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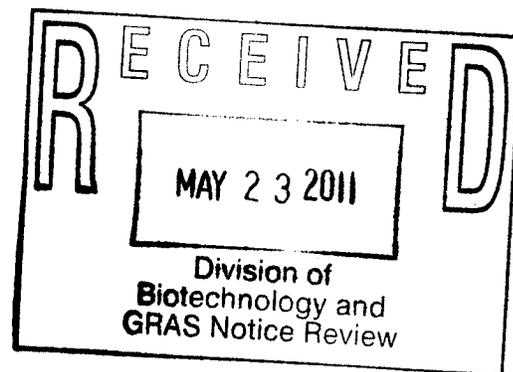


ORIGINAL SUBMISSION

000001

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

Dear Dr. Ditto:

Re: GRAS Notification for Phytic Acid (50% Solution)

In accordance with proposed 21 CFR §170.36 [Notice of a claim for exemption based on a Generally Recognized as Safe (GRAS) determination] published in the *Federal Register* [62 FR 18938 (17 April 1997)], I am submitting in triplicate, as the notifier [Tsuno Food Industrial Co., Ltd., 94 Shinden, Katsuragi, Ito-gun, Wakayama 649-7194, Japan], a Notice of the determination, on the basis of scientific procedures, that phytic acid (50% solution), produced by Tsuno Food Industrial Co., Ltd., as defined in the enclosed documents, is GRAS under specific conditions of use as a food ingredient, and therefore, is exempt from the premarket approval requirements of the *Federal, Food, Drug and Cosmetic Act*. Information setting forth the basis for the GRAS determination, which includes detailed information on the notified substance, information on self-limiting levels of use, and a summary of the basis for the GRAS determination, as well as a consensus opinion of an independent panel of experts in support of the safety of phytic acid (50% solution) under the intended conditions of use, also are enclosed for review by the agency.

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

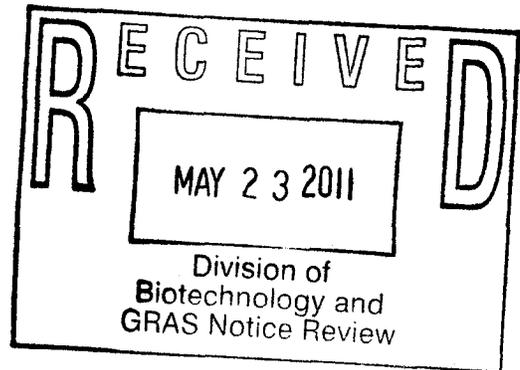
Sincerely,

(b) (6)

Mrs. Fumi Tsuno *fumi@tsuno.co.jp*
President
Tsuno Food Industrial Co., Ltd.

MAY 19, 2011

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GRAS Exemption Claim for Phytic Acid (50% Solution)

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

Submitted by: Tsuno Food Industrial Co., Ltd.
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May 16, 2011

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GRAS Exemption Claim for Phytic Acid (50% Solution)

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I GRAS EXEMPTION CLAIM

I.A Claim of Exemption from the Requirement for Premarket Approval Pursuant to Proposed 21 CFR §170.36(c)(1) [62 FR 18938 (17 April 1997) (U.S. FDA, 1997)]

Phytic acid (50% solution) has been determined to be Generally Recognized as Safe (GRAS) by Tsuno Food Industrial Co., Ltd. (Tsuno) for use in a variety of traditional food products and supplements, consistent with Section 201(s) of the *Federal Food, Drug, and Cosmetic Act*. This determination is based on scientific procedures as described in the following sections. Therefore, the use of phytic acid (50% solution) in foods and supplements as described below is exempt from the requirement of premarket approval.

Signed,

(b) (6)

5/19/11

Date

I.B Name and Address of Notifier

Ms. Fumi Tsuno
Tsuno Food Industrial Co., Ltd.
94 Shinden, Katsuragi,
Ito-gun, Wakayama 649-7194
Japan

I.C Common Name of the Notified Substance

The common name of the notified substance is phytic acid (50% solution).

I.D Conditions of Intended Use in Food

I.D.1 Intended Uses of Phytic Acid (50% Solution) and Levels of Use

Tsuno intends to market phytic acid (50% solution), produced from defatted rice bran, as a food ingredient for use at levels providing up to 0.2% phytic acid in selected food categories. Phytic acid (50% solution) also is proposed for use in dietary supplement softgels (to limit solubility reduction and provide strength to capsules) at a maximum level of 8.0%. The individual

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proposed food-uses and maximum use-levels for the individual components of phytic acid (50% solution) are summarized in Table I.D.1-1

Food Category	Proposed Food Uses	Use-Level (%)*
Beverages and Beverage Bases	Energy, Sports, and Isotonic Drinks	0.2
	Non-Milk Based Meal Replacements	0.2
Milk Products	Milk Based Meal Replacements	0.2
	Yogurt Shots ¹	0.2
Processed Vegetables and Vegetable Juices	Frozen Vegetables	0.2
	Pickles	0.2
Supplement Products	Softgels	8.0

¹ No codes for yogurt shots were identified and therefore, yogurt and fruit smoothies drinks were employed as a surrogate codes for this food use.

I.D.2 Estimated Consumption of Phytic Acid (50% Solution) Based upon Intended Uses

Estimates for the intake of phytic acid (50% solution) were based on the intended food uses and use levels in conjunction with food consumption data included in the National Center for Health Statistics' (NCHS) 2003-2004 and 2005-2006 National Health and Nutrition Examination Surveys (NHANES) (CDC, 2006, 2009; USDA, 2009).

In order to evaluate the impact of the proposed technical uses in supplement products, the results of the NHANES dietary supplement survey were combined with the 24-hour recall data. In the dietary supplement survey, the same respondents described above answered a variety of questions pertaining to their consumption of supplement products. Included in these questions is the name and nature of the supplement product, its ingredients, and how often the respondent has consumed the supplement over the previous 30-day period. One of the descriptors for the supplement products is the form in which they are provided, which allows for only those supplement products provided as softgels to be isolated for the purpose of this assessment. In order to ensure that the most conservative estimate of intake was generated, the list of dietary supplement products was expanded to include forms similar to softgels such as caplets and capsules.

As the dietary supplement survey is intended to be used to calculate intake of individual ingredients, only the amount of active ingredient is detailed as opposed to the size of the softgels themselves. As a result, to estimate the intake of phytic acid resulting from the proposed use in softgels, each softgel was assumed to weigh 1,000 mg. The intake of the softgels was then based on the reported softgels consumed per day and averaged across the previous 30 days based on the respondents' reported frequency of consumption. These data were then combined with the dietary intake data to allow for the estimation of total intake.

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The percentage of the total U.S. population identified as potential consumers was 58.1% (9,707 actual users identified). Consumption of all proposed food and supplement uses, calculated according to the assumptions described above, by the total U.S. population resulted in estimated mean all-person and all-user intakes of phytic acid of 137 and 223 mg/person/day, respectively, equivalent to 2.2 and 3.7 mg/kg body weight/day, respectively, on a body weight basis. The 90th percentile all-person and all-user intakes of phytic acid from all proposed food and supplement uses by the total population were 320 and 610 mg/person/day, respectively, or 5.6 and 9.4 mg/kg body weight/day, respectively.

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

I.E Basis for the GRAS Determination

Pursuant to Title 21, Section 170.30 of the *Code of Federal Regulations* (CFR) (U.S. FDA, 2010), phytic acid (50% solution) has been determined to be GRAS on the basis of scientific procedures. This GRAS determination is based on data generally available in the public domain pertaining to the safety of phytic acid, as discussed herein, and on consensus among a panel of experts who are qualified by scientific training and experience to evaluate the safety of the phytic acid (50% solution) ingredient as a component of food [see Appendix A and B, entitled “Expert Panel Consensus Report Regarding the Generally Recognized as Safe (GRAS) Status of Phytic Acid (50% Solution) for Use in Foods and Supplements” and “Expert Panel Consensus Statement Concerning the Generally Recognized as Safe (GRAS) Status of Phytic Acid (50% Solution) as an Ingredient in Food and Dietary Supplements Following Changes in Use Levels”, respectively].

At the request of Tsuno, an Expert Panel (“the Expert Panel”) of independent scientists, qualified by their relevant national and international experience and scientific training to evaluate the safety of food ingredients, was specially convened to conduct a critical and comprehensive evaluation of the available pertinent data and information, and to determine whether the intended uses of phytic acid (50% solution) in foods and supplements is safe and suitable and would be GRAS based on scientific procedures.

The Panel consisted of the following qualified scientific experts: Professor Joseph F. Borzelleca, Ph.D. (Virginia Commonwealth University School of Medicine), Professor Robert J. Nicolosi, Ph.D. (University of Massachusetts Lowell), and Professor John Thomas, Ph.D. (Indiana University School of Medicine).

GRAS EXEMPTION CLAIM FOR PHYTIC ACID (50% SOLUTION)

The Expert Panel convened on behalf of Tsuno independently and collectively, and critically evaluated the data and information summarized herein and concluded that the intended uses in traditional foods and supplements described herein for phytic acid (50% solution), meeting appropriate food-grade specifications and manufactured according to current Good Manufacturing Practice (cGMP), are safe and suitable and GRAS based on scientific procedures. It also is the Expert Panel's opinion that other qualified and competent scientists reviewing the same publicly available toxicological and safety information would reach the same conclusion.

Phytic acid (50% solution) is GRAS based on scientific procedures for its intended use as a food ingredient; therefore, it is excluded from the definition of a food additive, and thus, may be marketed and sold for its intended purpose in the U.S. without the promulgation of a food additive regulation under Title 21 of the CFR.

I.F Availability of Information

The data and information that serve as the basis for this GRAS Notification will be sent to the U.S. Food and Drug Administration (FDA) upon request, or will be available for review and copying at reasonable times at the offices of:

Tsuno Food Industrial Co., Ltd.
94 Shinden, Katsuragi,
Ito-gun, Wakayama 649-7194
Japan

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

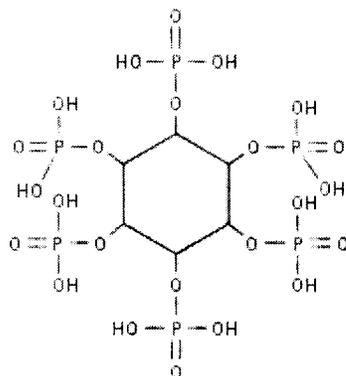
II. DETAILED INFORMATION ABOUT THE SOURCE AND IDENTITY OF THE SUBSTANCE

II.A Source and Identity

Phytic acid is identified by the chemical formula $C_6H_{18}O_{24}P_6$ and a corresponding molecular weight of 660.03 g/mol. Phytic acid is freely soluble in water, slightly soluble in ethanol, and almost insoluble in anhydrous ether, chloroform, and n-hexane.

Common or Usual Name:	Phytic acid (50% solution)
Trade Name:	Phytic acid (50% solution)
Chemical Abstracts Service (CAS) Number:	83-86-3
Empirical Formula and Formula Weight:	$C_6H_{18}O_{24}P_6$
Molecular Weight:	660.03 g/mol

Figure II.A-1 Pictorial Representation of Phytic Acid



II.B Method of Manufacture

A schematic diagram of the general manufacturing process employed to produce phytic acid (50% solution) is illustrated in Figure II.B-1. The production of phytic acid (50% solution) involves the addition of diluted sulfuric acid to defatted food-grade rice bran to dissociate phytate from iron and protein complexes. The solution undergoes centrifugation, filtration to remove impurities, neutralization with sodium hydroxide, and dilution with water. The diluted solution is decolorized, and sulfuric acid is added to dissociate the bound minerals from phytate to release phytic acid. The phytic acid-containing solution undergoes pH adjustment, ion-exchange, decolorization, and vacuum concentration to achieve a 50% concentration. All processing aids used in the manufacture of phytic acid (50% solution) are used in compliance with appropriate federal regulations as indicated in Table II.B-1.

GRAS EXEMPTION CLAIM FOR PHYTIC ACID (50% SOLUTION)

Figure II.B-1 Schematic Overview of the Manufacturing Process for Phytic Acid (50% Solution)

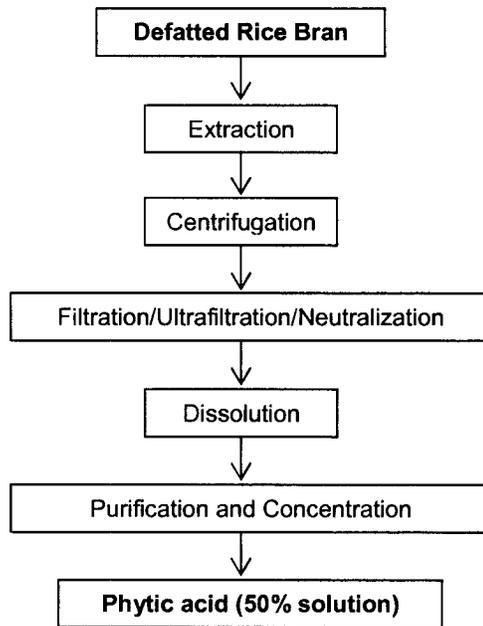


Table II.B-1 List of Processing Aids Used in the Manufacture of Phytic Acid (50% Solution)

Processing Aid	Function	Reference to Appropriate Use in Food
Sulfuric acid	pH control agent and processing aid	21 CFR §184.1095 (U.S. FDA, 2010)
Sodium hydroxide	pH control agent	21 CFR § 184.1763 (U.S. FDA, 2010)
Activated carbon	Decolorizing agent and filtration aid	No specific regulations pertaining to activated carbon; however, activated carbon is permitted for use as a filtration aid in the treatment of water for the following: 1. the manufacture of distilled alcoholic beverages following treatment with ion-exchange resins (21 CFR §173.25 - U.S. FDA, 2010); and 2. the fermentation production of citric acid by <i>Candida lipolytica</i> (21 CFR §173.165 - U.S. FDA, 2010)
Filters	Purification	21 CFR §177.1655 (U.S. FDA, 2010) 21 CFR §177.2250 (U.S. FDA, 2010)
Ion-exchange resins	Purification	21 CFR §173.25 (U.S. FDA, 2010)

GRAS EXEMPTION CLAIM FOR PHYTIC ACID (50% SOLUTION)

II.C Specifications and Analytical Data

Phytic acid (50% solution) is produced in accordance with cGMP, and in order to ensure a consistent and safe product, Tsuno has established food-grade specification parameters for the final ingredient. The product specifications for phytic acid (50% solution) presented in Table II.C-1.

