane metabolites in urine, was delayed significantly ($p<0.001$) when cooked broccoli was consumed (peak plasma time= 6 hr) versus fresh broccoli (1.6 hr) indicative of the lag time for bioconversion and absorption from the gastrointestinal tract. Bioavailability was 37% of the isothiocyanate-equivalents in fresh broccoli and significantly greater than the amount in cooked broccoli (3.4%; $p<002$). In an earlier study that evaluated glucosinolate bioavailability from consumption of 13 cruciferous vegetables (isothiocyanate content of the broccoli meal was 1.1 mg iberin, <0.3 mg erucin, 9.2 mg sulforaphane, and 6.7 mg indolylmethylisothiocyanate) and six condiments, eight different isothiocyanate mercapturic acid metabolites were found in urine samples. Absorption from consumption of fresh vegetables and condiments was higher overall (bioavailability: 8.2-113%) as compared to cooked vegetables (bioavailability: 1.8- 43%) as measured by total isothiocyanate metabolites in 24 hr urine samples (Vermuelen et al. 2006).

Rungapamestry et al. (2007) determined the bioavailability and absorption of isothiocyanates from 150 g lightly cooked broccoli (microwaved 2.0 min) or fully cooked broccoli (microwaved 5.5 min) showed that yield of total sulforaphane metabolites in urine samples was about 3-fold higher after consumption of lightly cooked broccoli than fully cooked broccoli. Interactions with allyl isothiocyanates added to the meal matrix did not significantly

influence the hydrolysis of glucoraphanin or the amount of excretion of sulforaphane metabolites. The results were consistent with increased sulforaphane bioavailability from glucosinolates in broccoli following partial vs. complete inactivation of myrosinase by microwave cooking times.

Petri et al. (2003) compared the absorption of isothiocyanates from broccoli (Class 1 glucosinolates) and onion extracts (Class 2 s) in 10-cm intestinal segments of six volunteers. Intestinal isothiocyanate permeability was estimated from the luminal disappearance and intestinal metabolism of sulforaphane in human proximal jejunum following single-pass perfusion of the intestinal segment with broccoli or onion extracts. After administration of the perfusion solution (onion or broccoli extract), the perfusate was quantitatively collected on ice in 10 min fractions and immediately frozen at ≤20°C for analysis of isothiocyanate content. The results showed that isothiocyanates from onion and broccoli extract permeated the enterocytes in the perfused jejunal segment and at the "physiologically relevant dietary concentrations tested," the average percentage of sulforaphane absorbed was 74 ± 29%. In addition, the authors reported that a proportion of sulforaphane was conjugated and excreted back into the lumen as sulforaphane-glutathione and this finding of intrahepatic recirculation may help to explain the differential bioavailability of isothiocyanates from similar types of diets rich in glucosinolates.

Ye et al. (2002) determined the rate and extent of absorption of isothiocyanates in four volunteers given 200 μmol broccoli sprout isothiocyanates containing sulforaphane, iberin and erucin. The authors observed rapid absorption of the isothiocyanates and peak concentrations in plasma, serum and erythrocytes were attained by 1 hr post-dosing. The cumulative excretion of isothiocyanate metabolites indicative of total absorption at 8 hr was 58.3 ± 2.8% of the dose.

Shapiro et al. (2001) evaluated the bioavailability of isothiocyanates in a series of experiments with four healthy volunteers given broccoli sprout glucosinolates or isothiocyanates. The extent of absorption, measured by urinary excretion of total isothiocyanate metabolites after dosing, showed significantly higher bioavailability of isothiocyanates versus glucosinolates. Urinary isothiocyanate conjugate excretion peaked within 8 hr post-dosing and decreased in a non-first-order fashion. Another study with 11 subjects given fresh broccoli sprouts that contained 100 μmol glucosinolates (~44.5 mg) showed significantly higher absorption of isothiocyanates estimated from amounts of isothiocyanate metabolites in urine from the group that chewed the sprouts, versus the group that swallowed them whole; a result that was consistent with findings of others that myrosinases in fresh sprouts released by plant maceration facilitates absorption by converting the glucosinolates to isothiocyanates which are absorbed more efficiently.

Shapiro et al. (1998) studied glucosinolates absorption in 18 healthy volunteers given broccoli in which myrosinase had been heat-inactivated. They found that only low amounts (10-20%) of total isothiocyanate conjugates, largely dithiocarbamates (mercapturic acids and related compounds), according to the authors, were recovered in the urine relative to the potential amount of isothiocyanates calculated from the amounts of glucosinolates in the broccoli. In another experiment, broccoli treated with myrosinase and given to the volunteers showed 47% recovery of isothiocyanate conjugates in urine that demonstrated the role of predigestion with myrosinase in maximizing bioavailability of isothiocyanates generated from glucosinolates. The authors noted that microbiota in the intestine play a pivotal role in conversion of glucosinolates to isothiocyanates for absorption from the gastrointestinal tract based on observations from additional studies in which the bowel microbiota of the volunteers were reduced by mechanical cleansing and administration of antibiotics with the result that conversion of isothiocyanates from ingested glucosinolates (-myrosinase) was reduced to minimal levels.

b. Animal Studies

Bheemreddy and Jeffery 2007 determined the absorption and disposition of purified broccoli seed glucoraphanin in adult male F344 rats given either saline or 65 mg glucoraphanin/kg bw either by i.p. injection or by gavage. Urine and feces were then collected every 4 hr for the first 12 hr and at 24, 36, 48, 60, and 70 hr post treatment. Five percent of the oral dose of glucoraphanin was recovered in urine, showing that some is absorbed intact and excreted unmetabolized, which is unlike its fate in humans. No glucoraphanin was found in feces suggesting that it may also be metabolized to other compounds in the gastrointestinal tract. Total urinary sulforaphane metabolites accounted for 20 and 45% of oral and intraperitoneal doses, respectively, including sulforaphane mercapturic acid (12.5 and 2.0%), free sulforaphane (0.65 and 0.77%), sulforaphane nitrile (2.0 and 1.4%), and erucin (0.1 and 0.1%), respectively for oral and peritoneal routes. The total amount of sulforaphane conjugate excreted in 36 hr by the i.p.-treated rats was significantly less than rats dosed orally (0.67 ±0.06 μmol) and accounted for an absorption of only ~2% of the glucoraphanin dose by this route of administration. isothiocyanates from oral doses of glucoraphanin could be detected in urine for up to 36 hr.

Zhang (2010) cited two studies that showed that more than 90% of 25 or 250 μmol/kg (2.5 or 24.8 mg/kg) of [14C]-labeled AITC was absorbed and approximately 80 % of the administered amounts were recovered in the urine as the mercapturic acid conjugate in mice and rats.

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3. Distribution

Although the data on isothiocyanate distribution in humans is limited to one study (Cornblatt et al. 2007), five animal studies evaluated the distribution of glucosinolates and isothiocyanate metabolites (Cornblatt et al. 2007; Brusewitz et al. 1977; IARC 1985; two studies cited in Zhang 2010). Furthermore, numerous pharmacokinetic studies in humans indicate that isothiocyanates are rapidly absorbed, metabolized, and eliminated within 24-36 hr after ingestion. Thus, accumulation of isothiocyanates or their conjugates is unlikely in humans.

a. Human Studies

Cornblatt et al. (2007) administered a freeze-dried aqueous broccoli sprout preparation containing 200 μmol sulforaphane (35.4 mg) to eight women 50 min prior to undergoing elective reduction mammoplasty. Sulforaphane metabolites were detected in the endothelial cells from breast tissue, urine and plasma but the highest concentration was found in the urine. The average time from ingestion of the dose to removal of the breast tissue sample used to quantify sulforaphane was approximately 100 minutes.

b. Animal Studies

Cornblatt et al. (2007) administered an oral 150 μmol dose of sulforaphane (26.6 mg) to 10-week-old female Sprague–Dawley rats. As early as 30 minutes later, sulforaphane metabolites could be detected in mammary tissue.

Brusewitz et al (1977) orally administered a cysteine conjugate of ^{14}C-radiolabeled benzylisothiocyanate in CFHB Wistar rats (20 mg/kg) and beagle dogs (5 mg/kg). Over 3 d, a mean of 92.4 ± 5.8% of the radioactivity was excreted in the urine of the rats, 5.6 ± 1.7% in the feces and 0.4% in the expired air. Less than 0.5 ± 0.04% was retained in the carcass. In dogs, after 3 d, a mean of 86.3% of the radioactivity was excreted in the urine and 13.2% in the feces. These results suggest little to no accumulation of benzylisothiocyanate in rats and dogs after oral administration. The authors also mentioned that, for an unpublished whole body autoradiography study, the gastrointestinal tract, liver and kidneys, which are directly associated with the absorption and elimination of the isothiocyanates, had the highest levels of radioactivity for dogs and rats, and that significant amounts of radioactivity were also found in the blood (veins/arteries) of these two species (percent not mentioned).

In Fischer 344 rats and B6C3F1 mice administered radiolabeled allyl isothiocyanate intravenously at 25 mg/kg bw, the greatest concentration of radioactivity was found in the

urinary bladder (male rats and mice) and kidneys (male mice) after 15 min (IARC 1985). The bladders of males contained 5-10 times more radioactivity versus bladders of females.

Zhang (2010) cited two studies that showing that more than 90% of 25 or 250 μmol/kg (2.5 or 24.8 mg/kg) of [^{14}C]-labeled AITC was absorbed in mice and rats and approximately 80 % of administered doses was recovered in the urine as the mercapturic acid conjugate.

c. Summary

Sulforaphane metabolites have been detected in breast tissue, urine and plasma in humans. Metabolites of other glucosinolates can be found in the mammary glands, urine, feces, bladder and expired air, plus the gastrointestinal tract, kidney, liver and bloodstream of rats. GSL metabolites have been detected in the urine, urinary badder, and kidneys of mice. In dogs, GSL metabolites have been measured in urine and feces.

4. Metabolism

Eighteen clinical studies (Cramer et al. 2012; Cramer et al. 2011; Fahey et al. 2012; Li et al. 2011; Vermuelen et al. 2008, Vermuelen et al. 2006; Cornblatt et al. 2007; Rungapamestry et al. 2007; Ye et al. 2002; Shapiro et al. 2001, Shapiro et al. 1998; Conaway et al. 2000; Getahun and Chung 1999; Mennicke et al. 1988; Brusewitz et al. 1977; Egner et al. 2011; Clarke et al. 2011a; Clarke et al. 2011b) and two animal studies (Bheemreddy et al. 2007; Lai et al. 2010) evaluated the metabolism of glucosinolates. Fifteen of the 18 clinical studies have been discussed in Section V.A.2 and used analytical procedures that quantified total isothiocyanate metabolites in the blood or urine (Cramer et al. 2012; Cramer et al. 2011; Fahey et al. 2012; Li et al. 2011; Vermuelen et al. 2008; Vermuelen et al. 2006; Cornblatt et al. 2007; Rungapamestry et al. 2007; Ye et al. 2002; Shapiro et al. 2001, Shapiro et al. 1998; Conaway et al. 2000; Getahun and Chung 1999;). Five additional clinical studies and two animal studies that evaluated glucosinolate metabolism are discussed below (Mennicke et al. 1988; Brusewitz et al. 1977; Egner et al. 2011; Clarke et al. 2011a; Clarke et al. 2011b; Bheemreddy et al. 2007; Lai et al. 2010). In general, glucosinolates are metabolized to mercapturic acid-containing isothiocyanate conjugates in both animals and humans, however it is noteworthy that unconjugated glucosinolate was detected in the urine of rats suggesting that they may be less efficient at metabolizing glucosinolates to their corresponding isothiocyanate.

a. Human Studies

Egner et al (2011) used isotope dilution mass spectrometric assay to quantify individual isothiocyanate conjugates in urine samples from 50 volunteers that consumed either a

glucoraphanin-rich broccoli powder beverage treated with or without myrosinase. Measurement of urine samples collected from volunteers and analyzed for glucosinolates and isothiocyanates showed that sulforaphane-mercapturic acid was the major conjugate in urine but that sulforaphane, and sulforaphane-cysteine were also present (concentrations not reported in the publication). A previous pilot study also revealed presence of sulforaphane-glutathione and sulforaphane-cysteinylglycine in urine but at only trace levels "substantially less than 0.1%" of the total pool of excreted sulforaphane equivalents such that these minor metabolites were not included in the main study. The presence of these unique sulforaphane conjugates in human urine has not been independently confirmed in our review of the literature. Surprisingly, this study is also the only available publication that reported the absorption and elimination of glucoraphanin as an intact glucosinolate present in urine samples from only the volunteers that were given glucoraphanin rich powder without myrosinase treatment; although the authors noted that the levels were less than 3% of the administered dose and about 10% of the excreted dose.

Clarke et al. (2011a, 2011b) used quantitative HPLC-MS/MS analysis to detect and quantitate glucosinolates and isothiocyanate conjugates in blood and urine samples from subjects that consumed glucosinolates/ isothiocyanates from extracts of broccoli sprouts or broccoli supplements. In the plasma, the most abundant metabolite for sulforaphane and erucin was the cysteine–glycine conjugate, representing ~85 and ~70% of the total amounts collected. Similarly, the most abundant urinary metabolites for sulforaphane and erucin, were the mercapturic acid conjugates. Free sulforaphane and the sulforaphane-cysteine conjugate were also found in urine. Data from their study suggested that erucin and sulforaphane were interconvertible *in vivo* based on observations of increases in erucin conjugates in urine at levels greater than found in the broccoli sprouts given to the volunteers.

Mennicke et al. (1988) evaluated metabolism of benzyl isothiocyanate in volunteers fed garden cress by infrared spectroscopy and ^{1}H – nuclear magnetic resonance and found benzyl isothiocyanate-mercapturic acid was excreted in urine. Free benzyl isothiocyanate was also found in a urine sample of one volunteer given benzyl isothiocyanate. The methods were not sensitive enough to detect other metabolites.

Similar findings of mercapturic acid as the major metabolite of benzyl isothiocyanate were reported by Brusewitz et al. (1977). Radiolabeled benzyl isothiocyanate was rapidly conjugated when incubated with glutathione (GSH). The corresponding cysteine conjugate was formed when the GSH or cysteinylglycine conjugates of benzyl isothiocyanate were incubated with rat liver or kidney homogenates. The corresponding mercapturic acid conjugate was formed following incubation of the cysteine conjugate of benzyl isothiocyanate in the presence of acetyl-

CoA. The authors of this early study concluded that mercapturic acid biosynthesis may be an important route of metabolism of certain isothiocyanates.

b. Animal Studies

Bheemreddy et al. (2007) administered 150 μmol/kg of glucoraphanin (65.5 mg/kg) was administered to F344 rats by gavage or i.p injection, and urine and feces were collected every four hr for the first 12 hr and at 24, 36, 48, 60, and 70 hr post post-treatment. Five percent of the orally administered dose was recovered intact in urine and no glucoraphanin or sulforaphane was found in feces, suggesting that further metabolism of these substances into compounds not identified by the analytical method occurred in the gastrointestinal tract. Metabolites recovered from the respective oral or intraperitoneal (i.p.) doses included sulforaphane mercapturic acid conjugate (12.5 and 2%), free sulforaphane (0.65 and 0.77%), sulforaphane nitrile (2 and 1.4%), and erucin (0.1 and 0.1%), as fractions of the oral and i.p doses respectively. Both glucoraphanin and glucoerucin were identified in bile following intravenous glucoraphanin administration indicating the interconvertability of these glucosinolates. The authors concluded that orally administered glucoraphanin in rats is absorbed intact, undergoes enterohepatic circulation, and is hydrolyzed in the gastrointestinal tract. Analysis of urine from both groups of glucoraphanin-treated rats identified intact glucoraphanin, the reduced analogue glucoerucin, and several metabolites.

Lai et al. (2010) dosed male F344 rats with 150 μmol sulforaphane/kg bw (26.6 mg) or glucoraphanin (30.4% pure; 19.9 mg glucoraphanin administered) directly in the cecum and measured total isothiocyanate equivalents in the plasma of mesenteric tissue surrounding small intestine. Isothiocyanates were formed in the mesenteric plasma of rats treated with either glucoraphanin or sulforaphane, with a faster time course observed for the appearance of sulforaphane in the mesenteric plasma.

5. Excretion

Nine clinical studies (Cramer and Jeffery 2011; Cramer et al. 2012; Clarke et al. 2011a; Egner et al. 2011; Vermuelen et al. 2008; Ye et al. 2002; Shapiro et al. 2001; Conaway et al. 2000; Mennicke et al. 1988) and one animal study showed that isothiocyanate metabolites of glucosinolates derived from foods or dietary glucosinolate supplements are rapidly excreted (Cornblatt et al. 2007).

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a. Human Studies

Cramer and Jeffery (2011) conducted a cross-over study with 4 healthy male volunteers to determine bioavailability of sulforaphane from air-dried broccoli sprouts (12.4 mg sulforaphane) or 2 g of a glucoraphanin rich broccoli powder (-myrosinase) alone (capable of generating 21.3 mg sulforaphane in the presence of myrosinase) or a combination of both ingredients. Urine collection was separated into 3 discrete intervals for evaluation of kinetics of excretion of sulforaphane conjugates. Urine was collected for the first 6 hr after meal ingestion (T1), from 6–12 hr post-ingestion (T2), and from 12–24 hr post-ingestion (T3). Significant differences were seen among dietary groups, with substantial levels of sulforaphane conjugates excreted during T1 for both the combination meal and the broccoli sprouts (44% and 37% of total conjugates excreted within 24 hr, respectively), but less than 10% of the conjugates were excreted during this first 6-hr period from those receiving the broccoli powder meal (-myrosinase). Only 22% of the total amount of conjugates recovered in 24 hr was excreted in T3 period from either the broccoli sprouts or combination meal while 39% of conjugates from the broccoli powder meal were excreted during this same period. The delay in excretion of sulforaphane conjugates in urine is consistent with the requirement for hydrolysis of glucosinolates in the broccoli powder by commensal bacteria before they are available for absorption, distribution and excretion.

A follow-up study by Cramer et al. (2012) evaluated the absorption of sulforaphane from the glucoraphanin-rich myrosinase-deficient broccoli powder in combination with fresh broccoli sprouts, which contain myrosinase. The 4 healthy volunteers participated in a crossover study wherein each consumed a base diet consisting of dry cereal and yogurt. To the diet was added either sprouts (equivalent to 70 mmol sulforaphane, or 12.4 mg), a glucoraphanin-containing powder (equivalent to 120 mmol sulforaphane, or 21.3 mg), both supplements, or a control diet without sulforaphane. Each supplemented diet was administered with a one week separation period between them. Appearance of isothiocyanate conjugates in urine was delayed following the broccoli powder meal compared with the sprout and combination meals that contained myrosinase to produce isothiocyanates. Urine collection was separated into 3 discrete intervals for evaluation of excretion of sulforaphane conjugates. Urine was collected for the first 6 h after meal ingestion, from 6 to 12 h and from 12 to 24 h post-ingestion. Significant differences were observed between the dietary groups and 61 and 62% of the total sulforaphane conjugates recovered in 24 hr were excreted during the first 6 hr from individuals who received the combination meal or the broccoli sprout meal, respectively, but less than 22% of the total sulforaphane conjugates recovered over 24 hr was excreted during this first 6 hr period from those receiving the broccoli supplement alone (-myrosinase).

The time course of excretion of glucosinolate and isothiocyanate metabolites in urine was compared in a crossover study with 12 subjects (19-50 yr) that consumed either 40 g of broccoli sprouts (150 and 71 μmol glucoraphanin and glucoerucin, respectively, +myrosinase; 65.5 mg glucoraphanin and 29.9 mg glucoerucin) or broccoli supplement powder (121 and 40 μmol glucoraphanin and glucoerucin, respectively, -myrosinase; 52.8 mg glucoraphanin and 16.8 mg glucoerucin) (Clarke et al. 2011a). Urinary excretion of isothiocyanate metabolites peaked between 3 and 6 hr following dosing with the broccoli sprouts but the peak in urinary excretion was delayed until between the 6 and 12 hr time points with the broccoli powder that provided unhydrolyzed glucosinolates. The findings of this study were consistent with the expected lag time in hydrolysis of glucosinolates in the gastrointestinal tract prior to being available for absorption when compared to isothiocyanates preformed from action of myrosinase in the fresh broccoli.

Evaluation of urinary excretion rates was conducted as part of a bioavailability study of sulforaphane in 50 healthy volunteers given broccoli sprout beverages that contained either glucoraphanin or sulforaphane (349.2 mg glucoraphanin or 26.6 mg sulforaphane) generated in the same beverage by addition of myrosinase in a crossover study (Egner et al. 2011). Isotope-dilution mass spectrometry was used to measure levels of glucoraphanin, sulforaphane and sulforaphane conjugates in the urine samples. In volunteers given sulforaphane beverage 95% of the total sulforaphane metabolites were eliminated in urine within 12 hr, whereas when glucoraphanin was administered only 60% of the total sulforaphane metabolites were eliminated in the same time period. The initial elimination rate was about 10 times lower in the 12-hour level urine collections from subjects given the glucoraphanin beverage than the elimination rate in the group give the sulforaphane beverage (0.044 vs. 0.413 vs; $p < 0.001$). The authors noted that they were also able to detect and quantify the absorption and urinary elimination of glucoraphanin as a glucosinolate in humans noting that these levels reflected less than 3% of the administered dose and about 10% of the excreted dose. Similar excretion of unhydrolyzed glucosinolates have also been reported in rats by Cornblatt et al. 2007 and are rarely detected or reported in human studies.

In studies of bioavailability and absorption of sulforaphane from fresh (containing 1.8 mg sulforaphane) versus cooked broccoli (containing 26.8 mg glucoraphanin, 2.3 mg glucoiberin, and 45.6 mg glucobrassicins) given to 8 healthy men, Vermuelen et al. (2008) observed that although absorption of sulforaphane into blood was significantly delayed following ingestion of cooked broccoli (peak plasma time = 6 hr) when compared to ingestions of fresh broccoli isothiocyanates liberated by myrosinase (peak plasma time = 1.6 hr), there was no significant

difference in urinary excretion half-lives in volunteers given sulforaphane from either source (mean = 2.6 ± 0.4 and 2.4 ± 0.9 hr for fresh and cooked broccoli, respectively).

Ye and co-workers measured the excretion of isothiocyanates in four volunteers given 200 μmol broccoli sprout isothiocyanates (largely sulforaphane and small amounts of iberin and erucin; ~35 mg isothiocyanates) (Ye et al. 2002). Plasma concentration of isothiocyanates peaked 1 hr after feeding and then decreased with first order kinetics with a half-life of 1.77 ± 0.13 hr. Cumulative excretion at 8 h was 58.3 ± 2.8% of the dose, and clearance was 369 ± 53 mL/min which indicated active renal tubular secretion.

Urinary excretion rates following equimolar doses of glucosinolates and isothiocyanates were measured in 4 healthy male volunteers following ingestion of broccoli sprout extracts or isothiocyanates from the same extracts treated with myrosinase (Shapiro et al. 2001). Administering free isothiocyanates as extracts pre-treated with myrosinase showed peak excretion of isothiocyanate conjugates occurring within 4 hr after dosing, and 92.9 ± 1.1% of total isothiocyanates were excreted within 16 hr. In contrast, maximum excretion of urinary isothiocyanate conjugates following exposure to the glucosinolate-rich extract was delayed to 8 hr postdosing and only 79.5 ± 5.3% was recovered in urine by 16 hr after dosing. Volunteers fed four repeated 50 μmol doses of isothiocyanates (~8.9 mg isothiocyanates per dose) showed low degrees of the intra- and inter-subject variation in isothiocyanate conjugate excretion (coefficient of variation = 9%), and excretion was linear over a 25- to 200-μmol (~4.4 to ~35.5 mg) escalating dose range. Overall, the variation among subjects in excretion of isothiocyanate conjugates after isothiocyanate dosing (CV: 8.2%) was smaller than that after glucosinolate dosing (CV: 17.7%).

