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November 11, 2004

Division of Standards and Labeling Regulations
Office of Nutritional Products, Labeling and Dietary Supplements (HFS-820)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD
20740-3835

NOV 15 2004
OFB/FDA

Re: Premarket Notification for a New Dietary Ingredient (Arginine Silicate Inositol Complex)

Dear Sir or Madam:

Pursuant to Section 413(a) of the Federal Food, Drug, and Cosmetic Act and 21 CFR §190.6, on behalf of Nutrition 21, Inc. of Purchase, NY, please accept for filing the enclosed original and two copies of a New Dietary Ingredient Notification for Arginine Silicate Inositol (ASI) Complex. Nutrition 21, Inc., intends to market this ingredient for dietary supplement use.

The Notification includes a discussion of the basis upon which Nutrition 21, Inc. has concluded that its ingredient, ASI Complex, when used under the conditions recommended or suggested in the labeling of the dietary supplement, is reasonably expected to be safe. Included in the Notification are chemistry and manufacturing data; a description of the intended use; and a summary of the information supporting the safety of the ingredient. Copies of all referenced materials relevant to the safety of the ingredient have been provided.

We trust that the information provided meets the requirements of a New Dietary Ingredient Notification as indicated in 21 CFR §190.6. Please call, write or e-mail (jkomorowski@nutrition21.com) me should you have any questions regarding this submission.

Sincerely,

A handwritten signature in black ink that reads 'James Komorowski'. The signature is fluid and cursive, written in a professional style.

James Komorowski, M.Sc.
Vice President, Technical Services and Scientific Affairs

Encl.

**NEW DIETARY INGREDIENT NOTIFICATION FOR
ARGININE SILICATE INOSITOL (ASI) COMPLEX**

Submitted to: Division of Standards and Labeling Regulations
Office of Nutritional Products, Labeling and Dietary
Supplements
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD
20740-3835

Submitted by: Nutrition 21, Inc.
4 Manhattanville Road, Suite 202
Purchase, NY
10577

November 10, 2004

NEW DIETARY INGREDIENT NOTIFICATION FOR ARGININE SILICATE INOSITOL (ASI) COMPLEX

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**AMES BACTERIAL REVERSE MUTATION TEST OF
ARGININE INOSITOL POTASSIUM SILICATE COMPLEX**

(39 PAGES TOTAL)

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CONTAINS

TRADE SECRET

CONFIDENTIAL

COMMERICAL

INFORMATION

NEW DIETARY INGREDIENT NOTIFICATION FOR ARGININE SILICATE INOSITOL (ASI) COMPLEX

In accordance with the *Dietary Supplement Health and Education Act of 1994* (DSHEA), 21 U.S.C. §350b (a) (2), and with final regulations published in the *Federal Register* (1997, 62:49886-49892, 21 C.F.R. § 190.6) "Requirement for Premarket Notification", the following information is submitted by Nutrition 21, Inc. in support of a New Dietary Ingredient (NDI) Notification for Arginine Silicate Inositol (ASI) Complex.

Nutrition 21, Inc. intends to market ASI Complex as a dietary ingredient for supplement products in the United States. As per the statutes of the DSHEA, 21 U.S.C. § 350b (a) (2), Nutrition 21, Inc. will not introduce, market, distribute or sell ASI Complex until at least 75 days following official acknowledgement of the receipt of this notification by the U.S. Food and Drug Administration (FDA).

SECTION 1

1.1 The name and complete address of the distributor of the dietary ingredient

The distributor¹ of the dietary ingredient will be:

Nutrition 21, Inc.
4 Manhattanville Road, Suite 202
Purchase, NY
10577

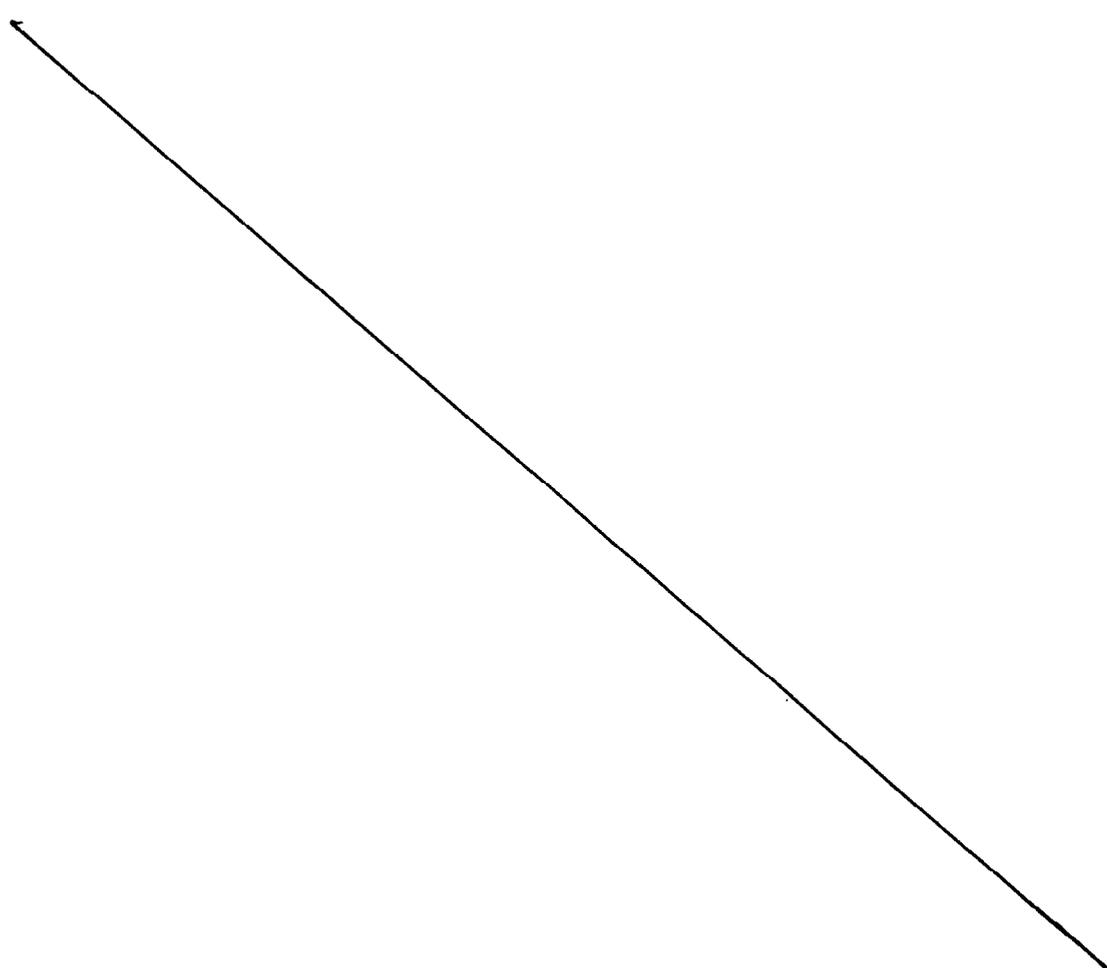
Contact: James R. Komorowski, M.Sc.
Vice President, Technical Services and Scientific Affairs
Tel: 914-701-4500