Analyses of 3 non-consecutive lots of phytic acid (50% solution) confirm that the material produced by the manufacturing process is consistent and complies with the product specifications. The analytical data also demonstrate the absence of any chemical impurities or microbiological contamination. Complete certificates of analysis for these lots are provided in Appendix C.

Specification Parameter	Specification	Method
Visual appearance	Slight-yellowish or brownish liquid	Visual examination
Phytic acid content (%)	48 to 52	Specific gravity ^a
Water content (%)	48 to 52	Calculation ^b
Heavy metals (as Pb) (ppm)	Less than 20	Colorimetric analysis (FCC, 2008 ^c)
Lead (ppm)	Less than 1	AAS (FCC, 2008)
Arsenic (ppm)	Less than 2	Arsenic limit test (FCC, 2008)
Total phosphorus (%)	13.5 to 14.6	UV spectrometry (FCC, 2008)
Inorganic phosphorus (%)	Not more than 1.0	Colorimetric analysis (FCC, 2008)
Chloride (%)	Not more than 0.04	Chloride limit test (FCC, 2008)
Sulfate (%)	Not more than 0.071	Sulfate limit test (FCC, 2008)
Microbiological Parameters		
Total aerobic bacterial count	Less than 500 CFU/mL	Chapter 3 (BAM, 2002 ^d)
Total mold and yeast count	Less than 500 CFU/mL	Chapter 18 (BAM, 2002)
<i>Escherichia coli</i> count	Less than 3 CFU/g	Chapter 4 (BAM, 2002)

AAS = atomic absorption spectrometry; CFU = colony forming unit; Pb = lead; ppm = parts per million; UV = ultraviolet

^a The purity of phytic acid (50% solution) is determined by measuring specific gravity and comparing it to a defined standard (value of 1.369).

^b Water content is not assayed directly. Tsuno measures the concentration of phytic acid, and all substances other than phytic acid are considered to be water.

^c FCC (2008). *Food Chemicals Codex, 6th edition*. Rockville (MD): United States Pharmacopeial Convention (USP).

^d BAM (2002). *Bacteriological Analytical Manual Online (BAM)*. College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety and Applied Nutrition (CFSAN). Available from: <http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/default.htm> [Page Last Updated: 04/13/2011].

GRAS EXEMPTION CLAIM FOR PHYTIC ACID (50% SOLUTION)

II.D Additional Compositional Characterization

II.D.1 Pesticide/Herbicide Analysis

The raw material used in the production of phytic acid (50%) solution is defatted rice bran. As such, the potential for the presence of residual pesticides and herbicides is addressed herein. Tsuno conducted an analysis of 222 pesticide/herbicide residues in the defatted rice bran used in the manufacture of phytic acid (50% solution). The classes of pesticides/herbicides analyzed included organochlorines, organophosphates, carbamates, chloroacetanilides, and diphenyl ethers, and none of the 222 residues analyzed were detected in the defatted rice bran (detection limit of 0.01 ppm). Results of these analyses are provided in Appendix C.

II.E Stability of Phytic Acid (50% Solution)

The stability of phytic acid (50% solution) has been assessed at various temperatures, and the results are discussed below.

II.E.1 Stability under Typical Storage Conditions

Phytic acid (50% solution) was stored at 25°C for up to 730 days, and at 10°C for up to 35 days. At 25°C, phytic acid (50% solution) was shown to be stable for at least 180 days, as inorganic phosphorus levels (representing the degradation products of phytic acid) were below the specification limit of 1%. At 10°C, the inorganic phosphorus content was well below the specification limit and remained constant for the duration of the study. Results are presented in Table II.E.1-1.

Temperature	Specification	Days									
		0	7	14	21	28	35	42	180	360	730
25°C	Not more than 1.0%	0.215	0.220	0.268	0.29	0.279	0.313	0.361	0.76	1.173	2.132
10°C		0.215	0.222	0.219	0.219	0.219	0.219	n/a	n/a	n/a	n/a

n/a = not applicable (not measured)

Results of the studies described above demonstrate the stability of phytic acid (50% solution) under the typical storage conditions and the intended conditions of use.

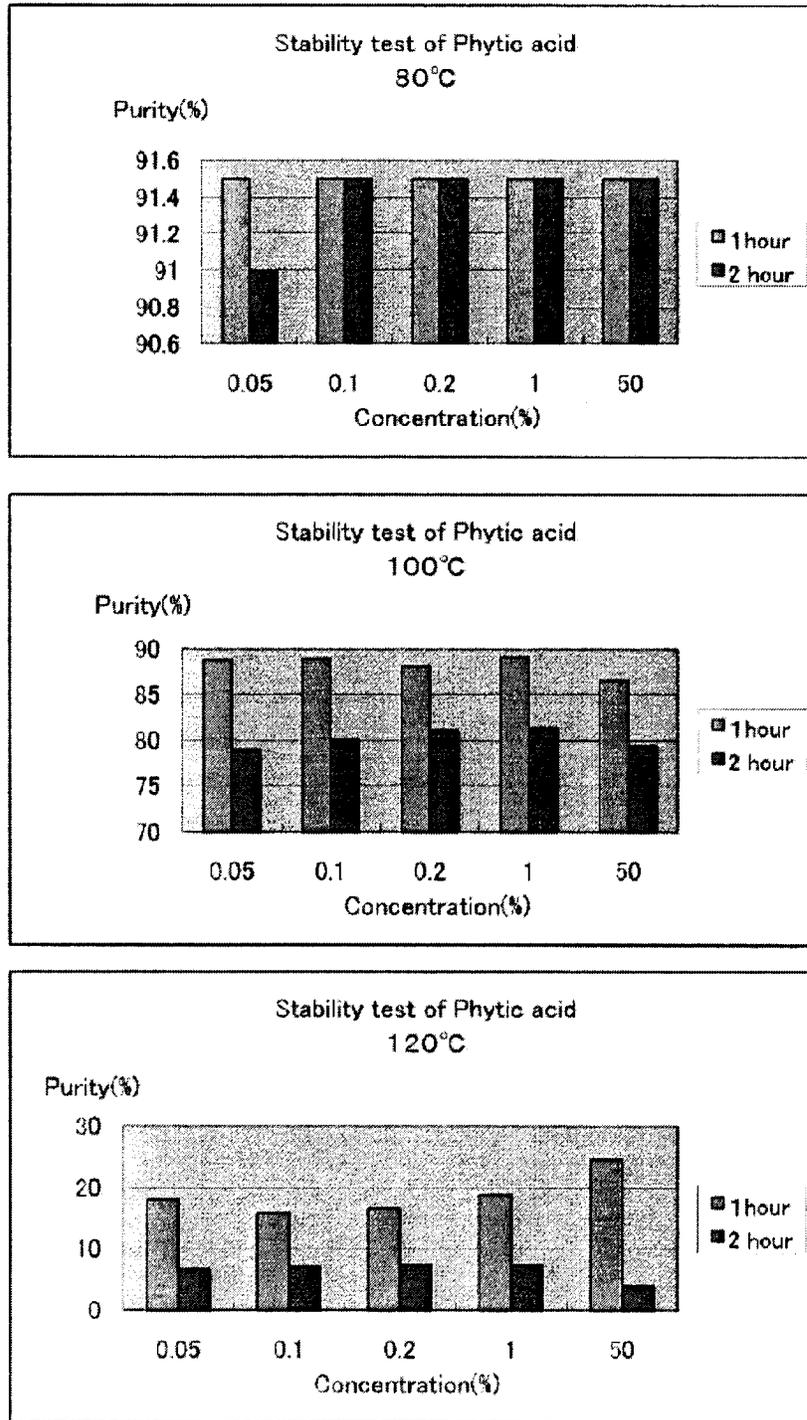
II.E.2 Stability at High Temperatures

The stability of phytic acid (50% solution) also was assessed at temperatures of 80, 100, and 120°C for 1 or 2 hours. At higher temperatures (100 and 120°C), the stability of the solution decreased substantially (Figure II.E.2-1); however, under the intended conditions of use, phytic acid (50%) solution will not be exposed to high temperatures for extended periods of time. The

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purity of phytic acid (50% solution) was slightly lower ($91.5 \times 50\% = 45.75\%$ phytic acid) than the product specification limits (48 to 52% phytic acid content) after exposure to heat (80°C) (Figure 3.6.2-1); however, the purity remained constant from 1 to 2 hours, indicating that the solution was stable during this time period.

Figure II.E.2-1 Stability of Phytic Acid Solutions at High Temperatures



III. SELF-LIMITING LEVELS OF USE

Phytic acid (50% solution) has a sour taste and thus, its use is self-limiting based on its organoleptic properties.

IV. BASIS FOR GRAS DETERMINATION

IV.A Documentation to Support the Safety of Phytic Acid (50% Solution)

The determination that phytic acid (50% solution) is GRAS is based on scientific procedures, and the information supporting the general recognition of the safety of the ingredient includes:

- data pertaining to the identity, intended use, and estimated intake of phytic acid;
- the natural occurrence of phytic acid in food and background dietary intakes;
- the metabolic fate of phytic acid; and
- toxicological and human studies conducted with phytic acid.

Moreover, these data were reviewed by a panel of experts, qualified by scientific training and experience to evaluate the safety of ingredients as components of food, who concluded that the intended uses of phytic acid (50% solution) are safe and suitable and would be GRAS based on scientific procedures [see Appendix A and B, entitled “Expert Panel Consensus Report Regarding the Generally Recognized as Safe (GRAS) Status of Phytic Acid (50% Solution) for Use in Foods and Supplements” and “Expert Panel Consensus Statement Concerning the Generally Recognized as Safe (GRAS) Status of Phytic Acid (50% Solution) as an Ingredient in Food and Dietary Supplements Following Changes in Use Levels”, respectively]. A summary of these data is presented herein.

IV.B Natural Occurrence and Background Dietary Consumption of Phytic Acid

Phytate, the salt of phytic acid, is the primary storage mechanism for phosphorus in plants. It is consumed by humans through the intake of the seeds of cereals and legumes, and can be found at up to 9% of dry matter (Szkudelski, 2005; Schlemmer *et al.*, 2009). Phytic acid in plants is primarily present as a mixed calcium, magnesium, and potassium salt (Fontaine *et al.*, 1946; Wang *et al.*, 1959; Zhou and Erdman, 1995). Phytate is stable at temperatures up to 100°C – *i.e.*, will not degrade during cooking, roasting, pressure-cooking, *etc.* (Schlemmer *et al.*, 2009). Although phytate, rather than phytic acid, is present in plants, all analytical procedures

GRAS EXEMPTION CLAIM FOR PHYTIC ACID (50% SOLUTION)

used to measure the phytate content of foods are based on the acidic dissociation of phytate to form phytic acid (Schlemmer *et al.*, 2009)¹.

A summary of the phytic acid content of commonly consumed foods is presented in Table IV.B-1.

Food	Phytic acid/Phytate content (%)^b
Cereals	
Oat	0.42 to 1.16
Rice	0.06 to 1.08
Rye	0.54 to 1.46
Wheat	0.39 to 1.35
Wheat bran	2.1 to 7.3
Wheat germ	1.14 to 3.91
Legumes	
Kidney beans	0.61 to 2.38
Lentils	0.27 to 1.51
Peas	0.22 to 1.22
Soybeans	1.0 to 2.22
Tofu	0.10 to 2.90
Nuts	
Almonds	0.35 to 9.42
Brazil nuts	0.29 to 6.34
Cashews	0.19 to 4.98
Hazelnuts	0.23 to 0.92
Macadamia nuts	0.15 to 2.62
Peanuts	0.17 to 4.47
Pecans	0.18 to 4.52
Walnuts	0.20 to 6.69

^a Data obtained from Schlemmer *et al.*, 2009

^b Data were reported either as phytic acid or phytate in different studies included in the review by Schlemmer *et al.* (2009).

Schlemmer *et al.* (2009) noted that the daily dietary intake of phytate varies depending on the type of diet consumed. Individuals consuming diets containing high levels of cereal, whole grain, and other phytate-rich foods (e.g., vegetarians) are reported to have higher levels of daily dietary phytate intake than those consuming diets low in plant-based foods.

¹ Some studies have reported the phytate content of foods; however, this value is calculated using dodecasodium phytate, which does not naturally occur in food but is commonly used as a calibration standard (Schlemmer *et al.*, 2009).

GRAS EXEMPTION CLAIM FOR PHYTIC ACID (50% SOLUTION)

Table IV.B-2 provides estimated daily intakes of phytic acid/phytate in North American and European populations (Schlemmer *et al.*, 2009). In male lacto-ovo-vegetarians in the U.S., phytate intakes are reported to be as high as 5,577 mg/day. Some European adults also consume large amounts of phytate, with reported levels reaching 2,191 mg/day in males >40 years of age in the United Kingdom, and 2,927 mg/day in Swedish adults consuming vegetarian diets. Given that the estimated intakes of phytic acid from the new proposed use levels are only a small fraction of background dietary intakes, it is not anticipated that the consumption of phytic acid (50%) solution under the intended conditions of use would present a safety concern.