In crossover studies with 12 healthy men fed fresh or steamed broccoli with nearly identical amounts of glucosinolates (~1.0-1.1 μmol/g fresh wt.; ~0.4-0.5 mg), Conaway et al. (2000) observed maximal excretion of urinary isothiocyanate conjugates within two to eight hours in both studies, but the peak excretion of isothiocyanate equivalents after ingestion of steamed broccoli was delayed compared with excretion after ingestion of isothiocyanates following myrosinase action in fresh broccoli. Shapiro et al. (1998) reported similar rates of urinary excretion of isothiocyanate conjugates in 8-hr collections urine from a volunteer given sequentially higher doses of broccoli homogenate that contained 25, 50, 100, or 200 μmol (~11, 22, 45, or 89 mg) of non-indole glucosinolates. Although the rate of excretion was not affected by the dose level of glucosinolates, a non-linear pattern was seen that did not obey first-order kinetics and the terminal half-life was about 35 hr. The curvilinear excretion pattern was hypothesized to result from competing factors including rate of glucosinolate hydrolysis in the

gastrointestinal tract with variable rates of absorption, biliary excretion and reabsorption of different glucosinolate metabolites with overlapping kinetic curves. A subsequent crossover study with 6 volunteers given a single dose of broccoli glucosinolates or isothiocyanates showed that the urinary excretion rate was much faster for isothiocyanates compared to glucosinolates (t½ of 12 hr for sulforaphane vs. ≥ 20 hr for the intact glucosinolates from broccoli) which is consistent with the lag in gastrointestinal digestion by gastrointestinal tract microbiota required for hydrolysis of isothiocyanates from glucosinolates to isothiocyanates.

Studies on excretion of metabolites of benzyl isothiocyanate (used pharmacologically to treat urinary tract infections) in urine of volunteers fed 25-27 g fresh garden cress, showed that benzylthiocarbamoylmercapturic acid was excreted in urine, reaching peak levels after 2-4 hr and exhibited undetectable levels after 12 hr (Mennicke et al. 1988). A total of 53.7 ± 5.9% of the dose administered was recovered and no other metabolites were detected.

b. Animal Studies

Cornblatt et al. 2007 administered 150 μmol of sulforaphane (26.6 mg) to female Sprague Dawley rats by gavage and collected blood samples from 3 animals at 0.5, 1, 2, 4, 8, 12, 24 and 48 hr after treatment and measured concentrations of sulforaphane metabolites in the rat plasma. Pharmacokinetic parameters were generated by fitting the plasma data to a non-compartmental model that yielded an AUC of 491 hr μmol/l, C_{max} of 60 μM, and an elimination half-life ($t_{1/2}$) of 6.7 hr. The longer $t_{1/2}$ time obtained in this study compared to what has been reported in humans may be attributable to the presence of sulforaphane metabolites that have not been found in humans.

B. Safety Studies

The various glucosinolates found in broccoli are distributed into four structural classes (Classes 1, 2, 3 and 4), only one of which occurs to significant levels in broccoli seeds (Class 1). This simplifies the safety assessment of the aqueous broccoli seed extract powder. Some glucosinolates and isothiocyanates are more biologically reactive than others in specific mutagenicity or genotoxicity assays; however, glucoraphanin, a Class 1 glucosinolate, tested negative in these assays. As discussed in section V.1, the presence of myrosinases can affect the glucosinolate metabolism and subsequent glucosinolate and isothiocyanate absorption. As mentioned in section V.A.5, the isothiocyanate conjugates of glucosinolates are excreted in the urine, exposing the bladder and urine to these compounds.. Studies in animals have reported some effects on the liver (increased liver weight) and thyroid glands (decreased weight, and reduced follicle size) for certain classes of isothiocyanates such as Class 2 and 3 glucosinolates,

but effects on bladder and kidneys are apparently minimal, in spite of their exposure to glucosinolates and/or their metabolites. It is important to note that Class 2 and 3 glucosinolates are present at low levels (0.004% - 0.006% and 0.02-0.04%, respectively) in the aqueous broccoli seed extract powder produced by Brassica Protection Products, LLC. These levels are not high enough to generate concern about exposure to Class 2 and 3 glucosinolates from ingestion of the aqueous broccoli seed extract. Therefore, the emphasis here is on class 1 glucosinolates.

1. Class 1 Glucosinolates and Corresponding Isothiocyanates

Class 1 glucosinolates include glucoraphanin and glucoerucin, which are found in high concentrations in broccoli seeds, sprouts and mature plants (Anon 2010; Melchini et al. 2010; Clarke et al. 2011a; b). Intact glucoraphanin is absorbed more slowly versus its isothiocyanate product. Glucoraphanin has been administered in short-term clinical studies at doses up to 800 μmol/day (349.2 mg); however, the duration of such studies was no longer than 2 wk, making it difficult to ascertain the safety of its ingestion after long periods of time.

a. Human Studies

Eighteen clinical studies of glucosinolate and isothiocyanate intake from broccoli sources in adults were found and reviewed (Table 15). Although the objective of these studies was to evaluate endpoints relevant to efficacy, six of the studies reported information that corroborates the safety of ingestion. The duration of the studies was relatively short, with no more than 2 weeks of intake, and the levels of glucosinolates and isothiocyanate administration ranged up to 534 mg/day (glucosinolates) or 35.5 mg/day (isothiocyanates). Two studies administered glucosinolates for longer than two weeks (Traka et al. 2008; Yanaka et al. 2009) and did not report safety information. The intakes and durations of exposure in the six studies that provided safety data ranged between 133.5 and 534.0 mg glucosinolates/day or between 3 and 33.7 mg isothiocyanates/day with the longest duration of exposure of 2 weeks. No adverse treatment findings were reported in clinical chemistry, urinalysis or hematology parameters. Some mild intolerance was reported at higher levels of ingestion; taste aversion to higher levels of isothiocyanates was seen in one study. Intolerance was not cited as a reason for withdrawal from any study and no test-article related serious adverse events were reported.

To assess the safety of a broccoli sprout extract, a large unblinded clinical study was undertaken in the province of Qidong, China (Qidong 2013, unpublished preliminary report) where beverages of glucoraphanin + sulforaphane rich broccoli sprout extract (600 μmole glucoraphanin and 40 μmole sulforaphane; 261.9 and 7.1 mg, respectively) or placebo were

administered to healthy subjects for 84 days. During the study, 50% of all subjects consumed all their allotted beverages and the remaining participants consumed at least 80 of the 84 allotted beverages, indicating excellent compliance. Blood samples were drawn immediately after consumption of the last consecutive daily ingestion of the beverages and the effects of the extract on blood chemistries was determined (Table 14). There was no difference in any of the clinical chemistry analyses between the two groups. No serious adverse events occurred. Only 10 adverse events occurred over the course of the study: 6 in the test article group and 4 in the placebo group. Adverse events in the test group consisted of vomiting (1), indigestion (4), and taste aversion (1). Adverse events in the placebo group consisted of indigestion (2) and taste aversion (2). The extract of 3 d old broccoli sprouts that was used in the Qidong study and the aqueous broccoli seed extract that is the subject of this GRAS have very similar glucosinolate profiles, and differ mainly in their quantitative content of glucoraphanin and glucoerucin (data not shown).

Table 14. Summary of Clinical Chemistry Analyses from Qidong 2013 Study

Analyte	Broccoli Sprout Beverage (n=136) (mean ± SD)	Placebo (n=131) (mean ± SD)	Units
Glucose	78±16	81±25	mg/dL
BUN	17±4	18±4	mg/dL
Creatinine	0.75±0.12	0.75±0.13	mg/dL
Na	140±2	140±2	mg/dL
CO_2	30.7±3.8	30.3±3.8	mg/dL
Ca	9.8±0.3	9.8±0.3	mg/dL
Total Bilirubin	0.49±0.21	0.51±0.16	mg/dL
Direct Bilirubin	0.10±0.09	0.10±0.08	mg/dL
Total Protein	7.7±0.5	7.6±0.5	g/dL
Albumin	4.6±0.2	4.6±0.2	g/dL
ALT	24±10	22±8	IU/L
AST	30±9	29±6	IU/L
Alkaline Phos	90±24	92±22	IU/L

In 2012, Cramer et al. evaluated effects from glucosinolates given to four healthy male subjects in a cross-over study. Subjects were given four meals, each separated by a 1 wk wash-out period. Meals contained either 12.4 mg sulforaphane as 42 g fresh 5 d old broccoli sprouts, 21.3 mg sulforaphane as 2 g broccoli powder, 33.7 mg sulforaphane as 42 g sprouts plus 2 g powder, or a control meal. Sulforaphane metabolites were detected in the urine, with a 24 hr urinary recovery of sulforaphane-mercapturic acid of 65, 60, and 24% of the dose ingested from

the combination (sprouts + powder), broccoli sprout, and broccoli powder meals, respectively. Plasma concentrations of total isothiocyanates were highest in the group receiving the combination meal. No adverse effects were reported in the study.

Cramer and Jeffery (2011) performed a cross-over study, similar to the one published by Cramer et al. (2012), where four healthy male subjects consumed each of four test meals, separated by a 2-3 d wash-out period. Subjects consumed 12.4 mg sulforaphane as 2 g of air-dried broccoli sprouts, 21.3 mg sulforaphane as 2 g broccoli powder alone, a combination of sprouts plus powder (33.7 mg sulforaphane), or a control meal. Mean values of liver function carried out at 24 h post-ingestion were similar across treatment and control groups for most parameters. One subject had high alanine aminotransferase levels 24 h after both the control and powder treatments and two other subjects had low alanine aminotransferase, and aspartate aminotransferase before and after consuming the test meals, suggesting that they may have had endogenously low levels of these liver enzymes altogether. Another subject exhibited low aspartate aminotransferase levels at 24 h after the powder treatment, but not after any other treatments or at other time points. Indirect bilirubin was borderline low at 0 h after the powder dosing in one of the individuals who exhibited both low alanine aminotransferase and aspartate aminotransferase at all time points measured. Two subjects had slightly elevated levels of albumin at 24 h after dosing with control treatment and powder treatment. Importantly, because the cause and significance of these variations from normal values was not apparent and were seen during non-treatment periods, they were not considered to be treatment-related.

Egner et al. (2011) fed 48 adult volunteers a glucosinolate-rich (349.2 mg glucoraphanin in 2.43 g powder) or isothiocyanate-rich (26.6 mg sulforaphane in 0.74 g powder) broccoli powder for 7 days per exposure period in a crossover study with participants receiving each diet with a 5-day washout period between interventions. Only two mild adverse events of nausea or vomiting associated with the odor or ingestion of the sulforaphane test article were reported. The authors reported that there were no serious events during the interventions and no abnormal clinical chemistry values were seen in blood samples collected on the last days of the two active intervention arms were found.

In a study conducted by Shapiro et al. (2006), twelve healthy subjects were hospitalized in a research unit for 17-19 days and fed a controlled diet devoid of crucifers and inducers of phase 2 enzymes. Cohorts of four subjects each received either broccoli-containing preparations or a control. After a 5 d run-in period, subjects were fed a placebo or broccoli sprout extracts containing approximately 33.3 mg glucosinolates, 133.5 mg glucosinolates, and 3 mg isothiocyanates, every 8 hr for 7 days. Abnormal hematology and chemistry values were reported

for subjects in the treatment groups, including low red cell count, low hemoglobin, borderline low hematocrit, low polymorphonuclear leukocytes, high lymphocytes, high monocytes, high eosinophils, high or borderline high reticulocytes, low prothrombin time, high aspartate aminotransferase, high alanine aminotransferase, high glucose, borderline high total triiodothyronine, borderline low free thyroxin, and high thyroid-stimulating hormone. However, the authors concluded that the findings were not biologically significant because there was no consistent pattern of adverse events associated with treatment. Furthermore, many of the changes occurred in both placebo and treatment groups or were seen during non-dosing periods.

Kensler et al. (2005) conducted a randomized, placebo-controlled chemoprevention study involving 200 healthy adults at high risk for hepatocellular carcinoma to determine the effects of consuming 1.3 or 174.6 mg glucoraphanin/day in the form of broccoli sprouts extracts for 2 weeks on overall safety and the excretion of aflatoxin and phenanthrene metabolites. No adverse events were recorded during the study and no abnormalities were found in the clinical chemistries. Furthermore, there were no differences between the two intervention arms in urinary levels of aflatoxin-N7-guanine adducts, indicating that the consumption of the broccoli sprout extract had no effect on the metabolism of aflatoxin and phenanthrene.

Table 15: Human Studies on Broccoli or Broccoli Extracts

Reference	Study type	Population	Test article and dose	Duration of exposure	Outcome	Other, safety-relevant observations
Clarke, et al. 2011	Randomized	12M/12F 19-50 yr	68 g broccoli sprouts/d [**95.3 mg glucoraphanin + 28.3 mg glucoerucin + 14.3 mg glucoiberin = 137.9 mg glucosinolates/d**] Or 6 Brocco-Max pills/d [~3 g freeze-dried broccoli sprouts] [**96.1 mg glucoraphanin + 32.0 mg glucoerucin + 13.4 mg glucoiberin = 141.5 mg glucosinolates/d**]	7 d with 3 d follow-up period	The amount of sulforaphane and glucoerucin metabolites excreted in urine was five-fold lower in subjects given the supplement (with no myrosinase) compared to sprouts. There was no change or accumulation in the amount of metabolites excreted after repeated 7 day consumption.	No safety information was reported.
Clarke, et al. 2011	Cross-over (2 phase), randomized, blinded	10F/6M 19-50 yr	12 subjects consumed 40 g fresh broccoli sprouts [**65.5 mg glucoraphanin + 29.9 mg glucoerucin = 95.5 mg glucosinolates**] and then 6 pills of a broccoli supplement (~ 3 g freeze-dried broccoli sprouts) [**52.8 mg glucoraphanin + 16.9 mg glucoerucin = 69.7 mg glucosinolates**]	1 d treatment 1 month wash-out 1 d alternate treatment	Bioavailability of sulforaphane and erucin is lower in subjects consuming broccoli supplements compared to fresh broccoli sprouts. The peaks in plasma concentrations and urinary excretion of the metabolites were also delayed in supplement consumption. Lower bioavailability and delayed appearance of metabolites in plasma and urine likely reflects the reliance on the microbiota in the colon for glucosinolate hydrolysis. Total metabolite concentrations in plasma were highest at 3 hours and 6 hours post consumption for sprouts and supplements, respectively and almost completely cleared from plasma by 24 hours.	No safety information was reported.

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Table 15: Human Studies on Broccoli or Broccoli Extracts

Reference	Study type	Population	Test article and dose	Duration of exposure	Outcome	Other, safety-relevant observations
Cornblatt, et al. 2007	Intervention only	8 F Healthy, undergoing reduction mammoplasty	Hot water extract of broccoli sprouts [**29 mg sulforaphane/d**]	1 d	Sulforaphane metabolites were measured in human breast tissue.	The extract was reported to be well tolerated.
Cramer, et al. 2011	Cross-over	4M 18-30 yr Healthy, normal body weight	2 g ground, dried broccoli sprouts [**12.4 mg sulforaphane/d**] Or 2 g broccoli powder [**21.3 mg sulforaphane/d**] Or Both [**33.7 mg sulforaphane/d**] Or Control meal	Four meals each separated by 2-3 d wash-out	Myrosinase from the sprouts enhanced sulforaphane absorption. Sulforaphane recovery at 24 hours from urine was 74%, 49% and 19% of the dose ingested from broccoli sprouts, combination and broccoli powder meals, respectively. Urinary and plasma isothiocyanates and their metabolites were delayed after ingestion of broccoli powder compared to sprouts and combination.	Hepatic function clinical chemistry parameters were evaluated 24 hours post-consumption; no differences were detected between control and test subjects for any parameter measured.
Cramer, et al. 2012	Cross-over	4M 18-30 yr Healthy	42 g fresh broccoli sprouts [**12.4 mg sulforaphane/d**] Or 2 g broccoli powder [**21.3 mg sulforaphane/d**] Or Both [**33.7 mg sulforaphane/d**] Or Control meal	Four meals each separated by 1 wk wash-out	Metabolites of sulforaphane were analyzed in blood and urine. The 24 hour urinary sulforaphane- mercapturic acid recovery was 65, 60 and 24% of the dose ingested from the combination, broccoli sprout and broccoli powder meals, respectively.	No safety information was reported.

Table 15: Human Studies on Broccoli or Broccoli Extracts

Reference	Study type	Population	Test article and dose	Duration of exposure	Outcome	Other, safety-relevant observations
Egner, et al. 2011 Kensler, et al. 2012	Randomized, cross-over	50 Adults Hepatitis B surface antigen-positive with normal liver function, living in a rural farming community in China	Reconstituted broccoli sprout powders rich in either **glucoraphanin [349.2 mg; in 2.43 g powder]** or **sulforaphane [26.6 mg; in 0.74 g powder]** per day	5 d run-in 7 d treatment 5 d wash-out 7 d treatment	Bioavailability, as measured by urinary excretion of sulforaphane and its metabolites was greater with the sulforaphane rich beverage (70%) compared to the glucoraphanin rich beverage (5%). With ingestion of sulforaphane, 95% of the sulforaphane plus conjugates was eliminated in the first 12 hours, compared to only 60% of the sulforaphane plus conjugates after glucoraphanin intake. The rate-limiting step for elimination of glucoraphanin is likely the initial hydrolysis step. Statistically significant increases in the levels of excretion of glutathione-derived conjugates of acrolein, crotonaldehyde and benzene were seen in individuals receiving all treatments compared to pre-intervention. Thus, broccoli sprouts may enhance detoxification of airborne pollutants.	With the exception of two drop-outs, the remaining 48 subjects consumed all beverages during the study. Two grade one adverse events were reported, nausea or vomiting associated with odor or ingestion of the sulforaphane beverage, respectively. There were no abnormal clinical chemistry values from blood samples collected on the last days of the two active interventions (day 12 and day 24) (data not reported).

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Table 15: Human Studies on Broccoli or Broccoli Extracts

Reference	Study type	Population	Test article and dose	Duration of exposure	Outcome	Other, safety-relevant observations
Fahey, et al. 2012	Intervention-only	18M/27F 21-87 yr Healthy adults	3 d old broccoli sprout aqueous extract **[89 mg glucosinolates/d** (containing 74.2 mg glucoraphanin)]	1 d	When single oral doses of glucosinolates were administered as broccoli sprout extracts to rural Chinese and racially mixed Baltimoreans, patterns of excretions of urinary dithiocarbamates were very similar but there was inter-individual variability from about 1% - 40% of dose. In contrast, administration of isothiocyanates, (largely sulforaphane) containing extracts resulted in uniformly high (70 – 90%) conversions to urinary dithiocarbamates.	No safety information was reported.
Gasper, et al. 2005	Randomized, three-phase, cross-over	9F/7M 18-46 yr	150 mL broccoli soup (homogenized florets) made from either standard broccoli (cultivar Marathon) [**2.9 mg sulforaphane/d**] or high-glucosinolate "super broccoli" [**9.2 mg sulforaphane/d**] Note: the concentration of sulforaphane in "super broccoli" is ~3x that of standard broccoli.	1 d each treatment 21 d wash-out between each treatment	The mean percentage of free sulforaphane and its thiol conjugates excreted in urine was about 82% for broccoli and 55% for super broccoli at 24 hours after consumption. After consumption of super broccoli soup, Cmax and AUC of sulforaphane-derived metabolites in the plasma were ~3x those after standard broccoli soup consumption. After 24 hours, only trace concentrations of sulforaphane metabolites were detected in the plasma.	No safety information was reported.

Table 15: Human Studies on Broccoli or Broccoli Extracts

Reference	Study type	Population	Test article and dose	Duration of exposure	Outcome	Other, safety-relevant observations
Kensler, et al. 2005	Randomized, placebo-controlled	200 healthy adults 25-65 yr	Hot water infusion of 3 d old broccoli sprouts containing either 1.3 or **174.6 mg glucoraphanin** (per day).	3 d run-in 2 wk treatment	Urinary levels of dithiocarbamates indicated striking inter-individual differences in bioavailability; excretion rates ranged from 1 – 45% of administered dose. The average amount of dithiocarbamate excreted by individuals consuming the high glucoraphanin beverage was 49 µmol/12 hours. This was about 12% of the administered dose. Sulforaphane bioavailability was consistent between doses within an individual.	One person randomized to the placebo arm dropped out of the study. The remaining subjects consumed all beverages during the study. No adverse events were reported in the study group nor were there any abnormal clinical chemistry values for blood samples collected on the last day of the study.

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Table 15: Human Studies on Broccoli or Broccoli Extracts

Reference	Study type	Population	Test article and dose	Duration of exposure	Outcome	Other, safety-relevant observations
Navarro, et al. 2009	Randomized, placebo controlled, cross-over.	33 men and 34 women (healthy), age 20 – 40 years	Basal diet (vegetable free) Basal diet supplemented with 7 or 14 g crucifers and basal diet with 7 g crucifers plus 4 g apiaceous vegetables. Total vegetable intake was between 5-10 servings per day and ranged from 284 – 662 g for the single-dose crucifer diet, 568 – 1,324 g for double-dose crucifer diet and 458 – 1,065g for the single dose crucifer plus apiaceous diet.	14 days	Serum glutathione S-transferase increased with cruciferous vegetable consumption compared to basal diet (10 and 13% for single and double dose) but did not differ between basal diet and crucifer plus apiaceous diet.	No safety information was reported.

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Table 15: Human Studies on Broccoli or Broccoli Extracts

Reference	Study type	Population	Test article and dose	Duration of exposure	Outcome	Other, safety-relevant observations
Riedl, et al. 2009	Placebo-controlled, dose-escalation	65 adults enrolled 21M/36F completed	Broccosprouts® broccoli sprout homogenates (25; 50; 75; 100; 125; 150; 175; 200 g) delivering: **66.75; 133.5; 200.25; 267.0; 333.75; 400.5; 467.25; or 534.0 mg glucosinolates/d, respectively** Or 200 g alfalfa sprouts control	3 d	Expression of Phase II enzymes significantly increased in nasal lavage fluid samples at doses above 100 g daily; no change in expression seen in the control subjects. Serum sulforaphane levels were undetectable in selected subjects before ingestion of test material but reached levels of 0.0115 – 0.0121 nmol at 24 hours after the final homogenate dose (analytical limit and data not shown).	Eight subjects discontinued due to inability to keep required visits; none reported dose intolerance of side effects as a factor. No SAEs reported. Discontinuing subjects: **200.25 mg**: 1 subject **267.0 mg**: 1 subject **333.75 mg**: 2 subjects **467.25 mg**: 3 subjects **534.0 mg**: 1 subject Tolerability: **400.5 mg**: large bowel movement (1) **467.25 mg**: nasal congestion (1) **534.0 mg**: soft stool (1); increased urination (1; lasted 3 d); flatulence (1; lasted 3 d and required medication)

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Table 15: Human Studies on Broccoli or Broccoli Extracts

Reference	Study type	Population	Test article and dose	Duration of exposure	Outcome	Other, safety-relevant observations
Rungapamestry, et al. 2007	Factorial, dietary intervention, 6 experimental meals/ volunteer over 3 wk	5M/7F Mean 38.4 yr Mean BMI 26.1 Healthy, non-smoking	150 g lightly cooked broccoli/d [**27.1 mg glucoraphanin/d**] Or 150 g fully cooked broccoli/d [**31.0 mg glucoraphanin/d**] Or 70 mL broccoli seed extract/d [(**0.5 mg sulforaphane + 17.7 mg sulforaphane nitrile)/d**] Plus 3 g English mustard with each meal	2 d each intervention	The influence of meal composition and cooking duration on isothiocyanate uptake was determined. The estimated yield of sulforaphane in vivo was about 3-fold higher after consumption of lightly cooked broccoli than fully cooked broccoli. The meal matrix did not significantly influence the hydrolysis of glucoraphanin and its secretion as sulforaphane.	No safety information was reported.
Shapiro, et al. 1998	Cross-over	4M 30-75 yr Healthy	250 g cooked frozen broccoli [**223 mg glucosinolates/d, of which 78.4 mg is glucoraphanin**]/d × 1 d	Inpatient: 2 d control diet Days 2-5: 250 g of a non-cruciferous vegetable (green beans, corn, tomatoes, or carrots) Days 6, 9, 12, 15: 250 g cruciferous vegetable (one vegetable per episode; kale, broccoli, green cabbage, or turnips)	Quantity of the dithiocarbamates excreted was related to the glucosinolate/ isothiocyanate profiles of the cruciferous vegetables consumed. Eating prepared horseradish containing graded doses of isothiocyanates (12.3 – 74 μmol) led to a rapid excretion of proportionate amounts of urinary dithiocarbamates with first-order kinetics. Broccoli with heat-inactivated myrosinase also led to proportionate but low recoveries of urinary dithiocarbamates. Broccoli treated with myrosinase was more completely converted to dithiocarbamates in vivo. When bowel microbiota were reduced by mechanical cleansing and antibiotics, conversion of glucosinolates became negligible.	No safety information was reported.

