Broccoli seed extract: Genotoxicity and subchronic toxicity studies

Yu Zhou a, Hui Yang a, Yongning Li a, B. Lynch b, Xudong Jia a, *

a Key Laboratory of Food Safety Risk Assessment of National Health and Family Planning Commission, China National Center for Food Safety Risk Assessment, Beijing 100021, China
b Intertek Scientific & Regulatory Consultancy, 2233 Argentia Rd., Suite 201, Mississauga, ON L5N 2X7, Canada

A R T I C L E   I N F O

Article history:
Received 24 June 2015
Received in revised form 6 August 2015
Accepted 7 August 2015
Available online 11 August 2015

Keywords:
Broccoli seed extract
Subchronic toxicity
Genotoxicity
Mouse sperm abnormality assay

A B S T R A C T

Potential health benefits have been attributed to broccoli consumption. Hence, there is potential for use of broccoli seed extract (BSE) in food or for use as a dietary supplement. To assess the potential safety of a BSE product, three genotoxicity experiments, including an Ames, in vivo mouse micronucleus, and in vivo mouse sperm abnormality assay, were carried out. BSE was subject to an acute oral toxicity test and was evaluated in a 30-day feeding study in rats. BSE showed no mutagenic activity in the Ames assay and no evidence of genotoxic potential in the in vivo assays at doses up to 10 g/kg body weight (bw). The LD₅₀ of BSE in rats was >10 g/kg bw/d. In the 30-day feeding study, in which BSE was administered in the diet to provide doses of 0, 0.3, 1.0, or 3.0 g/kg bw/d, no toxicological significant effects were noted on body weight, body weight gain, organ weights, or on the results of hematological, clinical chemistry and histopathological evaluations. The no-observed-adverse-effect level was considered to be 3.0 g/kg bw/d, the highest dose tested. Collectively, these results support the safe use of BSE as a food ingredient or product.

© 2015 Elsevier Inc. All rights reserved.

1. Introduction

The genus Brassica (family Brassicaceae or Cruciferae) includes many commonly eaten vegetables (in the form of top sprouts, stems, roots or oils) such as broccoli, cauliflower, Brussels sprout, cabbage, and others (Latté et al., 2011).

Consumption of broccoli provides an excellent natural source of dietary fiber, folate, minerals (calcium and potassium) and vitamins (A, C, E, and K). Other important components which have specifically been linked to the beneficial effects of broccoli consumption include the glucosinolates (Traka and Mithen, 2009; Latté et al., 2011; Dinkova-Kostova and Kostov, 2012; Veeranki et al., 2013). These are secondary plant products, the aglycones of which can be grouped into a number of different structural classes depending on the nature of the side chain present (Fahey et al., 2001). At least 16 glucosinolates are present in common broccoli cultivar samples (Vang et al., 2001; Vallet et al., 2003; Meyer and Adam, 2008; Latté et al., 2011). The most common of the glucosinolates present are glucoraphanin and glucobrassicin. Of the indole glucosinolates, glucobrassicin and neoglucobrassicin predominate. The exact nature of the glucosinolates present though is highly dependent upon the cultivar used, the habitat/environment present, and the stage of plant development (Kurilich et al., 1999; Vallet et al., 2003; Verkerk et al., 2009; Latté et al., 2011). The basic structure of the glucosinolate molecule, and associated potential side chains, is shown in Fig. 1.

Glucoraphanin is hydrolyzed by myrosinase intrinsic to gut microflora (Shapiro et al., 1998; Conaway et al., 2000; Shapiro et al., 2006) to the respective isothiocyanate sulforaphane (Fig. 2). Some of the glucoraphanin in broccoli may also be digested by myrosinase present within the plant cells, whereby the enzyme is released upon chewing or mechanical processing (Fahey et al., 2001). Also, glucoraphanin is absorbed to some extent intact and undergoes enterohepatic circulation with subsequent hydrolysis to sulforaphane in the lower gut (Bheemreddy and Jeffery, 2007).

Abbreviations: 2-AA, 2-Aminoanthracene; 2-NF, 2-nitrofluorene; 9-AA, 9-aminoacridine; BSE, broccoli seed extract; bw, body weight; FDA, United States Food and Drug Administration; GSH, glutathione; GST, glutathione transferase; LD₅₀, median lethal dose; NOAEL, no-observable-adverse-effect level; PCE, poly-chromatic erythrocytes; RBC, red blood cells; WBC, white blood cells.
* Corresponding author. 7 Panjiayuan Nanli, Beijing 100021, China. E-mail address: jiaxudong@cfsa.net.cn (X. Jia).

http://dx.doi.org/10.1016/j.yrtph.2015.08.003
0273-2300/© 2015 Elsevier Inc. All rights reserved.
Shapiro et al., 2006). Bioavailability and peak plasma concentrations of sulforaphane following oral consumption of broccoli are higher for fresh broccoli compared to cooked or steamed broccoli (Vermeulen et al., 2008).

Cruciferous vegetable consumption, specifically broccoli, has been associated with decreases in the risk of developing several chronic conditions, including various types of cancer (Kristal and Lampe, 2002; Kensler et al., 2005; Juge et al., 2007; Traka et al., 2008; Verkerk et al., 2009; Latté et al., 2011; Wu et al., 2013a,b). Broccoli also has been shown to possess anti-oxidant activity (Latté et al., 2011).

The apparent chemopreventive effect of broccoli and its extracts has been attributed in large part to the action of sulforaphane on both phase I and phase II metabolizing enzymes (Maheo et al., 1997; Talalay and Fahey, 2001; Latté et al., 2011; Boddupalli et al., 2012; Dinkova-Kostova and Kostov, 2012; James et al., 2012; Veerranki et al., 2013). Phase I enzymes, such as the CYP family, are responsible for the oxidation of many compounds to more polar, easily excreted metabolites (Dauterman, 1994). However, for many chemicals shown to be either carcinogenic in animals or humans (Williams et al., 2007; Macherey and Dansette, 2008), this oxidative metabolism is associated with the production of reactive intermediates which are capable of damaging DNA and which act as the ultimate carcinogen (Levi, 1994; Delclos and Kadlubar, 1997; Dragan, 1997; Caldwell and Mills, 1999; Clayson and Kitchin, 1999; Williams et al., 2007; Macherey and Dansette, 2008). Sulforaphane has been shown to competitively inhibit, or reduce, the activity of several isoforms of CYP enzymes (e.g., CYP1A1, CYP1A2, CYP2E1) (Clarke et al., 2008; Latté et al., 2011). Inhibition of these CYP enzymes can be an important mechanism, specifically for sulforaphane (Clarke et al., 2008), in the prevention of the formation of carcinogenic reactive intermediary metabolites (Higdon et al., 2007; Williams et al., 2007; Macherey and Dansette, 2008).