Table IV.B-2 Estimated Daily Intake of Phytic Acid/Phytate in North America and Europe^a	
Subpopulation	Daily Intake of Phytic Acid/Phytate (mg)^b
United States	
Infants (<1 year)	166±167
Children (1 to 3 years)	390±231
Children (4 to 5 years)	501±271
Females (18 to 24 years)	395±334
Females (19 to 35 years)	1,293±666 ^c
Female vegetarians	~1,250±450
Male vegetarians	~1,550±550
Male lacto-ovo-vegetarian	5,577
Male lacto-ovo-vegetarian	972
Canada	
Children (4 to 5 years)	250 – 320
Lacto-ovo diets – Asian immigrants	1487±791
Mexico	
Male–female (18 to 30 months)	1,666±650
Male–female (7 to 9 years)	3,380±1,070
United Kingdom	
Male (> 40 years)	1,436±755
Male–female	600 – 800
Male–female	504-848
Italy	
Male–female	219 (112 – 1,367)
Male–female (average Italian diet)	293 (265 – 320)
Sweden	
Male–female (35 to 76 years), Western-type diets	369 (230 – 530)
Male–female (35 to 76 years), vegetarian diets	1,146 (500 – 2,927)

Values are presented as mean ± SD or range.

^a Adapted from Schlemmer *et al.* (2009)

^b Depending on data published (Schlemmer *et al.*, 2009)

^c Held *et al.* (1988) reported a wide range of daily phytate intake, ranging from 198 to 3,098 mg/day, in American students and university faculty female staff members consuming self-selected diets.

IV.C Metabolic Fate of Phytic Acid

IV.C.1 Absorption

It has been suggested that orally administered phytic acid is not absorbed given that it is highly negatively charged at physiological pH, thus limiting its ability to cross the lipid bilayer of plasma membranes (Schlemmer *et al.*, 2009). Results from studies in rats and humans, however, suggest that absorption of orally administered phytic acid does occur, although the extent of absorption appears to differ between species.

In rats, 79% of radiolabeled phytic acid administered intragastrically has been reported to be absorbed (Sakamoto *et al.*, 1993). Following oral administration of [¹⁴C] phytate to rats, approximately 6% of the administered dose was recovered in the feces 48 hours after administration, suggesting that phytate is almost completely absorbed (*i.e.*, 94% of the total dose) when calcium intake is low (0.12% of the diet) (Nahapetian and Young, 1980). In contrast, high calcium intakes (0.93% of the diet) resulted in decreased phytate absorption, as indicated by the increased excretion of phytate in the feces (54% of total dose). The effect of calcium on phytate absorption is attributed to the insolubility of calcium phytate complexes at the pH of the gastrointestinal tract, thus reducing the absorption of phytate (Nahapetian and Young, 1980; Sandberg *et al.*, 1993).

Grases *et al.* (2000) reported a maximum absorbable amount of phytate of 20.9 mg/kg body weight/day in rats. The authors derived this value based on the constant maximum urinary excretion of phytate (*i.e.*, urinary concentrations of phytate reached a plateau) observed in rats administered phytate at a concentration of 182 mg/L in a liquid diet. Increasing the phytate concentration to 425 mg/L did not result in a further increase in urinary excretion.

In contrast to rats, humans appear to have a limited capacity to absorb dietary phytate (Grases *et al.*, 2001a). In a study conducted in 7 healthy subjects who consumed a phytate-poor diet for 3 days, the consumption of 1.4 g phytate (as dodecasodium salt from corn) increased plasma phytate levels at 2, 4, 6, and 8 hours (time of last measurement) after ingestion. Plasma phytate levels were significantly elevated at 4 and 6 hours compared to baseline levels, with peak levels reached at 4 hours after ingestion. The authors noted that the peak plasma levels indicated an overall low percentage of phytate absorption as only approximately 0.01% of the administered dose was present in the plasma at 4 hours.

In a separate experiment, the same subjects were given single doses of different phytate salts (*i.e.*, 400 to 3,200 mg of phytate as a calcium/magnesium or sodium salt) (Grases *et al.*, 2001a). Over an 8-hour period following phytate ingestion, approximately 0.16 mg of phytate (above baseline values) was estimated to be excreted in the urine (approximately 0.005 to 0.04% of the administered dose), providing further evidence that absorption of phytate is limited in humans. No significant differences in the urinary excretion profile of phytate were reported between the

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different phytate salts or doses. The absence of an increase in urinary phytic acid excretion following the consumption of 3,200 mg of phytate compared to 400 mg of phytate implies that the capacity for phytic acid absorption in humans is low. Additionally, given the lack of differences between the excretion of different forms of phytate, the authors suggested that phytate absorption likely occurs as its neutral protonated derivative (Grases *et al.*, 2001a). In a more recent report, Grases *et al.* (2005) noted that absorption of phytic acid occurs independently of the condition of the stomach (*i.e.*, full or fasted), suggesting that absorption likely occurs during intestinal transit.

The reasons for the differences in the extent of phytic acid absorption between humans and rats are unclear. One potential contributing factor is the reported interspecies variation in the activity of phytase, which catalyzes the hydrolysis of phytic acid. Iqbal *et al.* (1994) observed that phytase activity in the human small intestine was 30 times lower than the activity in the rat small intestine. It is possible that phytase is secreted from the rat small intestinal cells and hydrolyzes phytic acid, allowing for greater absorption than in the human small intestine, which has lower phytase activity. Another possibility is the presence of phytic acid carriers or active transporters in the rat gastrointestinal tract; however, these have not yet been detected.

IV.C.2 Distribution

Evidence indicates that dietary phytate is distributed to various organs in rats following absorption (Nahapetian and Young, 1980; Sakamoto *et al.*, 1993; Grases *et al.*, 2001b). For instance, a substantial amount of radioactivity was detected in the skeletal muscle (18.1%), skin (10.1%), liver (4%), and kidneys (2.2%) of male Fischer 344 rats 24 hours following oral administration of a single dose of [³H] phytic acid (Sakamoto *et al.*, 1993). Radioactivity also was detected in the esophagus, spleen, brain, lungs, heart, thymus, and testes at levels of <0.2% (limit of detection not specified). Grases *et al.* (2001b) reported the highest phytate concentrations in the brain, with lower levels in the kidneys, liver, and bone in female Wistar rats fed a standard non-purified diet containing 9 g phytate/kg diet.

Phytate levels in the brain, liver, kidneys, femur, and blood were reported to be significantly greater in male Sprague-Dawley rats fed a low-calcium diet compared to those fed a high-calcium diet 48 hours following oral administration of [¹⁴C] phytate (Nahapetian and Young, 1980). As discussed in Section IV.C.1, high dietary calcium has been shown to reduce the absorption of phytate.

IV.C.3 Metabolism

Findings in rats suggest that orally administered phytic acid is dephosphorylated to inositol and inositol monophosphate in the upper gastrointestinal tract. In the study reported by Sakamoto *et al.* (1993) (study design described in Section IV.C.1), ion exchange chromatography of plasma (1 h), gastric epithelial cells (1 h), and urine (12 h) after intragastric administration of

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radiolabeled phytic acid to rats revealed the presence of inositol and inositol monophosphate and the absence of higher inositol phosphates in the plasma and urine. In contrast, large amounts of inositol P₁₋₃ and small amounts of inositol P₄₋₆ were detected in gastric cells. Based on these findings, the authors proposed that phytic acid is absorbed intact in the stomach and is at least partially dephosphorylated during its transit through the gastric epithelial cells. The authors noted, however, that partial dephosphorylation in the gastric lumen and absorption of inositol P₁₋₅ was possible.

Nahapetian and Young (1980; study design described in Section IV.C.1) also reported complete metabolism of orally administered phytate in rats, as evidenced by the output of radioactivity in expired air as carbon dioxide. Approximately 50 to 60% of the radioactivity was recovered as carbon dioxide within 48 hours in rats administered phytate with a low-calcium diet, whereas only 25% of the radioactivity was recovered in the expired air in rats given a high-calcium diet.

In humans, endogenous phytases in the stomach and small intestine appear to be absent or to have low activity; consequently, limited degradation of phytate is expected to occur in the upper gastrointestinal tract (Iqbal *et al.*, 1994; Schlemmer *et al.*, 2009). While results from previous human studies have indicated that 37 to 66% of dietary phytate is degraded in the stomach and small intestine when the diet is rich in plant food phytases (McCance and Widdowson, 1935; Sandberg *et al.*, 1986, 1987; Sandberg and Andersson, 1988), these studies were conducted 20 to 75 years ago and current diets likely do not contain many phytases due to inactivation by food processing (*i.e.*, heat treatment).

Phytate hydrolysis in humans is hypothesized to occur mainly in the large intestine through the actions of microbial phytases (Schlemmer *et al.*, 2009). Results from studies in pigs, which are an excellent model of the human digestive system (Patterson *et al.*, 2008), support this hypothesis. In a study reported by Schlemmer *et al.* (2001), it was shown that phytate from an extruded diet with inactivated phytases fed to pigs was not hydrolyzed to a significant extent in the stomach or small intestine, and that hydrolysis of phytate occurred predominantly in the large intestine, with the major degradation products being DL-Ins(1,2,3,4,5)P₅ and DL-Ins(1,2,4,5,6)P₅, which are formed through the actions of the microbial 6- and 3-phytases. Given that phytases are generally inactive in the human diet due to food processing, the authors considered the degradation of phytase from the phytase-inactivated diet in pigs to be generally representative of phytate hydrolysis in the gastrointestinal tract of humans.

In addition to phytases, alkaline phosphatases (ALPs) can catalyze the dephosphorylation of inositol polyphosphates. Although the activity of endogenous ALPs is much higher than that of phytases in the large intestine, study findings indicate that ALPs do not contribute to inositol phosphate degradation to a relevant degree (Schlemmer *et al.*, 2009).

IV.C.4 Excretion

Findings from rat studies have indicated absorbed phytate is excreted in the urine (Sakamoto *et al.*, 1993; Shamsuddin, 1999; Grases *et al.*, 2000, 2001b). Low levels of unabsorbed phytate have been detected in the feces of rats (Nahapetian and Young, 1980; Wise and Gilbert, 1982; Sakamoto *et al.*, 1993; Joung *et al.*, 2007; Kim *et al.*, 2009).

Results from a human study also demonstrate the excretion of dietary phytate in the urine. In a study reported by Grases *et al.* (2001a), 0.16 mg of phytate (above baseline values) was estimated to be excreted in the urine over an 8-hour period following ingestion of 1.4 g phytate (as dodecasodium salt).

IV.D Toxicological Studies

IV.D.1 Acute Toxicity Studies

The oral LD₅₀ values for phytic acid was reported to be 405 (males) and 500 (females) mg/kg body weight/day in rats, and 900 (males) and 1,150 (females) mg/kg body weight in mice (Fujitani *et al.*, 1987; Ichikawa *et al.*, 1987).

Table IV.D.1-1 Acute Oral LD₅₀ Values for Phytic Acid		
Species (Strain, Sex)	Dose (mg/kg bw)	Reference
Mouse (Jcl:ICR, F)	1,150	Fujitani <i>et al.</i> , 1987
Mouse (Jcl:ICR, M)	900	Fujitani <i>et al.</i> , 1987
Rat (F344, F)	480	Ichikawa <i>et al.</i> , 1987
Rat (F344, M)	405 to 500	Ichikawa <i>et al.</i> , 1987

bw = body weight; F = female; M = male

IV.D.2 Repeated Dose Studies

A number of studies were conducted to investigate the effects of phytic acid, sodium phytate, and soybean protein isolate (SPI) in mice and rats (Hiasa *et al.*, 1992; Kamao *et al.*, 2000; Onomi *et al.*, 2004; Szkudelski, 2005; Lee *et al.*, 2006; Gaetke *et al.*, 2010). Although these were not traditional toxicological studies, the investigators evaluated endpoints related to safety (*e.g.*, food intake, body weight, organ weights) (Kamao *et al.*, 2000; Onomi *et al.*, 2004; Szkudelski, 2005; Lee *et al.*, 2006; Gaetke *et al.*, 2010). The results of these studies are discussed below, and summarized in Appendix D.

In a 12-week study, no adverse effects were observed at a concentration of 2.5% phytic acid in the drinking water (equivalent to 2,500 mg/kg body weight/day), but few parameters related to safety were assessed (mortality and body weight) as it was a dose range-finding study for a subsequent carcinogenicity study (see Section IV.D. 5 for study details; Hiasa *et al.*, 1992).

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Mortality was reported in rats administered doses greater than or equal to 5,000 mg/kg body weight/day.

In other studies in which food consumption, body weights, organ weights, and clinical chemistry parameters were evaluated, no adverse effects were reported at doses ranging from 1,000 to 1,623 mg phytic acid/kg body weight/day (Onomi *et al.*, 2004; Szkudelski, 2005; Lee *et al.*, 2006). At higher doses (2,300 to 5,000 mg/kg body weight/day), significantly decreased body weights compared to control animals were observed (Onomi *et al.*, 2004; Gaetke *et al.*, 2010). In addition, food consumption was reported to be significantly decreased in rats administered phytic acid at doses greater than or equal to 5,000 mg/kg body weight/day (Onomi *et al.*, 2004). These effects are not relevant to the safety of phytic acid (50% solution) under the intended conditions of use as the doses at which effects were reported are far higher than the estimated intakes of phytic acid from the proposed uses (all-user mean and 90th percentile intakes in the total population of 3.7 and 9.4 mg/kg body weight/day, respectively).

IV.D.3 Reproductive and Developmental Toxicity Studies

In a study conducted to investigate the effects of phytic acid on fetal development, pregnant Jcl:ICR mice (21 to 24/group) were orally administered 10 mL/kg body weight/day of a 0, 1.6, 3.1, or 6.31% aqueous solution of 50% phytic acid (equivalent to 0, 80, 155, and 315 mg/kg body weight/day of phytic acid) *via* gavage on gestation days 7 to 15 (Ogata *et al.*, 1987). All fetuses were removed on day 18 of gestation and examined for external and skeletal anomalies. Parameters evaluated included mortality, maternal body weights, maternal absolute and relative organ weights (liver, heart, spleen, kidney, lung, adrenal glands, and ovaries), number of corpora lutea and implantations per litter, incidence of early and late resorptions, fetal body weights, and the incidence of external and skeletal fetal malformations.

