REPORT OF THE EXPERT PANEL ON THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF BARLEY BETAFIBER

INTRODUCTION

We, the undersigned, an independent panel of recognized experts (the Expert Panel), qualified by our scientific training and relevant national and international experience in evaluating the safety of food and food ingredients, were requested by the Health and Food Technologies business unit of Cargill, Incorporated (Cargill) to determine the safety and the Generally Recognized As Safe (GRAS) status of barley betafiber (≥70% pure barley β-glucan) for use in specified foods as a nutritional supplement (source of fiber), as a thickening agent, a texturizing agent, a humectant and a fat replacer at levels consistent with current Good Manufacturing Practice (cGMP).

A comprehensive search of the scientific literature for health effects, safety and toxicity of barley β-glucan through 30 November 2002 was conducted by Dr. Albert Bar (Bioresco Ltd.) and this was made available to the Expert Panel. Dr. Bar prepared a confidential, critical analysis of the available information concerning barley β-glucan and the conditions under which barley betafiber is produced. Cargill provided this analysis to the Expert Panel. The Expert Panel independently and then collectively critically evaluated materials submitted by Cargill and other materials deemed appropriate or necessary.

This report is a summary of the materials critically evaluated by the Expert Panel and provides the basis for the unanimous conclusion of the Expert Panel concerning the safety and GRAS status of barley betafiber under the conditions of intended use.

BACKGROUND/HISTORY

Barley and oats are rich in soluble fiber components, in particular beta glucan. Beta glucan (β-glucan), a fiber-type polysaccharide, has been shown to have blood cholesterol lowering effects as well as regulating effects on blood glucose levels. Food uses of barley have historically been limited in comparison to uses for oats. The availability of a purified barley β-glucan extract offers food manufacturers greater flexibility in developing fiber-supplemented foods that provide a health benefit to the consumer.

Barley β-glucan is intended for addition to food primarily for its nutritional value, i.e., as a source of dietary fiber. Because of its purity and ease of formulation, the ≥70% barley β-glucan product, barley betafiber, can be used to supplement a broad array of food products.

The maximum use level of barley β-glucan for the foods included in the GRAS determination was chosen so as to achieve an intake of 3 g pure β-glucan per serving of

food. This amount (i.e, 3 g β-glucan) represents 12% of the Daily Reference Value (DRV) of dietary fiber, and the daily intake of oat β-glucan soluble fiber included in the qualifying requirements for the "Soluble Fiber From Whole Oats and Coronary Heart Disease Health Claim" (FDA, 1997). While barley currently doesn't qualify as a source of soluble fiber under this health claim, the requirements may eventually be amended to include whole barley and the β-glucan isolate as qualifying sources of β-glucan if adequate evidence is submitted to the FDA substantiating the efficacy of barley β-glucan in lowering cholesterol.

Barley betafiber is obtained from food-grade barley by water extraction at elevated temperature. Starch is removed during the extraction process by treatment with alpha-amylase from *Bacillus licheniformis* and *Bacillus amyloliquefaciens*, enzymes which are GRAS for use in food manufacturing processes. The extracted β-glucan is recovered by precipitation with denatured ethanol that is suitable for food production. A higher molecular weight product (HMW) with a weight average molecular weight of 800,000 – 1,000,000 Daltons is made utilizing only one enzyme treatment. Lower molecular weight (LMW) β-glucan with a weight average molecular weight range of 100,000 – 300,000 Daltons is prepared using two enzyme steps (see description provided in Figure 1).

Both the HMW and LMW isolates contain ≥70% β-glucan, 2 - 12% protein, and smaller amounts (<3%) of each of sugars, lipids and inorganic salts. Using AOAC Official Methods of fiber analysis, the product has a total dietary fiber content of about 75 - 85%.