¹ Nutrition 21 will contract the manufacturing of the ingredient to a qualified manufacturer to be produced in accordance with Good Manufacturing Practices (GMP).

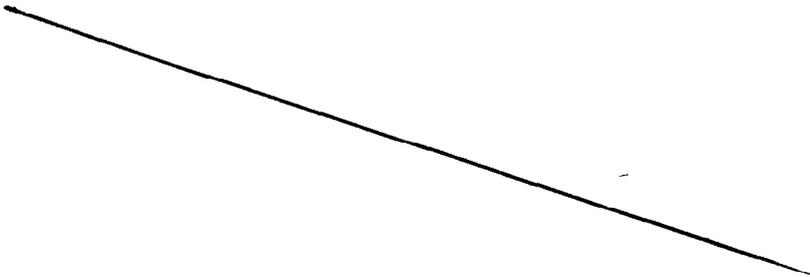
SECTION 2

2.1 The name of the dietary ingredient

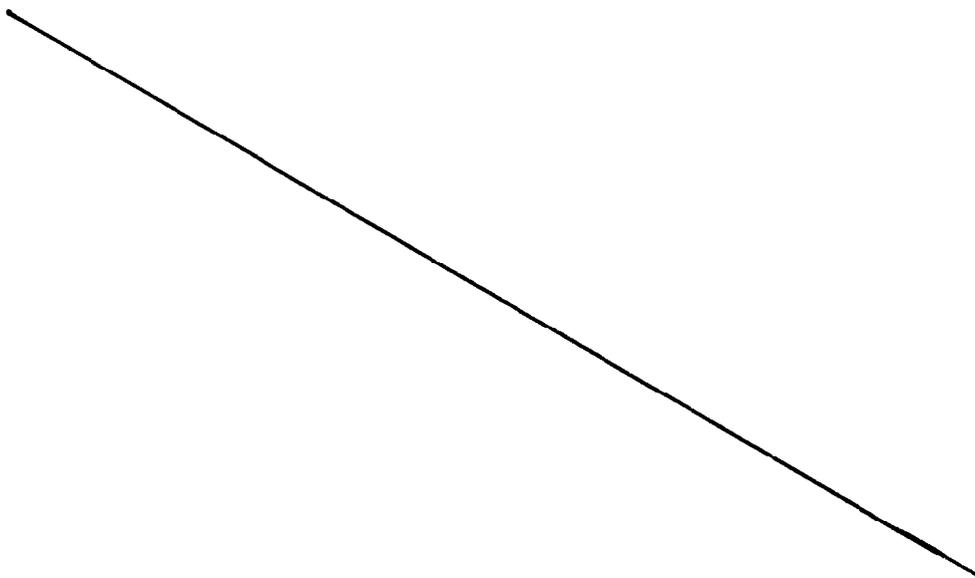
The new dietary ingredient is ASI Complex.



2.2 Manufacturing Information



**PRIVILEGED AND CONFIDENTIAL
DO NOT DISCLOSE OR REPRODUCE**



SECTION 3

3.1 Description of the dietary supplement

Description of the dietary supplement or dietary supplements that contain the dietary ingredient including (i) the level of dietary ingredient in the dietary supplement, and (ii) the conditions of use recommended or suggested in the labeling of the dietary supplement, or if no conditions of use are recommended or suggested in labeling of the dietary supplement, the ordinary conditions of use of the supplement.

ASI Complex will be marketed for use in products meeting the definition of "dietary supplement" in Section 201(ff) of the *Federal Food, Drug, and Cosmetic Act*, and will be clearly labeled and promoted as a dietary supplement. ASI Complex is currently intended to be sold in the form of oral tablets containing 750 mg of the arginine:silicon:inositol complex. Similar levels of the ingredient may be used in other dietary supplement formulations.

Using the levels outlined in the product specifications, under the current intended conditions of use of ASI Complex, each tablet will provide approximately 360, 60, and 187.5 mg of arginine, silicon, and inositol, respectively. Consumption of 2 tablets per day will be suggested or recommended, resulting in a recommended maximum daily consumption of 1,500 mg ASI Complex, or approximately 21.4 mg ASI Complex/kg body weight for a 70 kg person. The product label will indicate that ASI Complex is recommended for use in adults only, and that the product should not be used by pregnant or lactating women. The amounts of ASI Complex and its constituent dietary ingredients provided per tablet, per day, and per kilogram body weight are outlined in Table 1 below.

Ingredient	Per Serving	Per Day	
	mg/tablet	mg/day ^a	mg/kg bw/day ^b
ASI Complex	750	1,500	21.4
Total active ingredients	607.5^c	1,215	17.36
L-Arginine	360	720	10.29
Silicon	60	120	1.71
Inositol	187.5	375	5.36
Total inactive ingredients	142.5^d	285	4.04

^a Based on a recommended daily serving of 2 tablets/day

^b Based on an average body weight of 70 kg

^c Calculated using active ingredient levels outlined in the product specifications

^d ASI Complex also will contain inactive ingredients, such as water and dicalcium phosphate, which will bring the mass of each tablet to 750 mg

SECTION 4

The history of use or other evidence of safety establishing that the dietary ingredient, when used under the conditions recommended or suggested in the labeling of the dietary supplement, will reasonably be expected to be safe, including any citation to published articles or other evidence that is the basis on which the distributor or manufacturer has concluded that the dietary supplement will reasonably be expected to be safe.