In addition to modulating the activity of CYP enzymes, sulforaphane has also been demonstrated in vitro and in vivo to up-regulate the activity of phase II detoxification enzymes, including NAD(P)H-quinone reductase and glutathione transferase (GST) (Talalay and Fahey, 2001; Cornblatt et al., 2007; Clarke et al., 2008;
Latté et al., 2011; Boddupalli et al., 2012; Dinkova-Kostova and Kostov, 2012; James et al., 2012; Veeranki et al., 2013). Up-regulation of GST activity is thought to involve the KEAP1/Nrf2/ARE gene product pathway (Talalay and Fahey, 2001; Herr and Büchner, 2010; Latté et al., 2011; Boddupalli et al., 2012; Dinkova-Kostova and Kostov, 2012).

GST enzymes catalyze the conjugation of the reduced form of glutathione (GSH), through nucelophilic attack, to the electrophilic centers, usually carbon, sulfur, or nitrogen atoms, of xenobiotic substrates for the purpose of detoxification (Levi, 1994; Clayson and Kitchin, 1999; Williams et al., 2007; Macherey and Dansette, 2008). This prevents the interaction of these electrophilic centers with cell components such as protein and DNA. Depletion of cellular stores of GSH has been associated with organ toxicity, genetic damage, and cancer development (Meister, 1991; Pastore et al., 2003; Kalinina et al., 2014). Hence, consumption of glucoraphanin (sulfurphane precursor) in broccoli, or its extract, could potentially have beneficial effects on cellular GSH status.

In broccoli, the highest concentrations of glucoraphanin are present in the seeds. These concentrations decline rapidly such that only small amounts are present in market-ready broccoli sprouts (Trenerry et al., 2006; Gu et al., 2012; Chaudhary et al., 2014; Brassica Protection Products LLC, 2005). As a result, for the purposes of augmenting dietary concentrations of glucosinolates, and of sulforaphane specifically, broccoli seeds and their extract appear as a promising commercial source of these substances. To this end, Brassica Protection Products LLC, has developed a proprietary process to manufacture an aqueous extract of broccoli seeds containing a minimum of 13% glucoraphanin content. Briefly, the manufacturing process is as follows: broccoli seeds from non-genetically modified American-grown Brassica oleracea var. italic a Plenc (Brassicaceae) are boiled, charcoal filtered, and centrifuged then concentrated to about 20% solids, after which maltodextrin is added and the product spray dried.

Given the potential utility of developing an extract from broccoli seeds that would be a rich source of sulforaphane, and noting the relative absence of safety studies on broccoli seed or its extract per se, it was considered prudent to conduct a series of studies to demonstrate the safety of such a product for possible use in food. Reported herein are the results of a battery of genotoxicity studies, including an Ames assay, an in vivo mouse micronucleus test, and an in vivo mouse sperm abnormality assay, as well as of an acute toxicity and repeated dose 28-day toxicity study conducted in rats. These studies support the safe use of broccoli seed extract (BSE) in food or as a dietary supplement.

2. Materials and methods

All aspects in this project involving animal care, use, and welfare were performed in compliance with the United States Food and Drug Administration (FDA) principles of Good Laboratory Practice and in accordance with the FDA Guidance for Industry and Other Stakeholders, “Toxicological Principles for the Safety Assessment of Food Ingredients Redbook 2000” (U.S. FDA, 2000). All animal study protocols have been approved by the Office of Laboratory Animal Welfare, China National Center for Food Safety Risk Assessment (Beijing, China). Full details of the methods have been previously reported in Yang and Jia (2014).

2.1. Test substance

BSE, provided by Intertek Testing Services Limited, Shanghai, Beijing Chaoyang Branch, was a pale yellow powder and stored at room temperature for testing. The BSE (Batch No. 201308119) was manufactured by Brassica Protection Products LLC and was considered stable for a period of three years.

The product contains ~70% carbohydrates (up to 35% maltodextrin), 9.6% protein, 7% moisture and 12.8% ash. Glucoraphanin comprises about 13% of the product (i.e., ~19% of the carbohydrate present). Other glucosinolates are also present in the BSE and include gluconapin (~1.1%), sinigrin (~0.07%). The total glucosinolate content of the BSE tested was about 13–15%.

2.2. Animals and housing conditions

The experimental animals - healthy Kunming mice (SPF grade) and Wistar rats (SPF grade) were purchased from the Laboratory Animal Center of the Academy of Military Medical Sciences of China PLA, Certificate of Conformity No.: SCXK 2012-0004. Animals were reared within the Animal Rooms, under a barrier environment, of the Institute of Medical Experimental Animals of the Chinese Academy of Medical Sciences.

Co60 irradiated SPF-grade mouse/rat maintenance feeds were purchased from the Experimental Animal Center of the Academy of Military Medical Sciences for the acute toxicity test, bone marrow cell micronucleus test and mouse sperm malformation test. For the 30-day subchronic toxicity test, Co60 irradiated mouse/rat special feeds were supplied by Beijing HFK Bio-Technology Co., Ltd.