No mortality was reported to occur in the low-dose and control groups. Two out of 22 dams (9.1%) in 3.1% group and 15 out of 24 dams (62.5%) in the 6.31% group died during the study (statistical significance not reported); the reasons for death were not discussed. Piloerection and dormancy were reported to be observed in some surviving high-dose animals (number not reported). No significant differences in the rate of maternal body weight gain were reported in all phytic acid groups compared to the control; however, the authors noted that there was a trend for decreased maternal body weight gain in the mid- and high-dose groups (significance not reported). Other maternal effects were reported to include a statistically significant decrease in absolute heart weights in the low- and high-dose groups compared to controls. Absolute right adrenal gland weights in the mid-dose group and relative right adrenal gland weights in the mid- and high-dose groups also were reported to be significantly increased compared to controls; however, the authors reported that there was no significant dose-response relationship. No significant macroscopic findings were reported in all phytic acid groups.

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Oral administration of phytic acid was reported to have no significant effects on the number of live fetuses, number of corpora lutea per litter, number of implantations per litter, incidence of early resorptions, and number of live fetuses per litter. A significant increase in the incidence of late resorption was reported in the low-dose group compared to the control; however, the relevance of these findings is questionable as the standard deviation for the mean incidence values was larger than the actual mean (*i.e.*, 3.8 ± 4.2). In addition, no significant effects on late resorption were reported in the mid- and high-dose groups. Fetal body weights were reported to be significantly decreased in a dose-dependent manner in males exposed to phytic acid compared to controls, while a significant decrease in fetal body weight was reported in females at the mid-dose. The authors considered the increase in late resorption and fetal weight loss to be a result of maternal toxicity. No significant effects on the incidence of external or skeletal malformations were reported in all phytic acid-exposed groups. The authors reported a dose-dependent, but non-significant, effect of phytic acid on ossification of forelimb and hindlimb phalanxes, and post-lumbar vertebrae.

In the same report, Ogata *et al.* (1987) reported the findings of a preliminary study in which pregnant mice (5 mice/group, strain not reported) were administered a single dose of 10 mL/kg body weight of a 6.3 or 12.5% aqueous phytic acid solution (equivalent to 630 and 1,250 mg/kg body weight of phytic acid, respectively) on the 9th day of gestation to examine potential external and skeletal malformations in their fetuses. No external malformations were observed; however, fusion of lumbar vertebrae was observed in 5 fetuses from 1 dam at the 12.5% level. These findings are difficult to interpret as no control group was included and that the effects on lumbar vertebrae fusion were only observed in 1 litter.

Although maternal toxicity was observed at the 3.1% and 6.31% levels in the study reported by Ogata *et al.* (1987), these findings are of little relevance to the safety of phytic acid (50% solution) for its proposed uses given the differences in absorption of orally administered phytic acid between rodents and humans. Furthermore, phytic acid has been safely consumed in the diet at higher levels than the estimated exposure to phytic acid from the proposed uses in food and dietary supplements; therefore, toxicity from the addition of phytic acid (50% solution) to the diet is not expected.

While no additional studies on the potential reproductive and developmental toxicity of phytic acid were identified in the literature, several studies conducted on related phosphate compounds have been conducted. No signs of reproductive or developmental toxicity were reported following gavage administration of sodium acid pyrophosphate, sodium hexametaphosphate, and sodium tripolyphosphate in experimental animals (Food and Drug Research Laboratories, Inc., 1973a,b, 1974). In these studies, pregnant mice, rats, guinea pigs, and rabbits were administered doses of up to 370 mg/kg body weight/day for up to 10 days during gestation with no maternal toxicity or effects on implantation reported. Additionally,

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exposure to these compounds did not affect the incidence of abnormalities in soft or skeletal tissues. Summaries of these studies are presented in Appendix E.

Additionally, one study was identified in the literature in which the effects of inositol on reproduction and development were assessed in rats (Ershoff, 1946). Evidence indicates that orally administered phytic acid is dephosphorylated to inositol and inositol monophosphate in the upper gastrointestinal tract of rats (Section IV.C.3). No growth effects were observed among female rats (strain and number per group not reported) fed diets containing 1% inositol (approximately 2 g/kg body weight/day) for 60 days prior to mating. Moreover, no effects on reproductive performance, litter size, or weight of offspring were observed, and no gross structural abnormalities were detected in the offspring following continued feeding of the inositol diet to the dams.

IV.D.4 Mutagenicity and Genotoxicity Studies

IV.D.4.1 *In vitro* Studies

Several mutagenicity studies have been reported on phytic acid in prokaryotic and eukaryotic test systems (Ishidate *et al.*, 1984; Seifried *et al.*, 2006). In reverse mutation studies in *Salmonella typhimurium* strains TA92, TA1535, TA100, TA 1537, TA 94, and TA98, phytic acid (50% solution) up to a maximum dosage of 10 mg/plate in the presence and absence of metabolic activation did not result in any increased revertants (Ishidate *et al.*, 1984). Similarly, negative results were reported in a mouse lymphoma cells treated with up to 5,000 µg/mL of phytic acid (Seifried *et al.*, 2006), and no chromosomal aberrations were reported in Chinese hamster ovary cells following treatment with 2 mg/mL of phytic acid (Ishidate *et al.*, 1984).

In an unpublished study, phytic acid was reported to induce chromosomal aberrations in Chinese hamster cells at a high concentration (concentration not reported) (Ogata *et al.*, 1987). These findings cannot be interpreted given the lack of information on the study design, and are in contrast to the negative results reported in 2 published chromosomal aberration studies (Ishidate *et al.*, 1984).

IV.D.4.2 *In vivo* Studies

Phytic acid was reported to be non-genotoxic in a mouse bone marrow micronuclei test in which ddY mice (6/group) were administered a single dose of 60 mg/kg body weight or 4 doses of 30 mg/kg body weight in 24-hour intervals *via* intraperitoneal injection (Ishidate *et al.*, 1988).

IV.D.5 Carcinogenicity Studies

The potential carcinogenic effects of phytic acid in rats have been investigated by Hiasa *et al.* (1992), Hirose *et al.* (1994), and Takaba *et al.* (1994). Phytic acid was reported to have no significant effects on tumor incidence when fed to female Sprague-Dawley rats (10/group) at a

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dietary level of 2% [approximately 2,000 mg/kg body weight/day² (U.S. FDA, 1993)] for 32 weeks (Hirose *et al.*, 1994). Similarly, no significant effects on the incidence of papillary or nodular hyperplasia, papillomas, and carcinomas in the urinary bladder were reported in male F344 rats (15 to 16/group) fed a diet containing 2% phytic acid (approximately 877 mg/kg body weight/day) for 32 weeks compared to control rats fed a basal diet (Takaba *et al.*, 1994).

In a 108-week study, male and female F344 rats (60/sex/group) were given drinking water containing 0 (control), 1.25, or 2.5% of an aqueous solution of phytic acid (equivalent to approximately 0, 275, and 572 mg phytic acid/kg body weight/day for males, respectively, and 0, 302, and 605 mg phytic acid/kg body weight/day for females, respectively) (Hiasa *et al.*, 1992). Oral administration of phytic acid was reported to have no significant effects on hematology and clinical chemistry parameters or absolute and relative organ weights. A significant increase in the incidence of red blood cells (RBC) in the urine was reported in males and females that received phytic acid compared to their respective controls; no other significant differences in urinalysis parameters were reported.

The incidence of renal papillary necrosis, as well as the calcification of the renal papillae and non-renal pelvic area, was reported to be significantly higher in the female phytic acid-groups compared to the control group; however, these effects were not observed to the same extent in male rats. Males that received the 2.5% solution were reported to exhibit a significant increase in the incidence of mild or moderate hyperplasia in the renal pelvis compared to controls. Histopathological findings in the kidney are summarized in Table IV.D.5-1 below.

Group	Necrosis of renal papillae	Calcification of renal papillae	Calcification of non-renal pelvic area	Hyperplasia in renal pelvis		
				±	+	++
Males						
Control	0 (0)	0 (0)	0 (0)	0 (0)	1	0 (0)
1.25%	1 (1.7)	0 (0)	0 (0)	2 (3.4)	9 (15.3)*	0 (0)
2.5%	1 (1.8)	3 (5.3)	3 (5.3)	6 (10.5)*	21 (36.8)**	0 (0)
Females						
Control	0 (0)	0 (0)	0 (0)	0 (0)	2 (3.4)	0 (0)
1.25%	6 (10.3)*	6 (10.3)*	26 (44.8)**	2 (3.4)	5 (8.6)	0 (0)
2.5%	10 (18.2)**	17 (30.9)**	25 (45.5)**	2 (3.6)	6 (10.3)	2 (3.6)

± = mild; + = moderate; ++ = severe

Values in parentheses denote the incidence of the finding.

* Significantly different from the control group (P<0.05)

** Significantly different from the control group (P<0.01)

² Estimated using the 'Conversion Table for Test Chemical Treatment Doses Used in PAFA' (U.S. FDA, 1993). For a test chemical treatment, a dietary concentration of 2% is approximately equivalent to an intake of 2,000 mg/kg body weight/day in young rats.

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No significant differences in the incidence of neoplasms in the testes, prostate, uterus, pituitary gland, adrenal glands, or in leukemia were observed between the phytic acid and control groups. Kidney papillomas were reported in 3 females in the 1.25% group, in 4 females in the 2.5% group, and in 3 males in the 2.5% group; none were observed in their respective controls (see Table IV.D.5-2)³.

Group	Females	Males
Control	0 (0)	0 (0)
1.25%	3 (5.2)	0 (0)
2.5%	4 (7.3)	3 (5.3)

Values in parentheses denote the percentage of animals in which kidney papillomas were observed.

The authors suggested that the induction of kidney papillomas in female rats was likely due to the necrosis and calcification in the renal papillae. Nephrocalcinosis is a common disorder in female rats, and may be induced by increasing dietary phosphorus levels, which result in a decrease in the calcium:phosphate ratio of the diet (Ritskes-Hoitinga and Beynen, 1992). The dietary calcium:phosphate ratio is a major determinant of nephrocalcinosis, as a negative association between the calcium:phosphate ratio of the diet and the appearance of nephrocalcinosis in rats has been reported (Forbes, 1963; Mars *et al.*, 1988; Ritskes-Hoitinga *et al.*, 1991). Thus, the observed calcification in the renal papillae may be attributed to the high phosphorus levels in the drinking water consumed by the rats in the phytic acid groups.

In the study reported by Hiasa *et al.* (1992), the increased incidence of nephrocalcinosis in female but not male rats exposed to phytic acid compared to their respective controls also is consistent with the scientific literature. It has been well-documented that female rats fed a nephrocalcinogenic diet develop more severe nephrocalcinosis than male rats, and it is suggested that these sex differences are related to the influence of estrogens, although the underlying mechanism has not been fully elucidated (Forbes, 1963; Mars *et al.*, 1988; Ritskes-Hoitinga *et al.*, 1991).

As mentioned, in addition to nephrocalcinosis, necrosis of the renal papillae occurred in female rats fed phytic acid. These findings are consistent with results from other studies in which high levels of dietary phosphorus induced both nephrocalcinosis and necrosis (Forbes, 1963; Mars *et al.*, 1988; Ritskes-Hoitinga *et al.*, 1991; Matsuzaki *et al.*, 1997). The RBC observed in the urine of phytic acid-fed rats was considered to result from hemorrhage in the necrotic renal papillae.

³ In the Hiasa *et al.* (1992) publication, the tabular summary of the histological findings in the renal papillae and pelvis indicates that there were no significant differences in the incidence of kidney papilloma among groups; however, in the text, the authors report that there were "no statistically significant differences between groups in the incidence of neoplasms, except of kidney tumors".

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Mild or moderate hyperplasia reported in the renal pelvis of male rats fed phytic acid in the drinking water also was attributed to the high phosphorus levels consumed.

Given that no significant differences in the incidence of neoplasms in the testes, prostate, uterus, pituitary gland, adrenal glands, or in leukemia were reported between the phytic acid and control groups, and that the papillomas and histopathological effects observed in the kidneys of phytic acid-exposed rats were attributed to the high phosphorus levels consumed by the rats rather than to the toxicity of phytic acid *per se*, the results of the study reported by Hiasa *et al.* (1992) do not provide evidence of a carcinogenic effect of orally administered phytic acid at the levels tested. The findings of the studies reported by Hirose *et al.* (1994) and Takaba *et al.* (1994), in which no carcinogenic effects attributed to phytic acid were reported at doses of approximately 2,000 or 877 mg/kg body weight/day, respectively, support the conclusions regarding the Hiasa *et al.* (1992) study.

It also should be noted that humans are less susceptible to calcium:phosphorus imbalance compared to rats (Schauss *et al.*, 2009). The dietary intake of phosphorus in the U.S. is estimated to range from approximately 1 to 2.2 g/day, and the Institute of Medicine (IOM) has determined the Tolerable Upper Intake Level (UL) to be 4,000 mg/day (IOM, 1997). Based on the chemical formula for phytic acid, the consumption of 1.0 g of phytic acid is calculated to provide an equivalent dose of 0.28 g phosphorus. The estimated 90th percentile all-user intake of phytic acid resulting from the proposed food and supplement uses of phytic acid (50% solution) was determined to be 610 mg/person/day, which would provide approximately 172 mg/person/day of phosphorus. Given that the estimated combined intake of phosphorus from the proposed uses of phytic acid (50% solution) and the background diet is well-below the UL established by the IOM for phosphorus, it is not expected that the increased phosphorus intake from the consumption of phytic acid would result in any adverse effects in humans.

A number of investigators have suggested that phytic acid may have possible anti-mutagenic, anti-tumor, and/or anti-cancer effects in animals and humans (Vucenik *et al.*, 1993; Shamsuddin, 1995; Shamsuddin and Vucenik, 1999; Fox and Eberl, 2002; Singh and Agarwal, 2005); however, the relevance of these studies to the overall safety of phytic acid is limited and therefore are not further discussed.