The overall safety of ASI Complex is supported by the results of product-specific toxicology and related testing (see Section 4.1). The toxicology of ASI Complex has been investigated in oral studies in rats, including a single-dose study and a repeated-dose study of 8 weeks in duration. Additionally, mutagenicity studies were performed that examined the potential for genotoxic activity in the Ames test, a chromosome aberration assay, and a bone marrow micronucleus study.

In addition to toxicity data pertaining to ASI Complex, the safety of the individual components of ASI Complex (*i.e.*, arginine, silicon, and inositol) also has been assessed and is supported by the results of toxicology and related testing, clinical studies, and by data on their consumption directly in conventional food or as marketed as dietary supplements or over-the-counter products (see Sections 4.2 to 4.4). Furthermore, the Generally Recognized as Safe (GRAS) status of arginine and inositol, as well as the approved use of silica (silicon dioxide) and a variety of its salts as food additives, also provide supporting evidence for the safe use of ASI Complex as a dietary supplement.

4.1 Arginine Silicate Inositol (ASI) Complex

4.1.1 Acute Toxicity Study

In an acute toxicity study of ASI Complex, a single oral dose of 2,000 mg/kg body weight in 0.5% aqueous methylcellulose vehicle was administered to Sprague-Dawley rats (6/sex) (Devine, 2003). The study was conducted in accordance with Good Laboratory Practices (GLP), and the protocol was based on the Organization for Economic Co-operation and Development (OECD) guidelines for acute oral toxicity testing (OECD, 1992). Body weight, clinical signs, necropsy findings and mortality were all evaluated, and the animals were monitored for 14 days following dosing. All animals were sacrificed on Day 15 and then necropsied.

No deaths were reported, and there were no gross lesions reported following necropsy. Clinical signs of toxicity were limited to salivation, respiratory depressions, diarrhea, and tremors, which all resolved within 24 hours of dosing. Body weight was reported to increase in all rats over the

14-day observation period, with the exception of 1 female. Based on the results of this study, the LD₅₀ for ASI Complex in the rat can be considered to be greater than 2,000 mg/kg body weight, the highest dose tested in this study.

4.1.2 Repeated Dose Toxicity

ASI Complex was orally administered to male and female insulin-resistant JCR:LA-cp obese rats for 4- and 8-week periods, respectively, to determine the potential effect on metabolism and vascular function (Russell, 2003). Rats (8 to 10/group) were given access to a basic diet (obese control group), and the diets of the treatment groups were supplemented with a level of 1.81 g ASI Complex/kg diet or 1 g arginine hydrochloride/kg diet, such that each treatment group received an equivalent dose of arginine. Based on the body weights of the animals, these levels provided doses of 130 and 117 mg ASI Complex/kg body weight/day for males and females, respectively, and doses of 85 and 55 mg arginine hydrochloride/kg body weight/day for males and females, respectively. In addition to the obese group of control rats, an additional group of lean JCR:LA-cp rats was also used as a control (lean control group).

There were no deaths and no clinical signs of toxicity reported in any of the experimental animals. Treatment with ASI Complex or arginine hydrochloride was reported to have no significant effect on food intake in either sex relative to controls; however, the female rats treated with ASI Complex were reported to have a significantly lower rate of weight gain relative to females treated with arginine hydrochloride and relative to the obese female controls. There were no differences in weight gain between any of the male groups. The authors suggested that the difference in weight gain between male and female ASI Complex-treated rats might be due to the hormonal status and estrus cycling of the females. Obese rats treated with ASI Complex or arginine hydrochloride were reported to have significantly decreased levels of serum cholesterol, phospholipids, and triglycerides, relative to obese control rats. Urinary albumin excretion was not significantly different between groups of obese rats; however, it was significantly increased in each group of obese rats relative to lean control rats, which indicates that this effect occurred independent of arginine supplementation *via* ASI Complex or arginine hydrochloride. All obese rats were reported to have the presence of increased volume of Bowman's space and increased fissures and sclerosis relative to the lean control rats, which also indicates that this effect was independent of arginine supplementation. ASI Complex was reported not to have any adverse effect on bone metabolism, vascular function, or on renal or hepatic histology.

4.1.3 Genotoxicity Studies

The genotoxic potential of ASI Complex was examined in 3 studies, including an Ames bacterial mutagenicity assay, an *in vitro* chromosome aberration assay, and an *in vivo* mouse bone marrow micronucleus study. All 3 of these studies were conducted according to OECD protocols and were consistent with U.S. FDA Redbook guidelines.

4.1.3.1 Ames Assay

In the Ames assay, the potential mutagenicity of ASI Complex was investigated in *Salmonella typhimurium* strains TA97a, TA98, TA100, TA102, and TA1535, in the presence or absence of metabolic activation (S9) (Xu, 2003). This study was conducted in accordance with GLP and guidelines of the International Conference on Harmonisation (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH, 1997), OECD (1997), and U.S. Environmental Protection Agency (U.S. EPA, 1998). Following a range-finding toxicity study, the highest concentration selected in the presence of S9 was 5,000 $\mu\text{g}/\text{plate}$ due to a lack of toxicity. In the absence of S9, however, severe toxicity was observed at a concentration of 5,000 $\mu\text{g}/\text{plate}$; therefore a concentration of 4,000 $\mu\text{g}/\text{plate}$ was selected as the highest concentration. ASI Complex did not induce an increase in revertant colony numbers of TA98, TA100, TA102, or TA1535 strains at the highest doses tested; however, an equivocal response was reported in the TA97a strain in the presence and absence of S9, with less than a 2-fold increase in the number of revertant colonies and a tendency to a dose-response. As a result, an additional assay was performed in the TA97a strain only. Concentrations of 1,000, 1,500, 2,000, 2,500, or 3,000 $\mu\text{g}/\text{plate}$ in the presence of S9, and 500, 1,000, 2,000, 3,000, or 4,000 $\mu\text{g}/\text{plate}$ in the absence of S9, were selected in the second assay in TA97a. Results of the second assay were negative at all of the concentrations tested, clearly indicating ASI Complex was non-mutagenic in the Ames assay.