2.3. Genotoxicity studies

2.3.1. Bacterial reverse mutation assay (Ames test)

This test employed 4 Salmonella typhimurium histidine-deficient test strains, namely TA97, TA98, TA100 and TA102. The S9 microsomal fraction of rat liver homogenate was used as the metabolic activation system (source: purchased from Lphase biotech, Beijing, China). This was prepared according to previous methods (Mortelmans and Zeiger, 2000). Five doses, including 62, 185, 556, 1,667, and 5000 µg/dish were to be tested based on the results of a preliminary toxicity test. One g of BSE was weighed and autoclaved at 120 degrees C° (0.103 MPa, 20 min), sterile water was added to meter the volume to 20 mL in order to obtain a dose level of 5000 µg/dish. The remaining doses were prepared by three-fold serial dilution to obtain a total of five dose groups. The untreated control, solvent control (the solvent was sterile water) and positive controls were prepared concurrently with the treated groups. The standard mutagens were used as positive controls, including sodium azide (NaN₃, 1.5 µg/plate) for TA100 without S9, 2-aminofluorene (2-AF, 10 µg/plate) for TA97, TA98, and TA100 with S9, 4-nitro-o-phenylenediamine (4-NOPD, 20 µg/plate) for TA97 and TA98 without S9, mitomycin (MMC, 2.5 µg/plate) for TA102 with S9, and 8-dihydroxyanthraquinone (DHAQ, 50 µg/plate) for TA102 without S9.

To 0.1 mL test strain enrichment broth, 0.1 mL test article solution (or control) and 0.5 mL S-9 mixture (when metabolic activation was required) was added to the top agar layer; after mixing thoroughly, it was poured into the culture medium plate at the bottom. After culturing at 37 °C for 48 h, the number of revertant colonies per dish was counted manually. A positive result was determined where the revertant colony counts were greater than 2-fold those of the solvent control and there existed a clear dose–response relationship. The entire test was repeated once under the same conditions.

2.3.2. In vivo mouse bone marrow micronucleus assay

Fifty healthy Kunming mice (SPF grade) (25 of each sex) mice, weighing 25–30 g, were randomized by body weight (bw) into five groups, five sex in each group. Mice were treated by oral gavage (20 mL/kg bw) with either distilled water (negative control), or with BSE at a dose of 2.5, 5.0, or 10.0 g/kg bw, twice within a 24-h...
interval. Cyclophosphamide (40 mg/kg bw) was used as positive con-
trol. The high dose solution was prepared using 50.0 g BSE dis-
solved in distilled water to a 100 mL volume. Lower doses were
prepared through a two-fold serial dilution with distilled water.

Six hours following the second gavage treatment, animals were
euthanized and the sternum aseptically removed. The contents of
the spinal canal were squeezed out and diluted with calf serum,
then smeared onto slides. After fixation with methanol and Giemsa
staining, red blood cells (RBC) and polychromatic erythrocytes
(PCE) were observed under microscopy. The number of PCE was
counted from 200 RBC in each animal and the ratio of PCE/RBC was
calculated. For each animal, 1000 PCE were examined and the
incidence of micronucleated PCE recorded.

2.3.3. Mouse sperm malformation assay

Fifty sexually mature male healthy Kunming mice (SPF grade)
mice, weighing 30–35 g, were randomized into five groups, 10 in
each group. Treatment groups included a negative control (distilled
water), a positive control (40 mg/kg bw cyclophosphamide), and
three BSE treatment groups (2.5, 5.0, and 10.0 g/kg bw), all
administered by oral gavage. The high dose formulation was pre-
pared using 50.0 g BSE dissolved in distilled water to meter the
volume to 100 mL. The lower doses were prepared by two-fold
serial dilution with distilled water. The volume of intragastric
gavage was 20 mL/kg bw. Animals were treated once daily for
five successive days. Thirty days following the last dose, five mice
were randomly selected from each group and sacrificed by cervical
dislocation. The bilateral epididymides were harvested and cut into
sections for histological examination. The spermatozoa with morphological
abnormalities were counted based on evaluation of 1000 sperma-
tozoa per animal for the calculation of malformation rates.

2.4. Rat studies

2.4.1. Acute toxicity study

The conventional method (Horn, 1956) was used to assess the
acute oral toxicity of BSE. Forty (40) healthy Wistar rats, weighing
from 180.0 to 220.0 g, were randomized into four dose groups by
body weight; each group included 10 rats, half male and half fe-
male. The test article was prepared into the desired concentrations
from 180.0 to 220.0 g, were randomized into four dose groups by
body weight; each group included 10 rats, half male and half fe-
male. The test article was prepared into the desired concentrations
administered by oral gavage. The high dose formulation was pre-
pared using 50.0 g BSE dissolved in distilled water to a 100 mL volume. Lower doses were
prepared by two-fold serial dilution with distilled water. The volume of intragastric
gavage was 20 mL/kg bw. Animals were treated once daily for
five successive days. Thirty days following the last dose, five mice
were randomly selected from each group and sacrificed by cervical
dislocation. The bilateral epididymides were harvested and cut into
sections for histological examination. The spermatozoa with morphological
abnormalities were counted based on evaluation of 1000 sperma-
tozoa per animal for the calculation of malformation rates.

2.4.2. Thirty-day feeding study

After acclimating to the laboratory environment for one week,
40 male and 40 female weanling Wistar rats weighing approxi-
mately 70–75 g were randomized into four groups (10/sex/group)
including one control group and three treatment groups dosed at
0.33, 1.0, and 3.0 g/kg bw/d (equivalent to 33-, 100- and 300-fold of
the recommended human dose).

2.4.2.1. In-life observations. Over the course of the study, each of
the animals was reared in a single cage with free access to water.
General clinical observations were recorded daily. Body weights
and food consumption were measured weekly.

2.4.2.2. Hematology and clinical chemistry. On study day 30, rats
were anesthetized with 3% sodium pentobarbital solution after
16–18 h fast and blood was collected from the tail vein. Routine
hematologic parameters such as RBC, hemoglobin, platelet count,
white blood cells (WBC) count, and leucocyte differential counts
were measured with a Coulter Diff Hematology Analyzer (Beckman
Coulter Corporation) using whole blood stabilized by the antico-
gulant ethylenediaminetetraacetic acid. Clinical chemistry was
analyzed with an automatic clinical analyzer (Hitachi 7080, Hitachi
High-Technologies Corporation) to determine serum alanine
aminotransferase (ALT), aspartate aminotransferase (AST), alkaline
phosphatase, total protein, albumin, glucose, blood urea nitrogen,
creatinine, cholesterol, and triglyceride.