IV.E Studies in Humans

Numerous studies examining oral phytate supplementation in humans were identified in the scientific literature. The majority of the studies, however, did not include assessment of safety-related endpoints, but rather focused on the effects of acute dietary intake of phytate on mineral absorption. In these studies, subjects were provided up to 2,340 mg phytic acid/day in foods with no adverse events attributed to phytic acid (Turnlund *et al.*, 1985; Hallberg *et al.*, 1987; Heaney *et al.*, 1991; Bohn *et al.*, 2004; Kim *et al.*, 2009). The results of these studies are further discussed in Section IV.G.

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The metabolic fate of phytic acid in humans was reported by Grases *et al.* (2001a); Joung *et al.* (2007); and Kim *et al.* (2009). Seven (7) healthy subjects ingested single oral doses of up to 3,200 mg phytate (as a calcium/magnesium or sodium salt) with no adverse events reported (Grases *et al.*, 2001a). Likewise, no adverse events were reported in healthy young and elderly women following the consumption of low- or high-phytate diets (providing daily intakes of approximately 682 to 782 mg phytate/day and 1,587 to 1,723 mg phytate/day, respectively) for a period of 10 days (Joung *et al.*, 2007; Kim *et al.*, 2009).

IV.F Additional Safety Considerations

IV.F.1 Potential Effects on Mineral Bioavailability

Phytic acid is a highly negatively-charged molecule under physiological pH (Schlemmer *et al.*, 2009); therefore, it has the ability to form chelates with positively-charged multivalent cations, such as iron, zinc, magnesium, and calcium, in the gastrointestinal tract (Cheryan, 1980; Szkudelski, 1995; Bohn *et al.*, 2004; Fredlund *et al.*, 2006). Since these phytate-mineral complexes are insoluble at the neutral pH of the small intestine, dietary phytic acid may affect the absorption of minerals and trace elements (Bohn *et al.*, 2004).

The inhibitory effects of dietary phytate on mineral absorption and bioavailability have been examined in numerous human studies (*e.g.*, Turnlund *et al.*, 1984, 1985; Hallberg *et al.*, 1987; Heaney *et al.*, 1991; Hurrell *et al.*, 1992; Sandström *et al.*, 2000; Lind *et al.*, 2003; Bohn *et al.*, 2004; Fredlund *et al.*, 2006; Joung *et al.*, 2007; Kim *et al.*, 2009). Significant decreases in the absorption of iron, zinc, magnesium, and calcium were reported in healthy subjects consuming dietary phytate at levels ranging from 50 to 2,340 mg/day in several single-meal and short-term studies (*i.e.*, up to 15 days in duration) (Turnlund *et al.*, 1985; Hallberg *et al.*, 1987; Heaney *et al.*, 1991; Hurrell *et al.*, 1992; Bohn *et al.*, 2004; Fredlund *et al.*, 2006; Joung *et al.*, 2007).

Results from single-meal studies demonstrated that the inhibitory effects of phytate on iron and zinc absorption are dose-dependent (Hurrell *et al.*, 1992; Fredlund *et al.*, 2006). In contrast, dietary phytate was reported to have no significant effects on iron and zinc bioavailability in longer-term studies (*i.e.*, 21 days to 6 months) wherein healthy infants and adults consumed phytate at doses ranging from 17 to 2,640 mg/day (Sandström *et al.*, 2000; Lind *et al.*, 2003).

Despite the influence of dietary phytate on mineral absorption reported in short-term studies, the addition of phytic acid under the intended conditions of use is not expected to adversely affect long-term mineral bioavailability for several reasons. First, phytic acid is naturally-occurring in many foods, resulting in background dietary intakes of 300 to 2,600 mg/day (Schlemmer *et al.*, 2009), and the addition of a small amount of phytic acid to the diet from the proposed food and supplement uses will only result in minor increases in intake (223 and 610 mg/person/day at the mean and 90th percentile, respectively), which should not have an additional impact on mineral absorption.

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Secondly, daily consumption of 1 to 2 g phytate with balanced diets was reported to have no effects on mineral status in previous human studies (Walker *et al.*, 1948; Cullumbine *et al.*, 1950). Results from more recent studies also have demonstrated that the adverse effects of phytate on mineral absorption only occur when consumed in large quantities (*i.e.*, up to 4,569 mg/day) in combination with a mineral-poor diet (Kelsay, 1987; Harland *et al.*, 1988; Torre *et al.*, 1991; Sandström *et al.*, 2000; Harinarayan *et al.*, 2004). Given that the U.S. population generally consumes adequate amounts of minerals, such as calcium, iron, magnesium, and zinc, in the diet (IOM, 1997, 2001, 2011), it is not anticipated that the addition of phytic acid (50% solution) to the proposed food and supplement uses will affect mineral status.

Many of the studies conducted to investigate phytic acid's inhibitory actions on mineral absorption were single-meal or short-term studies (*i.e.*, up to 15 days) (Turnlund *et al.*, 1985; Hallberg *et al.*, 1987; Heaney *et al.*, 1991; Hurrell *et al.*, 1992; Bohn *et al.*, 2004; Fredlund *et al.*, 2006; Joung *et al.*, 2007), and thus, do not reflect the effects of chronic phytic acid consumption. In addition, the complex nature of the human diet should be considered when evaluating the effects of phytic acid on mineral absorption. There are numerous dietary factors that affect mineral absorption, many of which improve absorption and thus oppose the effects of phytic acid. For example, dietary ascorbic acid can enhance the absorption of non-heme iron (Davidsson *et al.*, 1997). Probiotic *Lactobacilli* also may counteract the effects of phytic acid as they are a source of the enzyme phytase, which catalyzes the degradation of phytic acid (Famularo *et al.*, 2005). It also has been shown that endogenous digestive microflora are a source of phytase (Haros *et al.*, 2007, 2009); however, the secretion of phytases into the gut lumen or the hydrolysis of phytate in the gut has not yet been observed in humans (Hambidge *et al.*, 2008). In contrast, other dietary factors, such as fiber, cadmium, and polyphenols, have been reported to inhibit mineral absorption (Torre *et al.*, 1991; Lönnerdal, 2000; Zijp *et al.*, 2000). Given the multi-factorial effect of the diet on mineral bioavailability, the intake of phytic acid from the intended uses is not anticipated to have adverse effects on long-term mineral bioavailability.

Findings from recent human studies have suggested that there may be adaptation to long-term or habitual high-phytic acid intakes, leading to an increased absorption of minerals over time (Sandström *et al.*, 2000; Lind *et al.*, 2003; Joung *et al.*, 2007; Kim *et al.*, 2009). These results are corroborated by animal studies in which inhibitory effects of phytate on iron absorption were observed in short-term, but not long-term, studies (Hunter, 1981). Similarly, there is evidence of adaptation after increasing or reducing the intake of bioavailable minerals, such as zinc (Hambidge *et al.*, 2008). The short duration of the available human studies therefore may not have allowed enough time for up- or down-regulation of absorption to occur in response to a change in the amount of bioavailable mineral in the study diet.

Based on the discussion above, the consumption of phytic acid (50% solution) added to the diet from the intended uses is not expected to be of concern with respect to mineral bioavailability.

IV.G Summary and Basis for GRAS Conclusion

The GRAS determination for the use of phytic acid (50% solution) as a food ingredient is based on scientific procedures. Phytic acid (50%) solution is intended for use at levels providing up to 0.2% phytic acid in selected food categories, including energy, sports, and isotonic drinks, milk and non-milk based meal replacements, yogurt shots, frozen vegetables, and pickles. It also is proposed for use in dietary supplement softgels (to limit solubility reduction and provide strength to capsules) at a maximum level of 8.0%. Under the intended conditions of use, the all-user mean and 90th percentile intakes of added phytic acid in the total population are estimated to be 223 and 610 mg/day, respectively (3.7 and 9.4 mg/kg body weight/day, respectively).

Phytic acid (50% solution) is produced from food-grade defatted rice bran. The manufacture of phytic acid (50% solution) involves the addition of diluted sulfuric acid to the defatted rice bran to dissociate phytate from iron and protein complexes. The phytate solution is diluted with water, and sulfuric acid is then added to dissociate the bound minerals from phytate to release phytic acid. Downstream steps in the manufacturing process include filtration, decolorization, and ion-exchange to remove protein residues, metallic cations, and other impurities. Phytic acid (50% solution) is manufactured in accordance with cGMP and meets appropriate food-grade specifications. Lot samples are routinely assayed to verify compliance with specifications. In addition, analysis of the defatted rice bran starting material indicates the absence of residual pesticides and herbicides. All reagents and processing aids used in the manufacture of phytic acid (50% solution) are food-grade materials.

The safety of phytic acid (50% solution) under the intended conditions of use is supported by its natural occurrence in numerous foods commonly consumed in the diet. Phytate, the salt of phytic acid, is the primary storage form for phosphorus in plants. It is consumed by humans through the intake of the seeds of cereals and legumes, and can be found at up to 9% of dry matter (Szkudelski, 2005; Schlemmer *et al.*, 2009). The average daily intake of phytic acid (as phytate) is estimated to be approximately 1,300 mg/day in U.S. adults (Schlemmer *et al.*, 2009). In male lacto-ovo-vegetarians in the U.S., phytate intakes are reported to be as high as 5,577 mg/day. Some European adults also consume large amounts of phytate, with reported levels reaching 2,191 mg/day in males >40 years of age in the United Kingdom, and 2,927 mg/day in Swedish adults consuming vegetarian diets. Given that the estimated intakes of phytic acid from the new proposed use levels are only a small fraction of background dietary intakes, it is not anticipated that the consumption of phytic acid (50%) solution under the intended conditions of use would present a safety concern.

Safety also is corroborated by the findings of a number of repeated-dose studies conducted in rodents that included the assessment of endpoints related to safety. In these studies, no adverse effects were reported in rats fed phytic acid in the diet or in drinking water at doses of 1,000 to 2,500 mg/kg body weight/day for 14 days to up to 12 weeks (Hiasa *et al.*, 1992; Kamao *et al.*, 2000; Onomi *et al.*, 2004; Szkudelski, 2005; Lee *et al.*, 2006; Gaetke *et al.*, 2010).

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Several mutagenicity studies have been conducted on phytic acid in prokaryotic and eukaryotic test systems (Ishidate *et al.*, 1984; Seifried *et al.*, 2006). Phytic acid was reported to be non-mutagenic in the bacterial reverse mutation assay, with and without metabolic activation (Ishidate *et al.*, 1984). Similarly, negative results were reported in a mouse lymphoma assay (Seifried *et al.*, 2006) and a chromosomal aberration assay conducted in Chinese hamster ovary cells (Ishidate *et al.*, 1984). Phytic acid also was reported to be non-genotoxic in a mouse bone marrow micronuclei test (Ishidate *et al.*, 1988).

The results of two 32-week studies, as well as a 108-week study conducted in rats, also do not provide evidence of carcinogenicity attributable to phytic acid at the levels tested. Phytic acid was reported to have no effects on carcinogenicity when provided in the diet at a level of 2% (equivalent to approximately 877 or 2,000 mg/kg body weight/day) for 32 weeks in 2 rat studies (Hirose *et al.*, 1994; Takaba *et al.*, 1994). In the 108-week carcinogenicity study, rats given 1.25 or 2.5% phytic acid in drinking water (equivalent to approximately 275 and 572 mg phytic acid/kg body weight/day for males, respectively, and 302 and 605 mg phytic acid/kg body weight/day for females, respectively) did not exhibit an increase in the incidence of neoplasms in the testes, prostate, uterus, pituitary gland, adrenal glands, or in leukemia compared to control rats (Hiasa *et al.*, 1992). Kidney papillomas, accompanied by necrosis and calcification in the renal papillae of female rats exposed to phytic acid, occurred as a result of the high phosphorus levels in the drinking water consumed by the rats in the phytic acid groups. Hyperplasia in the renal pelvis of male rats given phytic acid in the drinking water also was attributed to the high phosphorus levels consumed.

Furthermore, the findings reported in the Hiasa *et al.* (1992) study are of little relevance to humans given that humans are less susceptible to calcium:phosphorus imbalance (Schauss *et al.*, 2009), and that the addition of phytic acid (50% solution) from the proposed food and supplement uses would only result in a minor increase in phosphorus consumption. Specifically, the estimated 90th percentile all-user intake of phytic acid was determined to be 610 mg/person/day, which would provide approximately 172 mg/person/day of phosphorus. The intake of phosphorus from the proposed uses of phytic acid (50% solution) and background diet is well-below the UL established by the IOM (1997) for phosphorus (*i.e.*, 4 g/day); thus, it is not expected that the increased phosphorus intake from the consumption of phytic acid would result in any adverse effects in humans.

Although dietary phytate has been shown to affect mineral absorption in short-term studies, the addition of phytic acid to the proposed food uses is not expected to adversely affect long-term mineral bioavailability for several reasons: 1) the addition of a small amount of phytic acid to the diet from the proposed food uses will only result in small increases in intake (223 and 610 mg/day at the mean and 90th percentile compared to background dietary intakes of 1,300 mg/day in the U.S.); 2) the consumption of a varied diet with adequate protein and mineral intake protects against the effect of phytate on mineral utilization; 3) a number of dietary

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factors affect mineral absorption, many of which improve absorption and thus oppose the effects of phytic acid; 4) evidence suggests that there may be adaptation to long-term or habitual high-phytic acid intakes, leading to increased absorption of minerals.

Together, the above data provided support the conclusion that the consumption of phytic acid (50% solution) under the intended conditions of use would not be expected to produce adverse effects in consumers.