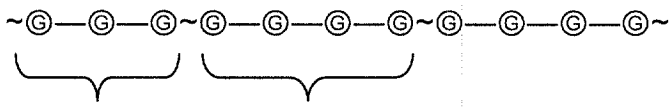
CHARACTERIZATION OF BARLEY β-GLUCAN

Chemical Identity

β-glucan from barley (*Hordeum vulgare*) is not a distinct chemical substance. It is a mixture of essentially unbranched, linear, mixed-linkage (1→3),(1→4)-β-D-glucans (see conformation below). Barley and oat derived β-glucans contain about 70% (1→4) and 30% (1→3) linkages (Woodward & Fincher, 1983; Saulnier et al., 1994). Blocks of typically three or four (1→4)-linked glucosyl units are connected by (1→3) linkages. Thus, β-glucan might be considered as a series of (1→3)-linked cellotriosyl and cellotetrosyl units (Woodward et al., 1983 a, b, 1988; Buliga & Brant, 1986). However, blocks of 5 - 11 (1→4)-linked glucosyl units are also present in lower proportions (Woodward et al., 1983).

The chemical names for barley β-glucan are: β-D-Glucan, (1→3),(1→4)- β-D-Glucan, and β-Glucosylglucan. The CAS number for mixed-linkage (1→3),(1→4)- β-D-glucans is 55965-23-6; the empirical formula is: $(C_6H_{10}O_5)_n$. The common or usual name for the ≥ 70% purity barley β-glucan product is barley betafiber.

β-Glucan Conformation



Cellotriosyl unit

Cellotetrosyl unit

Ⓖ = glucose

— = β-(1→ 4) linkage

~ = β-(1→ 3) linkage

Molecular Weight

The molecular weight (MW) of β-glucan from barley has been reported to range from about 500,000 to 3,330,000 Daltons depending upon cultivars and applied extraction procedures (reviewed by Fastnaught, 2001; Beer et al., 1997; Gómez et al., 1997).[1] Lower values of 80,000 - 300,000 (Woodward et al., 1983a, b; Wood et al., 1991b; Saulnier et al., 1994; Huth et al., 2000) have also been reported.

For processed foods, the molecular weight distributions of β-glucan from oat bran products have been reported to range from <250,000 Daltons to >1,000,000 Daltons with a significant fraction of the molecular weight distribution less than 250,000 for foods such as oat muffins (Kerckhoffs et al. 2003). Molecular weight distributions for barley β-glucan in beverages and cereal analyzed internally by Cargill indicate a decrease in molecular weight from 1,000,000 Daltons (starting material) to approximately 300,000 Daltons and lower in the finished good (e.g., citrus-type beverage). The weight average molecular weight range of the β-glucan which is the subject of the present Report is approximately 100,000 –1,000,000 Daltons.

Physicochemical properties

Barley betafiber is a viscous, soluble fiber. The degree of water solubility depends upon conditions (heat, shear) and molecular weight/viscosity. For β-glucan from barley a higher viscosity than for carboxymethyl cellulose (CMC) (both in 1% solution) has been reported (Lee et al., 1995). However, the viscosity of barley-derived β-glucan varies in a wide range depending upon barley cultivar and the applied extraction method

[1] The determination of molecular weights is hampered by the formation of labile molecular aggregates. There is a suggestion that this aggregation is enhanced at higher temperature (70 °C) (Gómez et al., 1997).