2.4.2.3. Necropsy and histopathology. At study termination, all an-
imals were weighed and euthanized for complete gross necropsy.
The liver, kidney, spleen, stomach, duodenum, heart, thymus, ad-
renals, testes, or ovary were collected and weighed. Organ-to-body
weight ratios (relative organ weight) were calculated. Gross ex-
amination of the liver, spleen, kidneys, stomach, duodenum, testes
or ovaries of animals in each dose groups was carried out. Following
fixation in 10% formalin, organs and tissues from each animal were
embedded in paraffin, sectioned, stained with hematoxylin and
eosin. If no significant lesions were found, histopathological ex-
aminations were conducted only in the high-dose and control
groups. If pathological changes were observed, histological exam-
ination of the corresponding organs were also carried out in the
mid- and low-dose groups.

2.5. Statistical analyses

Statistical analysis of the experimental data was performed us-
ing SPSS software. If the variance in each group was homogeneous,
one-way ANOVA was carried out using $\alpha = 0.05$ as the significance
level, while the mean was compared between each dose group and
control group using Dunnett's test. If homogeneity of variance was
not met, other statistical methods such as Brown--Forsythe test or
the Welch test were employed. The $X^2$-test was used for numera-
tion data.

The data from the in vivo mouse micronucleus assay were
analyzed using Poisson's distribution. The Wilcoxon rank sum test
was carried out for statistical processing of data from the mouse
sperm abnormality assay.

3. Results

3.1. Genotoxicity tests

3.1.1. Bacterial reverse mutation test

Based on the results of the initial toxicity-mutation assay (data
not shown), it was determined that the maximum concentration to
be tested in the confirmatory assay was 5000 µg/plate. No positive
mutagenic responses were observed with BSE in any of the tester
strains in the presence or in the absence of S9 activation in both test
phases. Data for the confirmatory assay are presented in Table 1.
Additionally, neither precipitate nor toxicity was observed. Treat-
ment with the positive control agents resulted in the expected large
fold increases in the number of revertant colonies. All negative and
positive control results were within the range of historical control
values.

3.1.2. In vivo mammalian cell micronucleus test

The mouse bone marrow micronucleus assay showed that PCE/
RBC ratios were not significantly altered by treatment with BSE at
doses of up to 10 g/kg bw. This indicates that the BSE was not
cytotoxic to bone marrow following oral exposure (Table 2). There were no statistically significant differences in micronucleus frequency between all test article dose groups and the negative control groups ($p > 0.05$). The micronucleus frequency in both male and female positive control groups was significantly higher than in the negative control group ($p < 0.05$) indicating the conduct of a valid test.

3.1.3. Mouse sperm malformation assay

As shown in Table 3, the sperm malformation rate was significantly higher in the positive control group compared to the negative control group ($p < 0.05$). The differences in sperm malformation rate between each test article dose group and negative control group, however, showed no statistical significance ($p > 0.05$) indicating a lack of effect of BSE on the mouse sperm malformation rate. Likewise, there was no indication of treatment with BSE on the rate or incidence of any specific sperm abnormality.

Taken together, the results of the in vivo micronucleus and sperm abnormality assays show no evidence of genotoxic activity of BSE either in somatic or germ cells.

3.2. Rat studies

3.2.1. Acute toxicity

In the 14 days following treatment of male and female rats with a bolus dose of 1.00, 2.15, 4.64, or 10.00 g/kg bw BSE, no obvious abnormalities were observed.

### Table 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/dish)</th>
<th>Revertant colonies per plate (mean ± standard deviation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test article</td>
<td>62</td>
<td>116.3 ± 4.2</td>
</tr>
<tr>
<td></td>
<td>185</td>
<td>135.7 ± 14.0</td>
</tr>
<tr>
<td></td>
<td>556</td>
<td>132.3 ± 18.5</td>
</tr>
<tr>
<td></td>
<td>1667</td>
<td>137.0 ± 28.5</td>
</tr>
<tr>
<td></td>
<td>5000</td>
<td>145.3 ± 20.6</td>
</tr>
<tr>
<td>Unreated control</td>
<td>155.7 ± 7.0</td>
<td>140.0 ± 27.2</td>
</tr>
<tr>
<td>Solvent control</td>
<td></td>
<td>134.0 ± 11.5</td>
</tr>
<tr>
<td>Positive controls</td>
<td></td>
<td>47.0 ± 10.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>38.3 ± 5.8</td>
</tr>
</tbody>
</table>

### Table 2

<table>
<thead>
<tr>
<th>Sex</th>
<th>Dose (g/kg bw)</th>
<th>PCE counts (counts/each)</th>
<th>PCE/RBC (%)</th>
<th>Micronucleus counts (counts/each)</th>
<th>Micronucleus frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>0.0</td>
<td>108.2 ± 3.7</td>
<td>54.1</td>
<td>1.6 ± 1.1</td>
<td>1.6</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>108.0 ± 4.4</td>
<td>54.0</td>
<td>1.8 ± 0.4</td>
<td>1.8</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>108.8 ± 3.8</td>
<td>53.4</td>
<td>1.6 ± 0.5</td>
<td>1.6</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>107.2 ± 4.5</td>
<td>53.6</td>
<td>1.6 ± 0.9</td>
<td>1.6</td>
</tr>
<tr>
<td>Cyclophosphamide$^a$ 40 mg/kg bw</td>
<td>0.0</td>
<td>107.2 ± 6.1</td>
<td>53.6</td>
<td>1.8 ± 0.8</td>
<td>1.8</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>107.2 ± 6.0</td>
<td>53.6</td>
<td>1.6 ± 0.9</td>
<td>1.6</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>108.2 ± 4.9</td>
<td>54.1</td>
<td>1.6 ± 0.9</td>
<td>1.6</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>107.4 ± 5.0</td>
<td>53.7</td>
<td>1.6 ± 0.9</td>
<td>1.6</td>
</tr>
</tbody>
</table>

### Table 3

<table>
<thead>
<tr>
<th>Dose (g/kg bw)</th>
<th>Number of various types of sperm malformation (%) of total malformations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amorphous</td>
<td>Hookless</td>
</tr>
<tr>
<td>0.0</td>
<td>45 (60.8)</td>
</tr>
<tr>
<td>2.5</td>
<td>41 (41.7)</td>
</tr>
<tr>
<td>5.0</td>
<td>38 (52.1)</td>
</tr>
<tr>
<td>10.0</td>
<td>14 (26.9)</td>
</tr>
<tr>
<td>Cyclophosphamide$^a$ 40 mg/kg bw</td>
<td>120 (64.2)</td>
</tr>
</tbody>
</table>

$^a$p < 0.05.