Finally, the Expert Panel convened on behalf of Tsuno, independently and collectively, critically evaluated the data and information summarized above and concluded that the intended uses of phytic acid (50% solution), produced consistently with cGMP and meeting appropriate food-grade specifications described herein, are safe and suitable. Furthermore, the Expert Panel unanimously concluded that the intended uses of phytic acid (50% solution) are GRAS based on scientific procedures. It is also Tsuno's opinion that other qualified and competent scientists reviewing the same publicly available toxicological and safety information would reach the same conclusion. Therefore, Tsuno has concluded that phytic acid (50% solution) is GRAS under the intended conditions of use on the basis of scientific procedures; therefore, the ingredient is excluded from the definition of a food additive and thus may be marketed and sold for the uses designated above in the U.S. without the promulgation of a food additive regulation under Title 21 of the CFR.

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Part	Section §	Section Title
170—Food additives	170.30	Eligibility for classification as generally recognized as safe (GRAS).
173—Secondary direct food additives permitted in food for human consumption	173.25	Ion-exchange resins
	173.165	<i>Candida lipolytica</i>
177—Indirect food additives: Polymers	177.1655	Polysulfone resins
	177.2250	Filters, microporous polymeric
184—Direct food substances affirmed as generally recognized as safe	184.1095	Sulfuric acid
	184.1763	Sodium hydroxide

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Expert Panel Consensus Report Regarding the Generally Recognized as Safe (GRAS) Status of Phytic Acid (50% Solution) for Use in Foods and Supplements

August 26, 2010

INTRODUCTION

At the request of Tsuno Food Industrial Co. Ltd. (Tsuno), an Expert Panel (the "Panel") of independent scientists, qualified by their relevant national and international experience and scientific training to evaluate the safety of food ingredients, was specially convened to conduct a critical and comprehensive evaluation of the available pertinent data and information, and determine whether, under the conditions of intended use as a food ingredient, phytic acid (50% solution) would be Generally Recognized as Safe (GRAS), based on scientific procedures. The Panel consisted of the below-signed qualified scientific experts: Professor Joseph F. Borzelleca, Ph.D. (Virginia Commonwealth University School of Medicine), Professor Robert J. Nicolosi, Ph.D. (University of Massachusetts Lowell), and Professor John Thomas, Ph.D. (Indiana University School of Medicine).

The Panel, independently and collectively, critically examined a comprehensive package of scientific information and data compiled from the literature and other published sources. This information was presented in a dossier [Documentation Supporting the Evaluation of Phytic Acid (50% Solution) as Generally Recognized as Safe (GRAS) for Use as a Food Ingredient] that was submitted by Tsuno to the Panel. In addition, the Panel evaluated other information deemed appropriate or necessary, including data and information provided by Tsuno. The data evaluated by the Panel included information pertaining to the method of manufacture and product specifications, analytical data, intended use levels in specified food products, consumption estimates for all intended uses, and a comprehensive assessment of the available scientific literature pertaining to the safety of phytic acid (50% solution).

Following independent, critical evaluation of such data and information, the Panel unanimously concluded that under the conditions of intended use in traditional foods described herein, phytic acid (50% solution), meeting appropriate food-grade specifications, and manufactured in accordance with current Good Manufacturing Practice (cGMP), is GRAS based on scientific procedures. A summary of the basis for the Panel's conclusion, excluding confidential data and information, is provided below.

SUMMARY AND BASIS FOR GRAS

Tsuno intends to market phytic acid (50% solution), produced from rice bran, as a food ingredient in the United States (U.S.) for use at levels providing up to 0.02% phytic acid in selected food categories, including energy, sports, and isotonic drinks, milk and non-milk based meal replacements, yogurt shots, , frozen vegetables, and pickles. It also is proposed for use in dietary supplement softgels at a maximum level of 8.0%.

Phytic acid (50% solution) is produced from food-grade defatted rice bran. The manufacture of phytic acid (50% solution) involves the addition of diluted sulfuric acid to the defatted rice bran to dissociate phytate from iron and protein complexes. The phytate solution is diluted with water, and sulfuric acid is then added to dissociate the bound minerals from phytate to release phytic acid. Downstream steps in the manufacturing process include filtration, decolorization, and ion-exchange to remove protein residues, metallic cations, and other impurities. Phytic acid (50% solution) is manufactured in accordance with cGMP and meets appropriate food grade specifications. Lot samples are routinely assayed to verify compliance with specifications. In addition, analysis of the defatted rice bran starting material indicates the absence of residual pesticides and herbicides. All reagents and processing aids used in the manufacture of phytic acid (50% solution) are food-grade materials. The specifications for phytic acid (50% solution) are provided in Appendix A.

Phytate, the salt of phytic acid, is the primary storage form for phosphorus in plants. It is consumed by humans through the intake of the seeds of cereals and legumes, and can be found at up to 9% of dry matter (Szkudelski, 2005; Schlemmer *et al.*, 2009). The average daily intake of phytic acid and other inositol phosphates from a Western diet is reported to range from 300 to 2,600 mg/day (Schlemmer *et al.*, 2009). In addition, the phytic acid content of the Mediterranean diet, often recommended for heart health, is reported to range from 700 to 1,400 mg/day (Miralpeix and Quer, 1989).

The consumption of phytic acid (50% solution) from all proposed food uses was estimated using the National Center for Health Statistics' (NCHS) 2003-2004 and 2005-2006 National Health and Nutrition Examination Surveys (NHANES) (CDC, 2006; USDA, 2009). Consumption data and information pertaining to the individual proposed food-uses of phytic acid (50% solution) were used to estimate the all-person and all-user intakes of phytic acid for specific demographic groups and for the total U.S. population. Collectively, on an all-user basis, the mean intake of phytic acid by the total U.S. population from all proposed food and supplement uses was estimated to be 50 mg/person/day or 0.8 mg/kg body weight/day. The heavy consumer (90th percentile) all-user intake of phytic acid by the total U.S. population from all proposed food and supplement uses was estimated to be 88 mg/person/day or 1.3 mg/kg body weight/day.

The safety of phytic acid (50% solution) under the intended conditions of use is supported by its natural occurrence in numerous foods commonly consumed in the diet, resulting in background

dietary intakes that are approximately 1,300 mg/day in U.S. adults (Schlemmer *et al.*, 2009). The addition of phytic acid (50% solution) to the proposed foods and supplements will only increase phytic acid intakes by a minor amount (50 and 88 mg/person/day at the mean and 90th percentile, respectively).

Safety also is corroborated by the findings of a number of repeated-dose studies conducted in rodents that included the assessment of endpoints related to safety. In these studies, no adverse effects were reported in rats fed phytic acid in the diet or in drinking water at doses of 1,000 to 2,500 mg/kg body weight/day for 14 days to up to 12 weeks (Hiasa *et al.*, 1992; Kamao *et al.*, 2000; Onomi *et al.*, 2004; Szkudelski, 2005; Lee *et al.*, 2006; Gaetke *et al.*, 2010).

In addition, several mutagenicity studies have been conducted on phytic acid in prokaryotic and eukaryotic test systems (Ishidate *et al.*, 1984; Seifried *et al.*, 2006). Phytic acid was reported to be non-mutagenic in the bacterial reverse mutation assay, with and without metabolic activation (Ishidate *et al.*, 1984). Similarly, negative results were reported in a mouse lymphoma assay (Seifried *et al.*, 2006) and a chromosomal aberration assay conducted in Chinese hamster ovary cells (Ishidate *et al.*, 1984). Phytic acid also was reported to be non-genotoxic in a mouse bone marrow micronuclei test (Ishidate *et al.*, 1988).

The results of two 32-week studies, as well as a 108-week study conducted in rats, also do not provide evidence of carcinogenicity attributable to phytic acid at the levels tested. Phytic acid was reported to have no effects on carcinogenicity when provided in the diet at a level of 2% (equivalent to approximately 877 or 2,000 mg/kg body weight/day) for 32 weeks in 2 rat studies (Hirose *et al.*, 1994; Takaba *et al.*, 1994). In the 108-week carcinogenicity study, rats given 1.25 or 2.5% phytic acid in drinking water (equivalent to approximately 275 and 572 mg phytic acid/kg body weight/day for males, respectively, and 302 and 605 mg phytic acid/kg body weight/day for females, respectively) did not exhibit an increase in the incidence of neoplasms in the testes, prostate, uterus, pituitary gland, adrenal glands, or in leukemia compared to control rats (Hiasa *et al.*, 1992). Kidney papillomas, accompanied by necrosis and calcification in the renal papillae of female rats exposed to phytic acid, occurred as a result of the high phosphorus levels in the drinking water consumed by the rats in the phytic acid groups. Hyperplasia in the renal pelvis of male rats given phytic acid in the drinking water also was attributed to the high phosphorus levels consumed. The addition of phytic acid (50% solution) from the proposed food uses would only result in a minor increase in phosphorus consumption, and thus, both of these effects are of little relevance to humans.

Although dietary phytate has been shown to affect mineral absorption in short-term studies, the addition of phytic acid to the proposed food uses is not expected to adversely affect long-term mineral bioavailability for several reasons: 1) the addition of a small amount of phytic acid to the diet from the proposed food uses will only result in minor increases in intake (50 and 88 mg/day at the mean and 90th percentile compared to background dietary intakes of 1,300 mg/day in the U.S.); 2) the consumption of a varied diet with adequate protein and mineral intake protects

against the effect of phytate on mineral utilization; 3) a number of dietary factors affect mineral absorption, many of which improve absorption and thus oppose the effects of phytic acid; 4) evidence suggests that there may be adaptation to long-term or habitual high-phytic acid intakes, leading to increased absorption of minerals.

From a critical evaluation of the available scientific data, it is concluded that phytic acid (50% solution) is safe for its intended use in food. The data and information summarized in this dossier demonstrate that phytic acid (50% solution), meeting appropriate food-grade specifications, would be GRAS based on scientific procedures under the intended conditions of use in foods, as described herein.

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CONCLUSION

We, the Expert Panel, have, independently and collectively, critically evaluated the data and information summarized above and conclude that the intended uses of phytic acid (50% solution), meeting appropriate food-grade specifications presented in the supporting dossier [Documentation Supporting the Evaluation of Phytic Acid (50% Solution) as Generally Recognized as Safe (GRAS) for Use as a Food Ingredient] and manufactured consistent with current Good Manufacturing Practices (cGMP), are safe and suitable.

We further conclude that the intended uses of phytic acid (50% solution) meeting appropriate food-grade specifications presented in the supporting dossier and manufactured consistent with cGMP are GRAS based on scientific procedures.

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

(b) (6)

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Appendix A

Product Specifications for Phytic Acid (50% Solution)

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Draft for Discussion

Product Specifications for Phytic Acid (50% Solution)		
Specification Parameter	Specification	Method
Visual appearance	Slight-yellowish or brownish liquid	Visual examination
Phytic acid content (%)	48 to 52	TSUNO TEST I ^a
Water content (%)	48 to 52	Calculation ^b
Heavy metals (as Pb) (ppm)	Less than 20	Colorimetric analysis (FCC, 2008 ^c)
Lead (ppm)	Less than 1	AAS (FCC, 2008)
Arsenic (ppm)	Less than 2	Arsenic limit test (FCC, 2008)
Total phosphorus (%)	13.5 to 14.6	UV spectrometry (FCC, 2008)
Inorganic phosphorus (%)	Not more than 1.0	Colorimetric analysis (FCC, 2008)
Chloride (%)	Not more than 0.04	Chloride limit test (FCC, 2008)
Sulfate (%)	Not more than 0.071	Sulfate limit test (FCC, 2008)
Microbiological Parameters		
Total aerobic bacterial count	Less than 500 CFU/mL	Chapter 3 (BAM, 2002 ^d)
Total mold and yeast count	Less than 500 CFU/mL	Chapter 18 (BAM, 2002)
<i>Escherichia coli</i> count	Less than 3 CFU/g	Chapter 4 (BAM, 2002)

AAS = atomic absorption spectrometry; CFU = colony forming unit; Pb = lead; ppm = parts per million; UV = ultraviolet

^a Internal method.

^b Water content is not assayed directly. Tsuno measures the concentration of phytic acid, and all substances other than phytic acid are considered to be water.

^c FCC (2008). *Food Chemicals Codex, 6th edition*. Rockville (MD): United States Pharmacopeial Convention (USP).

^d BAM (2002). *Bacteriological Analytical Manual Online (BAM)*. College Park (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety and Applied Nutrition (CFSAN), 2001-2005. Available from: <http://www.cfsan.fda.gov/~ebam/bam-toc.html> [Jan. 2001, Last Updated: Oct. 3, 2005].

APPENDIX B

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Expert Panel Consensus Statement Concerning the Generally Recognized as Safe (GRAS) Status of Phytic Acid (50% Solution) as an Ingredient in Food and Dietary Supplements Following Changes in Use Levels

As independent experts qualified by relevant national and international experience and scientific training to evaluate the safety of food ingredients, we, the undersigned, Professor Joseph F. Borzelleca, Ph.D. (Virginia Commonwealth University School of Medicine), Professor Robert J. Nicolosi, Ph.D. (University of Massachusetts Lowell), and Professor John Thomas, Ph.D. (Indiana University School of Medicine), were requested by Tsuno Food Industrial Co. Ltd. (Tsuno), as an Expert Panel to evaluate the impact of changes to the use levels on the Generally Recognized as Safe (GRAS) status of phytic acid (50% solution) under the conditions of intended use in conventional foods and dietary supplements.

Previously, the safety and GRAS status of phytic acid (50% solution) for use in foods and dietary supplements were critically evaluated by the Expert Panel. The Expert Panel concluded that the use of phytic acid (50% solution) at specified levels in the intended foods (maximum use level of 0.02%) and dietary supplements (maximum use level of 8%) was safe and GRAS based on scientific procedures. The mean and 90th percentile all-user intake of phytic acid by the total U.S. population from all previously intended food and supplement uses was estimated to be 50 mg/person/day (0.8 mg/kg body weight/day) and 88 mg/person/day (1.3 mg/kg body weight/day), respectively. Tsuno has since modified the intended use levels for their ingredient, with the use level increasing to 0.2% in the intended foods. A complete summary of the new intended use levels for phytic acid (50% solution) is presented in Table 1.