Table 15: Human Studies on Broccoli or Broccoli Extracts

Reference	Study type	Population	Test article and dose	Duration of exposure	Outcome	Other, safety-relevant observations
Shapiro, et al. 2001	Cross-over	4M Healthy 43-53 yr	~0.1 mg isothiocyanates or **~49 mg glucosinolates/d** for 3 d	Inpatient: 3 d wash-out 3 d treatment 3 d wash-out 3 d alternate treatment	Urinary dithiocarbamate excretion increased after administration of broccoli sprout glucosinolates or isothiocyanates.	No safety information was reported.
	Intervention only	4 M 21-53 yr	**3 mg isothiocyanates/d** or **6 mg isothiocyanates/d** or **12 mg isothiocyanates/d** or **24 mg isothiocyanates/d** (estimated from MW of 120)	Inpatient: 2 d wash-out Medium dose of isothiocyanate on days 2, 4, 6, and 8 Low dose of isothiocyanate on d 11 Moderately high dose of isothiocyanate on d 14 Highest dose of isothiocyanate on d 17	Excretion of dithiocarbamates was linear over the escalating dose range.	1 subject withdrew from the study; no cause was reported. No other safety information was reported.
	Intervention only	2M/9F 23-75 yr	11.8 g fresh broccoli sprouts [**44.5 mg glucosinolates/d**] for 1 d	Outpatient: 1 d wash-out 1 d treatment	Urinary dithiocarbamate excretion rose from a mean of 0.21 µmol (third baseline) to 55.8 µmol during the 24 h after dosing.	No safety information was reported.
	Intervention only	2M/1F 44-75 yr	12 g fresh broccoli sprouts [**48.1 mg glucosinolates/d** for 1 d on two occasions]	Outpatient: 1 d wash-out 1 d treatment 1 d wash-out 1 d treatment	Dithiocarbamate excretion was higher when intact sprouts were chewed thoroughly rather than swallowed whole. Studies indicate that isothiocyanates are more bioavailable than glucosinolates, which must first be hydrolyzed. Chewing exposes the glucosinolates to plant myrosinase and significantly increases dithiocarbamate excretion.	No safety information was reported.

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Table 15: Human Studies on Broccoli or Broccoli Extracts

Reference	Study type	Population	Test article and dose	Duration of exposure	Outcome	Other, safety-relevant observations
Shapiro, et al. 2006	Randomized, placebo-controlled, double-blind	12 healthy subjects Four subjects per treatment	**33.3 mg glucosinolates/d** Or **133.5 mg glucosinolates/d** Or **3 mg isothiocyanates/d**	5 d run-in 7 d treatment	Three subjects receiving 33.3 mg glucosinolates/d excreted 10.1, 16.3 and 27.1% of the total dose. Three subjects receiving 133.5 mg glucosinolates/d excreted 7.7, 20.1, and 31.1% of the total dose. Three subjects receiving 3 mg isothiocyanates/d excreted 68.9, 70.1 and 72.9% of the total dose. [Isothiocyanates appear to be more bioavailable versus glucosinolates.]	All subjects were questioned daily and no significant or persistent symptomatic abnormalities were reported. Routine blood and urine analyses and well as tests of thyroid and liver function were obtained. No systematic, clinically significant, adverse effects attributed to sprout extract were seen.
Traka, et al. 2008	Randomized, parallel, dietary intervention	22 M 57-70 yr Diagnosis of high-grade prostatic intraepithelial neoplasia	400 g broccoli/wk [**28.3 mg glucoraphanin + 9.3 mg glucoiberin = 37.6 mg glucosinolates/d**] or 400 g peas/wk in addition to normal diet	12 mo	No changes in plasma PSA levels were seen. Gene expression patterns in the prostate were studied; consuming broccoli interacts with the GSTM1 (glutathione S-transferase mu1) genotype to result in complex changes in signaling pathways associated with inflammation and carcinogenesis in the prostate. This is hypothesized to play a role in reducing the risk of prostate cancer.	No safety information was reported.

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Table 15: Human Studies on Broccoli or Broccoli Extracts

Reference	Study type	Population	Test article and dose	Duration of exposure	Outcome	Other, safety-relevant observations
Yanaka, et al. 2009	Randomized, placebo-controlled	19M/28F *Helicobacter pylori*-infected patients Mean age 54.5 yr	70 g broccoli sprouts [**183.3 mg glucoraphanin/d**] Or 70 g alfalfa sprouts	8 wk treatment 8 wk follow-up	Intervention with broccoli sprouts, but not placebo, decreased the levels of urease measured by the urea breath test and H. pylori stool antigen (both biomarkers of H. pylori colonization) and serum pepsinogens 1 and 11 (biomarkers of gastric inflammation).	No safety information was reported.
Ye, et al. 2002	Intervention only	4M Healthy	Aqueous extract of broccoli sprouts [**35.5 mg isothiocyanates/d**]	1 d	Isothiocyanates (largely sulforaphane) were absorbed rapidly, reached peak concentrations in plasma, serum and crythrocytes at 1 h after feeding and declined first-order kinetics (half-life of 1.77 ± 0.13 h). Cumulative excretion at 8 h was 58.3% of the dose. Clearance was by active renal tubular secretion.	No safety information was reported.

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b. Animal Studies

Glucoraphanin that was isolated and purified from broccoli seeds was given to six beagles (3M/3F) at 200 mg/kg per day for 3 days via capsules in a preliminary tolerance study (IITRI Project No. 2078-005-001, unpublished study). No animals died or were found moribund. Vomiting within 0.5-1 hr following dosing was the only adverse finding. All animals lost modest amounts of weight during the study (1-4% of predose weight), but this finding was not considered test-article related by the study authors. Two animals had transient loss of appetite evidenced by reduced feed intake on day 1, but recovered thereafter; no clear treatment-related effects on feed consumption were seen. Both glucoraphanin and sulforaphane were detected in plasma samples and sulforaphane accounted for 0.04 to 0.65% of the dose.

In a second study, after a washout period of 4 wk, the same beagles were given a dose of 5 mg sulforaphane/kg per day for 3 day via gavage in a water solution after a 4 wk washout period (IITRI Project No. 2078-005-001, unpublished study). No dogs died or were killed moribund, but there was a higher incidence of vomiting in this study versus the glucoraphanin dosed in capsules. Modest weight loss was noted over the course of the study, but it was <10% of the predose weight. Weight loss was greater in this study versus the glucoraphanin study and feed intake was diminished on day 1 and thus, oral administration of sulforaphane was not considered to be tolerable to the test animals. After day 1, all dogs ate on days 2 and 3; no clear treatment-related effects on feed consumption were seen. Plasma determinations detected only sulforaphane (268 to 408 ng/ml) in the samples from dogs dosed with sulforaphane. There were no clinical signs of toxicity observed in dogs given either sulforaphane or glucoraphanin. Dogs weren ot sacrificed at the completion of the dosing period.

In a 14-day oral gavage dosing study with glucoraphanin given to 5 groups of 5 male and female CD rats at dose levels of 0, 10 50, 100, and 500 mg/kg per day, no deaths or adverse clinical signs were observed throughout the study and glucoraphanin was well tolerated with no effects on mean body weights, body weight gain food consumption, clinical chemistry or gross or histopathology findings (IITRI Project Number 2078-006-001, unpublished study). The only effect seen was an increase in absolute and relative testes weights in males in the highest 2 dosage groups. There was no associated histopathology that indicated an adverse treatment-related effect. In addition, the testes weights in the control animals were lower than background weights reported for this strain of rats and may have accounted for the higher weights in the treated groups (Gur and Waner 1993). The only other finding from histopathology was cardiomyopathy, a common finding in rat toxicology studies, which occurred in 5/5 males and 1/5 females in the vehicle control group but was absent in all males and females in the highest dose group. All animals receiving glucoraphanin were found to have glucoraphanin in plasma samples that increased corresponding to increasing dose levels.

c. In vitro Studies

The sodium salt of glucoraphanin was evaluated for mutagenicity in *Salmonella typhimurium* test strains *TA98*, *TA100*, *TA1535*, *TA1537* and *Escherichia coli* strain *WP2 uvrA*, in the presence and absence of Arochlor-induced rat liver fraction S9 (BioReliance Study Number AB41VP.503.BTL, unpublished). Glucoraphanin was initially tested at 1.5, 5.0, 15, 50, 150, 500, 1500, and 5000 µg/plate. In initial assays, a slight, non-dose-related increase in revertants was observed in strain TA98 without added S9. This result did not meet the criteria required to be considered a positive finding. No positive mutagenic responses were found with the remaining test conditions. Confirmatory assays were performed with doses from 15-5000 µg/plate. No positive mutagenic responses were observed in the confirmatory assays. Glucoraphanin was concluded to be non mutagenic in the bacterial reverse mutation assay. However, the ability of the bacterial strains or the S9 liver activation system used in this standard application of the Ames test to hydrolyze glucoraphanin to sulforaphane was not demonstrated and the negative result is inconclusive. Results from genotoxicity studies reported by Kassie and Knasmüller (2000) indicate that rat liver S9 homogenate can function to inactivate and possibly mask mutagenic properties observed with some isothiocyanates so the relevance of the negative results in the classic test incorporating a rat liver S9 homogenate are problematic for substances like glucosinolates that are specifically hydrolyzed to active isothiocyanates only by the specific enzyme myrosinase.

Glucoraphanin sodium salt was tested in an *in vitro* chromosomal aberration assay using Chinese hamster ovary cells with or without addition of an Arochlor-treated rat liver S9 homogenate at doses ranging from 625-5000 µg glucoraphanin/mL (BioReliance Study Number AB41VP.331.BTL, unpublished). The percentage of cells with numerical aberrations in the test article-treated group was not significantly increased above that of the solvent control at any dose level. Although this test result indicated lack of mutagenicity for glucoraphanin *per se*, the ability for the S9 homogenate to hydrolyze glucose to form the corresponding bioactive metabolite sulforaphane was not demonstrated in the study and the negative result is inconclusive for potential genotoxicity of the corresponding isothiocyanate from glucoraphanin.

Glucoraphanin sodium salt was evaluated for its *in vivo* ability to induce micronucleated polychromatic erythrocytes in male and female Hsd:ICR mouse bone marrow cells at doses ranging from 500-2000 mg/kg (TRL 2007, unpublished). Following a single oral dose, glucoraphanin (and the sulforaphane metabolite that is anticipated to be formed in the mouse gastrointestinal tract) did not cause a clastogenic effect and was considered to be negative for micronuclei formation in erythrocytes. The lack of clastogenicity *in vivo* is consistent with the negative results for glucoraphanin in the CHO and Ames tests.

d. Conclusion

Glucoraphanin does not exhibit *in vitro* genotoxic activity in Ames assays and chromosomal aberration tests in CHO cells. However, lack of treatment with myrosinase and the absence of demonstration that the sulforaphane active metabolite was produced by the S9 metabolic activation system indicate that these systems do not address potential genotoxicity of the sulforaphane metabolite of glucoraphanin. It is noteworthy that some glucosinolates are mutagenic only in the absence of hydrolysis by myrosinase (Kassie and Knasmüller 2000); therefore, lack of mutagenicity for glucoraphanin in the highly sensitive Ames and CHO mutation assays may indicate a lack of genotoxic potential. The lack of clastogenicity in the *in vivo* mouse micronucleus test following the oral route of administration is consistent with this conclusion because of the absence of genotoxicity of both glucoraphanin and its sulforaphane metabolite that would be formed in the gastrointestinal tract and absorbed by the test animals.

C. Summary of the Safety Evaluation of Glucosinolates

- Published studies document that glucosinolates and corresponding isothiocyanate products do not accumulate in the body following their absorption. Isothiocyanates in blood are rapidly conjugated and excreted in urine. Animal studies have shown that the levels of ingested glucosinolates and their corresponding metabolites are undetectable by 36 hr.

- Published human studies with Class 1 glucosinolates from broccoli found no treatment-related adverse effects and when clinical endpoints were reported, no adverse effects were seen.

- Unpublished pilot studies in animals indicate that although the administration of glucoraphanin or sulforaphane to beagles caused a modest loss in weight and a transient loss of appetite, there were no clinical signs of toxicity. In rats administered up to 500 mg glucoraphanin /kg bw for 14 days, no adverse effects on body weights, weight gain, feed consumption, clinical chemistry or histopathology were found. Testes weight was increased in the two highest dose groups compared to control.

- Glucoraphanin was not genotoxic in the Ames *Salmonella typhimurium* gene mutation test or in an *in vitro* CHO chromosome aberration assay. Negative results for genotoxicity were also observed in an *in vivo* bone marrow micronucleus test in mice administered of up to 2000 mg glucoraphanin /kg bw by gavage.

- Glucosinolates and corresponding isothiocyanates in broccoli are demonstrated to be safe for consumption at the proposed levels of ingestion from human and animal studies and *in vitro* and *in vivo* genotoxicity studies.

D. Physiological Effects from Glucosinolates and Corresponding Isothiocyanates

In vitro and animal studies evaluating the metabolic and physiological effects of glucosinolates and corresponding isothiocyanates have found that cruciferous plants in the human diet are capable of modulating metabolism and providing chemoprotective effects from carcinogens and environmental toxicants. These effects include the induction of cytochrome P450 (CYP450) enzymes (Phase I metabolism), increases in quinone reductase and glutathione *S*-transferases (Phase II metabolism), and modulation of multidrug resistance proteins (MRPs; "Phase III" transport). The phase II detoxication enzymes, such as quinone reductase and glutathione-*S*-transferase, that are stimulated by isothiocyanates serve to conjugate reactive carcinogenic intermediates which inhibits their activity and facilitate their excretion from the body (Abdull Razis et al. 2010; 2011a,b; 2012a,b; Fimognari et al. 2008). However, human epidemiology studies have not provided consistent evidence to support the existence of clinically relevant outcomes related to these apparent beneficial effects at the cellular and enzymatic level (Verhoeven et al. 1996; Murillo and Mehta 2001; Higdon et al. 2007; Hayes et al. 2008). Although many reactions facilitate the inactivation and excretion of carcinogenic substances, there are concerns that specific metabolic reactions may result in activation of some carcinogenic agents. The cytochrome CYP1A family of genes function primarily to code for enzymes that oxidize various xenobiotic agents such as polycyclic aromatic hydrocarbons (PAHs), including the carcinogen benzo[a]pyrene (BaP), into reactive epoxides that have been associated with *in vitro* and *in vivo* toxic, mutagenic and carcinogenic effects in cellular and animal systems. Importantly, the epoxide metabolites are further and relatively rapidly metabolized to –OH groups that are more readily conjugated by glutathione and excreted from the body (Vang et al. 2001; Munday and Munday 2004; Uno et al. 2004; Melchini and Traka 2010).

In studies with CYP1A-deficient and wild type (CYP1A+) mice that were dosed orally with 125 mg BaP/kg/day (Uno et al. 2004), the CYP1A(- /-) deficient mice that received BaP died after 28 days with severe immunosuppression, while CYP1A(+ /+) wild-type mice fed for 1 year on this same BaP diet survived and remained as healthy as untreated wild-type mice. This was an unexpected finding that conflicted with *in vitro* and data from animal model systems suggesting that the CYP1A enzyme family may act by metabolically activating PAHs into active carcinogens; thus, absence of CYP1A was anticipated to show less toxicity in the test animals in contrast to the elevated toxicity observed. The authors concluded that the function of inducible CYP1A in detoxication of PAHs was apparently much more important in the living animals than the metabolic activation and formation of epoxide intermediates which are observed in model systems (Uno et al., 2004).

An overview of the many studies on the range of physiological and metabolic effects for each of the major classes of glucosinolates and corresponding isothiocyanates is presented in the

following sections. This overview is intended to illustrate a range of physiological effects that are peripherally related to evaluation of safety because isothiocyanates affect cytochrome and related enzymatic activities. However, because many of these effects have been reported in *in vivo* or *in vitro* model systems, the relevance to human health and safety remains undefined until sufficient data become available from large scale, double-blind, and placebo controlled clinical trials with various cohorts of both healthy and health-compromised human subjects to allow the non-clinical systems to be validated.

1. Class 1 Glucosinolates and Isothiocyanates

Glucoraphanin and glucoerucin, which are interconvertible in the liver (Melchini et al. 2010), have received the greatest attention in research studies because of the relatively high level of physiological activity that is associated with the isothiocyanate metabolite sulforaphane, a potent activator of the Phase II metabolic enzymes that conjugate and inactivate reactive agents in the cell. Sulforaphane also up-regulates the expression of different cytochrome P450 genes that are important in metabolism of PAHs and other environmental toxicants (Uno et al. 2004; Fimognari et al. 2008; Abdull Razis et al. 2010; 2011a,b; 2012a,b). Various glucosinolates and isothiocyanates have been found to increase activity and/or levels of CYP450 enzymes, stimulate quinone reductase activity, increase glutathione *S*-transferases, decrease androgen receptor expression in prostate cells and activate aryl hydrocarbon receptor. These effects on metabolic enzymes typically manifest within a few hours of exposure in the various indicator systems and indicate a rapid effect of the compound on its target. However, the clinical implications, if any, of these reported *in vitro* effects are unclear, especially because it is not known whether the glucosinolates and/or their metabolites are bioavailable at these molecular targets in the body.

a. Glucoraphanin

Glucoraphanin (GR) is reported to moderate multiple Phase I and Phase II enzyme systems in the lung, liver, colon, kidney, bladder and skin of rats. Phase II enzymes were slightly increased and the Phase-I enzymes P450 (CYP) isoforms CYP1A1/2, CYP3A1/2 and CYP2E were strongly induced in livers from rats dosed orally with 120 or 240 mg/kg bw glucoraphanin (Perocco et al. 2006). GR upregulates or induces methoxyresorufin *O*-dealkylase and ethoxyresorufin-*O*-dealkylase (Abdull Razis et al. 2010; 2011a; 2012a). Addition of myrosinase to form the isothiocyanate sulforaphane abolished these effects. GR causes an increase in cytosolic quinone reductase protein levels in rat liver slices and the effect was more pronounced when myrosinase was added. Glutathione *S*-transferase was increased in rat lung slices (Abdull Razis et al. 2011b) and addition of myrosinase to the medium increased the inductive effect. Epoxide hydrolase was increased in rat liver slices by glucoraphanin (Abdull Razis et al. 2011a). NAD(P)H-quinone oxidoreductase-1 was increased in rat colon, lung and liver (Lai et al. 2008;

Zhu et al. 2010) by glucoraphanin. This effect has also been reported for the kidney, bladder, and skin (Fimognari et al. 2008).

b. Glucoerucin

Glucoerucin affects enzymes in rat liver and lung. Glucoerucin induced activity of CYP1A1, CYP1B1, CYP1A2, quinone reductase, epoxide hydrolase, plus ethoxy- and methoxy-resorufin-*O*-dealkylase in rat liver slices (Abdull Razis et al. 2010); the effects in liver were seen after at least 6 hr exposure (Abdull Razis et al. 2010; 2011a; 2012a). Ethoxyresorufin-*O*-dealkylase was induced in rat lung slices (Abdull Razis et al. 2011b).Glucoerucin was also reported to act as an antioxidant by reacting directly with hydrogen peroxide (Barillari et al. 2005).

c. Sulforaphane

Sulforaphane, the isothiocyanate released from glucoraphanin by myrosinase, affects various enzymes in specific organs of the rat. CYP1A1 and CYP1A2 were affected by sulforaphane in rat liver slices (Perocco et al. 2006; Abdull Razis et al. 2010). CYP1B1 is affected by sulforaphane in lung slices (Hanlon et al. 2008a). Sulforaphane did not alter levels of methoxy- or ethoxyresorufin dealkylase, or 7-benzyloxyquinoline demethylase in rat lung (Abdull Razis et al. 2011b) but inhibited ethoxyresorufin *O*-deethylase activity in rat liver when given at 15 mg/kg (88.2 μmol/kg) in drinking water for 15 days (Hanlon et al. 2008b). Glutathione-*S*-transferase was modulated by sulforaphane in rat liver and lung slices (Abdull Razis et al. 2011a and 2011b; Abdull Razis et al. 2010; Hanlon et al. 2009a and 2009b); this effect took at least 2 hr to manifest in rat liver (Abdull Razis et al. 2012a). Glutathione and epoxide hydrolase levels were affected by sulforaphane in rat liver slices (Abdull Razis et al. 2010; 2011a). NADPH:quinone oxidoreductase (*NQO1*) and heme oxygenase-1 (*HO-1*) were stimulated by sulforaphane in mammary gland tissue in Sprague Dawley rats, with maximal NQO1 enzyme activity observed by 24 hr post-dosing (Cornblatt et al. 2007). Sulforaphane increased levels of UDP-glucuronosyl transferase and epoxide hydrolase in rat liver slices (Abdull Razis et al. 2011a). Quinone reductase and glutathione *S*-transferase are affected in the forestomach, duodenum and urinary bladder when dosing sulforaphane at 40 μmol/kg bw per day for 5 days in rats (Munday and Munday 2004). Quinone reductase is also induced by sulforaphane in rat liver and lung (Zhang et al. 1992; Hanlon et al. 2009a and 2009b; Abdull Razis et al. 2010; Abdull Razis et al. 2011b); this effect took 6 hr to manifest in rat liver (Abdull Razis et al. 2012a). Sulforaphane can compete with benzo[a]pyrene for binding to the aryl hydrocarbon (Ah) receptor and antagonizes benzo[a]pyrene activation of the Ah receptor in mouse hepatoma H1L1.1c2 cells (Abdull Razis et al. 2012b).

In contrast to results observed in rats, sulforaphane did not induce glutathione-*S*-transferase in human liver slices (Hanlon et al. 2009b). Sulforaphane significantly down-regulated cytochrome P-450 3A4 (CYP3A4) expression in human primary hepatocytes. CYP3A4 is responsible for the hepatic and intestinal metabolism of sterols, procarcinogens and over 50% of pharmaceutical compounds used clinically (Zhou et al. 2007; Fimognari et al 2008). Sulforaphane also decreased levels of total and phosphorylated androgen receptor (AR) protein in human prostate cancer cells (Kim and Singh 2009). This effect was mediated via inhibition of AR transcription but not via inhibition of protein synthesis. AR promoter activity was inhibited as well. Intracellular and secreted levels of prostate-specific antigen, an AR-regulated gene product, were also decreased by sulforaphane.

Sulforaphane at 12 μM induced reactive oxygen species formation in HCT116 human colon cancer cells (Kim et al. 2010). Sulforaphane did not alter levels of methoxy- or ethoxyresorufin dealkylase, or 7-benzyloxyquinoline demethylase in human liver slices at concentrations up to 25 μM (Hanlon et al. 2008a). It was a major and very potent inducer of glutathione S-transferase and NADPH (quinone-acceptor) oxidoreductase activities (Kassahun et al. 1997).

d. Erucin

Like sulforaphane, erucin affects various enzymes in specific organs of the rat. Erucin upregulated and induced activity of quinone reductase in the forestomach, duodenum and urinary bladder at 40 μmol/kg bw per day for 5 days in rats and glutathione *S*-transferase in the forestomach, duodenum and urinary bladder (Munday and Munday 2004), and in rat liver and lung slices (Abdull Razis et al. 2011; Abdull Razis et al. 2010; Hanlon et al. 2009a and 2009b; Hanlon et al. 2008b). . CYP1A1, CYP1B1, CYP1A2, and methoxyresorufin-*O*-dealkylase are affected by erucin in rat liver slices (Abdull Razis et al. 2010; Hanlon et al. 2008a, b). It did not inhibit ethoxyresorufin *O*-deethylase activity in rat lung slices (Abdull Razis et al. 2011). Erucin did not affect methoxy- or ethoxyresorufin dealkylase, or 7-benzyloxyquinoline demethylase in rat liver slices at concentrations up to 25 μM but decreased activity was observed at 50 μM (Hanlon et al. 2008b).Glutathione-*S*-transferase is affected by erucin in rat liver and lung (Abdull Razis et al. 2011; Abdull Razis et al. 2010; Hanlon et al. 2009a and 2009b); this effect takes at least 4 hr to manifest (Abdull Razis et al. 2012). Erucin also affects total glutathione levels, UDP-glucuronosyl transferase and epoxide hydrolase in rat liver slices (Abdull Razis et al. 2010; 2011a). Both erucin and sulforaphane elevated CYP1A1 mRNA levels in precision-cut rat liver slices following a 24-hr exposure, in a concentration-dependent fashion; in both cases, incubation times longer than 6 hr were required to attain a significant increase in enzyme levels. Benzo[a]pyrene caused a marked increase in CYP1A1 mRNA levels in rat slices that could be antagonized by either sulforaphane or erucin, with sulforaphane exhibiting the greatest activity.