$^a$ Plus/minus standard deviation.

$^a$ Positive control.
There were no statistically significant differences in body weights (Table 4) between the BSE-treated and control groups. A tendency to decreased food consumption was noted at the weekly intervals in high-dose males (Table 5). As shown in Table 6, the total food intake over the course of the 30-day study was decreased (p < 0.01) in males treated at the high dose of 3.00 g/kg bw/d. This finding, however, was associated with an increase in the food utilization rate (p < 0.01) since, despite the decreased food consumption in these animals, their body weight gain remained similar to the controls.

Several statistically significant changes in hematological and clinical chemistry parameters (p < 0.05) were noted in the 30-day study (Tables 7 and 8). Feeding of BSE at 0.33, 1.00, and 3.00 g/kg bw/d was associated with decreased hemoglobin in all male dose groups. Also in males, RBC counts were slightly decreased in the mid- and high-dose groups; WBC counts were slightly lower in the mid- and high-dose levels, and triglycerides were decreased in WBC differential counts in the high-dose group. All of these minor fluctuations in hematological parameters fell within the historical control range of the testing laboratory and showed no clear dose–response relationships. When compared with the control group, there were no statistically significant differences (p > 0.05) in WBC counts and differential counts, RBC counts and hemoglobin concentrations in treated females.

As shown in Table 8, albumin increased in the 0.33 and 3.00 g/kg bw/d dose group females; cholesterol decreased in males at the mid- and high-dose levels, and triglycerides were decreased in high-dose males when compared to the controls (p < 0.05). As with the observed changes in hematological parameters, the statistically significant differences in the clinical chemistry parameters all fell within the historical control range of the testing laboratory and the magnitude of change was minimal. There were no changes in parameters indicative of liver (AST, ALT) or kidney toxicity.

Following 30-days of treatment with BSE, the absolute and relative weights of the spleen and kidney increased in high-dose males (Table 9). Also, the absolute weight, but not the relative weight, of the testes was slightly reduced in high dose males.

### Table 4
Effects of broccoli seed extract on rat body weight.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Dose (g/kg bw/day)</th>
<th>Initial weight (g)</th>
<th>Week 1 (g)</th>
<th>Week 2 (g)</th>
<th>Week 3 (g)</th>
<th>Week 4 (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>0.00</td>
<td>74.2 ± 3.1a</td>
<td>126.6 ± 6.7</td>
<td>193.9 ± 10.2</td>
<td>253.0 ± 11.3</td>
<td>310.3 ± 13.4</td>
</tr>
<tr>
<td></td>
<td>0.33</td>
<td>74.0 ± 3.6</td>
<td>126.9 ± 7.0</td>
<td>195.3 ± 8.3</td>
<td>254.4 ± 14.3</td>
<td>315.8 ± 15.6</td>
</tr>
<tr>
<td></td>
<td>1.00</td>
<td>73.8 ± 4.5</td>
<td>126.3 ± 6.8</td>
<td>192.1 ± 7.8</td>
<td>256.0 ± 9.5</td>
<td>315.6 ± 10.9</td>
</tr>
<tr>
<td></td>
<td>3.00</td>
<td>73.7 ± 3.3</td>
<td>119.7 ± 5.9</td>
<td>185.3 ± 8.5</td>
<td>247.5 ± 10.1</td>
<td>306.2 ± 11.4</td>
</tr>
<tr>
<td>Female</td>
<td>0.00</td>
<td>73.2 ± 3.3</td>
<td>114.3 ± 6.7</td>
<td>151.2 ± 7.9</td>
<td>177.3 ± 11.8</td>
<td>204.8 ± 12.6</td>
</tr>
<tr>
<td></td>
<td>0.33</td>
<td>72.6 ± 3.1</td>
<td>115.5 ± 5.6</td>
<td>155.4 ± 6.7</td>
<td>183.3 ± 10.4</td>
<td>207.7 ± 8.0</td>
</tr>
<tr>
<td></td>
<td>1.00</td>
<td>72.9 ± 3.1</td>
<td>113.8 ± 3.9</td>
<td>154.8 ± 5.7</td>
<td>183.4 ± 7.3</td>
<td>205.8 ± 9.4</td>
</tr>
<tr>
<td></td>
<td>3.00</td>
<td>73.3 ± 3.3</td>
<td>109.4 ± 7.2</td>
<td>149.6 ± 8.2</td>
<td>178.1 ± 13.1</td>
<td>203.6 ± 14.0</td>
</tr>
</tbody>
</table>

* Plus/minus standard deviation.