In the course of assessing the impact of changes in the use levels of phytic acid (50% solution), the Expert Panel reviewed intake estimates for the previous GRAS uses alongside the new estimated exposures from the changes in use levels, information present in the original GRAS dossier [*i.e.*, data pertaining to the method of manufacture and product specifications of phytic acid (50% solution), supporting analytical data, and a comprehensive assessment of the available scientific literature pertaining to the safety of phytic acid], and any additional relevant information.

Following independent, critical evaluation of such data and information, the Expert Panel unanimously concluded that under the modified conditions of intended uses and use levels in foods described herein, phytic acid (50% solution), meeting appropriate food-grade specifications and manufactured in accordance with current good manufacturing practices (cGMP), is safe and suitable and GRAS based on scientific procedures. A summary of the basis for the Expert Panel's conclusion is provided below.

INTENDED USES AND ESTIMATED EXPOSURE OF PHYTIC ACID (50% SOLUTION)

The intended food uses and use levels for phytic acid (50% solution) are detailed in Table 1. The maximum use levels of phytic acid (50% solution) in energy, sports, and isotonic drinks, non-milk and milk-based meal replacements, yogurt shots, frozen vegetables, and pickles were increased from 0.02% to 0.2%. The maximum use level in dietary supplements remains unchanged (8.0%).

Food Category	Proposed Food Uses	Original Maximum Use Level (%)	New Maximum Use Level (%)
Beverages and Beverage Bases	Energy, Sports, and Isotonic Drinks	0.02	0.2
	Non-Milk Based Meal Replacements	0.02	0.2
Milk Products	Milk Based Meal Replacements	0.02	0.2
	Yogurt Shots ^a	0.02	0.2
Processed Vegetables and Vegetable Juices	Frozen Vegetables	0.02	0.2
	Pickles	0.02	0.2
Supplement Products	Softgels	8.0	8.0

^a No codes for yogurt shots were identified and therefore, yogurt and fruit smoothies drinks were employed as a surrogate codes for this food use.

The consumption of phytic acid (50% solution) from all original intended uses and use levels was estimated using the National Center for Health Statistics' (NCHS) 2003-2004 and 2005-2006 National Health and Nutrition Examination Surveys (NHANES) (CDC, 2006; USDA, 2009). Under the previous intended food and supplement uses, 58.1% of the total U.S. population was identified as potential consumers of phytic acid (50% solution) (9,707 actual users identified). On an all-user basis, the mean intake of phytic acid by the total U.S. population from all proposed food and supplement uses was estimated to be 50 mg/person/day or 0.8 mg/kg body weight/day. The heavy consumer (90th percentile) all-user intake of phytic acid by the total U.S. population from all proposed food uses was estimated to be 88 mg/person/day or 1.3 mg/kg body weight/day.

Using the same method for estimating the intake of phytic acid for the amended list of food uses and use levels, the change in the intended use levels for phytic acid resulted in a 4- to 7-fold increase in exposure. The revised intakes of phytic acid for all population groups are presented in Tables 2 and 3 on a per person and per kilogram body weight basis, respectively.

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Table 2 Estimated Daily Intakes of Phytic Acid Based on all New Proposed Food and Supplement Uses and Use-Levels in the United States (NHANES 2003-2004, 2005-2006)							
Population Group	Age (Years)	% Users	Actual # of Total Users	All-Person Consumption (mg/day)		All-User Consumption (mg/day)	
				Mean	90 th Percentile	Mean	90 th Percentile
Infants	0 to 2	39.9	763	55	106	120	252
Children	3 to 11	60.0	1,640	97	249	160	436
Female Teenagers	12 to 19	60.5	1,203	102	250	170	534
Male Teenagers	12 to 19	63.7	1,235	259	776	400	1142
Female Adults	>20	58.7	2,512	101	224	167	367
Male Adults	>20	61.3	2,354	186	520	289	887
Total Population	All ages	58.1	9,707	137	320	223	610

Table 3 Estimated Daily Per Kilogram Body Weight Intakes of Phytic Acid Based on all New Proposed Food and Supplement Uses and Use-Levels in the United States (NHANES 2003-2004, 2005-2006)							
Population Group	Age (Years)	% Users	Actual # of Total Users	All-Person Consumption (mg/kg bw/day)		All-User Consumption (mg/kg bw/day)	
				Mean	90 th Percentile	Mean	90 th Percentile
Infants	0 to 2	39.9	763	4.7	8.5	10.2	19.9
Children	3 to 11	60.0	1,640	3.4	9.4	5.6	13.9
Female Teenagers	12 to 19	60.5	1,203	1.7	4.2	2.9	8.1
Male Teenagers	12 to 19	63.7	1,235	3.9	11.5	6.0	18.6
Female Adults	>20	58.7	2,512	1.4	3.0	2.4	5.4
Male Adults	>20	61.3	2,354	2.2	6.0	3.4	9.8
Total Population	All ages	58.1	9,707	2.2	5.6	3.7	9.4

Thus, the new intended food uses and use levels for phytic acid (50% solution) resulted in an increase in estimated mean and 90th percentile all-user intakes of phytic acid by the total U.S. population from 50 and 88 mg/person/day, respectively, to 223 and 610 mg/person/day, respectively.

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DATA SUPPORTING THE SAFETY OF PHYTIC ACID (50% SOLUTION)

The determination of the safety of phytic acid (50% solution) under the conditions of intended use is based on information on the background dietary consumption of phytic acid, as well as the results of published toxicological studies of phytic acid.

Phytate, the salt of phytic acid, is the primary storage mechanism for phosphorus in plants, which is consumed by humans through the intake of the seeds of cereals and legumes, and can be found at up to 9% of dry matter (Szkudelski, 2005; Schlemmer *et al.*, 2009). The daily dietary intake of phytate varies depending on the type of diet consumed (Schlemmer *et al.*, 2009). Individuals consuming diets containing high levels of cereal, whole grain, and other phytate-rich foods (*e.g.*, vegetarians) are reported to have higher levels of daily dietary phytate intake than those consuming diets low in plant-based foods.

Table 4 provides estimated daily intakes of phytic acid/phytate in North American and European populations (Schlemmer *et al.*, 2009). In male lacto-ovo-vegetarians in the U.S., phytate intakes are reported to be as high as 5,577 mg/day. Some European adults also consume large amounts of phytate, with reported levels reaching 2,191 mg/day in males >40 years of age in the United Kingdom, and 2,927 mg/day in Swedish adults consuming vegetarian diets. Given that the estimated intakes of phytic acid from the new proposed use levels are only a small fraction of background dietary intakes, it is not anticipated that the consumption of phytic acid (50%) solution under the intended conditions of use would present a safety concern.

Table 4 Estimated Daily Intake of Phytic Acid/Phytate in North America and Europe^a	
Subpopulation	Daily Intake of Phytic Acid/Phytate (mg)^b
United States	
Infants (<1 year)	166±167
Children (1 to 3 years)	390±231
Children (4 to 5 years)	501±271
Females (18 to 24 years)	395±334
Females (19 to 35 years)	1,293±666 ^c
Female vegetarians	~1,250±450
Male vegetarians	~1,550±550
Male lacto-ovo-vegetarian	5,577
Male lacto-ovo-vegetarian	972
Canada	
Children (4 to 5 years)	250 – 320
Lacto-ovo diets – Asian immigrants	1487±791
Mexico	
Male–female (18 to 30 months)	1,666±650
Male–female (7 to 9 years)	3,380±1,070

Table 4 Estimated Daily Intake of Phytic Acid/Phytate in North America and Europe^a	
Subpopulation	Daily Intake of Phytic Acid/Phytate (mg)^b
United Kingdom	
Male (> 40 years)	1,436±755
Male–female	600 – 800
Male–female	504-848
Italy	
Male–female	219 (112 – 1,367)
Male–female (average Italian diet)	293 (265 – 320)
Sweden	
Male–female (35 to 76 years), Western-type diets	369 (230 – 530)
Male–female (35 to 76 years), vegetarian diets	1,146 (500 – 2,927)

Values are presented as mean ± SD or range.

^a Adapted from Schlemmer *et al.*, 2009

^b Depending on data published (Schlemmer *et al.*, 2009)

^c Held *et al.* (1988) reported a wide range of daily phytate intake, ranging from 198 to 3,098 mg/day, in American students and university faculty female staff members consuming self-selected diets.

The safety of phytic acid (50% solution) is supported by the findings of several repeated-dose studies conducted in rodents that included the assessment of endpoints related to safety. In these studies, no-observed-adverse-effect levels (NOAELs) ranging from 1,000 to 2,500 mg/kg body weight/day were derived

Moreover, the results of two 32-week studies, as well as a 108-week study conducted in rats, do not provide evidence of carcinogenicity attributable to phytic acid at the levels tested.

Phytic acid was reported to have no effects on carcinogenicity when provided in the diet at a level of 2% (equivalent to approximately 877 or 2,000 mg/kg body weight/day) for 32 weeks in male F344 (Takaba *et al.*, 1994) and female Sprague-Dawley (Hirose *et al.*, 1994) rats, respectively. In a 108-week carcinogenicity study, F344 rats given 1.25 or 2.5% phytic acid in drinking water (equivalent to approximately 275 and 572 mg phytic acid/kg body weight/day for males, respectively, and 302 and 605 mg phytic acid/kg body weight/day for females, respectively) did not exhibit an increase in the incidence of neoplasms in the testes, prostate, uterus, pituitary gland, adrenal glands, or in leukemia compared to control rats (Hiasa *et al.*, 1992). Although kidney papillomas, accompanied by necrosis and calcification in the renal papillae, were reported in female rats administered phytic acid, this effect was attributed to the high phosphorus levels in the drinking water consumed by the rats in the phytic acid groups.

Mild or moderate hyperplasia reported in the renal pelvis of male rats fed phytic acid in the drinking water also was attributed to the high phosphorus levels consumed. Given that the papillomas and histopathological effects observed in the kidneys of phytic acid-exposed rats were attributed to the high phosphorus levels consumed by the rats rather than to the toxicity of phytic acid *per se*, a NOAEL of 2.5% phytic acid in the drinking water (equivalent to

approximately 572 and 605 mg phytic acid/kg body weight/day for male and female rats, respectively), the highest dose tested, can be determined.

Based on the information supporting the safety of phytic acid that was previously reviewed and the fact that the changes in the intended uses and use levels of phytic acid (50% solution) resulted in estimated intakes of the ingredient that are below the background dietary intakes of phytic acid, the changes in the proposed use levels do not impact the safety or GRAS status of phytic acid (50% solution).

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CONCLUSION

We, the Expert Panel, have independently and collectively critically evaluated the data and information summarized above and conclude that the proposed uses of phytic acid (50% solution), meeting food-grade specifications and produced in accordance with current good manufacturing practices (cGMP), are safe.

We further conclude that the proposed uses of phytic acid (50% solution), meeting food-grade specifications and produced in accordance with cGMP are Generally Recognized as Safe (GRAS) by scientific procedures.

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

(b) (6)

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Date: Mar. 6, 2009

R E P O R T

(ANALYSIS CERTIFICATE)

This is to certify that we, the undersigned, inspector authorized by the TSUNO RICE FINE CHEMICALS research laboratories, examined the commodity, and obtained the following results.

Commodity : Phytic Acid(50% Solution)
Quantity : sample
Packing :
Lot No. : 04511
Date of assay : Mar. 5, 2009

R E S U L T

Description : Phytic Acid (50%solution)occurs as slight yellowish or brownish viscous liquid. It is a myo-inositol hexaphosphate.
Heavy metals : Not more than 20 p.p.m.
Arsenic : Not more than 2 p.p.m.
Phytic acid contents : 48.8 %
Total phosphor : 13.94 %
Inorganic phosphor : 0.199 %
Chloride : Not more than 0.04 %
Sulphate : Not more than 0.071 %
Microbiological:
Total aerobic bacterial count : To pass test
Total mold and yeast count : To pass test
E.coli : To pass test
Drum No. :

TSUNO RICE FINE CHEMICALS CO., LTD.

(b) (6)

Chief of Analysis Section

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Date: Nov. 6, 2009

R E P O R T

(ANALYSIS CERTIFICATE)

This is to certify that we, the undersigned, inspector authorized by the TSUNO RICE FINE CHEMICALS research laboratories, examined the commodity, and obtained the following results.

Commodity	:	Phytic Acid(50% Solution)
Quantity	:	sample
Packing	:	
Lot No.	:	04608
Date of assay	:	Nov. 5, 2009

R E S U L T

Description	:	Phytic Acid (50% solution) occurs as slight yellowish or brownish viscous liquid. It is a myo-inositol hexaphosphate.
Heavy metals	:	Not more than 20 p.p.m.
Arsenic	:	Not more than 2 p.p.m.
Phytic acid contents	:	49.8 %
Total phosphor	:	14.19 %
Inorganic phosphor	:	0.169 %
Chloride	:	Not more than 0.04 %
Sulphate	:	Not more than 0.071 %
Microbiological:		
Total aerobic bacterial count	:	To pass test
Total mold and yeast count	:	To pass test
E.coli	:	To pass test
Drum No.	:	

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TSUNO RICE FINE CHEMICALS CO., LTD.