4.1.3.2 Chromosome Aberration Assay

In order to evaluate the clastogenic potential of ASI Complex, the compound was tested in an *in vitro* chromosome aberration assay using Chinese hamster ovary (CHO) cells in both the presence and absence of an Aroclor-induced S9 activation system (Gudi and Rao, 2004). This study was conducted in accordance with GLP and guidelines of the OECD (1998a) and U.S. EPA (1998). Following a preliminary range-finding toxicity assay, the concentrations selected for the chromosome aberration assay ranged from 325 to 4,000 $\mu\text{g}/\text{mL}$. ASI Complex was incubated in the non-activated test system for 4 or 20 hours, and for 4 hours in the activated test system. All cells were harvested 20 hours after treatment initiation, allowing cells incubated for 4 hours a 16-hour recovery period; however, no recovery period followed the 20-hour incubation. At concentrations of $\geq 1,500 \mu\text{g}/\text{mL}$, a visible precipitate was reported in the treatment medium, and concentrations of $\leq 750 \mu\text{g}/\text{mL}$ were reported to be soluble in the treatment medium. Selection of concentrations for microscopic analysis was based on cell growth inhibition (*i.e.*, the lowest dose with at least 50% reduction in mitotic index, as well as the additional 2 lower doses) in all harvests. The results of the chromosomal aberration assay are summarized in Table 2 below.

Treatment Time (hr)	Recovery Time (hr)	Harvest Time (hr)	S9	Cell growth inhibition at highest dose scored ($\mu\text{g/mL}$)	Mitotic Index Reduction ^a	LED ^b for Structural Aberrations ($\mu\text{g/mL}$)	LED for Numerical Aberrations ($\mu\text{g/mL}$)
4	16	20	-	13% at 1,500	51%	None	None
20	0	20	-	None at 1,500	57%	None	None
4	16	20	+	None at 2,000	57%	None	None

^a Relative to solvent control at high dose evaluated for chromosome aberrations

^b Lowest effective dose

The percentage of ASI Complex-treated cells with structural or numerical aberrations was not significantly greater than that for cells treated with the solvent control (water) at any dose level ($p > 0.05$, Fisher's exact test). The authors concluded that ASI Complex did not induce structural or numerical chromosome aberrations in CHO cells.

4.1.3.3 *Micronucleus Assay*

In an attempt to evaluate the potential of ASI Complex to increase the incidence of micronucleated polychromatic erythrocytes, a micronucleus assay was conducted (Gudi and Krsmanovic, 2004). The study was conducted in accordance with ICH (1996, 1997) and OECD (1998b) guidelines. In the *in vivo* mouse bone marrow micronucleus assay, 5 groups of ICR mice (5/sex/group) were treated with a single intraperitoneal injection of ASI Complex at doses of 50, 100, or 200 mg/kg body weight, with 3 groups of mice receiving 200 mg/kg body weight (high dose), and one of these groups serving as a replacement group. Three additional groups of mice served as positive and negative controls. The 2 negative control groups (5/sex/group) were administered corn oil, while the positive control group (5/sex) was administered cyclophosphamide monohydrate at a dose of 50 mg/kg body weight. All doses were established on the basis of initial toxicity studies in ICR mice. The mice from 5 of the study groups, which included one negative control group, the positive control group, the 50 and 100 mg ASI Complex/kg body weight groups, and one high-dose group, were euthanized 24 hours after dosing, while mice in the other negative control and one of the remaining 200 mg ASI Complex/kg body weight groups were euthanized 48 hours after dosing. Bone marrow cells were collected from all euthanized mice and were examined microscopically for the presence of micronuclei. There were no deaths reported in any of the mice following dosing; however, clinical signs in ASI Complex-treated mice included piloerection in both sexes at all doses, and lethargy in 3 high-dose mice (1/15 males and 2/15 females). There were no clinical changes reported in any of the control mice. A summary of the results of the bone marrow and micronucleus assay of ASI Complex is presented in Table 3.

Table 3 Summary of Bone Marrow Micronuclei Analysis in ICR Mice for Arginine Silicate Inositol (ASI) Complex						
Treatment ^a	Sex	Number of mice	PCE/Total Erythrocytes (Mean +/- SD)	Change from Control (%)	Micronucleated Polychromatic Erythrocytes	
					Number per 100 PCEs (Mean +/- SD)	Number per PCEs Scored ^b
At 24 hours Post Dosing						
Corn Oil (Negative Control)						
Corn Oil	M	5	0.469 ± 0.06	N/A	0.8 ± 0.27	8 / 10,000
	F	5	0.507 ± 0.05	N/A	0.7 ± 0.27	7 / 10,000
ASI Complex						
50 mg/kg bw	M	5	0.470 ± 0.04	0	0.5 ± 0.00	5 / 10,000
	F	5	0.474 ± 0.04	-7	0.6 ± 0.22	6 / 10,000
100 mg/kg bw	M	5	0.432 ± 0.03	-8	0.4 ± 0.22	4 / 10,000
	F	5	0.461 ± 0.05	-9	0.5 ± 0.00	5 / 10,000
200 mg/kg bw	M	5	0.434 ± 0.09	-7	0.6 ± 0.22	6 / 10,000
	F	5	0.416 ± 0.06	-18	0.7 ± 0.27	7 / 10,000
Cyclophosphamide Monohydrate (Positive Control)						
50 mg/kg bw	M	5	0.356 ± 0.03	-24	23.5 ± 2.18	235 ^b / 10,000
	F	5	0.328 ± 0.02	-35	24.7 ± 3.77	247 ^b / 10,000
At 48-hours Post Dosing						
Corn oil	M	5	0.469 ± 0.08	N/A	0.6 ± 0.22	6 / 10,000
	F	5	0.438 ± 0.03	N/A	0.6 ± 0.22	6 / 10,000
ASI Complex						
200 mg/kg bw	M	5	0.441 ± 0.05	-6	0.6 ± 0.22	6 / 10,000
	F	5	0.458 ± 0.03	5	0.7 ± 0.22	7 / 10,000