### Table 5
Effects of broccoli seed extract on weekly feed consumption of rats.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Dose (g/kg bw/day)</th>
<th>Week 1 (g)</th>
<th>Week 2 (g)</th>
<th>Week 3 (g)</th>
<th>Week 4 (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>0.00</td>
<td>100.4 ± 7.5b</td>
<td>161.0 ± 9.0</td>
<td>186.5 ± 8.8</td>
<td>197.3 ± 6.1</td>
</tr>
<tr>
<td></td>
<td>0.33</td>
<td>90.9 ± 8.9</td>
<td>154.7 ± 8.4</td>
<td>185.8 ± 15.8</td>
<td>201.7 ± 8.4</td>
</tr>
<tr>
<td></td>
<td>1.00</td>
<td>93.5 ± 8.2</td>
<td>154.8 ± 6.3</td>
<td>185.6 ± 9.8</td>
<td>199.4 ± 11.6</td>
</tr>
<tr>
<td></td>
<td>3.00</td>
<td>82.7 ± 3.3*</td>
<td>146.0 ± 7.8*</td>
<td>181.8 ± 8.5</td>
<td>194.9 ± 10.4</td>
</tr>
<tr>
<td>Female</td>
<td>0.00</td>
<td>95.3 ± 8.9</td>
<td>133.2 ± 8.2</td>
<td>148.4 ± 9.3</td>
<td>153.4 ± 12.5</td>
</tr>
<tr>
<td></td>
<td>0.33</td>
<td>86.8 ± 6.0</td>
<td>132.2 ± 5.0</td>
<td>145.4 ± 4.1</td>
<td>152.3 ± 11.3</td>
</tr>
<tr>
<td></td>
<td>1.00</td>
<td>85.1 ± 3.6</td>
<td>130.5 ± 5.9</td>
<td>145.3 ± 7.0</td>
<td>158.2 ± 36.6</td>
</tr>
<tr>
<td></td>
<td>3.00</td>
<td>80.4 ± 6.2</td>
<td>123.9 ± 5.8</td>
<td>140.4 ± 9.7</td>
<td>149.4 ± 7.3</td>
</tr>
</tbody>
</table>

*p < 0.05.

* Plus/minus standard deviation.

3.2.2. Thirty-day feeding study

No mortality or treatment related adverse clinical findings occurred during the 30-day feeding study. The animals in all groups showed normal activities, normal growth and thick shiny hair. There were no statistically significant differences in body weights (Table 4) between the BSE-treated and control groups. A tendency to decreased food consumption was noted at the weekly intervals in high-dose males (Table 5). As shown in Table 6, the total food intake over the course of the 30-day study was decreased (p < 0.01) in males treated at the high dose of 3.00 g/kg bw/d. This finding, however, was associated with an increase in the food utilization rate (p < 0.01) since, despite the decreased food consumption in these animals, their body weight gain remained similar to the controls.

Several statistically significant changes in hematology and clinical chemistry parameters (p < 0.05) were noted in the 30-day study (Tables 7 and 8). Feeding of BSE at 0.33, 1.00, and 3.00 g/kg bw/d was associated with decreased hemoglobin in all male dose groups. Also in males, RBC counts were slightly decreased in the mid- and high-dose groups; WBC counts were slightly lower in the mid-dose (1.0 g/kg bw/d) group; lymphocytes decreased and neutrophils increased in WBC differential counts in the high-dose group. All of these minor fluctuations in hematological parameters fell within the historical control range of the testing laboratory.

As shown in Table 8, albumin increased in the 0.33 and 3.00 g/kg bw/d dose group females; cholesterol decreased in males at the mid- and high-dose levels, and triglycerides were decreased in high-dose males when compared to the controls (p < 0.05). As with the observed changes in hematological parameters, the statistically significant differences in the clinical chemistry parameters all fell within the historical control range of the testing laboratory and the magnitude of change was minimal. There were no changes in parameters indicative of liver (AST, ALT) or kidney toxicity.

Following 30-days of treatment with BSE, the absolute and relative weights of the spleen and kidney increased in high-dose males (Table 9). Also, the absolute weight, but not the relative weight, of the testes was slightly reduced in high dose males.

### Table 6
Effects of broccoli seed extract on overall food utilization efficiency after 4 weeks of treatment.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Dose (g/kg bw/day)</th>
<th>Body weight gain (g)</th>
<th>Total food intake (g)</th>
<th>Total food utilization rate (weight gain as % of total food intake)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>0.00</td>
<td>236.0 ± 12.1a</td>
<td>647.1 ± 27.3</td>
<td>36.5 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>0.33</td>
<td>241.8 ± 14.4</td>
<td>633.1 ± 34.0</td>
<td>38.2 ± 1.2**</td>
</tr>
<tr>
<td></td>
<td>1.00</td>
<td>241.8 ± 9.2</td>
<td>633.3 ± 30.3</td>
<td>38.2 ± 1.5**</td>
</tr>
<tr>
<td></td>
<td>3.00</td>
<td>232.5 ± 8.8</td>
<td>605.5 ± 25.7**</td>
<td>38.4 ± 0.7**</td>
</tr>
<tr>
<td>Female</td>
<td>0.00</td>
<td>131.5 ± 10.9</td>
<td>530.2 ± 34.3</td>
<td>24.8 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>0.33</td>
<td>135.1 ± 7.3</td>
<td>516.7 ± 17.6</td>
<td>26.1 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>1.00</td>
<td>132.9 ± 6.8</td>
<td>519.1 ± 44.7</td>
<td>25.7 ± 1.8</td>
</tr>
<tr>
<td></td>
<td>3.00</td>
<td>130.4 ± 12.2</td>
<td>496.1 ± 24.2</td>
<td>26.2 ± 1.6</td>
</tr>
</tbody>
</table>

**p < 0.01.

* Plus/minus standard deviation.
Effects of broccoli seed extract on hematological parameters in rats following 4 weeks of treatment.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Dose (g/kg bw/day)</th>
<th>Parameter</th>
<th>Value (± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>WBC counts (x10^9/L)</td>
<td>145 ± 1.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RBC counts (x10^12/L)</td>
<td>6.37 ± 0.25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hemoglobin (g/L)</td>
<td>141 ± 7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hematocrit (%)</td>
<td>76.8 ± 6.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hemoglobin (g/L)</td>
<td>0.8 ± 0.2</td>
</tr>
<tr>
<td>Male</td>
<td>0.00</td>
<td>Other cells (%)</td>
<td>22.4 ± 6.5</td>
</tr>
<tr>
<td></td>
<td>0.33</td>
<td>Neutrophils (%)</td>
<td>21.8 ± 7.7</td>
</tr>
<tr>
<td></td>
<td>1.00</td>
<td></td>
<td>19.4 ± 7.0</td>
</tr>
<tr>
<td></td>
<td>3.00</td>
<td></td>
<td>31.5 ± 7.6</td>
</tr>
<tr>
<td>Female</td>
<td>0.00</td>
<td></td>
<td>21.5 ± 8.1</td>
</tr>
<tr>
<td></td>
<td>0.33</td>
<td></td>
<td>21.1 ± 5.1</td>
</tr>
<tr>
<td></td>
<td>1.00</td>
<td></td>
<td>21.5 ± 8.2</td>
</tr>
<tr>
<td></td>
<td>3.00</td>
<td></td>
<td>21.5 ± 8.2</td>
</tr>
</tbody>
</table>

*p < 0.05, **p < 0.01.