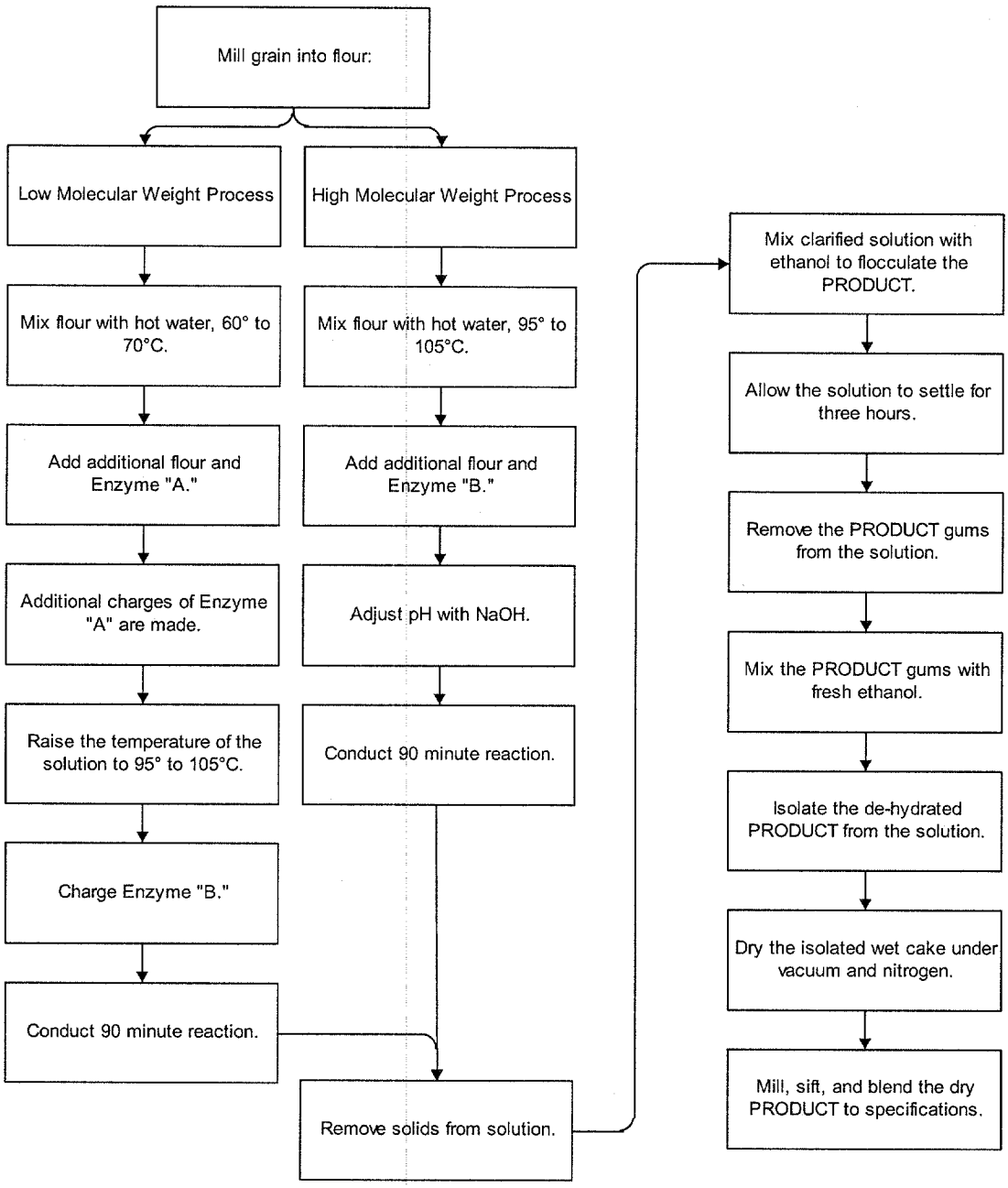
(Bhatty, 1987, 1992). A 1% aqueous solution of HMW β-glucan has a viscosity that corresponds approximately to that of 1.0-1.3% solution of guar gum (Zupfer, 2002). The LMW β-glucan has a significantly lower viscosity and thus is more suitable for food applications where gelling or thickening is not a desired functionality. Despite their differences in molecular weight, the HMW and LMW barley betafiber products have been determined to be equivalent in cholesterol lowering efficacy when administered to hamsters (Nicolosi, 2003).

MANUFACTURING PROCESS, SPECIFICATIONS, BATCH ANALYSES

Barley β-glucan is extracted from barley grain following the general outline in Figure 1 provided on the following page. The barley is cleaned and de-stoned before going through milling. The grain is milled into powder with a particle range of 50 - 1700 microns (this is particle size range of the flour). The final product, barley betafiber, has a particle size of ≤250 microns due to the size of the screen selected to filter the material during milling. The processing aids utilized in the process are food grade α-amylases, sodium hydroxide and denatured ethanol.

Compliance of barley betafiber with the proposed specification is supported by analyses of 5 batches of representative commercial product. The specifications and supporting analyses are provided on page 6 of this document.