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Chief of Analysis Section

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Table D-1 Summary of Repeated Dose Studies on Phytic Acid, Sodium Phytate, and Soybean Protein Isolate that Included Safety-Related Endpoints

Species (Strain), Sex, and Number of Animals	Route of Administration, Study Duration, and Study Objective	Chemical	Dose (mg/kg bw/d)	Reported Effects ^{a,b}		Reference
Phytic Acid						
Rat (Wistar) M 5 to 8/group	Oral (diet) 20 days [Provided in diet at concentrations of 0, 0.1, 0.2, 0.3, or 1%] Study conducted to assess the hormonal and metabolic effects of phytic acid	Phytic acid	0, 100, 200, 300, or 1,000 ^c	General condition/survival	NR	Szkudelski, 2005
				Food and water intake	NE	
				Body weight	NSD in body weight	
				Organ and tissue effects	NSD in relative liver, kidney, adrenal glands, hypophysis, or testis weights [≥100]	
				Hematology, clinical chemistry, and urinalysis	↑ blood glucose levels [≥200] ↑ serum T ₃ [All groups] ↓ serum T ₄ [200] ↓ T ₃ /T ₄ ratio [≥300] ↑ serum FFA [100, 200] ↓ serum iron [1,000] NSD in serum insulin, triglycerides, cholesterol, calcium, and magnesium ↓ liver triglycerides [≥100] ↑ liver glycogen [≥100] NSD in liver cholesterol, total sulfhydryl groups, and non-protein sulfhydryl groups [≥100] ↑ muscle glycogen [≥100] NSD in muscle cholesterol [≥100]	
Rat (F344) M, F 10/sex/group	Oral (drinking water) 12 weeks Provided in drinking water at concentrations of 0, 0.6, 1.25, 2.5, 5, or 10% Dose-range finding study for	Phytic acid	0, 600, 1,250, 2,500, 5,000, or 10,000 ^c	General condition/survival	All animals died [10,000] All M died and 1 F died [5,000]	Hiasa <i>et al.</i> , 1992
				Food and water intake	NR	
				Body weight	Less than 10% ↓ in body weight [M, F, ≥2,500] (SSNR)	
				Organ and tissue effects	NR	

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Table D-1 Summary of Repeated Dose Studies on Phytic Acid, Sodium Phytate, and Soybean Protein Isolate that Included Safety-Related Endpoints

Species (Strain), Sex, and Number of Animals	Route of Administration, Study Duration, and Study Objective	Chemical	Dose (mg/kg bw/d)	Reported Effects ^{a,b}		Reference
	carcinogenicity study (see Section II.D.5)			Hematology, clinical chemistry, and urinalysis	NR	
Sodium Phytate						
Mouse (diabetic KK) M 10/group	Oral (diet) 8 weeks Provided in diet at concentrations of 0, 0.5, or 1% Study conducted to assess the effect of sodium phytate on blood glucose levels	Sodium phytate	0, 653, or 1,351 [approximately equivalent to 0, 637, and 1,307 mg/kg bw/d phytic acid ^d , respectively]	General condition/survival Food and water intake Body weight Organ and tissue effects Hematology, clinical chemistry, and urinalysis	NR NSD in food intake [≥653] ↑ food efficiency ratio in all test groups [≥653] NSD in initial and final body weights [≥653] NSD in liver, kidney, heart, spleen, and epididymal fat pad weights [≥653] NSD in non-fasting and fasting blood glucose levels [653] ↓ non-fasting and fasting blood glucose levels [1,351] ↓ glucose tolerance at 30 min after glucose injection [≥653] NSD in glucose tolerance at 60 and 120 min after glucose injection [≥653] ↓ glycosylated HbA1c levels [≥653] NSD in serum insulin levels [≥653]	Lee <i>et al.</i> , 2006
Rat (Wistar) M 5/group	Oral (diet) 14 days Provided in high-sucrose diet at concentrations of 0, 0.02, 0.1, 0.5, 2.5, 5.0, or 10.0%	Sodium phytate	0, 20, 100, 500, 2,500, 5,000, or 10,000 ^c [approximately equivalent to 0, 13, 65, 324, 1,623, 3,247, and 6,494 mg/kg bw/d]	General condition/survival Food and water intake Body weight Organ and tissue effects	NR ↓ food intake [≥5,000] ↓ body weight [≥5,000] NR	Onomi <i>et al.</i> , 2004

Table D-1 Summary of Repeated Dose Studies on Phytic Acid, Sodium Phytate, and Soybean Protein Isolate that Included Safety-Related Endpoints

Species (Strain), Sex, and Number of Animals	Route of Administration, Study Duration, and Study Objective	Chemical	Dose (mg/kg bw/d)	Reported Effects ^{a,b}		Reference
	Study conducted to assess the effects of sodium phytate on hepatic and serum lipid status		phytic acid ^d , respectively]	Hematology, clinical chemistry, and urinalysis	↓ serum triglyceride, cholesterol levels [10,000] ↓ serum phospholipids [100, 500, ≥5,000] ↓ hepatic total lipids and triglycerides [≥13] ↓ hepatic cholesterol [100, ≥2,500] NSD in hepatic phospholipids concentrations in all test groups [≥13] ↓ hepatic malic enzyme activity [≥500] ↓ hepatic FAS activity [10,000]	
Rat (Sprague-Dawley, weanling) M 6/group	Oral (diet) 25 days [Provided in diet to provide phytic acid: Zn molar ratio of 60:1] Study conducted to assess the effects of yogurt added to diets high in phytic acid and adequate in Zn (basal diet providing 38 µg Zn/g diet)	Sodium phytate	0 or 2,300 ^c phytic acid	General condition/survival	NR	Gaetke <i>et al.</i> , 2010
				Food and water intake	↑ food efficiency ratio on days 10	
				Body weight	↓ body weight on days 10, 15, 20, and 25 ↓ body weight gain on days 5, 10, and 25	
				Organ and tissue effects	↓ bone Zn concentrations NSD in bone Cu and Pb concentrations	
				Hematology, clinical chemistry, and urinalysis	↓ plasma Zn concentrations NSD in plasma Cu and Pb concentrations	
Soybean Protein Isolate (SPI)						
Rat (Wistar) M 7/group	Oral (diet) 5 weeks Diets supplemented with 20% phytate-free soybean protein (PFS) or soybean protein isolate (SPI) All diets had equal mineral contents	SPI	20,382 (PFS) or 19,691 (SPI) [approximately equivalent to 590 or 30.6 mg/kg bw/d of phytic acid ^e , respectively]	General condition/survival	NR	Kamao <i>et al.</i> , 2000
				Food and water intake	NSD in food intake or food efficiency ratio	
				Body weight	NSD in body weight	
				Organ and tissue effects	NSD in tissue weights No abnormalities in tissues or organs NSD in mechanical bone strength and bone ash weight	

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Table D-1 Summary of Repeated Dose Studies on Phytic Acid, Sodium Phytate, and Soybean Protein Isolate that Included Safety-Related Endpoints

Species (Strain), Sex, and Number of Animals	Route of Administration, Study Duration, and Study Objective	Chemical	Dose (mg/kg bw/d)	Reported Effects ^{a,b}	Reference
	Study conducted to assess the effect of SPI on mineral status			<p>↑ BMD [SPI vs. PFS] NSD in femur Ca and Mg concentrations</p> <p>Hematology, clinical chemistry, and urinalysis</p> <p>NSD in absorption and retention ratios of Ca, Mg, P, and Fe at 2 weeks ↑ Zn absorption and retention ratio at 2 weeks [SPI vs. PFS] ↑ absorption and retention ratio of Ca, Mg, and Fe at 5 weeks [PFS vs. SPI] ↑ P absorption at 5 weeks [PFS vs. SPI] NSD in P retention ratio at 5 weeks [All groups] NSD in Zn absorption and retention ratio [PFS vs. SPI] NSD in plasma Ca and P ↓ plasma Mg [SPI vs. PFS] ↓ plasma ALP [SPI vs. PFS] NSD in plasma PTH and 25(OH)D₃ ↓ plasma 1α25(OH)₂D₃ [SPI vs. PFS]</p>	

↓ = decrease(d); ↑ = increase(d); 1α25(OH)₂D₃ = 1α-hydroxyvitamin D₃; 25(OH)D₃ = 25-hydroxyvitamin D₃; ALP = alkaline phosphatase; BMD = bone mineral density; Ca = calcium; F = female; FAS = fatty acid synthase; FFA = free fatty acids; HbA1c = glycosylated hemoglobin; M = male; Mg = magnesium; NE = not evaluated (parameter not assessed by the authors); NOAEL = no-observed-adverse-effect level; NR = not reported (parameter evaluated by the authors, but no data were provided); NSD = no significant difference; P = phosphorus; PTH = parathyroid hormone; T₃ = triiodothyronine; T₃ = thyroxine; Zn = zinc

^a unless stated otherwise, all reported effects are relative to control group(s)

^b numbers in [] correspond to the dose(s) at which the reported effects were observed

^c dose was calculated using the FDA's "Conversion Table for Test Chemical Treatment Dose Used in PAFA" (U.S. FDA, 1993).

^d based on the molecular formulas for sodium phytate and phytic acid (ChemIDplus, 2010a,b).

^e SPI was reported to contain 1 to 5% phytic acid, and the phytic acid content of PFS was estimated to be 0.15% (Kamao *et al.*, 2000).

Table E-1 Summary of Developmental Toxicity Studies on Related Phosphate Compounds

Species (Strain) and Number of Animals	Route of Administration and Exposure Period	Chemical	Dose (mg/kg bw/d)	Reported Effects at Lowest-Observed-Effect Level ^{a,b}		Reference
Sodium Acid Pyrophosphate						
Mouse (CD-1) 22 to 25 F/group	Oral (gavage) GD 6 to 15	Sodium acid pyrophosphate	0, 3.35, 15.6, 72.3, or 335	Maternal Effects	<ul style="list-style-type: none"> No maternal toxicity NSE on nidation 	FDA, 1973a
				Fetal Effects	<ul style="list-style-type: none"> NSE on fetal survival NSD in incidence of abnormalities in soft or skeletal tissues 	
				NOAEL	<ul style="list-style-type: none"> 335 mg/kg bw/d 	
Rat (Wistar) 21 to 24 F/group	Oral (gavage) GD 6 to 15	Sodium acid pyrophosphate	0, 1.69, 9.24, 42.95, or 169	Maternal Effects	<ul style="list-style-type: none"> No maternal toxicity NSE on nidation 	FDA, 1973a
				Fetal Effects	<ul style="list-style-type: none"> NSE on fetal survival NSD in incidence of abnormalities in soft or skeletal tissues 	
				NOAEL	<ul style="list-style-type: none"> 169 mg/kg bw/d 	
Hamster (golden) 20 to 22 F/group	Oral (gavage) GD 6 to 10	Sodium acid pyrophosphate	0, 1.66, 7.71, 35.8, or 166	Maternal Effects	<ul style="list-style-type: none"> No maternal toxicity NSE on nidation 	FDA, 1973a
				Fetal Effects	<ul style="list-style-type: none"> NSE on fetal survival NSD in incidence of abnormalities in soft or skeletal tissues 	
				NOAEL	<ul style="list-style-type: none"> 166 mg/kg bw/d 	
Rabbit (Dutch-belted) 9 to 11 F/group	Oral (gavage) GD6 to 18	Sodium acid pyrophosphate	0, 1.28, 5.95, 27.6, or 128	Maternal Effects	<ul style="list-style-type: none"> No maternal toxicity NSE on nidation 	FDA, 1973a
				Fetal Effects	<ul style="list-style-type: none"> NSE on fetal survival NSD in incidence of abnormalities in soft or skeletal tissues 	
				NOAEL	<ul style="list-style-type: none"> 128 mg/kg bw/d 	
Sodium Hexametaphosphate						
Mouse (CD-1) 20 to 21 F/group	Oral (gavage) GD 6 to 15	Sodium hexametaphosphate	0, 3.7, 17.2, 79.7., or 370	Maternal Effects	<ul style="list-style-type: none"> No maternal toxicity 	FDA, 1974
				Fetal	<ul style="list-style-type: none"> NSE on fetal survival 	

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Table E-1 Summary of Developmental Toxicity Studies on Related Phosphate Compounds						
Species (Strain) and Number of Animals	Route of Administration and Exposure Period	Chemical	Dose (mg/kg bw/d)	Reported Effects at Lowest-Observed-Effect Level ^{a,b}		Reference
				Effects	<ul style="list-style-type: none"> NSD in incidence of abnormalities in soft or skeletal tissues 	
				NOAEL	<ul style="list-style-type: none"> 370 mg/kg bw/d 	
Rat (Wistar) 20 F/group	Oral (gavage) GD 6 to 15	Sodium hexametaphosphate	0, 2.4, 11.1, 51.7, or 240	Maternal Effects	<ul style="list-style-type: none"> No maternal toxicity NSE on nidation 	FDA, 1974
				Fetal Effects	<ul style="list-style-type: none"> NSE on fetal survival NSD in incidence of abnormalities in soft or skeletal tissues 	
				NOAEL	<ul style="list-style-type: none"> 240 mg/kg bw/d 	
Sodium Tripolyphosphate, anhydrous						
Mouse (CD-1) 20 to 24 F/group	Oral (gavage) GD 6 to 15	Sodium tripolyphosphate, anhydrous	0, 2.4, 11, 52, or 238	Maternal Effects	<ul style="list-style-type: none"> No maternal toxicity NSE on nidation 	FDA, 1973b
				Fetal Effects	<ul style="list-style-type: none"> NSE on fetal survival NSD in incidence of abnormalities in soft or skeletal tissues 	
				NOAEL	<ul style="list-style-type: none"> 238 mg/kg bw/d 	
Rat (Wistar) 20 F/group	Oral (gavage) GD 6 to 15	Sodium tripolyphosphate, anhydrous	0, 1.7, 8, 37, or 170	Maternal Effects	<ul style="list-style-type: none"> No maternal toxicity NSE on nidation 	FDA, 1973b
				Fetal Effects	<ul style="list-style-type: none"> NSE on fetal survival NSD in incidence of abnormalities in soft or skeletal tissues 	
				NOAEL	<ul style="list-style-type: none"> 170 mg/kg bw/d 	
Hamster (golden) 20 to 21 F/group	Oral (gavage) GD 6 to 10	Sodium tripolyphosphate, anhydrous	0, 1.41, 6.5, 30, or 141	Maternal Effects	<ul style="list-style-type: none"> No maternal toxicity NSE on nidation 	FDA, 1973b
				Fetal Effects	<ul style="list-style-type: none"> NSE on fetal survival NSD in incidence of abnormalities in soft or skeletal tissues 	
				NOAEL	<ul style="list-style-type: none"> 141 mg/kg bw/d 	

F = female; GD = gestation day; NOAEL = no-observed-adverse-effect level; NSE = no significant effect; NSD = no significant differences

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