Plus/minus standard deviation.

Effects of broccoli seed extract on blood biochemistry parameters in rats following 4 weeks of treatment.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Dose (g/kg bw/day)</th>
<th>Parameter</th>
<th>Value (± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Alanine aminotransferase</td>
<td>0.00 36 ± 5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(U/L)</td>
<td>166 ± 34*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Aspartate aminotransferase</td>
<td>0.33 34 ± 4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(U/L)</td>
<td>155 ± 21</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Urea nitrogen (mmol/L)</td>
<td>1.00 33 ± 5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>150 ± 20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Creatinine (µmol/L)</td>
<td>3.00 32 ± 6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>149 ± 20</td>
</tr>
<tr>
<td>Female</td>
<td>0.00</td>
<td>Cholesterol (mmol/L)</td>
<td>0.00 30 ± 4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>152 ± 17</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Triglycerides (mmol/L)</td>
<td>0.33 32 ± 6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>163 ± 40</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Blood glucose (mmol/L)</td>
<td>1.00 34 ± 6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>181 ± 29</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total proteins (g/L)</td>
<td>3.00 41 ± 13</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>189 ± 59</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Albumin (g/L)</td>
<td>0.02 6.02 ± 0.62</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>57.3 ± 6.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2.13 ± 0.22</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2.79 ± 0.94</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5.03 ± 0.58</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>52.1 ± 3.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>28.9 ± 2.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3.02 ± 1.2</td>
</tr>
</tbody>
</table>

*p < 0.05, **p < 0.01.

Plus/minus standard deviation.

Relative to the controls (p < 0.05). Each of the organ weight values, however, fell within the historical control range of this laboratory. There were no statistically significant differences in the absolute or relative organ weights at any dose level in treated females.

At necropsy, macroscopic evaluations revealed no obvious abnormalities. The samples of liver, kidney, spleen, stomach, duodenum and testis or ovary tissues, obtained from 10 males and 10 females of the control and high-dose groups, revealed no changes associated with BSE treatment. In the liver of both animals in the control and high-dose groups, the normal structure of the liver was clearly identifiable; the lobules were neatly arranged; the hepatocytes showed normal morphology; and, the cells were generally in radial orientation. Some cells showed slightly hydropic degeneration. Only a few animals showed spotty necrosis of the hepatocytes (2/20 cases in control group, 1/20 cases in high-dose group). The normal structure of the kidneys was clearly identifiable in the control and high-dose animals; the nephrons were uniformly distributed; and, the structures of the renal glomerulus and Bowman’s capsules were clear without observable morphological abnormalities. Some convoluted renal tubules showed hydropic degeneration. Pyelactasis was observed in 1/20 rats in the controls, but in 0/20 in the high-dose group. The spleen of all animals evaluated was found to contain normal spleen tissue structure that was easily identifiable; the red pulp and white pulp showed clear structure. The splenic sinuses showed mild dilatation and congestion. Given the fact that the above pathological changes in the liver, kidneys and spleen are common lesions in these animals, and the differences between high-dose group and control group had no statistical significance, these changes were considered to be unrelated to BSE treatment.

Histological evaluation of the stomach, duodenum, testis and ovary revealed that, for both control and high-dose groups, the various layers of gastric and duodenal tissues were clearly identifiable; the mucosa and various submucosal layers showed normal
structure without bleeding, necrosis, or inflammatory cell infiltration. The testis had normal structure in which all stages of spermatogenic cells were visible without observable bleeding, necrosis or abnormalities in spermatid development. The ovaries had normal structure in which all stages of ovarian follicles and mature corpus luteum were visible without observable abnormality of follicular development, bleeding, or inflammation.

4. Discussion

The results of the present series of genotoxicity studies on a proprietary BSE produced by Brassica Protection Products LLC showed no evidence of mutagenic or clastogenic effects in either somatic or germ cells. The Ames test was negative in all tester strains to the limit dose of 5 mg/plate, there was no indication of micronucleus induction in the bone marrow cells of mice treated at very high doses (up to 10 g/kg bw), and there were no effects on the incidence of sperm abnormalities in mice treated at up to 10 g/kg bw. Although verification of exposure to target tissues in the in vivo assays was not conducted, it is known from previous research that the active ingredients in BSE, and their metabolites, are at least partly absorbed into systemic circulation in both animals (Bheemreddy and Jeffery, 2007) and man (Egger et al., 2011; Cramer and Jeffery, 2011; Fahey et al., 2012) thus indicating likely exposure of target tissues in the present series of studies. The lack of genotoxicity in vivo is further consistent with the protective effect of cruciferous vegetable consumption on cancers of the colon, a site of contact tissue for glucoraphanin and its colonic metabolites, in man (Wu et al., 2013a,b; Tse and Eslick, 2014).

As reviewed by Latte et al. (2011), broccoli (fresh and cooked), broccoli extracts and various components of broccoli extracts, including glucosinolates have been subject to extensive testing to assess potential genotoxic and anti-genotoxic activity. Genotoxicity/mutagenicity has been reported from in vitro studies conducted with broccoli extracts in the presence of myrosinase. DNA strand breaks and DNA adduct formation were reported after raw or steamed broccoli were orally administered to animals such as pigs, mice and rats, and an increase in genotoxicity was observed when raw broccoli was tested in feeding experiments conducted with Drosophila melanogaster. In studies conducted with isolated glucosinolates, the indole glucosinolate neoglucobrassicin and its degradation product, 1-methoxy indole-3-carbinol, were identified as mutagenic components in broccoli; however, such indoles are virtually absent in broccoli seeds. A lack of genotoxicity/mutagenicity of broccoli extracts and sulforaphane has also been reported in a number of other in vitro assays and studies conducted in experimental animals (Latté et al., 2011). Moreover, broccoli extracts and sulforaphane in particular, have been shown to be anti-genotoxic/anti-mutagenic when tested in combination with various known carcinogens (reviewed in Latté et al. (2011)). The results of many of the studies on broccoli, or its extract, given the complexity of the potential compositions of the products tested, may be of limited relevance to the BSE extract reported on here.