Figure 1. Barley Betafiber Production Process



Specifications & Analyses of Barley Betafiber

		HMW			*LMW*	
Analyte	**Specifications**	**Blend 1**	**Blend 2**	**Blend 3**	**Blend 1**	**Blend 2**
Assay (β-glucan)	≥70%[1)]	70.1	71.3	70.5	74.8	78.3
Moisture	<7%	4.1	3.7	3.4	7.2	5.1
Total ash	<3%[1)]	2.3	2.6	2.4	2.5	2.7
Ethanol	<1000 ppm	290	550	680	2500	2100
Lead	<0.5 ppm[1)]	0.07	0.15	0.10	0.036	0.020
Vomitoxin	<0.25 ppm[2)]	<0.25	<0.25	<0.25	<0.25	<0.25
Total mesophilic bacteria	<10,000 CFU/g	300	1500	4600	<100	200
Coliforms	<10 CFU/g	<3	<3	<10	<10	<10
Salmonella sp.	negative in 100 g	neg.	neg.	neg.	neg.	neg.
Staphylococci[3)] (coag. Positive)	<100 CFU/g	<100	<100	<100	<100	<100
Phytic Acid[3)]	<1%	< 0.477%	0.92%	N/A	N/A	N/A

1) On a dry matter basis.

2) Limit of detection.

3) Included for internal QA/QC but not specifications.

USES and ANTICIPATED EXPOSURE/INTAKE

Proposed Food Uses

Historical consumption

Barley is a traditional food with a defined reference amount per eating occasion of 45 g (dry) and 140 g (prepared). It is consumed mainly in the form of pearled barley and rolled flour and as such has a long history of safe use. In the US, an annual consumption of 0.6 kg (barley alone) is reported. The largest per capita food user of barley is Morocco with an annual per capita intake of 63 kg (reported between 1980 – 1995) (FAO, 2000). Relatively high intakes are reported also from other Maghreb countries.

In Maghreb countries (Morocco, Algeria, Libya, Tunisia), barley is used in a variety of traditional foods (bread, soup, porridge), resulting in an average intake of up to 172 g/person/day (Morocco). With this intake of barley, about 6 g/person/day of pure β-glucan is consumed. Importantly, the preparation of the mentioned foods involves baking or boiling for longer periods of time, which ensures extraction of β-glucan from its natural context (cell walls, complexes with proteoglycans). The physiological properties of β-glucan as a dietary fiber may, therefore, be found in these traditional foods as is intended to be achieved with the addition to processed foods of barley β-glucan as described in this GRAS determination.

Intended uses and intake

The intended uses of barley betafiber include: bars, beverages, bread (whole grain and specialty), breakfast cereals (RTE and cooked), cookies (lite), crackers (reduced fat), instant rice, macaroni products, muffins (reduced fat), salad dressings (lite), snack chips (reduced fat), soups, tortillas and taco shells, vegetarian patties/crumbles, and reduced fat yogurt. The maximum incorporation rate for each of these food applications is 3 g barley β-glucan per serving.

The estimated daily intake (EDI) of barley betafiber and barley β-glucan from the uses listed above was calculated for the US population using the dietary survey approach. Using this method, the estimated 2-day average intake of barley betafiber (users of age ≥ 2 years) from all proposed uses combined is 10.5 and 19.9 g/person/day for the mean and 90th percentile consumer, respectively. This corresponds to barley β-glucan intake levels of 7.3 g and 13.9 g/person/day. These are conservative intake estimates given the underlying assumption of the applied EDI calculation model that barley β-glucan is used simultaneously in all listed foods at the highest feasible concentration. The true average daily intake of barley betafiber will likely be far below the calculated levels.

The estimated barley betafiber intakes per eating occasion demonstrate that the consumption of barley β-glucan is evenly distributed over the day. At no eating occasion

are levels reached at which non-digestible, fermentable carbohydrates might produce intestinal symptoms (typically about 20 g single dose).

SAFETY/TOXICOLOGICAL STUDIES

The dietary safety of barley betafiber has been evaluated in 28-day feeding studies in rats and mice. Genotoxicity was studied in an *in vivo* bone marrow micronucleus assay. Human tolerance studies, in addition to outcomes from these studies, are discussed below.