M = male; F = female; N/A = not applicable

^aVolume of dose was 20 mL/kg body weight

^b Statistically significant, $p \leq 0.05$ (Kastenbaum-Bowman Tables)

Microscopic examination of bone marrow cells indicated that the group mean ratio of polychromatic erythrocytes (PCE) to total erythrocytes was reduced in most ASI Complex treatment groups (6 to 18%), with the exception of males treated with 50 mg ASI Complex/kg body weight and euthanized 24 hours after dosing (no change), and females treated with 200 mg/kg body weight euthanized 48 hours after dosing (5% increase). The authors reported that the reductions suggest that ASI Complex does not inhibit erythropoiesis. There was no significant increase in the number of micronucleated PCEs in ASI Complex-treated groups relative to controls in either male or female mice at any dose level or bone marrow collection time. The authors concluded that ASI Complex does not induce formation of micronucleated polychromatic erythrocytes in the mouse micronucleus assay.

4.2 Arginine

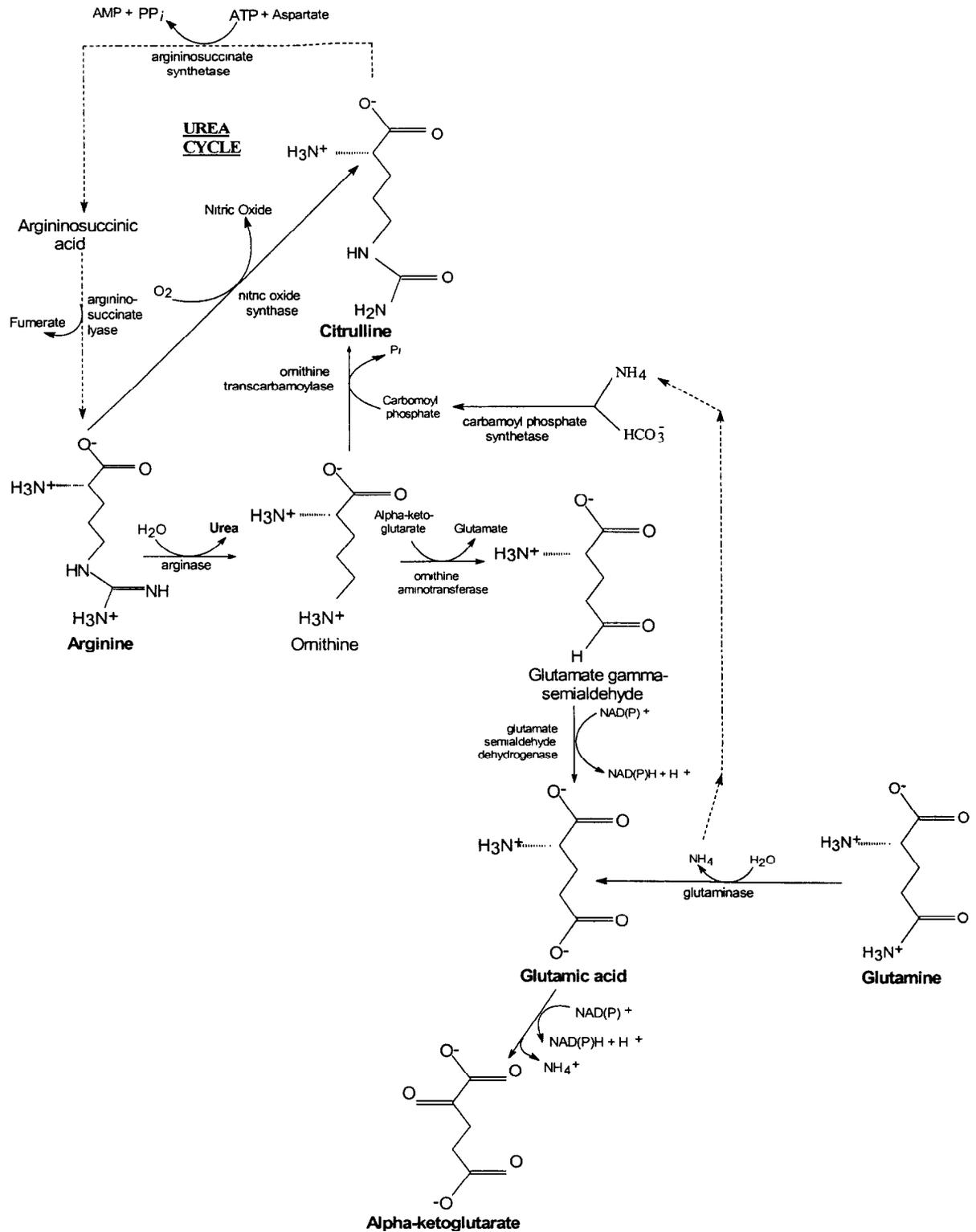
Arginine is an intermediate in the urea cycle, which is involved in eliminating toxic ammonia from the body as non-toxic urea (Kettner and Silbernagl, 1984; Rabier and Kamoun, 1995). In addition to being supplied *via* the diet, arginine also can be synthesized endogenously from citrulline, a urea cycle intermediate, in both the liver and kidney (FASEB, 1992). Although arginine is not an essential amino acid, it is considered conditionally indispensable given that a dietary source is required only when endogenous synthesis cannot meet metabolic needs (IOM, 2002). Arginine is consumed regularly in the diet as a constituent of plant and animal protein, and is approved for use as a food additive by the FDA (21 CFR §172.320); however, consumption of arginine as a food additive is insignificant compared to the amount consumed as a component of food proteins (Newberne *et al.*, 1998; FDA, 2004). Arginine is considered GRAS by the Flavor and Extract Manufacturers' Association (FEMA).