The authors are also aware that glucoraphanin, in the form of a sodium salt, derived from broccoli seeds, has been tested for genotoxic activity by researchers at Johns Hopkins Medical University under the Grants for Rapid Response Research (RAPID) program conducted under the auspices of the National Institutes of Health (Kapetanovic, 2009). The summary of these tests, the full reports remaining the intellectual property of the researchers involved, indicated no mutagenic activity in the Ames assay to a concentration of 5000 µg/plate, a lack of potential to induce chromosome aberrations in Chinese hamster ovary cells in vitro, and the absence of effect in an in vivo mouse bone marrow micronucleus assay at doses of up to 2000 mg/kg bw. The results of this series of studies are consistent with the results obtained with the BSE, containing 13% glucoraphanin, in the Ames assay and in the in vivo mouse micronucleus and sperm abnormality assays reported here. It is worth noting that BSE tested in the present Ames assay was autoclaved for 20 min (sterilization) prior to testing. As a result, it is possible that some of the glucosinolates may have been degraded; however, the results from the current study are consistent with the negative results from the RAPID program on semi-purified glucoraphanin.

The hydrolysis product of glucoraphanin, sulforaphane, has been assessed for potential to induce micronucleus formation in vitro (Fimognari et al., 2005a,b). In both studies, treatment of human lymphocytes with sulforaphane at concentrations up to 13 µM was non-genotoxic, and when added to cultures at a concentration of 30 µM, sulforaphane reduced the replicative index of the cells by more than 60% (Fimognari et al., 2005b), indicative of cytotoxicity.

In the toxicity study on BSE, there was no evidence of any untoward effects of dietary exposure to BSE at doses of up to 3 g/kg bw/d for 30 days. Several statistically significant changes in hematological and clinical chemistry were all minor in nature and fell within the historical control range of the testing laboratory and/or showed no clear-dose response relationships. A few statistically significant differences in either absolute and/or relative organ weight values were recorded, however, all again were of minor magnitude, generally limited to a single sex, within the historical control values for the testing laboratory, and were without histological or clinical pathology correlates. The results of the 30-day study provide no indication of any safety concerns of BSE. The no-observable-adverse-effect level (NOAEL) was considered to be 3.0 g/kg bw/d, the highest dietary dose tested.

The BSE product tested contains a minimum of 13% glucoraphanin. As such, the highest dose of glucoraphanin experienced by the rats in the 30-day study was on the order of 390 mg/kg bw/d (i.e., 0.13 × 3000 mg/kg bw/d). In the scientific literature, glucoraphanin has been tested for short-term toxicity in rats (Lai et al., 2008; Zhu et al., 2010). In the first study, male F344 rats (6/group) were administered semi-purified glucoraphanin extract derived from broccoli seeds (30.4% pure glucoraphanin) (Group 1) by gavage at doses of 0, 30, 60, 120, or 240 mg (as glucoraphanin)/kg body weight/d for four days (Lai et al., 2008). Also tested was a semi-purified glucoraphanin extract derived from arugula seeds (29.9% pure) (Group 2) and a purified extract derived from broccoli seeds (97% pure glucoraphanin) (Group 3) each at a dose of 240 mg glucoraphanin/kg body weight/d for four days by gavage. In this experiment, the only treatment-related effect noted in Group 1 was mild inflammation of the caecum in one animal at 30 mg/kg body weight/d, in three animals treated at 120 mg/kg body weight/d, and in 5/6 rats receiving 240 mg/kg body weight/d. Inflammation of the caecum was observed in five out of six animals in Group 2 and in three out of six animals in Group 3. Inflammation of the caecum was not observed in any of the control animals. Lai et al. (2008) further reported that the treatment with glucoraphanin from various sources was associated with an increase in NAD(P)H-quinone oxidoreductase (a phase II enzyme) activity in both lung and liver tissue.

In a study similar to Lai et al. (2008), semi-purified glucoraphanin from broccoli seeds (26.3% pure) was administered by gavage at a dose of 218.8 mg/kg body weight/d for four days to male F344 rats (4/group). This treatment regimen also produced inflammation in the caecum in all animals (two mild, two severe) (Zhu et al., 2010). Significant improvement in the inflammation was observed upon cessation of treatment during a four-day recovery period. Interestingly, inflammation of the caecum was not observed when the same glucoraphanin extract was incorporated in the diet
of male F344 rats at a inclusion rate 4.3% for four days, a dietary level that provided a similar dose to that administered by gavage. The authors suggested that the isothiocyanates formed from glucoraphanin by colonic microflora are known to be irritants at high concentrations, and that dietary administration likely resulted in lower concentrations reaching the caecum as compared to the bolus dose received from gavage administration. This is of significance to the study reported herein, as dietary exposure of rats to BSE providing 390 mg (as glucoraphanin)/kg bw/d for 30 days showed no evidence of irritation of the GI tract, including the caecum, although the caecum was not specifically examined histologically.

A summary of an unpublished 14-day oral toxicity study (part of the previously mentioned RAPID grant program of the NIH) conducted with glucoraphanin sodium salt indicated that doses up to 500 mg/kg bw/d (highest dose tested) were well-tolerated with no adverse histopathological findings. The only reported effect was of an increase in testis weights, with no accompanying histopathological changes, at doses of 100 and 500 mg/kg bw/d. The results of the 30-day feeding study with BSE (containing 13% glucoraphanin) also showed no indications of toxicity, and in the case of the BSE tested, had no biologically significant effects on testis weights and was not associated with any histological changes in this organ. The NOAEL in the 30-day study was established at 3.0 g/kg bw/d, many fold higher than likely human exposures from food sources or dietary supplement uses.

In summary, the results of the 30 day feeding study with BSE containing 13% glucoraphanin, together with the negative genotoxicity results, support the safety of this product for potential dietary consumption by humans, either in the form of food or dietary supplements.

Transparency document
Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.yrtph.2015.08.003.

References
155–194.