Erucin activated the aryl hydrocarbon (Ah) receptor and antagonized benzo[a]pyrene activation of the Ah receptor in mouse hepatoma H1L1.1c2 cells (Abdull Razis et al. 2012b).

Erucin did not induce glutathione-*S*-transferase in human liver slices (Hanlon et al. 2009b). It did not induce quinone reductase in human liver slices (Hanlon et al. 2009b). Erucin did not affect methoxy- or ethoxyresorufin dealkylase, or 7-benzyloxyquinoline demethylase in human liver slices at concentrations up to 25 μM but decreased activity was observed at 50 μM (Hanlon et al. 2008b).

Erucin also reacts directly with hydrogen peroxide as an antioxidant (Barillari et al. 2005).

E. Identification and Safety of Non-Glucosinolate Components in Broccoli Seed Extracts

Analysis of five lots of commercial aqueous broccoli seed extract powder indicated that the total glucosinolate content (which was comprised almost entirely of glucoraphanin and glucoerucin) ranged from 13-17%, w/w; the final product also contains ≤ 35.0%, w/w of maltodextrin; ≤ 2% silicon dioxide; ≤ 8.0% moisture, w/w; 9-11% protein, w/w; 12-14% ash, w/w; and ≤0.15% fat, w/w (see Table 6). This accounts for up to 79.2-87.2%, w/w, of the final commercial product, leaving a remaining unknown fraction of 12.8-20.8%, w/w; therefore, additional qualitative analyses were carried out in order to evaluate the probable identities of some of the unknown compounds.

A literature review was conducted to determine the types and identity of non-glucosinolate compounds that could be present in aqueous extracts of broccoli seeds by searching the NAPRALERT® natural products database for compound occurrence reports for *Brassica* species, with emphasis given to compounds occurring in *Brassica* seeds. The results of the search revealed 60+ non-glucosinolate compounds that could be potentially be present in aqueous extracts of broccoli seed. The exact masses of the compounds reported in the seeds of *Brassica* species were calculated, tabulated and compared against both ultraviolet (UV) absorption and mass spectrometric data that were generated on a representative commercial lot of Brassica's seed extract using HPLC with both UV-vis and Q-TOF (Quadrupole Time of Flight) mass spectrometric detection. The objective was to detect and identify as many possible constituents in the extract that could be tentatively matched to those compounds identified during the literature search on *Brassica* compounds. For those compounds which did not correspond to a known compound on the *Brassica* list, a reasonable attempt was made to assign a molecular formula and possible structure, based on a combination of exact masses and mass spectrometry fragmentation data in comparison against mass spectral databases.

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The test extract was selected based on having a representative profile of major UV-detectable components that were present in all five lots. The five major mass peaks per UV chromatographic peak were compared against the list of reported *Brassica* seed compounds identified using the NAPRALERT® database. The Agilent 1200 Q-TOF instrument used for the analyses is characterized by its ability to identify substances by mass and has limitations in identification of some compounds. This was an understood technical limitation of the study and the intent was to identify the possible presence of any compounds which might present a safety concern, rather than to quantitatively account for every single component.

The results of the HPLC-MS analyses of the aqueous broccoli seed extract powder suggested the presence of 56 major ions in the chromatogram that also corresponded to specific UV peaks; of these, tentative identities were assigned to 30 ions. Some of the UV peaks contained co-eluting substances. The non-glucosinolates that were tentatively identified consisted of sugars and various carbohydrates (*e.g.*, glucose, mannoheptulose and maltodextrin), several organic acids (*e.g.*, citric, malic, malonic, pantothenic, mesaconic acid; *alpha*-hydroxyglutaric acid, etc.) that are commonly found in plants, metabolic intermediates of cellular pyruvate or amino acid metabolic pathways (*e.g.*, isopropylmalic, methylmalonic acids), simple sugars (*e.g.*, glucuronic acid; *D*-glucose), flavonoids (*e.g.*, dihydrochalcone), low molecular weight phenolics (*e.g.*, 4-hydroxybenzoic acid/salicylic acid; gentisic acid/protocatechuic acid), metabolic products of glucosinolates (*e.g.*, phenyl isothiocyanate), and substances derived from maltodextrin (*e.g.*, mannoheptulose; gluconic acid). These compounds are ubiquitous in the food supply and do not present a safety concern.

1. Conclusion

The non-glucosinolate components present in the aqueous broccoli seed extract powder are typical plant primary or secondary metabolites, including carbohydrates, organic acids, low molecular weight phenolics, and other compounds that are involved in plant structure and function. Reasonable breakdown products of maltodextrin were also detected. As these ubiquitous compounds already occur at significant levels in the food supply, the presence of these substances in the aqueous broccoli seed extract powder are not expected to pose any safety concern.

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APPENDIX I1. Spherix Consulting, Inc., Broccoli Intake Report: Estimated Daily Intake of Broccoli by the U.S. Population from Foods, June 16, 2013

Spherix Consulting Inc.

BROCCOLI INTAKE REPORT

ESTIMATED DAILY INTAKE OF BROCCOLI BY THE U.S. POPULATION FROM FOODS

Spherix Consulting Incorporated
6/16/2013

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ESTIMATED DAILY INTAKE OF BROCCOLI BY THE U.S. POPULATION FROM FOODS

1.0 INTRODUCTION

Spherix Consulting Incorporated has completed dietary intake estimate of broccoli (raw or cooked combined), hereafter "broccoli R/C", by children and adults in the United States. Estimates for the intake of broccoli R/C were based on the food consumption data included in the National Center for Health Statistics' (NCHS) 2009-2010 National Health and Nutrition Examination Surveys (NHANES) (CDC, 2006; USDA, 2012; Bodner-Montville et al, 2006). Calculations for the mean and 90th percentile intakes were performed. The intakes were reported for the following population groups:

- children, ages 2 to 11 years (males, females, and all),
- adolescents, ages 12 to 18 years (males, females, and all),
- adults, ages 19 to 30 years (males, females, and all),
- adults, ages 31 to 50 years (males, females, and all),
- adults, ages 51 to 70 years (males, females, and all),
- adults, ages 71 years and older (males, females, and all),
- adults, ages 19 years and older (males, females, and all).

2.0 FOOD CONSUMPTION SURVEY DATA

2.1 Survey Description

The most recent National Health and Nutrition Examination Surveys (NHANES) for the years 2009-2010 are available for public use. NHANES are conducted as a continuous, annual survey, and are released in 2-year cycles. In 2009-2010, approximately 10,000 people across the U.S. completed the health examination component of the survey. Any combination of consecutive years of data collection is a nationally representative sample of the U.S. population. It is well established that the length of a dietary survey affects the estimated consumption of individual users and that short-term surveys, such as the typical 1-day dietary survey, overestimate consumption over longer time periods (Gregory et al, 1995). Because two 24-hour dietary recalls administered on 2 non-consecutive days (Day 1 and Day 2) are available from the NHANES 2009-2010 survey, these data were used to generate estimates for the current intake analysis.

The NHANES provide the most appropriate data for evaluating food-use and food-consumption patterns in the United States, containing 2 years of data on individuals selected *via* stratified multistage probability sample of civilian non-institutionalized population of the U.S. NHANES 2009-2010 survey data were collected from individuals and households *via* 24-hour dietary recalls administered on 2 non-consecutive days (Day 1 and Day 2) throughout all 4 seasons of the year. Day 1 data were collected in-person in the Mobile Examination Center (MEC), and Day 2 data were collected by telephone in the following 3 to 10 days, on different days of the week, to achieve the desired degree of statistical independence. The data were collected by first selecting Primary Sampling Units (PSUs), which were counties throughout the U.S. Small counties were combined to attain a minimum population size. These PSUs were segmented and households were chosen within each segment. One or more participants within a household were interviewed. Fifteen PSUs are visited each year. For the 2009-2010 NHANES, there were 13,272 persons selected; of these 10,253 were considered respondents to the MEC examination and data collection. 9754 of the MEC respondents provided complete dietary intakes for Day 1 and of those providing the Day 1 data, 8,405 provided complete dietary intakes for Day 2.

In addition to collecting information on the types and quantities of foods being consumed, NHANES 2009-2010 collected socioeconomic, physiological, and demographic information from individual participants in the survey, such as sex, age, height and weight, and other variables useful in characterizing consumption. The inclusion of this information allows for further assessment of food intake based on consumption by specific population groups of interest within the total population. Among those who completed food intake survey on both Day 1 and

Day 2, 8301 respondents also provided physiological information including age, sex and weight; of these 7738 were 2 years and older.

Sample weights were incorporated with NHANES 2009-2010 to compensate for the potential under-representation of intakes from specific population groups as a result of sample variability due to survey design, differential non-response rates, or other factors, such as deficiencies in the sampling frame (CDC, 2006; USDA, 2012).[1]

2.2 Statistical Methods

Consumption data from individual dietary records, detailing food items ingested by each survey participant, were collated by computer and used to generate estimates for the intake of broccoli R/C by the U.S. population. Estimates for the daily intake of broccoli R/C represent projected 2-day averages for each individual from Day 1 and Day 2 of NHANES 2009-2010 data; these average amounts comprised the distribution from which mean and percentile intake estimates were produced. Mean and percentile estimates were generated incorporating sample weights in order to provide representative intakes for the entire U.S. population. "All-person" intake refers to the estimated intake of broccoli R/C averaged over all individuals surveyed, regardless of whether they consumed food products containing broccoli R/C, and therefore includes "zero" consumers (those who reported no intake of food products containing broccoli R/C during the 2 survey days). "All-user" intake refers to the estimated intake of broccoli R/C by those individuals consuming food products containing broccoli R/C, hence the "all-user" designation. Individuals were considered users if they consumed 1 or more food products containing broccoli R/C on either Day 1 or Day 2 of the survey.

3.0 FOODS INCLUDED IN THE ANALYSES

The individual foods that contain broccoli R/C and the broccoli contents in foods employed in the current intake analysis are summarized in Appendix 1. Food codes were chosen from the Food and Nutrition Database for Dietary Studies (FNDDS). In FNDDS, the primary (usually generic) description of a given food is assigned a unique 8-digit food code (CDC, 2006; USDA, 2012). Broccoli R/C contents in foods were generated with predefined recipes for the foods, which are available in NHANES 2009-2010 database.

[1] A sample weight is assigned to each sample person. It is a measure of the number of people in the population represented by that sample person in NHANES, reflecting the unequal probability of selection, non-response adjustment, and adjustment to independent population controls.

4.0 FOOD SURVEY RESULTS

Only 19.7% of the U.S. population are considered user of foods containing broccoli R/C (see "adults 19 years and older" in Table 1). To avoid underestimating the broccoli consumption by those broccoli-eaters, therefore, instead of "all-person", the "all-user" total intakes of broccoli R/C by the U.S. by population group are calculated and the results are summarized in Table 1.

Table 1. Estimated "All-user" Daily Intake (EDI) of Broccoli, Raw or Cooked (Broccoli R/C), by Population Group (2009-2010 NHANES Data)

Population Group	N_u/N_p [A]	% users [B]	Average Intake (g/day)	90th Percentile Intake (g/day)
Children 2-11 years old				
Males	135/855	14.3	9.3	38.8
Females	110/814	13.7	9.7	30.4
All	245/1669	14.0	9.5	34.3
Adolescents 12-18 years old				
Males	50/486	10.2	13.6	53.8
Females	58/453	11.5	20.4	72.1
All	108/939	10.9	17.4	53.8
Adults 19-30 years old				
Males	71/480	19.2	10.7	29.9
Females	85/548	18.6	12.2	50.9
All	156/1028	18.9	11.4	36.5
Adults 31-50 years old				
Males	114/798	15.3	17.5	46.8
Females	200/938	22.3	15.7	38.9
All	314/1736	19.0	16.4	43.7
Adults 51-70 years old				
Males	125/789	18.3	16.4	43.7
Females	160/766	24.6	24.0	77.5
All	285/1555	21.5	20.8	67.9
Adults 71 years and older				
Males	71/374	19.1	15.2	61.5
Females	82/437	19.2	10.5	34.3
All	153/811	19.1	12.3	38.8
Adults 19 years and older				
Males	381/2441	17.5	15.3	43.6
Females	527/2689	21.8	17.2	51.1
All	908/5130	19.7	16.4	50.9

[A] Number of people consuming one or more foods containing broccoli, raw or cooked on either Day 1 or Day 2 of the survey over the number of people surveyed.
[B] Percent of user when sample weights are considered.

For broccoli-eaters, defined as all-users in this report, average people eat about 16.4 g/day and, at the 90th percentile, people eat about 50.9 g/day of broccoli (see "adults 19 years and older" in Table 1). Children (2-11 years old) eat much less of broccoli (9.5 g/day on average and 34.3 g/day at the 90th percentile). The driver in the calculation is the cooked broccoli since few people consume much of the raw vegetable and these small numbers are not statistically reliable. In addition, most broccoli-containing foods are cooked (Appendix 1).

Vegetable utilization figures from USDA that provide per capita intake do not account for waste and thus overestimate consumption; raw broccoli per capita calculation is particularly deceiving because we know from NHANES user data that very little of the raw broccoli sold is actually consumed. Nevertheless, processed broccoli data from the USDA are more likely to reflect actual consumption and they show: [2]

> Processed Broccoli Domestic Utilization: 828,100,000 lbs/year in Year 2011
> Per capita use: 2.7 lbs/year or 3.4 g/day per capita

Calculation of user consumption from this number can be estimated using the assumption that 19.7% of the population is broccoli eaters (NHANES 2009-2010 data); thus the average consumption is 17.3 g/day per user. This number agrees with the data (16.4 g/day on average for the broccoli eaters in "adults 19 years and older") we are seeing in NHANES 2009-2010.

5.0 DISCUSSION

The estimated daily intakes of broccoli R/C by the U.S. population with NHANES 2009-2010 data are compared with the results presented in Environ report of 1999 (Amann and Douglass 1999), which were calculated with 1994-96 USDA Continuing Survey of Food Intakes by Individuals (CSFII) data (Table 2). Current calculation found that there are 132 foods that contain broccoli R/C in comparison with 81 foods reported by Environ in 1999. Unlike current calculation, in which broccoli R/C contents in foods were generated from predefined recipes for the foods, which are available in NHANES 2009-2010 database; Environ (Amann and Douglass 1999) used its own "recipe file" with broccoli R/C contents in the foods, which were not documented in the report.

[2] Table 40 in Vegetables and Pulses Yearbook 2012. Available online at http://usda.mannlib.cornell.edu/MannUsda/viewDocumentInfo.do?documentID=1858

Environ (Amann and Douglass 1999) did not give a clear definition for "all-user" in the report; individuals were considered user if they consumed 1 or more food products containing broccoli R/C on either Day 1 or Day 2 of the survey or on both Day 1 and Day 2 of the survey. Assuming that the "all-user" definition in Environ 1999 report is the same as that defined in this report,[3] direct comparison between current estimates with the results reported in Environ report of 1999 is summarized in Table 2. It shows that, on average, the mean and 90th percentile broccoli R/C (based 2009-2010 survey data) consumption by US population (see "adults 19 years and older" in Table 2) are approximately 34.3% and 46.5%, respectively, of the intakes 15 years ago (Environ's estimates were based on 94-96 CSFII survey data).

[3] Individuals were considered users if they consumed 1 or more food products containing broccoli R/C on either Day 1 or Day 2 of the survey

Table 2. Estimated "All-user" Daily Intake (EDI) of Broccoli, Raw or Cooked (Broccoli R/C), by Population Group, Current Calculation with 2009-2010 NHANES Data Compared with Environ's Calculation with 94-96 CSFII data *				
Population Group	**Current Calculation**		**Environ's Calculation**	
	Average Intake (g/day)	**90th Percentile Intake (g/day)**	**Average Intake (g/day)**	**90th Percentile Intake (g/day)**
Children 2-11 years old				
Males	9.3	38.8	31.2	58.1
Females	9.7	30.4	32.2	83.0
All	9.5	34.3	31.7	72.2
Adolescents 12-18 years old				
Males	13.6	53.8	43.7	91.5
Females	20.4	72.1	37.3	91.5
All	17.4	53.8	40.2	91.5
Adults 19-30 years old				
Males	10.7	29.9	55.8	136.8
Females	12.2	50.9	48.1	91.5
All	11.4	36.5	51.1	116.3
Adults 31-50 years old				
Males	17.5	46.8	55.8	116.5
Females	15.7	38.9	42.8	91.5
All	16.4	43.7	49.1	116.3
Adults 51-70 years old				
Males	16.4	43.7	51.6	114.0
Females	24.0	77.5	41.2	91.5
All	20.8	67.9	45.4	92.8
Adults 71 years and older				
Males	15.2	61.5	45.7	96.9
Females	10.5	34.3	42.1	78.6
All	12.3	38.8	43.3	86.4
Adults 19 years and older				
Males	15.3	43.6	53.8	116.5
Females	17.2	51.1	43.3	91.2
All	16.4	50.9	47.8	109.4
CSFII represents USDA Continuing Survey of Food Intakes by Individuals				

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6.0 REFERENCES

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7.0 APPENDICES

Appendix 1.

Food codes of the foods that contain broccoli, preparation and broccoli contents in foods.

Food Code	Food Name	Preparation	Broccoli content %w/w
27311600	Beef, potatoes, and vegetables (including carrots, broccoli, and/or dark-green leafy), gravy (mixture)	cooked	7.58%
27311625	Beef, potatoes, and vegetables (including carrots, broccoli, and/or dark-green leafy), tomato-based sauce (mixture)	cooked	7.01%
27311635	Beef, potatoes, and vegetables (including carrots, broccoli, and/or dark-green leafy), cheese sauce (mixture)	cooked	7.10%
27311645	Beef, potatoes, and vegetables (including carrots, broccoli, and/or dark-green leafy), soy-based sauce (mixture)	cooked	10.97%
27313150	Beef, noodles, and vegetables (including carrots, broccoli, and/or dark-green leafy), soy-based sauce (mixture)	cooked	10.78%
27315330	Beef, rice, and vegetables (including carrots, broccoli, and/or dark-green leafy), cheese sauce (mixture)	cooked	8.27%
27315510	Beef, rice, and vegetables (including carrots, broccoli, and/or dark-green leafy), soy-based sauce (mixture)	cooked	12.88%
27320090	Sausage, noodles, and vegetables (including carrots, broccoli, and/or dark-green leafy), tomato-based sauce	cooked	1.40%
27320320	Pork, rice, and vegetables (including carrots, broccoli, and/or dark-green leafy), soy-based sauce (mixture)	cooked	12.87%
27341025	Chicken or turkey, potatoes, and vegetables (including carrots, broccoli, and/or dark-green leafy), gravy (mixture)	cooked	6.80%
27341035	Chicken or turkey, potatoes, and vegetables (including carrots, broccoli, and/or dark-green leafy), cream sauce, white sauce, or mushroom soup-based sauce (mixture)	cooked	5.64%
27341045	Chicken or turkey, potatoes, and vegetables (including carrots, broccoli, and/or dark-green leafy), cheese sauce (mixture)	cooked	7.02%
27341055	Chicken or turkey, potatoes, and vegetables (including carrots, broccoli, and/or dark-green leafy), tomato-based sauce (mixture)	cooked	5.77%
27343010	Chicken or turkey, noodles, and vegetables (including carrots, broccoli, and/or dark-green leafy), no sauce (mixture)	cooked	9.80%
27343950	Chicken or turkey, noodles, and vegetables (including carrots, broccoli, and/or dark-green leafy), cheese sauce (mixture)	cooked	16.41%
27345010	Chicken or turkey, rice, and vegetables (including carrots, broccoli, and/or dark-green leafy), no sauce (mixture)	cooked	9.85%
27345210	Chicken or turkey, rice, and vegetables (including carrots, broccoli, and/or dark-green leafy), gravy (mixture)	cooked	20.39%
27345310	Chicken or turkey, rice, and vegetables (including carrots, broccoli, and/or dark-green leafy), soy-based sauce (mixture)	cooked	12.88%
27345410	Chicken or turkey, rice, and vegetables (including carrots, broccoli, and/or dark-green leafy), cream sauce, white sauce, or mushroom soup-based sauce (mixture)	cooked	23.18%
27345440	Chicken or turkey, rice, and vegetables (including carrots, broccoli, and/or dark-green leafy), cheese sauce (mixture)	cooked	25.44%

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Food Code	Food Name	Preparation	Broccoli content %w/w
27350090	Fish, noodles, and vegetables (including carrots, broccoli, and/or dark green leafy), cheese sauce (mixture)	cooked	7.87%
27415100	Beef and vegetables (including carrots, broccoli, and/or dark-green leafy (no potatoes)), soy-based sauce (mixture)	cooked	14.81%
27415120	Beef, tofu, and vegetables (including carrots, broccoli, and/or dark-green leafy (no potatoes)), soy-based sauce (mixture)	cooked	14.02%
27420500	Pork and vegetables (including carrots, broccoli, and/or dark-green leafy), soy-based sauce (mixture)	cooked	14.80%
27443150	Chicken or turkey divan	cooked	18.89%
27445110	Chicken or turkey and vegetables (including carrots, broccoli, and/or dark-green leafy (no potatoes)), soy-based sauce (mixture)	cooked	14.80%
27445125	Chicken or turkey and vegetables (including carrots, broccoli, and/or dark-green leafy (no potatoes)), tomato-based sauce (mixture)	cooked	5.80%
27445180	Moo Goo Gai Pan	cooked	23.27%
27446400	Chicken or turkey and vegetables (including carrots, broccoli, and/or dark-green leafy (no potatoes)), cheese sauce (mixture)	cooked	10.63%
27450400	Shrimp and vegetables (including carrots, broccoli, and/or dark-green leafy (no potatoes)), no sauce (mixture)	cooked	36.67%
27450410	Shrimp and vegetables (including carrots, broccoli, and/or dark-green leafy (no potatoes)), soy-based sauce (mixture)	cooked	14.78%
27450600	Shellfish mixture and vegetables (including carrots, broccoli, and/or dark-green leafy (no potatoes)), soy-based sauce	cooked	14.78%
27450650	Shellfish mixture and vegetables (including carrots, broccoli, and/or dark-green leafy (no potatoes)), (mushroom) soup (mixture)	cooked	9.97%
27450740	Fish and vegetables (including carrots, broccoli, and/or dark-green leafy (no potatoes)), soy-based sauce (mixture)	cooked	14.47%
28110270	Sirloin beef with gravy, potatoes, vegetable (frozen meal)	cooked	12.94%
28110370	Salisbury steak with gravy, macaroni and cheese, vegetable (frozen meal)	cooked	18.94%
28140150	Chicken divan (frozen meal)	cooked	12.25%
28141050	Chicken patty parmigiana, breaded, with vegetable (diet frozen meal)	cooked	6.22%
28141630	Chicken cordon bleu with vegetable, rice (frozen meal)	cooked	4.09%
28141640	Chicken kiev with rice-vegetable mixture (frozen meal)	cooked	3.25%
28143030	Chicken and vegetable entree, oriental (diet frozen meal)	cooked	5.00%
28143150	Chicken and vegetable entree with noodles (diet frozen meal)	cooked	3.48%
28143190	Chicken in mushroom sauce, white and wild rice, vegetable (frozen meal)	cooked	12.07%
28144100	Chicken and vegetable entree with noodles and cream sauce (frozen meal)	cooked	9.68%
28150220	Flounder with chopped broccoli (diet frozen meal)	cooked	23.91%
28154010	Shrimp and vegetables in sauce with noodles (diet frozen meal)	cooked	5.96%
28160810	Livers, chicken, with vegetable (diet frozen meal)	cooked	69.03%
28340580	Chicken soup with vegetables (broccoli, carrots, celery, potatoes and onions), Oriental style	cooked	2.98%