28-d feeding study in rats

In a 28-day study (OECD 407), groups of Wistar rats received diets with 0, 1, 5 and 10% HMW barley betafiber (5 rats/sex/group). Considering that the applied barley β-glucan had a purity of about 64% (on a dry matter basis), the dietary concentrations of pure β-glucan was about 0.6, 3.2 and 6.4% in the different dose groups. The corresponding 28-day average intakes of pure β-glucan were 0, 0.5, 2.6 and 5.1 g/kg bw/d, respectively.

Individually-housed rats were examined twice daily for clinical signs of toxicity. Body weights and food consumption were determined in weekly intervals. Neurobehavioral functioning (motor activity assessment, functional observational battery) was examined in week 4 of the study. Standard hematological and clinical chemical parameters were measured in blood samples collected from the abdominal aorta at termination. Urinary parameters were not examined. On day 28, all surviving rats were killed and subjected to gross necropsy. The absolute and relative weights of main organs were determined. Organs and tissues were preserved for histopathological examination. The tissues and organs of all animals of the control and high dose group as well as the spleens of the low and mid-dose group were subjected to microscopic examination.

All animals survived until the end of the study. There were no clinical signs, reactions or changes in neurobehavioral functions that could be attributed to the treatment. Body weights and feed consumption did not differ between treated groups and controls. The hematological examination revealed a slight yet statistically significant increase of total white blood cell (WBC) counts in males of the low and mid-dose groups. Differential analyses of the WBCs attributed the increase to lymphocytes. In males of the high dose group, WBC counts were not significantly different from controls. No treatment-related differences or trends were seen for WBC counts in female rats. Red blood cell parameters and thrombocytes did not differ between treated groups and controls at any dose level.

The clinical chemistry values showed some significant differences, which, however, did not exhibit a clear dose-response relationship. In all cases the values remained within the limits of historical controls. No toxicological relevance was, therefore, attributed to

the observed decreases of total protein, albumin and calcium in females of the mid and high-dose group, and the observed increases of urea (mid- and high-dose group) and chloride (all dose groups) among the male animals.

The analysis of the organ weights revealed a significant increase of full and empty cecum weights in males and females of the mid and high dose groups. This cecal enlargement is commonly observed in rats (and mice) fed diets with high levels of low digestible, fermentable carbohydrates (polyols, lactose, dietary fibers). A significantly elevated relative spleen weight was noted in males of the low-dose group. However, morphological changes were not observed on histopathological examination. No toxicological relevance was, therefore, attributed to the increased spleen weight of male rats of the low-dose group.

The high dose level (10% barley β-glucan corresponding to an intake of 8 g/kg bw/d in male and female rats) was determined to be the No-Observed-Adverse-Effect Level (NOAEL) in this 28-day study (Delaney et al., 2003a; Jonker, 2002).

28-d feeding study in mice

Because of reports of an immune-stimulating effect of β-glucans (Delaney et al., 2003a), it could not be excluded *a priori* that the observed increase of WBC counts in rats of the low and mid-dose groups of the 28-day study represented a real, reproducible effect. This effect was not observed in the high dose group.

To better understand the significance of the increased WBC counts, groups of CD-1 mice were fed diets with 0% (1st control group with repeated blood collection), 0 (controls with blood collection at termination only), 1%, 5% and 10% HMW barley betafiber for 28 days (6 mice/sex/group, except for the 2nd control group which consisted of 24 mice/sex). The mouse rather than the rat was used in this study because it is the animal of choice for studying effects of xenobiotics on the immune system (Burns et al., 1995).

All animals were examined for clinical signs twice daily. Body weights and feed consumption were measured in weekly intervals. Blood was collected from the orbital plexus of all mice (except 2nd control group) on days 0, 14, 28 and 42. On day 28, 6 mice/sex/group (except 2nd control group) were sacrificed for pathologic examination. The remaining animals were kept on control diet for another 14 days (recovery period). The animals of the 2nd control group were killed on days 0, 14, 28 and 42 (6 mice/sex/time point) after blood had been drawn from the orbital plexus.

Standard hematological and clinical chemical parameters were determined in all blood samples. At termination, the kidneys, liver, spleen and thymus were weighed, examined macroscopically and processed for histopathological examination. In addition, bone marrow, GALT (gut associated lymphoid tissue), mesenteric lymph nodes and axillary lymph nodes were subjected to histopathological examination.