Since arginine can be produced endogenously, there is no set Recommended Daily Allowance (RDA) (IOM, 2002). In 2002, the Institute of Medicine (IOM) stated that there were insufficient data to establish an Upper Limit (UL) for any of the amino acids (IOM, 2002). As the average consumption of arginine in the human diet is 5.4 g per 100 g of protein (FASEB, 1992), and the mean and 90th percentile daily protein intakes for all individuals in the United States are 75.2 and 114.0 g, respectively, the mean daily intake of arginine from food is estimated to be 4.06 g, or 6.16 g for individuals in the 90th percentile (IOM, 2002). Additionally, doses of arginine of up to 21 g/day in dietary supplement form have been reported for treatment of cardiovascular dysfunction (PDRNS, 2001).

Each serving of ASI Complex will contain 360 mg of L-arginine, which is equivalent to a daily dose of 720 mg of arginine from ASI Complex under the current intended conditions of use. This is at least 82 and 88% lower than the mean and 90th percentile daily exposures from food, respectively, and up to 30-fold lower than daily exposures from other supplements containing arginine.

Arginine is an α -amino acid that is primarily absorbed through the intestinal mucosa following consumption (Nelson and Cox, 2000). Once absorbed, α -amino acids enter the portal blood and are transported into cells for incorporation into proteins by a variety of carrier systems of overlapping specificities (Kilberg, 1982). α -Amino acids are only found in the plasma in trace amounts, as the enzymes that catalyze the inclusion of amino acids into protein are extremely efficient (Nelson and Cox, 2000). Under normal conditions, α -amino acids that are not required for new protein synthesis undergo catabolism primarily in the liver (Nelson and Cox, 2000). Once in the liver, very little arginine leaves due to the high arginase activity present in the organ (Edmonds *et al.*, 1987). The catabolic pathway for arginine is outlined in Figure 2.

Figure 2 Catabolic Pathway for Arginine, Including the Urea Cycle



Adapted from Rabier and Kamoun (1995) and Nelson and Cox (2000)

Arginine is a glucogenic amino acid, as it forms α -ketoglutarate that may be converted to glucose *via* gluconeogenesis in the liver (Nelson and Cox, 2000). Following hepatic catabolism, arginine and arginine metabolites are subsequently excreted in the urine, as there is no storage of excess amino acids in humans (Nelson and Cox, 2000). Renal tubular resorption regulates the excretion of α -amino acids, which are conserved in the proximal tubules (Nelson and Cox, 2000). The daily urinary excretion of α -amino acids is approximately 20 to 150 mg/day in non-pregnant humans (Tietz, 1996).

L-arginine (as an arginine-glucose mixture heated for 30 minutes at 100°C) was tested for mutagenicity in *S. typhimurium* strains TA98 and TA100. Slightly elevated revertant counts were reported in TA100 without S9 activation, but negative results were reported in TA98 with and without metabolic activation, as well as in TA100 with metabolic activation (Aeschbacher *et al.*, 1981).

The potential effect of chronic administration of L-arginine was examined in male and female Sprague Dawley rats (Tsubuku *et al.*, 2004). L-arginine was administered to rats (n=75) in a standard diet at levels of 0, 1.25, 2.5, or 5% *ad libitum* for a period of 13 weeks, beginning at 6 weeks of age. The authors calculated the dose of L-arginine ingested by the rats in the 5% treatment group to be approximately 3.3 and 3.9 g/kg body weight/day for male and female rats, respectively. The rats were examined for clinical signs of toxicity twice daily during the dosing period and daily for 5 weeks after dosing. Ophthalmologic examination, hematology, blood chemistry analysis, pathology, histopathology, and urinalysis also were conducted at various time points during and after the dosing period. No compound-related changes were observed in either male or female rats, and the researchers established a no-observed-adverse-effect level (NOAEL) for both sexes of 5% L-arginine, or approximately 3.3 to 3.9 g L-arginine/kg body weight/day, the highest dose tested.

A review conducted by Preli *et al.* (2002) investigated the results of 22 studies examining the potential effect of L-arginine supplementation on vascular health in hypercholesterolemic rabbits. Rabbits administered L-arginine *ad libitum* in drinking water at doses up to 2.25% [providing approximately 3,937 mg/kg body weight (FDA, 1993)] for up to 14 weeks were not reported to have compound-related adverse effects (Jeremy *et al.*, 1996). Similar results were reported in hypercholesterolemic knockout mice administered 2.25% L-arginine *ad libitum* in drinking water [providing approximately 5,625 mg/kg body weight (FDA, 1993)] for 6 months (Aji *et al.*, 1997). Furthermore, apolipoprotein E-deficient mice (*i.e.*, mice with reduced endothelium-derived nitric oxide activity) were not reported to have any compound-related adverse effects following administration of 6% L-arginine in drinking water [providing approximately 15,000 mg/kg body weight (FDA, 1993)] for up to 8 weeks (Maxwell *et al.*, 2001).

Due to the limited availability of preclinical safety studies on L-arginine, studies investigating L-arginine salts are also included to support the safety of L-arginine. L-arginine hydrochloride is reported to have an oral LD₅₀ of 12,000 mg/kg body weight in the rat (RTECS, 2000). Additionally, rats orally administered arginine hydrochloride at a dose of 1,000 mg/kg body weight/day for up to 7 days were reported to exhibit no adverse effects (Drago *et al.*, 1984). Similarly, administration of L-arginine hydrochloride in the diet at doses of up to 4,500 mg/kg body weight/day for 15 days also was without adverse effects (Ronnenberg *et al.*, 1991).

Intraperitoneal administration of L-arginine hydrochloride to pregnant rats during Days 1 to 6 of gestation at a dose of 15 mg/kg body weight/day was reported to result in 5 resorption sites and hind limb malformations in 43% of fetuses (Naidu, 1973). The authors reported that it was difficult to determine whether arginine was embryotoxic or whether toxicity was possibly due to accumulated degradation products or consequences of secondary amino acid imbalance.