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Food Code	Food Name	Preparation	Broccoli content %w/w
28355450	Seafood soup with potatoes and vegetables (including carrots, broccoli, and/or dark-green leafy)	cooked	2.27%
28355470	Seafood soup with vegetables (including carrots, broccoli, and/or dark-green leafy (no potatoes))	cooked	3.46%
32105040	Egg omelet or scrambled egg, with dark-green vegetables	cooked	10.88%
32105045	Egg omelet or scrambled egg, with cheese and dark-green vegetables	cooked	9.40%
32105059	Egg omelet or scrambled egg, with ham or bacon, and dark-green vegetables	cooked	9.76%
32105081	Egg omelet or scrambled egg, with ham or bacon, cheese, and dark-green vegetables	cooked	8.27%
32105116	Egg omelet or scrambled egg, with sausage and dark-green vegetables	cooked	9.41%
32105117	Egg omelet or scrambled egg, with sausage, cheese, and dark-green vegetables	cooked	8.27%
41812500	Tofu and vegetables (including carrots, broccoli, and/or dark-green leafy vegetables (no potatoes)), with soy-based sauce (mixture)	cooked	13.37%
58126270	Turnover, chicken- or turkey-, and cheese-filled, no gravy	cooked	25.00%
58127110	Vegetables in pastry	cooked	5.01%
58127200	Croissant sandwich, filled with broccoli and cheese	cooked	15.03%
58127220	Croissant sandwich, filled with chicken, broccoli, and cheese sauce	cooked	6.04%
58130320	Lasagna, meatless, with vegetables	cooked	2.24%
58134630	Tortellini, cheese-filled, meatless, with vegetables and vinaigrette dressing	cooked	21.75%
58136110	Lo mein, NFS	cooked	13.05%
58136120	Lo mein, meatless	cooked	14.41%
58136130	Lo mein, with shrimp	cooked	13.20%
58136140	Lo mein, with pork	cooked	13.02%
58136150	Lo mein, with beef	cooked	12.96%
58136160	Lo mein, with chicken	cooked	13.08%
58146160	Pasta with vegetables, no sauce or dressing	cooked	9.02%
58147350	Macaroni, creamed, with vegetables	cooked	7.52%
58160200	Rice with vegetables (including carrots, broccoli, and/or dark-green leafy), no sauce, NS as to fat added in cooking	cooked	10.11%
58160202	Rice with vegetables (including carrots, broccoli, and/or dark-green leafy), no sauce, fat not added in cooking	cooked	10.10%
58160204	Rice with vegetables (including carrots, broccoli, and/or dark-green leafy), no sauce, fat added in cooking	cooked	9.83%
58160220	Rice with vegetables, tomato-based sauce (mixture)	cooked	3.67%
58161400	Rice, brown, with vegetables (including carrots, broccoli, and/or dark-green leafy), no sauce, NS as to fat added in cooking	cooked	8.51%
58161402	Rice, brown, with vegetables (including carrots, broccoli, and/or dark-green leafy), no sauce, fat not added in cooking	cooked	8.71%
58161404	Rice, brown, with vegetables (including carrots, broccoli, and/or dark-green leafy), no sauce, fat added in cooking	cooked	8.51%
58301110	Vegetable lasagna (frozen meal)	cooked	21.31%
58303100	Rice, with broccoli, cheese sauce (frozen side dish)	cooked	37.04%

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Food Code	Food Name	Preparation	Broccoli content %w/w
58304400	Linguini with vegetables and seafood in white wine sauce (diet frozen meal)	cooked	8.74%
58305250	Pasta with vegetable and cheese sauce (diet frozen meal)	cooked	25.99%
58409000	Noodle soup, with fish ball, shrimp, and dark green leafy vegetable	cooked	3.78%
71507040	White potato, stuffed, baked, peel not eaten, stuffed with broccoli and cheese sauce	cooked	29.43%
71507100	White potato, stuffed, baked, peel not eaten, stuffed with chicken, broccoli and cheese sauce	cooked	22.76%
71508040	White potato, stuffed, baked, peel eaten, stuffed with broccoli and cheese sauce	cooked	24.18%
71508100	White potato, stuffed, baked, peel eaten, stuffed with chicken, broccoli and cheese sauce	cooked	19.49%
71508120	White potato, stuffed with ham, broccoli and cheese sauce, baked, peel eaten	cooked	12.49%
72201100	Broccoli, raw	raw	100.00%
72201200	Broccoli, cooked, NS as to form, NS as to fat added in cooking	cooked	96.44%
72201201	Broccoli, cooked, from fresh, NS as to fat added in cooking	cooked	96.44%
72201202	Broccoli, cooked, from frozen, NS as to fat added in cooking	cooked	72.72%
72201210	Broccoli, cooked, NS as to form, fat not added in cooking	cooked	99.39%
72201211	Broccoli, cooked, from fresh, fat not added in cooking	cooked	99.39%
72201212	Broccoli, cooked, from frozen, fat not added in cooking	cooked	74.61%
72201220	Broccoli, cooked, NS as to form, fat added in cooking	cooked	96.44%
72201221	Broccoli, cooked, from fresh, fat added in cooking	cooked	96.44%
72201222	Broccoli, cooked, from frozen, fat added in cooking	cooked	72.72%
72201230	Broccoli, cooked, NS as to form, with cheese sauce	cooked	63.22%
72201231	Broccoli, cooked, from fresh, with cheese sauce	cooked	63.22%
72201232	Broccoli, cooked, from frozen, with cheese sauce	cooked	50.23%
72201240	Broccoli, cooked, NS as to form, with mushroom sauce	cooked	27.06%
72201241	Broccoli, cooked, from fresh, with mushroom sauce	cooked	50.00%
72201242	Broccoli, cooked, from frozen, with mushroom sauce	cooked	40.59%
72201250	Broccoli, cooked, NS as to form, with cream sauce	cooked	29.77%
72201251	Broccoli, cooked, from fresh, with cream sauce	cooked	55.52%
72201252	Broccoli, cooked, from frozen, with cream sauce	cooked	44.66%
72202010	Broccoli casserole (broccoli, noodles, and cream sauce)	cooked	16.04%
72202020	Broccoli casserole (broccoli, rice, cheese, and mushroom sauce)	cooked	26.27%
72202030	Broccoli, batter-dipped and fried	cooked	71.46%
72302000	Broccoli soup	cooked	5.00%
72302100	Broccoli cheese soup, prepared with milk	cooked	5.07%
75100250	Raw vegetable, NFS	raw	2.50%
75140500	Broccoli salad with cauliflower, cheese, bacon bits, and dressing	raw	18.45%

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Food Code	Food Name	Preparation	Broccoli content %w/w
75330100	Vegetable combination (including carrots, broccoli, and/or dark-green leafy), cooked, no sauce, NS as to fat added in cooking	cooked	38.39%
75330110	Vegetable combination (including carrots, broccoli, and/or dark-green leafy), cooked, no sauce, fat not added in cooking	cooked	39.80%
75330120	Vegetable combination (including carrots, broccoli, and/or dark-green leafy), cooked, no sauce, fat added in cooking	cooked	38.39%
75340000	Vegetable combinations, Oriental style, (broccoli, green pepper, water chestnut, etc) cooked, NS as to fat added in cooking	cooked	33.74%
75340010	Vegetable combinations, Oriental style, (broccoli, green pepper, water chestnuts, etc), cooked, fat not added in cooking	cooked	34.79%
75340020	Vegetable combinations, Oriental style, (broccoli, green pepper, water chestnuts, etc), cooked, fat added in cooking	cooked	33.74%
75340160	Vegetable and pasta combinations with cream or cheese sauce (broccoli, pasta, carrots, corn, zucchini, peppers, cauliflower, peas, etc.), cooked	cooked	17.72%
75440100	Vegetable combination (including carrots, broccoli, and/or dark-green leafy), cooked, with soy-based sauce	cooked	28.32%
75440300	Vegetable combinations (including carrots, broccoli, and/or dark-green leafy), cooked, with tomato sauce	cooked	15.39%
75440500	Vegetable combinations (including carrots, broccoli, and/or dark-green leafy), cooked, with cheese sauce	cooked	19.06%
75450500	Vegetable combination (including carrots, broccoli, and/or dark-green leafy), cooked, with cream sauce	cooked	15.57%
75450600	Vegetable combination (including carrots, broccoli, and/or dark-green leafy), cooked, with butter sauce	cooked	19.35%
75460700	Vegetable combinations (including carrots, broccoli, and/or dark-green leafy), cooked, with pasta	cooked	15.10%
75460800	Vegetable combinations (including carrots, broccoli, and/or dark-green leafy), cooked, with butter sauce and pasta	cooked	14.08%
75649100	Vegetable soup, cream of, made from dry mix, low sodium, prepared with water	cooked	0.71%
76102030	Broccoli, carrots and cheese, baby food, junior	cooked	29.96%
76604000	Broccoli and chicken, baby food, strained	cooked	50.00%
76607100	Potatoes with cheese and broccoli, baby food, toddler	cooked	50.00%

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APPENDIX I2. Spherix Consulting, Inc., Broccoli Seed Aqueous Extract Intake Report: Estimated Daily Intake of Broccoli Seed Aqueous Extract by the U.S. Population from All Proposed Uses in Foods, June 2013

BROCCOLI SEED AQUEOUS EXTRACT INTAKE REPORT

Estimated Daily Intake Of Broccoli Seed Aqueous Extract By The U.S. Population From All Proposed Uses In Foods

July 9, 2013

Prepared for

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ESTIMATED DAILY INTAKE OF BROCCOLI SEED AQUEOUS EXTRACT BY THE U.S. POPULATION FROM ALL PROPOSED USES IN FOODS

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Prepared for Brassica

ESTIMATED DAILY INTAKE OF BROCCOLI SEED AQUEOUS EXTRACT BY THE U.S. POPULATION FROM ALL PROPOSED USES IN FOODS

1.0 INTRODUCTION

Brassica intends to add broccoli seed aqueous extract (BSAE) to selected beverages in the U.S. food supply. The intended maximum use level is 115 mg BSAE per serving. A list of food products, default serving sizes and corresponding maximum use-level for BSAE are summarized in Appendix 1.

Spherix Consulting Incorporated has completed an assessment of the consumption of BSAE by the U.S. population resulting from the proposed uses of BSAE. Estimates for the intake of BSAE were based on the proposed food uses and maximum use level in conjunction with food consumption data included in the National Center for Health Statistics' (NCHS) 2009-2010 National Health and Nutrition Examination Surveys (NHANES) (CDC, 2006; USDA, 2012; Bodner-Montville et al, 2006). Calculations for the mean and 90th percentile intakes were performed for all proposed food uses of BSAE combined. The intakes were reported for the following population groups:

- children, ages 2 to 5 years,
- children, ages 6 to 12 years,
- teenagers, ages 13 to 19 years,
- adults, ages 20 years and up,
- total population (all age groups combined, excluding infants of 0-2 years),
- total population (all age groups combined).

2.0 FOOD CONSUMPTION SURVEY DATA

2.1 Survey Description

The most recent National Health and Nutrition Examination Surveys (NHANES) for the years 2009-2010 are available for public use. NHANES are conducted as a continuous, annual survey, and are released in 2-year cycles. In 2009-2010, approximately 10,000 people across the U.S. completed the health examination component of the survey. Any combination of consecutive years of data collection is a nationally representative sample of the U.S. population. It is well established that the length of a dietary survey affects the estimated consumption of individual users and that short-term surveys, such as the typical 1-day dietary survey, overestimate consumption over longer time periods (Gregory et al, 1995). Because two 24-hour dietary recalls administered on 2 non-consecutive days (Day 1 and Day 2) are available from the NHANES 2009-2010 survey, these data were used to generate estimates for the current intake analysis.

The NHANES provide the most appropriate data for evaluating food-use and food-consumption patterns in the United States, containing 2 years of data on individuals selected *via* stratified multistage probability sample of civilian non-institutionalized population of the U.S. NHANES 2009-2010 survey data were collected from individuals and households *via* 24-hour dietary recalls administered on 2 non-consecutive days (Day 1 and Day 2) throughout all 4 seasons of the year. Day 1 data were collected in-person in the Mobile Examination Center (MEC), and Day 2 data were collected by telephone in the following 3 to 10 days, on different days of the week, to achieve the desired degree of statistical independence. The data were collected by first selecting Primary Sampling Units (PSUs), which were counties throughout the U.S. Small counties were combined to attain a minimum population size. These PSUs were segmented and households were chosen within each segment. One or more participants within a household were interviewed. Fifteen PSUs are visited each year. For the 2009-2010 NHANES, there were 13,272 persons selected; of these 10,253 were considered respondents to the MEC examination and data collection. 9754 of the MEC respondents provided complete dietary intakes for Day 1 and of those providing the Day 1 data, 8,405 provided complete dietary intakes for Day 2.

In addition to collecting information on the types and quantities of foods being consumed, NHANES 2009-2010 collected socioeconomic, physiological, and demographic information from individual participants in the survey, such as sex, age, height and weight, and other variables useful in characterizing consumption. The inclusion of this information allows for further assessment of food intake based on consumption by specific population groups of interest within the total population. Among those who completed food intake survey on both Day 1 and

Day 2, 8301 respondents also provided physiological information including age, sex and weight; of these 7738 were 2 years and older.

Sample weights were incorporated with NHANES 2009-2010 to compensate for the potential under-representation of intakes from specific population groups as a result of sample variability due to survey design, differential non-response rates, or other factors, such as deficiencies in the sampling frame (CDC, 2006; USDA, 2012).[1]

2.2 Statistical Methods

Consumption data from individual dietary records, detailing food items ingested by each survey participant, were collated by computer using R to read and edit the SAS files, and Matlab to generate estimates for the intake of BSAE by the U.S. population. Estimates for the daily intake of BSAE represent projected 2-day averages for each individual from Day 1 and Day 2 of NHANES 2009-2010 data; these average amounts comprised the distribution from which mean and percentile intake estimates were produced. "All-person" intake refers to the estimated intake of BSAE averaged over all individuals surveyed, regardless of whether they consumed food products containing BSAE, and therefore includes "zero" consumers (those who reported no intake of food products containing BSAE during the 2 survey days). "All-user" intake refers to the estimated intake of BSAE by those individuals consuming food products containing BSAE, hence the "all-user" designation. Individuals were considered users if they consumed 1 or more food products containing BSAE on either Day 1 or Day 2 of the survey.

3.0 FOOD USAGE DATA

The individual proposed food uses for BSAE, default serving sizes and the corresponding maximum use-levels for BSAE (i.e., 115 mg per serving) employed in the current intake analysis are summarized in Appendix 1. Food codes representative of each proposed use were chosen from the Food and Nutrition Database for Dietary Studies (FNDDS). In FNDDS, the primary (usually generic) description of a given food is assigned a unique 8-digit food code (CDC, 2006; USDA, 2012).

[1] A sample weight is assigned to each sample person. It is a measure of the number of people in the population represented by that sample person in NHANES, reflecting the unequal probability of selection, non-response adjustment, and adjustment to independent population controls.

4.0 FOOD SURVEY RESULTS

The estimated "all-person" and "all-user" total intakes of BSAE from all proposed food uses of BSAE in the U.S. by population group are summarized in Table 1 and Table 2, respectively.

Table 1. Estimated "All-person" Daily Intake (EDI) of Broccoli Seed Aqueous Extract (BSAE) from All Proposed Uses in the U.S by Population Group (2009-2010 NHANES Data)

Population	N_u/N_p [A]	Percent users	Absolute EDI (mg BSAE/day)		Weight-based EDI (mg BSAE/kg/day)	
			Mean	90th Percentile	Mean	90th Percentile
Children, 2-5 years	100/764	13.1	17	28	1.1	1.7
Children, 6-12 years	242/1388	17.4	32	56	0.9	1.7
Teenagers, 13-19 years	355/1124	31.6	90	142	1.4	2.2
Adults, 20+ years	2224/5821	38.2	114	212	1.4	2.6
Total population (2+ years)	2921/9097 [B]	32.1	91	178	1.4	2.7
Total population	3564/9740 [B]	36.5	104	203	1.6	3.2

[A] Number of people consuming one or more foods containing BSAE on either Day 1 or Day 2 of the survey over the number of people surveyed.

[B]. 9704 respondents provided relevant dietary intakes for Day 1 and Day 2 and provided information including age; of these 9097 were 2 years and older, 235 were between the age of 1 and 2, and 408 were 0-1 year old.

Table 2. Estimated "All-user" Daily Intake (EDI) of Broccoli Seed Aqueous Extract (BSAE) from All Proposed Uses in the U.S by Population Group (2009-2010 NHANES Data)

Population	N_u/N_p [A]	Percent users	Absolute EDI (mg BSAE/day)		Weight-based EDI (mg BSAE/kg/day)	
			Mean	90th Percentile	Mean	90th Percentile
Children, 2-5 years	100/764	13.1	128	210	8.1	13.3
Children, 6-12 years	242/1388	17.4	186	321	5.0	9.5
Teenagers, 13-19 years	355/1124	31.6	285	450	4.4	6.9
Adults, 20+ years	2224/5821	38.2	298	555	3.7	6.8
Total population (2+ years)	2921/9097 [B]	32.1	284	554	4.3	8.3
Total population	3564/9740 [B]	36.5	283	554	4.5	8.8

[A] Number of people consuming one or more foods containing BSAE on either Day 1 or Day 2 of the survey over the number of people surveyed.
[B]. 9704 respondents provided relevant dietary intakes for Day 1 and Day 2 and provided information including age; of these 9097 were 2 years and older, 235 were between the age of 1 and 2, and 408 were 0-1 year old.

5.0 CONCLUSIONS

In summary, 32.1% of the total U.S. population of 2+ years was identified as consumers of BSAE from the proposed food uses. The mean intakes of BSAE by the all BSAE consumers ("all-user") from all proposed food uses were estimated to be 284 mg/person/day or 4.3 mg/kg body weight/day. The heavy consumer (90th percentile all-user) intakes of BSAE from all proposed food-uses were estimated to be 554 mg/person/day or 8.3 mg/kg body weight/day.

6.0 REFERENCES

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7.0 APPENDICES

Appendix 1. Food codes for selected beverages, default serving sizes and proposed maximum use level of broccoli seed aqueous extract (BSAE) in the foods.

Food Code	Food description	Serving size, g	Maximum use level, mg per serving
92301000	Tea, NS as to type, unsweetened	178	115
92301060	Tea, NS as to type, presweetened with sugar	178	115
92301080	Tea, NS as to type, presweetened with low calorie sweetener	178	115
92301100	Tea, NS as to type, decaffeinated, unsweetened	178	115
92301130	Tea, NS as to type, presweetened, NS as to sweetener	178	115
92301160	Tea, NS as to type, decaffeinated, presweetened with sugar	178	115
92301180	Tea, NS as to type, decaffeinated, presweetened with low calorie sweetener	178	115
92301190	Tea, NS as to type, decaffeinated, presweetened, NS as to sweetener	178	115
92302000	Tea, leaf, unsweetened	178	115
92302200	Tea, leaf, presweetened with sugar	178	115
92302300	Tea, leaf, presweetened with low calorie sweetener	178	115
92302400	Tea, leaf, presweetened, NS as to sweetener	178	115
92302500	Tea, leaf, decaffeinated, unsweetened	178	115
92302600	Tea, leaf, decaffeinated, presweetened with sugar	178	115
92302700	Tea, leaf, decaffeinated, presweetened with low calorie sweetener	178	115
92302800	Tea, leaf, decaffeinated, presweetened, NS as to sweetener	178	115
92304000	Tea, made from frozen concentrate, unsweetened	178	115
92304700	Tea, made from frozen concentrate, decaffeinated, presweetened with low calorie sweetener	178	115
92305000	Tea, made from powdered instant, presweetened, NS as to sweetener	178	115
92305010	Tea, made from powdered instant, unsweetened	178	115
92305040	Tea, made from powdered instant, presweetened with sugar	178	115
92305050	Tea, made from powdered instant, decaffeinated, presweetened with sugar	178	115
92305090	Tea, made from powdered instant, presweetened with low calorie sweetener	178	115

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Food Code	Food description	Serving size, g	Maximum use level, mg per serving
92305110	Tea, made from powdered instant, decaffeinated, presweetened with low calorie sweetener	178	115
92305180	Tea, made from powdered instant, decaffeinated, unsweetened	178	115
92305800	Tea, made from powdered instant, decaffeinated, presweetened, NS as to sweetener	178	115
92306000	Tea, herbal	178	115
92306020	Tea, herbal, presweetened with sugar	178	115
92306030	Tea, herbal, presweetened with low calorie sweetener	178	115
92306040	Tea, herbal, presweetened, NS as to sweetener	178	115
92306200	Bean beverage	28.8	115
92307000	Tea, powdered instant, unsweetened, dry	0.7	115
92307400	Tea, powdered instant, sweetened, NS as to sweetener, dry	1	115
92560000	Fruit-flavored thirst quencher beverage	240	115
92560100	Gatorade Thirst Quencher sports drink	244	115
92560200	Powerade sports drink	244	115
92565000	Fruit-flavored sports drink or thirst quencher beverage, low calorie	240	115
92565100	Gatorade G2 thirst quencher sports drink, low calorie	244	115
92565200	Powerade Zero sports drink, low calorie	244	115
92651000	Energy drink	240	115
94100200	Water, bottled, sweetened, with low or no calorie sweetener	500	115
94100300	Water, fruit flavored, sweetened, with high fructose corn syrup and low calorie sweetener	240	115
94210100	Propel Water	237	115
94210200	Glaceau Water	237	115

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APPENDIX C9. SRC Analytical Laboratories, SOP Number Chm-515: Elemental Analysis by Inductively Coupled Plasma Mass Spectrometry (ICP-MS), June 28, 2013

SRC Analytical Laboratories
Standard Operating Procedure Summary

Elemental Analysis by Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

SOP Number: Chm-515

Summary:

Several elements in aqueous solution may be quickly and easily determined using Inductively Coupled Plasma Atomic Mass Spectrometry (ICP-MS). The plasma is a stream of Argon gas that is ionized by an applied radio frequency field. Sample aerosols are injected into the plasma which subjects the atoms to temperatures of 6000 to 10,000°K. The plasma dissociates the sample into its constituent atoms or ions. The ions are extracted from the central channel of the plasma and pass into the mass spectrometer, where they are separated on the basis of their mass to charge ratio by a quadrapole analyzer. The ions passing through the mass spectrometer are counted by an electron multiplier detector in a computer based data handling system.
A variety of sample types can be analyzed after suitable preparation.

Scope and Detection Limit:

Contact the laboratory for a complete list of current detection limits.

Sample Requirements

Samples should be collected in plastic containers and preserved with nitric acid. A minimum of 100 mL of water sample or 10 g of solid sample is required.

Quality Control:

One of every 10 samples is analyzed in duplicate. At least one control sample is analyzed with each batch. Standards and blanks are analyzed after every 10 samples to check the calibration. An internal standard is added to each sample. All quality control checks must be within specified limits or corrective action is taken.

References:

This method follows Standard Methods for the Examination of Water and Wastewater, 22nd Edition, 2012 APHA-AWWA-WEF, Part 3125; without modification.

SRC Analytical Laboratories
Standard Operating Procedure Summary

Sample Preparation by Microwave Digestion

SOP Number: PRP-032

Summary:

Microwave digestion is used to prepare samples of different types for analysis. Prior to the digestion, several techniques (drying, grinding, and/or thermal ashing) are used to prepare the samples for digestion in the Microwave. These techniques will depend on the sample type and required parameters. Microwave digestion is used to prepare:

- Soil or sediment for metals
- Foodstuff samples that are oilyand food oil samples (e.g., food oil, egg lecithin, emulsions, etc.) for mercury and metals.

Scope and Detection Limit:

Detection limits are specific for each analyte as indicated in the analytical method summary.

Sample Requirements

Drying, grinding, and/or thermal ashing of samples may be performed to prepare the samples for digestion in the microwave.

Quality Control:

A control samples is prepared with each batch , if available. One of every 10 samples is prepared and analyzed in duplicate. A spiked sample is analyzed monthly.

References:

Follows US EPA 3052 with the following modifications:

- Hydrofluoric acid is not used during the digestions for metals and mercury.
- Microwave digestion temperature and time profiles differ from those in method.