No clinical signs or adverse reactions to the treatment were observed during the study. Body weights and feed consumption did not differ between treated groups and controls. The hematological and clinical chemical parameters did not reveal changes that could be attributed to the treatment. In particular, there were no differences for WBCs and lymphocytes. The absolute and relative weights of the examined organs were not affected by the treatment. The histopathological examination of the organs specified above did not reveal any abnormalities.

It is concluded that the high dose level (10% in the diet, corresponding to an intake of 19.0 and 23.6 barley β-glucan/kg bw/d in male and female mice, respectively) is the NOAEL with regard to the observed parameters (Delaney et al., 2003b; van Zijverdem & Jonker, 2002).

In vivo genotoxicity: micronucleus test

A standard *in-vivo* bone marrow micronucleus test (OECD 475) was conducted in male mice (Charles River CD-1). Groups of animals received single oral doses of HMW barley betafiber of 74, 222, 666 and 2000 mg/kg bw by gavage. A negative (vehicle) control group and a positive (mitomycin C) control group were included as well. The animals were killed after 24 and 48 hours (5 mice/group/occasion). Since no clinical signs of toxicity were observed at any dose level, only the animals of the high dose group and the negative and positive controls were subjected to examination of their bone marrow cells. No increase in the number of micronucleated polychromatic erythrocytes was found in response to the barley betafiber treatment. Under the conditions of the assay, barley β-glucan did not increase the incidence of micronuclei in bone-marrow cells (Delaney et al., 2003d; de Vogel, 2003).

Human tolerance studies

Human tolerance studies with barley betafiber have not been conducted. However, barley and barley-derived products with β-glucan (barley flour, barley bran) have been administered to human volunteers under controlled conditions in several studies on the potential beneficial effects of these products. In none of these studies were adverse effects reported that could be attributed to the ingestion of barley or barley-derived products. Mild intestinal symptoms (flatulence, bloating) were reported from a study with the ingestion of 129 g/d barley flour (providing 42 g total dietary fiber) and a 12-week study with the ingestion of barley bread (providing 39 g/d total dietary fiber of which 5 g/d β-glucan) (Newman et al., 1989b; Pick et al., 1998)[2]. However, no such effects were reported in a

[2] In the study with 42 g/d total dietary fiber, 14 male volunteers supplemented their diets for 28 days with either barley flour, wheat flour or wheat bran. About half of the subjects reported feelings of fullness, bloating and gas. These complaints were reported to subside in some of the cases by the third and fourth weeks (Newman et al., 1989b). In the 12-week study incorporating 39 g total dietary fiber/day into the diet of eleven type-2 diabetics, minor flatulence was reported by 6 subjects which, however, diminished after the first 2-3 weeks of the study (Pick et al., 1998).

study in which the participants consumed 100 g/d whole barley flour (providing an estimated 33 g total dietary fiber) for 4 weeks (Narain, 1992).

Allergenicity

Celiac disease is an autoimmune disease triggered in genetically predisposed individuals by gluten ingestion. Gluten is a ubiquitous component of cereals and as such is broadly found in many foods. In genetically susceptible individuals, gluten ingestion results in intestinal mucosa damage and malabsorption of essential nutrients. Currently, a life-long gluten-free diet is the only therapy available.

In order to ensure that people with celiac disease can recognize barley beta-glucan as a food component which may contain gluten, the name of the product under which it will appear in food ingredient lists, should always refer to its barley origin (i.e., "barley β-glucan" or "barley betafiber" and not just "β-glucan" or "betafiber").

This labeling will also be relevant for those few people who suffer from a barley allergy (Bonadonna et al., 1999; Vidal & González-Quintela, 1995; Armentia et al., 1993).

DISCUSSION and SUMMARY

The following considerations support the safety of barley betafiber under the conditions of intended use:

(1) The product contains only components of barley, substances that are formed from it by the action of the applied enzymes, or residues from the applied solvents (water, ethanol) and processing aids.

The applied solvents, i.e., water and denatured ethanol, and processing aids meet appropriate specifications for use in food production.

(2) β-Glucan is widely present in numerous grains and other plants. It is thus consumed not only with barley but also with other cereals and edible plants. Adverse effects due to the consumption of β-glucans with such foods are not known. Particularly relevant in this regard is the safe use for more than 10 years of oat-derived β-glucan isolates (e.g., Oatrim with a β-glucan content of up to 15%).