L-arginine has been administered orally in a variety of clinical studies investigating conditions such as cardiovascular disease, diabetes, and erectile dysfunction, among others. The vascular effects of dietary L-arginine supplementation were investigated in two separate literature reviews conducted by Preli *et al.* (2002) and Cheng and Baldwin (2001). These publications reviewed the results of 39 clinical trials. The clinical trials included double blind, placebo-controlled studies, some of which were crossover in design, and ranged in duration from 1 day to 6 months. Dosing regimes for L-arginine supplementation in healthy volunteers included a single dose of 7 g, a daily dose of 21 g for 30 days, or 9 g/day for up to 6 months (Adams *et al.*, 1995, 1997; Lerman *et al.*, 1998). No significant compound-related adverse effects were reported in any of the reviewed trials; however, side effects were reported in a few patients and included gastrointestinal complaints such as diarrhea, nausea, and abdominal cramping. In placebo-controlled trials, the reported side effects were not significantly different between L-arginine and placebo treatment groups. Of the 39 trials reviewed, 28 indicated that oral L-arginine supplementation provided significant benefit to vascular health, as evidenced by decreased platelet aggregation and adhesion, decreased monocyte adhesion, and endothelium-dependant vasodilation.

Luiking *et al.* (1998) orally administered L-arginine to 8 healthy male volunteers to investigate the effect of L-arginine supplementation on esophageal motility and gallbladder dynamics. Volunteers were administered a dose of 30 g L-arginine/day for a period of 8 days. Although this is not a traditional safety study, no adverse effects were reported by the authors, and this study provides support that L-arginine is well tolerated in humans at doses of up to 30 g/day for a period of 8 days.

In a study of 24 hypercholesterolemic patients orally administered 14 g of L-arginine/day for a period of 12 weeks, no significant changes in plasma insulin, growth hormone, or biochemical

parameters measured to assess hepatic and renal function were reported (Chan *et al.*, 2000). The hepatic and renal safety parameters included serum glutamine oxaloacetic transaminase, serum glutamic pyruvic transaminase, alkaline phosphatase, albumin, blood urea nitrogen, and creatinine. Additionally, there were no adverse effects reported in any of the patients.

Park *et al.* (1992) reported that oral supplementation of L-arginine at a dose of 30 g/day for a period of 3 days to patients with breast cancer (number not specified) significantly increased their rate of tumor protein synthesis. Subsequent studies have failed to replicate these findings and moreover, a number of preclinical studies have reported that L-arginine supplementation suppresses tumor growth (Milner and Stepanovich, 1979; Tachibana *et al.*, 1985; Barbul, 1986).

Barbul *et al.* (1990) investigated the effect of arginine hydrochloride on wound healing and lymphocyte immune response in 12 healthy, non-smoking volunteers. Subjects were orally administered a dose of 30 g arginine hydrochloride/day (equivalent to 24.8 g of L-arginine) for 2 weeks and effects were compared with a placebo group (sex not specified; n=12). Mild hyperchloremic acidosis was reported (significance not discussed), along with a significant increase in serum chloride and significant compensatory decrease in serum potassium, calcium, and bicarbonate. No significant effect was reported on either blood urea nitrogen or creatinine. Side effects were reported in 6 of 12 treatment subjects and included bloating, mild anorexia, and diarrhea; however, they were reportedly not severe enough to interfere with regular activities. One patient in the placebo group also experienced the aforementioned side effects. The authors reported that the side effects were due to osmotic load, and recommended that subjects should space out supplementation over the course of the day, as timing of supplementation was not rigorously controlled in this study.

L-arginine is reported to interact with cyclosporin, ibuprofen, organic nitrates, sildenafil citrate, and the herb yohimbe (PDRNS, 2001). The antinaturetic effect of cyclosporin may be counteracted by arginine, while the effects of organic nitrates, sildenafil citrate, and yohimbe may be potentiated. Additionally, arginine is reported to increase the absorption of ibuprofen if taken concomitantly (PDRNS, 2001). Arginine should not be consumed by individuals with argininemia, a genetic disorder resulting from a deficiency in arginase (Brusilow and Horwich, 1989; PDRNS, 2001). Clinical manifestations of the disorder include recurrent vomiting, seizures, spastic diplegia, psychomotor retardation, and delayed physical growth (Kang *et al.*, 1983). Arginine also is contraindicated in individuals with hypersensitivity to any component of an arginine-containing preparation (PDRNS, 2001).

4.3 Silicon

As the second most abundant element on earth, silicon is consumed regularly in the diet in a variety of foods such as grains, root vegetables, fruits, beer, and several types of meats,

including pork, beef, chicken, and lamb, and also in water, milk, coffee, and tea (Pennington, 1991, Merck, 2001). Additionally, refined and processed foods are high in silicon, and silicate additives have been increasingly used in prepared foods and confections as anti-caking agents (T.J. Clark & Co., 2004). Most dietary forms of silicon are poorly absorbed despite the fact that silicon is readily available from a variety of foods (PDRNS, 2001; Jugdaohsingh *et al.*, 2002). Silicon that is absorbed is mainly excreted in the urine (PDRNS, 2001).

Silicon dioxide, and a number of its silica salts, including calcium silicate, tricalcium silicate, magnesium silicate, silica aerogel, aluminum calcium silicate, sodium calcium aluminosilicate, and sodium metasilicate, are approved for use as food additives and/or are considered GRAS by the FDA (21 CFR §160.105; §169.179; §173.310; §182.1711; §182.2729). In addition, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) has approved potassium aluminum silicate and aluminum silicate as anticaking agents.

Silicon does not occur freely in nature, and is present as silica or as silicate (Merck, 2001). The silicon present in ASI Complex is supplied by potassium silicate, which is added to the product to provide a level of silicon of 60 mg/tablet, or 120 mg silicon/day under the recommended conditions of use of the product. This level is similar to the level of silicon present in the over-the-counter product, GAVISCON® Regular Strength Antacid Tablets, which contains 20 mg magnesium trisilicate/tablet (PDR, 2003). Under the recommended conditions of use of up to 16 tablets/day, the daily dose of magnesium trisilicate from GAVISCON® Regular Strength Antacid Tablets would be 320 mg, providing 103.36 mg silicon/day.