RO June 28/2013

APPENDIX C12. PhytoLab GmbH & Co. KG, Report for Contaminants 12027702-20001, Broccoli Seed Extract, November 17, 2010

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PhytoLab

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Date: 17.11.10

Cust.No: 92315

SAL-akkreditiertes Prüflaboratorium Reg.Nr.: SAL-BY-G 104-03-0

Report for contaminants

Report-No.: 12027702- 20001
Cust.-lot: (b) (6)
Sample labelling: Broccoli Seed Extract
Sample description: 3 x approx. 100 g fine powder
Packing: PE-bag
Receipt of sample: 2.11.10

Test	Unit	Direction for testing	Limits	Testresult
Sample preparation for pesticide test, according to § 64 LFGB L 00.00-34 Modul E2		EG396/05 081010		
Organophosphorus pesticides, according to § 64 LFGB L 00.00-34 (GC/FPD)		EG396/05 081010		Conform
Acephate	mg/kg		0.02	Not det.
Azinphos-ethyl	mg/kg		0.02	Not det.
Bromophos-ethyl	mg/kg		0.05	Not det.
Bromophos-methyl	mg/kg		0.01	Not det.
Bromfenvinphos-cis	mg/kg			Not det.
Bromfenvinphos-trans	mg/kg			Not det.
Bromfenvinphos, total	mg/kg		0.01	Not det.
Carbophenothion	mg/kg		0.01	Not det.
Chlormephos	mg/kg		0.01	Not det.
Chlorpyrifos (-ethyl)	mg/kg		0.05	Not det.
Chlorpyrifos-methyl	mg/kg		0.05	Not det.
Chlorothiophos	mg/kg		0.01	Not det.
Coumaphos	mg/kg		0.01	Not det.
Cyanofenphos	mg/kg		0.01	Not det.
Cyanophos	mg/kg		0.01	Not det.
Chlorfenvinphos-cis (E)	mg/kg			Not det.
Chlorfenvinphos-trans (Z)	mg/kg			Not det.
Chlorfenvinphos, total	mg/kg		0.02	Not det.
Diazinon	mg/kg		0.01	Not det.

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Report for contaminants

Report-No.: 12027702- 20 001
Cust.-lot: (b) (6)
Sample labelling: Broccoli Seed Extract

Test	Unit	Direction for testing	Limits	Testresult
Dichlofenthion	mg/kg		0.01	Not det.
Dichlorvos	mg/kg		0.01	Not det.
Dimefox	mg/kg		0.01	Not det.
Dioxathion	mg/kg		0.05	Not det.
Ditalimphos	mg/kg		0.01	Not det.
Dimethoate	mg/kg			Not det.
Dmethoate	mg/kg			Not det.
Dimethoate, total	mg/kg		0.02	Not det.
Edifenphos	mg/kg		0.01	Not det.
Ethion	mg/kg		0.01	Not det.
Ethoprophos	mg/kg		0.02	Not det.
Etrimfos	mg/kg		0.01	Not det.
EPN	mg/kg		0.01	Not det.
Fenamiphos, total	mg/kg		0.02	Not det.
Fenchlorphos, total	mg/kg		0.01	Not det.
Fenitrothion	mg/kg		0.01	Not det.
Fenthion, total	mg/kg		0.01	Not det.
Fonofos	mg/kg		0.01	Not det.
Formothion	mg/kg		0.02	Not det.
Fensulfothion, total	mg/kg		0.01	Not det.
Heptenophos	mg/kg		0.01	Not det.
Iprobenfos	mg/kg		0.01	Not det.
Isocarbofos	mg/kg		0.01	Not det.
Isofenphos, total	mg/kg		0.01	Not det.
Jodofenfos	mg/kg		0.01	Not det.
Leptophos	mg/kg		0.01	Not det.
Mecarbam	mg/kg		0.05	Not det.
Mephosfolan	mg/kg		0.01	Not det.
Methacrifos	mg/kg		0.05	Not det.
Methamidophos	mg/kg		0.02	Not det.
Methidathion	mg/kg		0.02	Not det.
Monocrotophos	mg/kg		0.01	Not det.
Malaoxon	mg/kg			Not det.
Malathion	mg/kg			Not det.
Malathion & Malaoxon	mg/kg		0.02	Not det.
Mevinphos-E	mg/kg			Not det.
Mevinphos-Z	mg/kg			Not det.
Mevinphos, total	mg/kg		0.01	Not det.
Phenkapton	mg/kg		0.01	Not det.
Phenthoate	mg/kg		0.01	Not det.
Phorat, total	mg/kg		0.05	Not det.
Phosalone	mg/kg		0.05	Not det.
Phosfolene	mg/kg		0.01	Not det.

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Report for contaminants

Report-No.: 12027702- 20001
Cust.-lot: (b) (6)
Sample labelling: Broccoli Seed Extract

Test	Unit	Direction for testing	Limits	Testresult
Pirimiphos-ethyl	mg/kg		0.01	Not det.
Pirimiphos-methyl	mg/kg		1	Not det.
Profenofos	mg/kg		0.05	Not det.
Propetamphos	mg/kg		0.01	Not det.
Prothiofos	mg/kg		0.01	Not det.
Pyraclophos	mg/kg		0.01	Not det.
Pyrazophos	mg/kg		0.05	Not det.
Pyridaphenthion	mg/kg		0.01	Not det.
Parethion (-ethyl)	mg/kg		0.05	Not det.
Paraoxon (-ethyl)	mg/kg		0.01	Not det.
Parathion-methyl	mg/kg			Not det.
Paraoxon-methyl	mg/kg			Not det.
Parathion-methyl & Paraoxon-methyl	mg/kg		0.02	Not det.
Phosphamidon-E	mg/kg			Not det.
Phosphamidon-Z	mg/kg			Not det.
Phosphamidon, total	mg/kg		0.01	Not det.
Quinalphos	mg/kg		0.05	Not det.
Salithion (Dioxabenzophos)	mg/kg		0.01	Not det.
Sulfotep	mg/kg		0.01	Not det.
Sulprofos	mg/kg		0.01	Not det.
Tetrachlorvinphos	mg/kg		0.01	Not det.
Thiometon	mg/kg		0.01	Not det.
Tolclofosmethyl	mg/kg		0.5	Not det.
Triazofos	mg/kg		0.01	Not det.
Trichloronate	mg/kg		0.01	Not det.
TEPP	mg/kg		0.01	Not det.
Terbufos	mg/kg		0.01	Not det.
Zinophos (Thionazin)	mg/kg		0.01	Not det.
Confirmation of the results of organophosphorus pesticides, SOP 720115				
Organochlorine pesticides, according to § 64 LFGB L 00.00-34 (Eluate 1 + 2/GC/ECD)		EG396/05 081010		Conform
Benfluralin	mg/kg		0.05	Not det.
Binapacryl	mg/kg		0.05	Not det.
Bromoxynil-octanoate	mg/kg			Not det.
Bromoxynil, incl. salz/ester	mg/kg		0.05	Not det.
Chlorfenapyr	mg/kg		0.05	Not det.
Chlorfenson	mg/kg		0.01	Not det.
Chloroneb	mg/kg		0.01	Not det.
Chlorthal-dimethyl	mg/kg		0.5	Not det.

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Report for contaminants

Report-No.: 12027702- 20001
Cust.-lot: (b) (6)
Sample labelling: Broccoli Seed Extract

Test	Unit	Direction for testing	Limits	Testresult
Chlorthalonil	mg/kg		3	Not det.
Chlorothion	mg/kg		0.01	Not det.
Chlordane, cis	mg/kg			Not det.
Chlordane, trans	mg/kg			Not det.
Oxychlordane	mg/kg		0.01	Not det.
Chlordane, total	mg/kg		0.01	Not det.
Dichlobenil	mg/kg		0.05	Not det.
Dichlofluanid	mg/kg		0.01	Not det.
Dicloran	mg/kg		0.1	Not det.
Dicofol	mg/kg		0.02	Not det.
Dinitramine	mg/kg		0.01	Not det.
DDD-o,p'	mg/kg			Not det.
DDD-p,p'	mg/kg			Not det.
DDE-o,p'	mg/kg			Not det.
DDE-p,p'	mg/kg			Not det.
DDt-o,p'	mg/kg			Not det.
DDT-p,p'	mg/kg			Not det.
DDT, total	mg/kg		0.05	Not det.
Dieldrin	mg/kg			Not det.
Aldrine	mg/kg			Not det.
Dieldrin & Aldrin	mg/kg		0.01	Not det.
Endrin	mg/kg		0.01	Not det.
Ethalfluralin	mg/kg		0.02	Not det.
Etridiazol	mg/kg		0.05	Not det.
Endosulfan, alpha	mg/kg			Not det.
Endosulfan, beta	mg/kg			Not det.
Endosulfan sulfate	mg/kg			Not det.
Endosulfan, total	mg/kg		0.05	Not det.
Fenson	mg/kg		0.01	Not det.
Flubenzimine	mg/kg		0.01	Not det.
Fluchloralin	mg/kg		0.01	Not det.
Flumetralin	mg/kg		0.01	Not det.
Halfenprox	mg/kg		0.01	Not det.
Hexachlorbenzene	mg/kg		0.01	Not det.
HCH, alpha	mg/kg			Not det.
HCH, beta	mg/kg			Not det.
HCH, delta	mg/kg			Not det.
HCH, epsilon	mg/kg			Not det.
HCH, total (without lindane)	mg/kg		0.01	Not det.
Heptachlor	mg/kg			Not det.
Heptachlorepoxide, cis	mg/kg			Not det.
Heptachlorepoxide, trans	mg/kg			Not det.
Heptachlor, total	mg/kg		0.01	Not det.

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Report for contaminants

Report-No.: 12027702- 20001
Cust.-lot: (b) (6)
Sample labelling: Broccoli Seed Extract

Test	Unit	Direction for testing	Limits	Testresult
Isobenzan	mg/kg		0.01	Not det.
Isodrin	mg/kg		0.01	Not det.
Isopropalin	mg/kg		0.01	Not det.
Ioxynil-Octanoat	mg/kg			Not det.
Ioxynil, incl. salt/ester	mg/kg		0.05	Not det.
Ketoendrin, delta	mg/kg		0.01	Not det.
Lindane	mg/kg		0.01	Not det.
Methoxychlor	mg/kg		0.01	Not det.
Mirex	mg/kg		0.01	Not det.
Nitrapyrin	mg/kg		0.01	Not det.
Nitrofen	mg/kg		0.01	Not det.
Octachlorstyrol	mg/kg		0.01	Not det.
Oxyfluorfen	mg/kg		0.05	Not det.
Pendimethalin	mg/kg		0.05	Not det.
Pentachloroanisol	mg/kg		0.01	Not det.
Pentachlorobenzene	mg/kg		0.01	Not det.
Pilfenate	mg/kg		0.01	Not det.
Profluralin	mg/kg		0.01	Not det.
Quintozene (Pentachloronitrobenzene)	mg/kg			Not det.
Pentachloroaniline	mg/kg			Not det.
Methylpentachlorophenylsulfide	mg/kg		0.01	Not det.
Quintozene, total (Sum of Quintozene and Pentachloroaniline)	mg/kg		0.02	Not det.
S-421	mg/kg		0.01	Not det.
Tecnazene	mg/kg		0.05	Not det.
Tetrachloroaniline-2,3,5,6	mg/kg		0.01	Not det.
Tetradifon	mg/kg		0.02	Not det.
Trialiat	mg/kg		0.1	Not det.
Trifluralin	mg/kg		0.5	Not det.
Vinclozolin	mg/kg		0.05	Not det.
Pyrethroids, according to § 64 LFGB L 00.00-34 (Eluate 1+2/GC/ECD)		EG396/05 081010		Conform
Acrinathrin	mg/kg		0.05	Not det.
Bifenthrine	mg/kg		0.2	Not det.
Cyfluthrine	mg/kg		0.05	Not det.
Cyhalothrine-(lambda)	mg/kg		0.1	Not det.
Cypermethrin, total	mg/kg		1	Not det.
Cyphenothrin	mg/kg		0.01	Not det.
Deltamethrin	mg/kg		0.1	Not det.
Fenpropathrin	mg/kg		0.01	Not det.
Flucythrinate	mg/kg		0.05	Not det.
Fluvalinate-(tau)	mg/kg		0.01	Not det.

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Report for contaminants

Report-No.: 12027702- 20001
Cust.-lot: (b) (6)
Sample labelling: Broccoli Seed Extract

Test	Unit	Direction for testing	Limits	Testresult
Fenvalerate RR/SS	mg/kg		0.02	Not det.
Fenvalerate RS/SR	mg/kg		0.02	Not det.
Permethrin, total	mg/kg		0.05	Not det.
Transfluthrin	mg/kg		0.01	Not det.
N-containing and further pesticides, according to § 64 LFGB L 00.00-34 (GC-MS)		EG396/05 081010		Conform
Alachlor	mg/kg		0.1	Not det.
Allethrin	mg/kg		0.01	Not det.
Azinphos-methyl	mg/kg		0.05	Not det.
Bromopropylate	mg/kg		0.05	Not det.
Buprofezin	mg/kg		0.05	Not det.
Diflubenzuron	mg/kg		1	Not det.
Fipronil	mg/kg			Not det.
Fipronil-dasulfinyl	mg/kg			Not det.
Fipronil, total	mg/kg		0.02	Not det.
Piperonyl butoxide	mg/kg		3	Not det.
Procymidone	mg/kg		0.02	Not det.
Propargit	mg/kg		0.01	Not det.
Propiconazole	mg/kg		0.05	Not det.
Pyrethrins, total	mg/kg		1	Absent
Pyridaben	mg/kg		0.05	Not det.
Phosmet	mg/kg			Not det.
Phosmet, total	mg/kg		0.05	Not det.
N-containing and further pesticides, group 2 according to § 64 LFGB L 00.00-34 (GC-MS)		EG396/05 081010		Conform
Bromacil	mg/kg		0.01	Not det.
Chlorobenzilate	mg/kg		0.02	Not det.
Etoxazol	mg/kg		0.02	Not det.
Famophos	mg/kg		0.01	Not det.
Fenarimol	mg/kg		0.02	Not det.
Fenazaquin	mg/kg		0.01	Not det.
Kresoxim-methyl	mg/kg		0.05	Not det.
Nepropamid	mg/kg		0.05	Not det.
Picoxystrobin	mg/kg		0.05	Not det.
Pyrifenox	mg/kg		0.01	Not det.
Pyriproxyfen	mg/kg		0.05	Not det.
Terbutylazine	mg/kg		0.05	Not det.
Trifloxystrobin	mg/kg		0.05	Not det.

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Report for contaminants

Report-No.: 12027702- 20001
Cust.-lot: (b) (6)
Sample labelling: Broccoli Seed Extract

Test	Unit	Direction for testing	Limits	Testresult
Further Pesticides, according to § 64 LFGB L 00.00-34, (GC-MS/MS), Screening		EG396/05 081010		Conform
Acetochlor	mg/kg		0.01	Not det.
Ancymidol	mg/kg		0.01	Not det.
Aconazole	mg/kg		0.01	Not det.
Benodanil	mg/kg		0.01	Not det.
Benzoylprop-ethyl	mg/kg		0.01	Not det.
Bromocyclen	mg/kg		0.01	Not det.
Bromuconazole, total	mg/kg		0.05	Not det.
Butachlor	mg/kg		0.01	Not det.
Captafol	mg/kg		0.02	Not det.
carbofuran	mg/kg			Not det.
Carbofuran, total	mg/kg		0.02	Not det.
Carbofuran, 3-hydroxy	mg/kg			Not det.
Carfentrazon-ethyl, total	mg/kg		0.01	Not det.
Chinomethionat	mg/kg		0.01	Not det.
Chlorfenprop-methyl	mg/kg		0.01	Not det.
Chlorpropham	mg/kg		0.05	Not det.
Chlorpropylate	mg/kg		0.01	Not det.
Clodinafop, total	mg/kg		0.02	Not det.
Crufomat	mg/kg		0.01	Not det.
Cycloat	mg/kg		0.01	Not det.
Cyprofuram	mg/kg		0.01	Not det.
Desmetryn	mg/kg		0.01	Not det.
Diflufenican	mg/kg		0.05	Not det.
Dimethachlor	mg/kg		0.02	Not det.
Dimethenamid, total	mg/kg		0.01	Not det.
Dimethipin	mg/kg		0.1	Not det.
Dinobuton	mg/kg		0.01	Not det.
Diclofop-methyl	mg/kg			Not det.
Diclofop, total (calculated as Diclofop-methyl)	mg/kg		0.1	Not det.
Fenothiocarb	mg/kg		0.01	Not det.
Fenpiclonil	mg/kg		0.01	Not det.
Flamprop-isopropyl	mg/kg		0.01	Not det.
Flamprop-methyl	mg/kg		0.01	Not det.
Fluorodifen	mg/kg		0.01	Not det.
Flurprimidol	mg/kg		0.01	Not det.
Isocarbamide	mg/kg		0.01	Not det.
Chlordecone	mg/kg		0.02	Not det.
Methoprotryne	mg/kg		0.01	Not det.
Molinate	mg/kg		0.05	Not det.
Nitrothal-isopropyl	mg/kg		0.01	Not det.

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Report for contaminants

Report-No.: 12027702- 20001
Cust.-lot: (b) (6)
Sample labelling: Broccoli Seed Extract

Test	Unit	Direction for testing	Limits	Testresult
Paclobutrazol	mg/kg		0.02	Not det.
Pentanochlor	mg/kg		0.01	Not det.
Perthane	mg/kg		0.01	Not det.
Phenothrin	mg/kg		0.05	Not det.
Profluralin	mg/kg		0.01	Not det.
Prometon	mg/kg		0.01	Not det.
Propachlor, total	mg/kg		0.3	Not det.
Propanil	mg/kg		0.1	Not det.
Pyraflufen-ethyl	mg/kg		0.02	Not det.
Swep	mg/kg		0.01	Not det.
Tetrasul	mg/kg		0.01	Not det.
Tiocarbazil	mg/kg		0.01	Not det.
Tridiphane	mg/kg		0.01	Not det.
Trietazine	mg/kg		0.01	Not det.
Triticonazole	mg/kg		0.01	Not det.
Further Pesticides, according to § 64 LFGB L 00.00-34 (GC-MS/MS)		EG396/05 081010		Conform
Acionifen	mg/kg		0.05	Not det.
Butralin	mg/kg		0.02	Not det.
Ethofumesat, total	mg/kg		0.05	Not det.
Etofenprox	mg/kg		0.2	Not det.
Fenoxycarb	mg/kg		0.05	Not det.
Indoxacarb, total	mg/kg		0.3	Not det.
Isoprothiolan	mg/kg		0.01	Not det.
Prometryne	mg/kg		0.01	Not det.
Propyzamide	mg/kg		0.02	Not det.
Tebuconazole	mg/kg		1	Not det.
Terbacil	mg/kg		0.01	Not det.
Tolylfluanid	mg/kg		1	Not det.
Pesticides, SOP 720515 (LC-MS/MS), Screen		EG396/05 081010		Conform
Acetamipride	mg/kg		0.01	Not det.
Ametryn	mg/kg		0.01	Not det.
Aminocarb	mg/kg		0.01	Not det.
Atrazine	mg/kg		0.05	Not det.
Azoxystrobin	mg/kg		5	Not det.
Aldicarb	mg/kg			Not det.
Aldicarb-sulfon	mg/kg			Not det.
Aldicarb-sulfoxid	mg/kg			Not det.
Aldicarb, total	mg/kg		0.02	Not det.
Benalaxyl, total	mg/kg		0.05	Not det.

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Report for contaminants

Report-No.: 12027702- 20001
Cust.-lot: (b) (6)
Sample labelling: Broccoli Seed Extract

Test	Unit	Direction for testing	Limits	Testresult
Bendiocarb	mg/kg			Not det.
Bendiocarb, total	mg/kg		0.01	Not det.
Benoxacor	mg/kg		0.01	Not det.
Bifenox	mg/kg		0.05	Not det.
Bitertanol	mg/kg		0.05	Not det.
Boscalid	mg/kg		1	Not det.
Bromacil	mg/kg		0.01	Not det.
Bromuconazole, total	mg/kg		0.05	Not det.
Bupirimat	mg/kg		0.05	Not det.
Cadusafos	mg/kg		0.01	Not det.
Carbaryl	mg/kg		0.05	Not det.
carbofuran	mg/kg			Not det.
Carbofuran, total	mg/kg		0.02	Not det.
Carbofuran, 3-hydroxy	mg/kg			Not det.
Carbosulfan	mg/kg		0.05	Not det.
Chloroxuron	mg/kg		0.05	Not det.
Chromafenozid	mg/kg		0.01	Not det.
Clomazone	mg/kg		0.01	Not det.
Cyanazin	mg/kg		0.01	Not det.
Cyclanilid	mg/kg		0.05	Not det.
Cymoxanil	mg/kg		0.05	Not det.
Cyprodinil	mg/kg		0.05	Not det.
Desmedipham	mg/kg		0.05	Not det.
Dicrotophos	mg/kg		0.01	Not det.
Diethofencarb	mg/kg		0.05	Not det.
Difenoxuron	mg/kg		0.01	Not det.
Dimethomorph	mg/kg		0.05	Not det.
Dinoterb	mg/kg		0.05	Not det.
Dodemorph	mg/kg		0.01	Not det.
Dodin	mg/kg		0.2	Not det.
Daminozid	mg/kg			Not det.
Dimethylhydrazin-1,1	mg/kg			Not det.
Daminozid, total	mg/kg		0.02	Not det.
Demeton-S-methyl	mg/kg			Not det.
Demeton-S-methyl-sulfon	mg/kg			Not det.
Oxydemeton-methyl	mg/kg			Not det.
Oxydemeton-methyl, total	mg/kg		0.01	Not det.
Dimethoate	mg/kg			Not det.
Omethoate	mg/kg			Not det.
Dimethoate, total	mg/kg		0.02	Not det.
Disulfoton	mg/kg			Not det.
Disulfoton-sulfoxid	mg/kg			Not det.
Disulfoton-sulfon	mg/kg			Not det.

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Report for contaminants

Report-No.: 12027702- 20001
Cust.-lot: (b) (6)
Sample labelling: Broccoli Seed Extract

Test	Unit	Direction for testing	Limits	Testresult
Disulfoton, total	mg/kg		0.02	Not det.
Ethoprophos	mg/kg		0.02	Not det.
Ethoxyquin	mg/kg		0.05	Not det.
Etoxazol	mg/kg		0.02	Not det.
Ethiofencarb	mg/kg			Not det.
Ethiofencarb-Sulfon	mg/kg			Not det.
Ethiofencarb-Sulfoxid	mg/kg			Not det.
Ethiofencarb, total	mg/kg		0.01	Not det.
Famophos	mg/kg		0.01	Not det.
Fenobucarb	mg/kg		0.01	Not det.
Fenoxaprop, total	mg/kg		0.1	Not det.
Fenoxycarb	mg/kg		0.05	Not det.
Fenpropidine	mg/kg		0.05	Not det.
Fenpropimorphe	mg/kg		0.05	Not det.
Fenpyroximat	mg/kg		0.05	Not det.
Flazasulfuron	mg/kg		0.01	Not det.
Florasulam	mg/kg		0.01	Not det.
Fluazinam	mg/kg		0.05	Not det.
Flufenoxurone	mg/kg		0.05	Not det.
Fluopicolid	mg/kg		0.01	Not det.
Fuberidazol	mg/kg		0.05	Not det.
Furathiocarb	mg/kg		0.05	Not det.
Fensulfothion	mg/kg			Not det.
Fensulfothion-oxon	mg/kg			Not det.
Fensulfothion-oxon-sulfon	mg/kg			Not det.
Fensulfothion-sulfon	mg/kg			Not det.
Fensulfothion, total	mg/kg		0.01	Not det.
Hexaflumuron	mg/kg		0.01	Not det.
Hexythiazox	mg/kg		0.5	Not det.
Hydroxythiabendezol-5	mg/kg		0.01	Not det.
Imidacloprid	mg/kg		0.5	Not det.
Iprovalicarb	mg/kg		0.05	Not det.
Isofenphos-methyl	mg/kg		0.01	Not det.
Isofenphos, total	mg/kg		0.01	Not det.
Isoprocarb	mg/kg		0.01	Not det.
Isoxaflutole, total	mg/kg		0.05	Not det.
Iodosulfuron-methyl, total	mg/kg		0.02	Not det.
Lufenuron	mg/kg		0.2	Not det.
Mepronil	mg/kg		0.05	Not det.
Metalaxyl	mg/kg		0.2	Not det.
Metamitron	mg/kg		0.1	Not det.
Metazachlor	mg/kg		0.3	Not det.
Methoxyfenozid	mg/kg		0.02	Not det.