Barley is a traditional food with long history of safe use. In Maghreb countries (Morocco, Algeria, Libya, Tunisia), barley is used in a variety of traditional foods (bread, soup, porridge), resulting in an average intake of up to 172 g/person/day (Morocco). With this intake of barley, about 6 g/person/day of pure β-glucan is consumed. Importantly, the preparation of the mentioned foods involves baking or boiling for longer periods of time, which ensures extraction of β-glucan from its natural context (cell walls, complexes with proteoglycans). The physiological properties of β-glucan as a dietary fiber may therefore be found in these traditional foods as is intended with the addition of barley betafiber to processed foods.

(3) β-glucan is partially depolymerised under certain conditions of food manufacturing as demonstrated by MW analysis of finished goods containing oat and barley β-glucan. It is also known that β-Glucan from oat with a high molecular weight (2200 – 2600 kDa) is depolymerised progressively during intestinal passage in pigs to products with a MW of 90 – 350 kDa (Johansen et al., 1993). Thus, consumers have historically consumed β-glucan within a range of molecular weights that spans the molecular weight range of the barley betafiber isolate.

(4) The subchronic toxicity of HMW barley betafiber (produced by the method described in this Report) has been examined in 28-day toxicity studies in rats and mice. In these studies, the highest dose level tested (10% in the diet corresponding to an intake of about 21 and 8 g/kg bw/d in mice and rats, respectively) was the NOAEL.

(5) Numerous studies have been conducted in animals (mainly in rats and hamsters) and humans on the potential plasma cholesterol lowering effect of different barley-derived products with elevated β-glucan content. The attenuating effects of such products on the glycemic and insulinemic response to food have been investigated as well.

The test diets in the animal studies contained up to 75% of the test products and were administered for up to six weeks. Although the studies were not designed to test toxicological end-points, it is noteworthy that behavioural changes, impaired growth, or other manifestations of an underlying toxicity were not observed.

In the human studies in which barley β-glucan was consumed at levels of up to 5 g/d for a period of 12 weeks, no adverse effects were noted. Only in two studies were mild intestinal symptoms (flatulence, bloating) recorded upon daily ingestion of 39 - 42 g barley-derived dietary fiber. These effects were reported to dissipate soon after the initiation of the studies.

Barley betafiber intake from the intended uses results in a conservatively-estimated average intake level of 10.5 g/d (equivalent to 7.3 g/d β-glucan). The intake calculations also demonstrate that the consumption of barley betafiber would be evenly distributed throughout the day, with similar intakes at main meals and in-between snacks. This eating pattern decreases the likelihood that gastrointestinal intolerance would ensue.

(6) Phytic acid is a natural component of cereal grains with a known inhibiting effect on the intestinal absorption of certain minerals (e.g., calcium). Analyses of barley β-glucan have shown that phytic acid is not concentrated in the extraction and isolation process of barley β-glucan. Analysis of representative commercial batches of barley betafiber revealed a phytic acid content of less than 1%.

CONCLUSION

The Expert Panel has independently and collectively critically evaluated the available information concerning barley β-glucan and barley betafiber summarized in this report and unanimously concludes that barley betafiber, meeting appropriate food grade specifications, is safe and suitable as a food ingredient for the uses described herein. The Expert Panel further unanimously concludes that this barley betafiber is Generally Recognized As Safe (GRAS) based on scientific procedures and supported by evidence of safe use of barley in food prior to 1958.

Joseph F. Borzelleca, Ph.D.
Professor Emeritus
Pharmacology and Toxicology
Medical College of Virginia

08 July 2003
Date

David Kritchevsky, Ph.D.
Caspar Wistar Scholar, Wistar Institute
University of Pennsylvania

10 July 03
Date

Michael Levitt, M.D.
Associate Chief of Staff for Research
Veterans Affairs Medical Center
Professor of Medicine
University of Minnesota

15 July 03
Date

Robert Nicolosi, Ph.D,
Director of the Center for Health Sciences
University of Massachusetts Lowell

7/17/03
Date