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Report for contaminants

Report-No.: 12027702- 20 001
Cust.-lot: (b) (6)
Sample labelling: Broccoli Seed Extract

Test	Unit	Direction for testing	Limits	Testresult
Metolachlor, total	mg/kg		0.05	Not det.
Metosulame	mg/kg		0.01	Not det.
Metribuzin	mg/kg		0.1	Not det.
Mercaptodimethur (Methiocarb)	mg/kg			Not det.
Mercaptodimethur-sulfon	mg/kg			Not det.
Mercaptodimethur-sulfoxid	mg/kg			Not det.
Mercaptodimethur, total (Methiocarb)	mg/kg		0.1	Not det.
Nicosulfuron	mg/kg		0.05	Not det.
Nitralin	mg/kg		0.01	Not det.
Nuarimol	mg/kg		0.01	Not det.
Ofurace	mg/kg		0.01	Not det.
Oxadiazon	mg/kg		0.05	Not det.
Oxadixyl	mg/kg		0.01	Not det.
Oxamyl	mg/kg		0.01	Not det.
Pencycuron	mg/kg		0.05	Not det.
Phoxime	mg/kg		0.01	Not det.
Primisulfuron-methyl	mg/kg		0.01	Not det.
Prochloraz, total	mg/kg		0.05	Not det.
Promecarb	mg/kg		0.01	Not det.
Propazine	mg/kg		0.01	Not det.
Propham	mg/kg		0.05	Not det.
Propoxur	mg/kg		0.5	Not det.
Pymetrozin	mg/kg		0.02	Not det.
Pyraclostrobin	mg/kg		0.1	Not det.
Pyridate	mg/kg		0.05	Not det.
Pyrimidifen	mg/kg		0.01	Not det.
Quinoxyfen	mg/kg		0.02	Not det.
Rimsulfuron	mg/kg		0.05	Not det.
Rotenone	mg/kg		0.01	Not det.
Sebuthylazin	mg/kg		0.01	Not det.
Simeconazol	mg/kg		0.01	Not det.
Spinosad, gesamt	mg/kg		2	Not det.
Spiroxamine	mg/kg		0.05	Not det.
Sulfentrazone	mg/kg		0.01	Not det.
Sulfosulfuron	mg/kg		0.05	Not det.
Clethodim	mg/kg			Not det.
Sethoxydim	mg/kg			Not det.
Sethoxydim, total	mg/kg		0.5	Not det.
Tebufenpyrad	mg/kg		0.05	Not det.
Tebutam	mg/kg		0.01	Not det.
Teflubenzuron	mg/kg		0.5	Not det.
Terbumeton	mg/kg		0.01	Not det.
Terbutryn	mg/kg		0.01	Not det.

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Report for contaminants

Report-No.: 12027702- 20001
Cust.-lot: (b) (6)
Sample labelling: Broccoli Seed Extract

Test	Unit	Direction for testing	Limits	Testresult
Tetramethrin	mg/kg		0.01	Not det.
Thiacloprid	mg/kg		0.1	Not det.
Thiofanox	mg/kg			Not det.
Thiofanox-sulfon	mg/kg			Not det.
Thiofanox-sulfoxid	mg/kg			Not det.
Thiofanox, total	mg/kg		0.01	Not det.
Tricyclszole	mg/kg		0.05	Not det.
Tridemorph	mg/kg		0.05	Not det.
Triforin	mg/kg		0.05	Not det.
Tarbufos	mg/kg		0.01	Not det.
Terbufos-sulfoxid	mg/kg		0.01	Not det.
Tarbufos-sulfon	mg/kg		0.01	Not det.
Clothianidine	mg/kg		0.02	Not det.
Thiametoxam	mg/kg			Not det.
Thiamatoxam, total	mg/kg		0.2	Not det.
Vamidothion	mg/kg			Not det.
Vamidothion-sulfoxid	mg/kg			Not det.
Vamidothion, gesamt	mg/kg		0.01	Not det.
2,4-D, total	mg/kg		0.05	Not det.
3,4,5-Trimethacarb (Landrin)	mg/kg		0.01	Not det.
Surface treatment means, according to § 64 LFGB L 00.00-34 (GC-MS/MS)		EG396/05 081010		Conform
Biphenyl	mg/kg		0.01	Not det.
Diphenylamine	mg/kg		0.05	Not det.
Imazalil (Enilconazole)	mg/kg		0.05	Not det.
O-Phenylphenol	mg/kg		0.05	Not det.
Conazole fungicides, according to § 64 LFGB L 00.00-34 (GC-MS/MS)		EG396/05 081010		Conform
Cyproconazole	mg/kg		0.05	Not det.
Diclobutrazole	mg/kg		0.01	Not det.
Difenoconazole	mg/kg		0.2	Not det.
Diniconazole	mg/kg		0.05	Not det.
Epoxiconazole	mg/kg		0.05	Not det.
Etaconazole	mg/kg		0.01	Not det.
Fluquinconazole	mg/kg		0.05	Not det.
Flusilazole	mg/kg		0.02	Not det.
Flutriafol	mg/kg		0.05	Not det.
Hexaconazole	mg/kg		0.02	Not det.
Metconazole	mg/kg		0.02	Not det.

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Report for contaminants

Report-No.: 12027702- 20001
Cust.-lot: (b) (6)
Sample labelling: Broccoli Seed Extract

Test	Unit	Direction for testing	Limits	Testresult
Myclobutanil	mg/kg		0.02	Not det.
Penconazole	mg/kg		0.05	Not det.
Propiconazole	mg/kg		0.05	Not det.
Tebuconazole	mg/kg		1	Not det.
Tetraconazole	mg/kg		0.02	Not det.
Triflumizole	mg/kg		0.1	Not det.
Triadimefon	mg/kg			Not det.
Triadimenol	mg/kg			Not det.
Triadimefon & triadimenol	mg/kg		0.1	Not det.
Uniconazole	mg/kg		0.01	Not det.

The pesticide test results comply with the maximum limits of the current regulation described above.

Information on the method employed and on method characteristics is available to customers on request. The results in the analytical report apply to the supplied sample. Copying of the analytical report is only permissible without any modification of the document.

The residues of processed products should be recalculated to unprocessed raw material considering processing (e.g. concentration after extraction).
The specified limits are maximum residue levels (MRL) according to Regulation (EC) No 396/2005 for Broccoli - Brassica oleracea (0241010)

Vestenbergsgreuth, 17.11.10
(b) (6)

Elmar Häfner
(b) (6)

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APPENDIX C13. PhytoLab GmbH & Co. KG, Report No. 13494940-20001, Certificate of Analysis for Broccoli Seed Extract Powder, January 23, 2012

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PhytoLab GmbH & Co.KG Dutendorfer Straße 5-7 91487 Vestenbergsgreuth

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Date: 23.01.12

Cust.No: 92315

SAL-akkreditiertes Prüflaboratorium
Reg.Nr.: SAL-BY-G 104-09-09

Certificate of analysis

Report-No.: 13494940- 20001
Cust.-lot: (b) (6)
Sample labelling: Broccoli Seed Extract Powder
Sample description: approx. 300 g yellow powder
Packing: 3 x plastic bag
Receipt of sample: 23.12.11

Test	Unit	Direction for testing	Maximum limit	Testresult
Sample preparation for pesticide test, according to § 64 LFGB L 00.00-34 Modul E2		EG396/05 110811		
Organophosphorus pesticides, according to § 64 LFGB L 00.00-34 (GC/FPD)		EG396/05 110811		Conform
Acephate	mg/kg		0.02	Not det.
Azinphos-ethyl	mg/kg		0.02	Not det.
Bromophos-ethyl	mg/kg		0.05	Not det.
Bromophos-methyl	mg/kg		0.01	Not det.
Bromfenvinphos-cis	mg/kg			Not det.
Bromfenvinphos-trans	mg/kg			Not det.
Bromfenvinfos, total	mg/kg		0.01	Not det.
Carbophenothion	mg/kg		0.01	Not det.
Chlormephos	mg/kg		0.01	Not det.
Chlorpyrifos (-ethyl)	mg/kg		0.05	Not det.
Chlorpyrifos-methyl	mg/kg		0.05	Not det.
Chlorothiophos	mg/kg		0.01	Not det.
Coumaphos	mg/kg		0.01	Not det.
Cyanofenphos	mg/kg		0.01	Not det.
Cyanophos	mg/kg		0.01	Not det.
Chlorfenvinphos-cis (E)	mg/kg			Not det.
Chlorfenvinphos-trans (Z)	mg/kg			Not det.
Chlorfenvinphos, total	mg/kg		0.02	Not det.
Diazinon	mg/kg		0.01	Not det.

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Certificate of analysis

Report-No.: 13494940- 20001
Cust.-lot: (b) (6)
Sample labelling: Broccoli Seed Extract Powder

Test	Unit	Direction for testing	Maximum limit	Testresult
Dichlofenthion	mg/kg		0.01	Not det.
Dichlorvos	mg/kg		0.01	Not det.
Dimefox	mg/kg		0.01	Not det.
Dioxathion	mg/kg		0.05	Not det.
Ditalimphos	mg/kg		0.01	Not det.
Dimethoate	mg/kg			Not det.
Omethoate	mg/kg			Not det.
Dimethoate, total	mg/kg		0.02	Not det.
Edifenphos	mg/kg		0.01	Not det.
Ethion	mg/kg		0.01	Not det.
Ethoprophos	mg/kg		0.02	Not det.
Etrimfos	mg/kg		0.01	Not det.
EPN	mg/kg		0.01	Not det.
Fenamiphos, total	mg/kg		0.02	Not det.
Fenchlorphos, total	mg/kg		0.01	Not det.
Fenitrothion	mg/kg		0.01	Not det.
Fenthion, total	mg/kg		0.01	Not det.
Fonophos	mg/kg		0.01	Not det.
Formothion	mg/kg		0.02	Not det.
Fensulfothion, total	mg/kg		0.01	Not det.
Heptenophos	mg/kg		0.01	Not det.
Iprobenfos (IBP)	mg/kg		0.01	Not det.
Isocarbophos	mg/kg		0.01	Not det.
Isofenphos, total	mg/kg		0.01	Not det.
Iodofenphos	mg/kg		0.01	Not det.
Leptophos	mg/kg		0.01	Not det.
Mecarbam	mg/kg		0.05	Not det.
Mephosfolan	mg/kg		0.01	Not det.
Methacrifos	mg/kg		0.05	Not det.
Methamidophos	mg/kg		0.02	Not det.
Methidathion	mg/kg		0.02	Not det.
Monocrotophos	mg/kg		0.01	Not det.
Malaoxon	mg/kg			Not det.
Malathion	mg/kg			Not det.
Malathion & Malaoxon	mg/kg		0.02	Not det.
Mevinphos-E	mg/kg			Not det.
Mevinphos-Z	mg/kg			Not det.
Mevinphos, total	mg/kg		0.01	Not det.
Phenkapton	mg/kg		0.01	Not det.
Phenthoate	mg/kg		0.01	Not det.
Phorate, total	mg/kg		0.05	Not det.
Phosalone	mg/kg		0.05	Not det.
Phosfolan	mg/kg		0.01	Not det.

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Certificate of analysis

Report-No.: 13494940- 20001
Cust.-lot: (b) (6)
Sample labelling: Broccoli Seed Extract Powder

Test	Unit	Direction for testing	Maximum limit	Testresult
Pirimiphos-ethyl	mg/kg		0.01	Not det.
Pirimiphos-methyl	mg/kg		1	Not det.
Profenofos	mg/kg		0.05	Not det.
Propetamphos	mg/kg		0.01	Not det.
Prothiofos	mg/kg		0.01	Not det.
Pyraclofos	mg/kg		0.01	Not det.
Pyrazophos	mg/kg		0.05	Not det.
Pyridaphenthion	mg/kg		0.01	Not det.
Parathion (-ethyl)	mg/kg		0.05	Not det.
Paraoxon (-ethyl)	mg/kg		0.01	Not det.
Parathion-methyl	mg/kg			Not det.
Paraoxon-methyl	mg/kg			Not det.
Parathion-methyl & Paraoxon-methyl	mg/kg		0.02	Not det.
Phosphamidon-E	mg/kg			Not det.
Phosphamidon-Z	mg/kg			Not det.
Phosphamidon, total	mg/kg		0.01	Not det.
Quinalphos	mg/kg		0.05	Not det.
Salithion (Dioxabenzofos)	mg/kg		0.01	Not det.
Sulfotep	mg/kg		0.01	Not det.
Sulprofos	mg/kg		0.01	Not det.
Tetrachlorvinphos	mg/kg		0.01	Not det.
Thiometon	mg/kg		0.01	Not det.
Tolclofos-methyl	mg/kg		0.5	Not det.
Triazophos	mg/kg		0.01	Not det.
Trichloronat	mg/kg		0.01	Not det.
TEPP (tetraethyl pyrophosphate)	mg/kg		0.01	Not det.
Terbufos	mg/kg		0.01	Not det.
Zinophos (Thionazin)	mg/kg		0.01	Not det.
Confirmation of the results of organophosphorus pesticides, SOP 720115				
Organochlorine pesticides, according to § 64 LFGB L 00.00-34 (Eluate 1 + 2/GC/ECD)		EG396/05 110811		Conform
Benfluralin	mg/kg		0.05	Not det.
Binapacryl	mg/kg		0.05	Not det.
Bromoxynil octanoate	mg/kg			Not det.
Bromoxynil, incl. salt and ester	mg/kg		0.05	Not det.
Chlorfenapyr	mg/kg		0.05	Not det.
Chlorfenson	mg/kg		0.01	Not det.
Chloroneb	mg/kg		0.01	Not det.
Chlorthal-dimethyl	mg/kg		0.5	Not det.

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Certificate of analysis

Report-No.: 13494940- 20001
Cust.-lot: (b) (6)
Sample labelling: Broccoli Seed Extract Powder

Test	Unit	Direction for testing	Maximum limit	Testresult
Chlorthalonil	mg/kg		3	Not det.
Chlorothion	mg/kg		0.01	Not det.
Chlordane, cis	mg/kg			Not det.
Chlordane, trans	mg/kg			Not det.
Oxychlordane (octachlor epoxide)	mg/kg		0.01	Not det.
Chlordane, total	mg/kg		0.01	Not det.
Dichlobenil	mg/kg		0.05	Not det.
Dichlofluanid	mg/kg		0.01	Not det.
Dicloran	mg/kg		0.1	Not det.
Dicofol	mg/kg		0.02	Not det.
Dinitramine	mg/kg		0.01	Not det.
DDD-o,p'	mg/kg			Not det.
DDD-p,p'	mg/kg			Not det.
DDE-o,p'	mg/kg			Not det.
DDE-p,p'	mg/kg			Not det.
DDT-o,p'	mg/kg			Not det.
DDT-p,p'	mg/kg			Not det.
DDT, total	mg/kg		0.05	Not det.
Dieldrin	mg/kg			Not det.
Aldrine	mg/kg			Not det.
Dieldrin & Aldrin	mg/kg		0.01	Not det.
Endrin	mg/kg		0.01	Not det.
Ethalfluralin	mg/kg		0.02	Not det.
Etridiazole	mg/kg		0.05	Not det.
Endosulfan, alpha	mg/kg			Not det.
Endosulfan, beta	mg/kg			Not det.
Endosulfan-sulfate	mg/kg			Not det.
Endosulfan, total	mg/kg		0.05	Not det.
Fenson	mg/kg		0.01	Not det.
Flubenzimine	mg/kg		0.01	Not det.
Fluchloralin	mg/kg		0.01	Not det.
Flumetralin	mg/kg		0.01	Not det.
Halfenprox	mg/kg		0.01	Not det.
Hexachlorobenzene (HCB)	mg/kg		0.01	Not det.
HCH, alpha	mg/kg			Not det.
HCH, beta	mg/kg			Not det.
HCH, delta	mg/kg			Not det.
HCH, epsilon	mg/kg			Not det.
HCH, total (without Lindane)	mg/kg		0.01	Not det.
Heptachlor	mg/kg			Not det.
cis-Heptachlorepoxide	mg/kg			Not det.
trans-Heptachlorepoxide	mg/kg			Not det.
Heptachlor, total	mg/kg		0.01	Not det.

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Report-No.: 13494940- 20001
Cust.-lot: (b) (6)
Sample labelling: Broccoli Seed Extract Powder

Test	Unit	Direction for testing	Maximum limit	Testresult
Isobenzan	mg/kg		0.01	Not det.
Isodrin	mg/kg		0.01	Not det.
Isopropalin	mg/kg		0.01	Not det.
Ioxynil octanoate	mg/kg			Not det.
Ioxynil, incl. salt and ester	mg/kg		0.05	Not det.
Ketoendrin, delta	mg/kg		0.01	Not det.
Lindane (HCH, gamma)	mg/kg		0.01	Not det.
Methoxychlor	mg/kg		0.01	Not det.
Mirex	mg/kg		0.01	Not det.
Nitrapyrin	mg/kg		0.01	Not det.
Nitrofen	mg/kg		0.01	Not det.
Octachlorostyrene	mg/kg		0.01	Not det.
Oxyfluorfen	mg/kg		0.05	Not det.
Pendimethalin	mg/kg		0.05	Not det.
Pentachloroanisole	mg/kg		0.01	Not det.
Pentachlorobenzene	mg/kg		0.01	Not det.
Plifenate	mg/kg		0.01	Not det.
Profluraline	mg/kg		0.01	Not det.
Quintozene (Pentachloronitrobenzene)	mg/kg			Not det.
Pentachloroaniline	mg/kg			Not det.
Methylpentachlorophenylsulfide	mg/kg		0.01	Not det.
Quintozene, total (Sum of Quintozene and Pentachloroaniline)	mg/kg		0.02	Not det.
S421 (octachlorodipropyl ether)	mg/kg		0.01	Not det.
Tecnazene	mg/kg		0.05	Not det.
Tetradifon	mg/kg		0.01	Not det.
Tri-allate	mg/kg		0.1	Not det.
Trifluralin	mg/kg		0.5	Not det.
Vinclozolin	mg/kg		0.05	Not det.
2,3,5,6-Tetrachloraniline	mg/kg		0.01	Not det.
Pyrethroids, according to § 64 LFGB L 00.00-34 (Eluate 1 + 2/GC/ECD)		EG396/05 110811		Conform
Acrinathrin	mg/kg		0.05	Not det.
Bifenthrin	mg/kg		0.2	Not det.
Cyfluthrin	mg/kg		0.05	Not det.
Cyhalothrin-(lambda)	mg/kg		0.1	Not det.
Cypermethrin, total	mg/kg		1	Not det.
Cyphenothrin	mg/kg		0.01	Not det.
Deltamethrin	mg/kg		0.1	Not det.
Fenpropathrin	mg/kg		0.01	Not det.
Flucythrinate	mg/kg		0.05	Not det.
Tau-Fluvalinat	mg/kg		0.01	Not det.

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Report-No.: 13494940- 20001
Cust.-lot: (b) (6)
Sample labelling: Broccoli Seed Extract Powder

Test	Unit	Direction for testing	Maximum limit	Testresult
Fenvalerate RR/SS	mg/kg		0.02	Not det.
Fenvalerate RS/SR	mg/kg		0.02	Not det.
Permethrin, total	mg/kg		0.05	Not det.
Transfluthrin	mg/kg		0.01	Not det.
N-containing and further pesticides, according to § 64 LFGB L 00.00-34 (GC-MS)		EG396/05 110811		Conform
Alachlor	mg/kg		0.1	Not det.
Allethrin	mg/kg		0.01	Not det.
Azinphos-methyl	mg/kg		0.05	Not det.
Bromopropylate	mg/kg		0.01	Not det.
Buprofezin	mg/kg		0.05	Not det.
Diflubenzuron	mg/kg		1	Not det.
Fipronil	mg/kg			Not det.
Fipronil-desulfinyl	mg/kg			Not det.
Fipronil, total	mg/kg		0.02	Not det.
Piperonyl butoxide	mg/kg		3	Not det.
Procymidone	mg/kg		0.02	Not det.
Propargite	mg/kg		0.01	Not det.
Propiconazole	mg/kg		0.05	Not det.
Pyrethrins, total	mg/kg		1	Absent
Pyridaben	mg/kg		0.05	Not det.
Phosmet	mg/kg			Not det.
Phosmet, total	mg/kg		0.05	Not det.
N-containing and further pesticides, group 2 according to § 64 LFGB L 00.00-34 (GC-MS)		EG396/05 110811		Conform
Bromacil	mg/kg		0.01	Not det.
Chlorobenzilate	mg/kg		0.02	Not det.
Etoxazole	mg/kg		0.02	Not det.
Famophos (Famphur)	mg/kg		0.01	Not det.
Fenarimol	mg/kg		0.02	Not det.
Fenazaquin	mg/kg		0.01	Not det.
Kresoxim-methyl	mg/kg		0.05	Not det.
Napropamide	mg/kg		0.05	Not det.
Picoxystrobin	mg/kg		0.05	Not det.
Pyrifenox	mg/kg		0.01	Not det.
Pyriproxyfen	mg/kg		0.05	Not det.
Terbuthylazine	mg/kg		0.05	Not det.
Trifloxystrobin	mg/kg		0.05	Not det.

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Report-No.: 13494940- 20001
Cust.-lot: (b) (6)
Sample labelling: Broccoli Seed Extract Powder

Test	Unit	Direction for testing	Maximum limit	Testresult
Further Pesticides, according to § 64 LFGB L 00.00-34, (GC-MS/MS), Screening		EG396/05 110811		Conform
Acetochlor	mg/kg		0.01	Not det.
Ancymidol	mg/kg		0.01	Not det.
Azaconazole	mg/kg		0.01	Not det.
Benodanil	mg/kg		0.01	Not det.
Benzoylprop-ethyl	mg/kg		0.01	Not det.
Bromocyclen	mg/kg		0.01	Not det.
Bromuconazole, total	mg/kg		0.05	Not det.
Butachlor	mg/kg		0.01	Not det.
Captafol	mg/kg		0.02	Not det.
Carfentrazone-ethyl, total	mg/kg		0.01	Not det.
Chinomethionat	mg/kg		0.01	Not det.
Chlorfenprop-methyl	mg/kg		0.01	Not det.
Chlorpropham	mg/kg		0.05	Not det.
Chlorpropylate	mg/kg		0.01	Not det.
Clodinafop, total	mg/kg		0.02	Not det.
Crufomat	mg/kg		0.01	Not det.
Cycloat	mg/kg		0.01	Not det.
Cyprofuram	mg/kg		0.01	Not det.
Carbofuran	mg/kg			Not det.
Carbofuran, 3-hydroxy	mg/kg			Not det.
Carbofuran, total	mg/kg		0.02	Not det.
Desmetryn	mg/kg		0.01	Not det.
Diflufenican	mg/kg		0.05	Not det.
Dimethachlor	mg/kg		0.02	Not det.
Dimethenamid, total	mg/kg		0.01	Not det.
Dimethipin	mg/kg		0.1	Not det.
Dinobuton	mg/kg		0.01	Not det.
Diclofop-methyl	mg/kg			Not det.
Diclofop, total (calculated as Diclofop-methyl)	mg/kg		0.1	Not det.
Fenothiocarb	mg/kg		0.01	Not det.
Fenpiclonil	mg/kg		0.01	Not det.
Flamprop-isopropyl	mg/kg		0.01	Not det.
Flamprop-methyl	mg/kg		0.01	Not det.
Fluorodifen	mg/kg		0.01	Not det.
Flurprimidol	mg/kg		0.01	Not det.
Isocarbamid	mg/kg		0.01	Not det.
Chlordecone	mg/kg		0.02	Not det.
Methoprotryne	mg/kg		0.01	Not det.
Molinate	mg/kg		0.05	Not det.
Nitrothal-isopropyl	mg/kg		0.01	Not det.

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Report-No.: 13494940- 20001
Cust.-lot: (b) (6)
Sample labelling: Broccoli Seed Extract Powder

Test	Unit	Direction for testing	Maximum limit	Testresult
Paclobutrazol	mg/kg		0.02	Not det.
Pentanochlor	mg/kg		0.01	Not det.
Perthane	mg/kg		0.01	Not det.
Phenothrin	mg/kg		0.05	Not det.
Profluralin	mg/kg		0.01	Not det.
Prometon	mg/kg		0.01	Not det.
Propachlor, total	mg/kg		0.3	Not det.
Propanil	mg/kg		0.1	Not det.
Pyraflufen-ethyl	mg/kg		0.02	Not det.
Swep	mg/kg		0.01	Not det.
Tetrasul	mg/kg		0.01	Not det.
Tiocarbazil	mg/kg		0.01	Not det.
Tridiphane	mg/kg		0.01	Not det.
Trietazine	mg/kg		0.01	Not det.
Triticonazole	mg/kg		0.01	Not det.
Further Pesticides, according to § 64 LFGB L 00.00-34 (GC-MS/MS)		EG396/05 110811		Conform
Aclonifen	mg/kg		0.05	Not det.
Butralin	mg/kg		0.02	Not det.
Chlorbenside	mg/kg		0.01	Not det.
Dicapthon	mg/kg		0.01	Not det.
Ethofumesate, total	mg/kg		0.05	Not det.
Etofenprox	mg/kg		0.2	Not det.
Fenobucarb	mg/kg		0.01	Not det.
Fenoxycarb	mg/kg		0.05	Not det.
Indoxacarb, total	mg/kg		0.3	Not det.
Iprodione	mg/kg		0.1	Not det.
Isoprothiolane	mg/kg		0.01	Not det.
Lenacil	mg/kg		0.1	Not det.
Metribuzin	mg/kg		0.1	Not det.
Nitralin	mg/kg		0.01	Not det.
Prometryn	mg/kg		0.01	Not det.
Propyzamide	mg/kg		0.02	Not det.
Silafluofen	mg/kg		0.01	Not det.
Tebuconazole	mg/kg		1	Not det.
Terbacil	mg/kg		0.01	Not det.
Tolylfluanid	mg/kg		1	Not det.

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Report-No.: 13494940- 20001
Cust.-lot: (b) (6)
Sample labelling: Broccoli Seed Extract Powder

Test	Unit	Direction for testing	Maximum limit	Testresult
Pesticides, SOP 720515 (LC-MS/MS), Screening		EG396/05 110811		Conform
Acetamiprid	mg/kg		0.01	Not det.
Ametryn	mg/kg		0.01	Not det.
Aminocarb	mg/kg		0.01	Not det.
Atrazine	mg/kg		0.05	Not det.
Azoxystrobin	mg/kg		5	Not det.
Aldicarb	mg/kg			Not det.
Aldicarb-sulfone (Aldoxycarb)	mg/kg			Not det.
Aldicarb-sulfoxide	mg/kg			Not det.
Aldicarb, total	mg/kg		0.02	Not det.
Benalaxyl, total	mg/kg		0.05	Not det.
Bendiocarb	mg/kg			Not det.
Bendiocarb, total	mg/kg		0.01	Not det.
Benoxacor	mg/kg		0.01	Not det.
Bifenox	mg/kg		0.05	Not det.
Bitertanol	mg/kg		0.05	Not det.
Boscalid	mg/kg		5	Not det.
Bromacil	mg/kg		0.01	Not det.
Bromuconazole, total	mg/kg		0.05	Not det.
Bupirimate	mg/kg		0.05	Not det.
Cadusafos	mg/kg		0.01	Not det.
Carbaryl	mg/kg		0.05	Not det.
Carbosulfan	mg/kg		0.05	Not det.
Chloroxuron	mg/kg		0.05	Not det.
Chromafenozide	mg/kg		0.01	Not det.
Clomazone	mg/kg		0.01	Not det.
Cyanazine	mg/kg		0.01	Not det.
Cyclanilide	mg/kg		0.05	Not det.
Cymoxanil	mg/kg		0.05	Not det.
Cyprodinil	mg/kg		0.05	Not det.
Carbofuran	mg/kg			Not det.
Carbofuran, 3-hydroxy	mg/kg			Not det.
Carbofuran, total	mg/kg		0.02	Not det.
Desmedipham	mg/kg		0.05	Not det.
Dicrotophos	mg/kg		0.01	Not det.
Diethofencarb	mg/kg		0.05	Not det.
Difenoxuron	mg/kg		0.01	Not det.
Dimethomorph	mg/kg		0.05	Not det.
Dinoterb	mg/kg		0.05	Not det.
Dodemorph	mg/kg		0.01	Not det.
Dodine	mg/kg		0.2	Not det.
DEET-Diethyltoluamide	mg/kg		0.01	Not det.

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Report-No.: 13494940- 20001
Cust.-lot: (b) (6)
Sample labelling: Broccoli Seed Extract Powder

Test	Unit	Direction for testing	Maximum limit	Testresult
Daminozide	mg/kg			Not det.
Dimethylhydrazine-1,1	mg/kg			Not det.
Daminozide, total	mg/kg		0.02	Not det.
Demeton-S-methyl	mg/kg			Not det.
Demeton-S-methyl-sulfone	mg/kg			Not det.
Oxydemeton-methyl	mg/kg			Not det.
Oxydemeton-methyl, total	mg/kg		0.01	Not det.
Dimethoate	mg/kg			Not det.
Omethoate	mg/kg			Not det.
Dimethoate, total	mg/kg		0.02	Not det.
Disulfoton	mg/kg			Not det.
Disulfoton-sulfoxide	mg/kg			Not det.
Disulfoton-sulfone	mg/kg			Not det.
Disulfoton, total	mg/kg		0.02	Not det.
Ethoprophos	mg/kg		0.02	Not det.
Ethoxyquin	mg/kg		0.05	Not det.
Etoxazole	mg/kg		0.02	Not det.
Ethiofencarb	mg/kg			Not det.
Ethiofencarb-sulfone	mg/kg			Not det.
Ethiofencarb-sulfoxide	mg/kg			Not det.
Ethiofencarb, total	mg/kg		0.01	Not det.
Famophos (Famphur)	mg/kg		0.01	Not det.
Fenbuconazole	mg/kg		0.05	Not det.
Fenobucarb	mg/kg		0.01	Not det.
Fenoxaprop, total	mg/kg		0.1	Not det.
Fenoxycarb	mg/kg		0.05	Not det.
Fenpropidin	mg/kg		0.05	Not det.
Fenpropimorph	mg/kg		0.05	Not det.
Fenpyroximate	mg/kg		0.05	Not det.
Flazasulfuron	mg/kg		0.01	Not det.
Florasulam	mg/kg		0.01	Not det.
Fluazinam	mg/kg		0.05	Not det.
Flufenoxuron	mg/kg		0.05	Not det.
Flumioxazine	mg/kg		0.05	Not det.
Fluopicolid	mg/kg		2	Not det.
Fuberidazole	mg/kg		0.05	Not det.
Furathiocarb	mg/kg		0.05	Not det.
Fensulfothion	mg/kg			Not det.
Fensulfothion-oxon	mg/kg			Not det.
Fensulfothion-oxon-sulfone	mg/kg			Not det.
Fensulfothion-sulfone	mg/kg			Not det.
Fensulfothion, total	mg/kg		0.01	Not det.
Hexaflumuron	mg/kg		0.01	Not det.

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Report-No.: 13494940- 20001
Cust.-lot: (b) (6)
Sample labelling: Broccoli Seed Extract Powder

Test	Unit	Direction for testing	Maximum limit	Testresult
Hexythiazox	mg/kg		0.5	Not det.
Hydroxythiabendazole-5	mg/kg		0.01	Not det.
Imidacloprid	mg/kg		0.5	Not det.
Iprovalicarb	mg/kg		0.05	Not det.
Isofenphos-methyl	mg/kg		0.01	Not det.
Isofenphos, total	mg/kg		0.01	Not det.
Isoprocarb	mg/kg		0.01	Not det.
Isoxaflutole, total	mg/kg		0.05	Not det.
Iodosulfuron-methyl, total	mg/kg		0.02	Not det.
Lufenuron	mg/kg		0.2	Not det.
Mepronil	mg/kg		0.05	Not det.
Metamitron	mg/kg		0.1	Not det.
Metazachlor	mg/kg		0.3	Not det.
Methoxyfenozide	mg/kg		0.02	Not det.
Metolachlor, total	mg/kg		0.05	Not det.
Metosulam	mg/kg		0.01	Not det.
Metribuzin	mg/kg		0.1	Not det.
Metalaxyl, total	mg/kg		0.2	Not det.
Mercaptodimethur (Methiocarb)	mg/kg			Not det.
Mercaptodimethur-sulfone	mg/kg			Not det.
Mercaptodimethur-sulfoxide	mg/kg			Not det.
Mercaptodimethur, total (Methiocarb)	mg/kg		0.1	Not det.
Nicosulfuron	mg/kg		0.05	Not det.
Nitralin	mg/kg		0.01	Not det.
Nuarimol	mg/kg		0.01	Not det.
Ofurace	mg/kg		0.01	Not det.
Oxadiazon	mg/kg		0.05	Not det.
Oxadixyl	mg/kg		0.01	Not det.
Oxamyl	mg/kg		0.01	Not det.
Pencycuron	mg/kg		0.05	Not det.
Phoxim	mg/kg		0.01	Not det.
Primisulfuron-methyl	mg/kg		0.01	Not det.
Prochloraz, total	mg/kg		0.05	Not det.
Promecarb	mg/kg		0.01	Not det.
Propazine	mg/kg		0.01	Not det.
Propham	mg/kg		0.05	Not det.
Propoxur	mg/kg		0.5	Not det.
Pymetrozine	mg/kg		0.02	Not det.
Pyraclostrobin	mg/kg		0.1	Not det.
Pyridate, total	mg/kg		0.05	Not det.
Pyrimidifen	mg/kg		0.01	Not det.
Quinoxyfen	mg/kg		0.02	Not det.
Rimsulfuron	mg/kg		0.05	Not det.

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Report-No.: 13494940- 20001
Cust.-lot: (b) (6)
Sample labelling: Broccoli Seed Extract Powder

Test	Unit	Direction for testing	Maximum limit	Testresult
Rotenone	mg/kg		0.01	Not det.
Sebuthylazine	mg/kg		0.01	Not det.
Simazine	mg/kg		0.01	Not det.
Simeconazole	mg/kg		0.01	Not det.
Spinosad, total	mg/kg		2	Not det.
Spiroxamine	mg/kg		0.05	Not det.
Sulfentrazone	mg/kg		0.01	Not det.
Sulfosulfuron	mg/kg		0.05	Not det.
Clethodim	mg/kg			Not det.
Sethoxydim	mg/kg			Not det.
Sethoxydim, total	mg/kg		0.5	Not det.
Tebufenpyrad	mg/kg		0.05	Not det.
Tebutam	mg/kg		0.01	Not det.
Teflubenzuron	mg/kg		0.5	Not det.
Terbumeton	mg/kg		0.01	Not det.
Terbutryn	mg/kg		0.01	Not det.
Tetramethrin	mg/kg		0.01	Not det.
Thiacloprid	mg/kg		0.1	Not det.
Tricyclazole	mg/kg		0.05	Not det.
Tridemorph	mg/kg		0.05	Not det.
Triforine	mg/kg		0.01	Not det.
Terbufos	mg/kg		0.01	Not det.
Terbufos-sulfoxide	mg/kg		0.01	Not det.
Terbufos-sulfone	mg/kg		0.01	Not det.
Clothianidin	mg/kg		0.02	Not det.
Thiametoxam	mg/kg			Not det.
Thiametoxam, total	mg/kg		0.2	Not det.
Thiofanox	mg/kg			Not det.
Thiofanox-sulfone	mg/kg			Not det.
Thiofanox-sulfoxide	mg/kg			Not det.
Thiofanox, total	mg/kg		0.01	Not det.
Vamidothion	mg/kg			Not det.
Vamidothion-sulfoxide	mg/kg			Not det.
Vamidothione, gesamt	mg/kg		0.01	Not det.
2,4-D, total	mg/kg		0.05	Not det.
3,4,5-Trimethacarb (Landrin)	mg/kg		0.01	Not det.
Carbamates and further substances, SOP 720523 (LC-MS/MS)		EG396/05 110811		Conform
Bifenazate	mg/kg		0.01	Not det.
Carbendazim	mg/kg			Not det.
Benomyl	mg/kg			Not det.
Carbendazim, total	mg/kg		0.1	Not det.

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Report-No.: 13494940- 20001
Cust.-lot: (b) (6)
Sample labelling: Broccoli Seed Extract Powder

Test	Unit	Direction for testing	Maximum limit	Testresult
Fludioxonil	mg/kg		0.05	Not det.
Flurochloridone	mg/kg		0.1	Not det.
Methomyl	mg/kg			Not det.
Thiodicarb	mg/kg			Not det.
Methomyl, total	mg/kg		0.02	Not det.
Napropamide	mg/kg		0.05	Not det.
Phenmedipham	mg/kg		0.05	Not det.
Prosulfocarb	mg/kg		0.05	Not det.
Pyrimethanil	mg/kg		0.05	Not det.
Pirimicarb	mg/kg			Not det.
Desmethyl pirimicarb	mg/kg			Not det.
Desmethyl-formamido pirimicarb	mg/kg			Not det.
Pirimicarb, total	mg/kg		2	Not det.
Tebufenozide	mg/kg		0.5	Not det.
Thiabendazole	mg/kg		5	Not det.
Thiophanate-methyl	mg/kg		0.1	Not det.
Triflumuron	mg/kg		0.05	Not det.
Surface treatment means, according to § 64 LFGB L 00.00-34 (GC-MS/MS)		EG396/05 110811		Conform
Orthophenylphenol (2-Hydroxybiphenyl)	mg/kg		0.05	Not det.
Biphenyl (Diphenyl)	mg/kg		0.01	Not det.
Diphenylamine	mg/kg		0.05	Not det.
Imazalil (Enilconazole)	mg/kg		0.05	Not det.
Conazole fungicides, according to § 64 LFGB L 00.00-34 (GC-MS/MS)		EG396/05 110811		Conform
Cyproconazole	mg/kg		0.05	Not det.
Diclobutrazol	mg/kg		0.01	Not det.
Difenoconazole	mg/kg		0.2	Not det.
Diniconazole	mg/kg		0.05	Not det.
Epoxiconazole	mg/kg		0.05	Not det.
Etaconazole	mg/kg		0.01	Not det.
Fluquinconazole	mg/kg		0.05	Not det.
Flusilazole	mg/kg		0.02	Not det.
Flutriafol	mg/kg		0.05	Not det.
Hexaconazole	mg/kg		0.02	Not det.
Metconazole	mg/kg		0.02	Not det.
Myclobutanil	mg/kg		0.02	Not det.
Penconazole	mg/kg		0.05	Not det.
Propiconazole	mg/kg		0.05	Not det.

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Report-No.: 13494940- 20001
Cust.-lot: (b) (6)
Sample labelling: Broccoli Seed Extract Powder

Test	Unit	Direction for testing	Maximum limit	Testresult
Tebuconazole	mg/kg		1	Not det.
Tetraconazole	mg/kg		0.02	Not det.
Triflumizole	mg/kg		0.1	Not det.
Triadimefon	mg/kg			Not det.
Triadimenol	mg/kg			Not det.
Triadimefon & triadimenol	mg/kg		0.1	Not det.
Uniconazole	mg/kg		0.01	Not det.

The residues of processed products should be recalculated to unprocessed raw material considering processing (e.g. concentration after extraction) if the maximum residue limit is set for the unprocessed food.
The specified limits are maximum residue levels (MRL) according to Regulation (EC) No 396/2005 incl. 813/2011 dated 2011/08/11 for
Broccoli - Brassica oleracea (0241010)

The test results comply with the requirements described above.

Information on the method employed and on method characteristics is available to customers on request. The results apply to the supplied sample. Copying of the document is only permissible without any modification of the document.

Vestenbergsgreuth, 23.01.12
(b) (6)

Elmar Häfner
(b) (6)

APPENDIX P10. Ingredient Identity, Sampling of SGS™ Broccoli Seed Extract, II7.1-BPP.27, June 19, 2013

INGREDIENT IDENTITY GUIDANCE • DEVELOPMENT • COMPLIANCE®

PO BOX 1537, LAGUNA BEACH, CA 92652 | WWW.INGREDIENTIDENTITY.COM | P: 949 415-7795

Antony Talalay
President/CEO
Brassica Protection Products
250 S President Street Suite 2000
Baltimore, MD 21202

6/19/2013

RE: Sampling of SGS™ Broccoli Seed Extract
II7.1-BPP.27

Dear Tony :

This report provides information concerning the Lot size and sampling of **SGS™ Broccoli Seed Extract.**

Please let me know if you have any questions concerning this report.

Lot – Size and Definition

The concept of what constitutes a batch is found most directly within the regulations for the manufacture of Dietary Supplements. Other references to Lot are either associated with pharmaceutical products or other non-aligned products.

> *Batch means a specific quantity of a dietary supplement that is uniform, that is intended to meet specifications far identity, purity, strength, and com- position, and that is produced during a specified time period according to a single manufacturing record during the same cycle of manufacture.*

While **SGS** is not a Dietary Supplement, application of this definition is most appropriate since there is no definition for batch within the confines of regulations for foods.

Application of this definition parses the requirements down to uniformity, conformance with specifications and production during a specified time period. For **SGS** a Lot (synonymous with Batch according to the discussion of the regulations) is comprised of a single day's production of material. The process is controlled from beginning to end of each production day and thus the assurance of uniformity. In order to determine whether the material both uniform and in conformance with applicable specifications, the material is tested against established specifications.

Owing to the equipment constraints in manufacturing, each day's production results in numerous sub-units (internally called batches) that comprise the entirety of a day's production.

According to Batch Production information

> **One Lot has a theoretical yield of between 980 – 1,980 kg of SGS (dependent on amount of seed available and used)**

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Each batch (owing to the physical constraints of the equipment) has a theoretical yield of 125 kg

Therefore, theoretical yield for a single lot is comprised of between 7 – 16 of these subunits

All produced **SGS** is held in 25 kg units (drums). The number of drums from a given lot then is from 35 to 80.

Sampling

The sampling plan employed is based on the American National Standard (ANSI) criteria prepared by the Statistics Subcommittee Z1 (*ANSI/QSQ Z1.4-2008*). The plan employed is Single Normal, General Inspection Level II.

The ANSI Z1.4 sampling plan applied to the finished product out of each individual subunit (5 drums) results in a sampling of 3 drums per subunit.

The material drawn from each of the drums is composited and tested against specifications.

Therefore, for an individual lot, a total of between 21 and 48 drums are sampled from the 7 to 16 subunits that comprise each lot.

The sampling plan employed is to provide a stringent and statistically valid evaluation of the product.

Should you have any questions concerning this report, please let me know.

Sincerely,

(b) (6)

Jim Lassiter, President/COO
Ingredient Identity
jim@ingredientidentity.com

Attachment: Erucic Acid Test Results

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GENERALLY RECOGNIZED AS SAFE (GRAS) DETERMINATION FOR AQUEOUS BROCCOLI SEED EXTRACT POWDER

We, the members of the Expert Panel, qualified by scientific training and experience to evaluate the safety of food and food ingredients, have performed a comprehensive and critical review of available information and data on the safety and Generally Recognized As Safe (GRAS) status of the use of aqueous broccoli seed extract powder in selected food products for the general population. These data and information are summarized in the GRAS determination document, Generally Recognized As Safe (GRAS) Determination for Aqueous Broccoli Seed Extract Powder, produced by Spherix Consulting, Inc., for Brassica Protection Products LLC. This GRAS determination was made based on scientific procedures for GRAS determinations described under 21 CFR §170.30(b).

Based upon our review of the information and data available, we find that the intake of aqueous broccoli seed extract powder from the intended uses specified has been shown to be safe and GRAS, using scientific procedures, under the Federal Food, Drug, and Cosmetic Act, Section 201(s). To determine that aqueous broccoli seed extract powder is GRAS under the intended conditions of use, its safety has been demonstrated at the specified level of intake by experts qualified by both scientific training and experience to evaluate the safety of substances directly added to food, and this determination is based on generally available and accepted information.

The proposed use of this aqueous broccoli seed extract powder as an ingredient in food has been determined to be safe through scientific procedures set forth under 21 CFR §170.30(b) based on the following:

1. The aqueous broccoli seed extract powder is produced from non-genetically modified broccoli seed, complies with food-grade product specifications, and is standardized to a glucoraphanin content of 13-20%, w/w.
2. The Class 1 glucosinolates (GSLs) glucoraphanin and glucoerucin account for 99.6 % of the glucosinolate fraction of five commercial lots of the aqueous broccoli seed extract powder. The Class 2 (sinigrin, gluconapin, epigoitrin and progoitrin), Class 3 (gluconasturtiin), and Class 4 (glucobrassicin) GSLs comprise the remaining 0.4%.
3. Analyses of multiple production lots of aqueous broccoli seed extract powder document appropriate process control of contaminants (microbes, pesticides, and heavy metals).

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4. The proposed level of addition of aqueous broccoli seed extract powder to teas, energy drinks, thirst quenching drinks and non-carbonated flavored waters is 115 mg/serving. Using the National Center for Health Statistics' 2009-2010 National Health and Nutrition Examination Surveys data, 36.5% of the total U.S. population was identified as consumers of these beverages. An Estimated Daily Intake (EDI) calculation of the aqueous broccoli seed extract powder from its proposed uses indicates that the mean intake of the extract is 283 mg/person per day or 4.5 mg/kg body weight per day. The heavy consumer (90th percentile, all-user) intake is 554 mg/person per day or 8.8 mg/kg body weight per day.
5. At a typical content of 13 – 22% Class 1 GSLs (13-20% glucoraphanin, plus up to 2% glucoerucin) in the commercial aqueous broccoli seed extract powder, the mean intake of these glucosinolates would range between 37 – 62 mg/day (approximately 1 serving cooked broccoli) and the 90th percentile glucosinolate intake would range between 72 – 122 mg/day (approximately 3 servings cooked broccoli).
6. Estimates of intake for broccoli and other *Brassica* vegetables were derived using numerous food consumption surveys. Analyses indicate that the average and heavy broccoli consumers ingest 16.4 and 50.9 g/day, respectively. For reference, one serving of raw broccoli is 36 g and one serving of cooked broccoli is 78 g.
7. Raw broccoli is estimated to contain 85 mg total GSLs/100 g and cooked broccoli is estimated to contain 51 mg total GSLs/100 g. Therefore, usual glucosinolate intake based on broccoli and/or other *Brassica* vegetable consumption is in the range of 40–50 mg GSLs/day.
8. Regulatory guidance from various authorities in the U.S. and abroad recommend the consumption of 5-10 servings of vegetables per day.
9. The proposed use of aqueous broccoli seed extract powder results in mean consumer intake of glucosinolates at a level that is consistent with background consumption from broccoli. Heavy beverage consumers would ingest the amount of glucosinolates present in three servings of cooked broccoli per day. Therefore, these amounts of glucosinolates from the proposed uses are determined to be safe because they are equivalent to or less than exposure from normal diet and consistent with global authoritative dietary recommendations.
10. The safety of these levels of intake is corroborated by a critical review of human and animal studies documenting the metabolic fate, physiologic activity and lack of adverse health effects associated with ingestion of levels of glucosinolates exceeding the proposed levels of ingestion.

Determination of the GRAS status of aqueous broccoli seed extract powder under the intended conditions of use has been made through the deliberations of Thomas E. Sox, Ph.D.; Andrew Wallace Hayes, Ph.D., D.A.B.T.; and Roger Clemens, Dr.PH. These individuals are qualified by scientific training and experience to evaluate the safety of food and food ingredients. These experts have carefully reviewed and evaluated the publicly available information summarized in this document, including the safety of and human exposure to aqueous broccoli seed extract powder resulting from its intended use as an ingredient in selected foods, and have concluded:

> *There is no evidence in the available information on aqueous broccoli seed extract powder that demonstrates, or suggests reasonable grounds to suspect, a hazard to the public when aqueous broccoli seed extract powder is used at the intended use levels in the proposed applications. Aqueous broccoli seed extract powder is GRAS for use in selected foods as proposed by Brassica Protection Products LLC.*

Therefore, this aqueous broccoli seed extract powder is excluded from the definition of a food additive, and may be used in the U.S. without the promulgation of a food additive regulation by the FDA under 21 CFR.

It is our opinion that other experts qualified by training and/or experience to evaluate the safety of food and food ingredients would concur with these conclusions.

Roger Clemens, DrPH, CNS, FACN, FIFT
GRAS Expert Panel Member
School of Pharmacy
University of Southern California

Signature: (b) (6)

Date: July 10, 2013

A. Wallace Hayes, PhD, DABT, FATS, ERT
GRAS Expert Panel Member
Harvard School of Public Health

Signature: (b) (6)

Date: July 10, 2013

Thomas E. Sox, PhD, JD
GRAS Expert Panel Member
Senior Consultant
Spherix Consulting, Inc.

Signature: (b) (6)

Date: July 10, 2013

Claire Kruger, PhD, DABT
Scientific Advisor to the Panel
Spherix Consulting, Inc.

Signature: (b) (6)

Date: July 10, 2013

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SUBMISSION END