The daily human requirement for silicon is 3 to 5 mg, and the recommended intake is 5 to 10 mg/day; however, the average daily intake of silicon is 20 to 50 mg (Seaborn and Nielsen, 1993; Jugdaohsingh *et al.*, 2002), and due to its presence in a large variety of foods and food types (Pennington, 1991), it can reasonably be assumed that 90th percentile intakes could be much higher. In 2000, the IOM reported that there were inadequate safety data to establish a NOAEL for silicon (IOM, 2001). Moreover, there were insufficient data to establish an adequate intake (AI) or tolerable upper intake level (UL) in humans; however, the IOM reported that there was no evidence that silicon that occurs naturally in food or water produces adverse health effects (IOM, 2001).

Silica was reported to be non-genotoxic in the Rec Assay with *Bacillus subtilis* strains H17 and M45 when tested at concentrations of 0.005 to 0.5 M (Kanematsu *et al.*, 1980). Silica was similarly reported to be negative in a Rec Assay conducted by Kada *et al.* (1980).

McClendon *et al.* (1958) investigated the potentially toxic effect of silica supplementation in rats. Six different forms of silica, including 3 particle sizes of silica, silica gel, sodium metasilicate, and silica fibers, were administered to rats (10/group) at a level of 10% in the diet [providing

approximately 2,500 mg/kg body weight (FDA, 1993)] for 3 months. A control group (n=10) was administered the same diet, unsupplemented, under the same treatment conditions. Following the dosing period, all rats were sacrificed and histological preparations were made of the stomach, small and large intestine, liver, spleen, pancreas, adrenals, mesenteric lymph nodes, and lung. No lesions were reported in any of the organs examined.

Chronic oral administration of silica in the diet to 40 B6C3F₁ mice and Fisher rats was reported to have no compound-related adverse effects (Takizawa *et al.*, 1988). Mice and rats were administered silica at levels of 0, 1.25, 2.5, or 5% in the diet [providing approximately 0, 1,875, 3,750, or 7,500 mg/kg body weight in mice and 0, 1,250, 2,500, or 5,000 mg/kg body weight in rats, respectively (FDA, 1993)] for 21 and 24 months in mice and rats, respectively. Interim sacrifices were made in both species at 6 and 12 months. Although transient changes were observed in a number of the parameters tested, no significant effects were reported at the end of the 21- or 24-month dosing period relative to controls. Silica administration was therefore reported to have no significant effect on survival, body weight, food consumption, or clinical signs of toxicity, in either species at any dose, and there were no carcinogenic effects reported. Additionally, hematological and clinical chemistry examinations, as well as gross examination of the lungs, bronchus, heart, kidneys, liver, spleen, brain, stomach, colon, intestines, pancreas, adrenals, pituitary, thyroid, salivary glands, thymus, testes, prostate, bladder, ovaries, uterus, oviduct, femoral bones, mammary glands, skin, and subcutis revealed no significant changes relative to controls following chronic silica administration in either mice or rats. Based on the results of this study, silica is considered to have low oral toxicity following chronic administration.

Administration of supplemental dietary silicon to mares for 45 days after foaling at a level of 16.5 g/day was not reported to cause any adverse effects in either the mares or in their nursing foals (Lang *et al.*, 2001). The level of exposure to silicon *via* the maternal milk was not reported.

Clinical studies investigating the effects of silicon supplementation are limited; however, the available studies indicate that silicon, silica, and its salts are well tolerated (Van Dyck *et al.*, 1999; Jugdaohsingh *et al.*, 2000). Jugdaohsingh *et al.* (2000) investigated the pharmacokinetics of a soluble silica polymer, oligomeric silica, in 5 volunteers (3 male, 2 female). Each volunteer ingested a single dose of 1.2 mmol silicon in solution, equivalent to 33.7 mg silicon. There were no adverse effects reported by the authors. Similarly, no adverse effects were reported when the bioavailability of silicon from 3 different sources (*i.e.*, a silicon-rich diet, a horsetail supplement, and a silicon solution) were investigated in a single female volunteer (Van Dyck *et al.*, 1999). The silicon-rich diet provided an exposure of approximately 45 mg silicon/day for 31 days. The horsetail extract tablets provided an exposure of approximately 23 mg silicon as silicic acid/day for 7 days, and the silicon solution provided an

exposure of 10 mg silicon/day, also for 7 days. Each supplementation experiment was conducted on 3 separate occasions.

4.4 Inositol

Inositol is consumed regularly in the diet with an estimated intake from food of 1 g/day (PDRNS, 2001). Phytic acid is the major form of dietary inositol, and is found in a large variety of grains and legumes. Inositol-containing phospholipids also are commonly consumed as part of both plant and animal sources. Inositol is considered GRAS by the FDA (21 CFR §184.1370), and in agreement with 21 CFR §184.1(b)(1), inositol can be added to food as a nutrient supplement without limitation other than current Good Manufacturing Practices (GMP). Each serving of ASI Complex will contain 187.5 mg of inositol, providing a daily exposure of 375 mg inositol under the recommended conditions of use of the product. Therefore, exposure from the recommended daily dose of ASI Complex is less than the estimated daily intake of inositol from food.

Dietary inositol is readily absorbed from the small intestine, and is used in a wide variety of metabolic processes in tissues throughout the body. Inositol can cross the blood-brain barrier (Kofman *et al.*, 1998), and therefore has been investigated for its potential in the treatment of psychiatric disorders (*e.g.*, depression, panic, obsessive-compulsive disorder) (Levine, 1997; Colodny and Hoffman, 1998). Pre-clinical behavioral and anticarcinogenicity studies have demonstrated that inositol has low oral toxicity in laboratory animals (Einat *et al.*, 1999a,b; Hecht *et al.*, 2001). No adverse effects were reported in rats at doses of up to 10% inositol in the diet [providing approximately 5,000 mg/kg body weight (FDA, 1993)], or in mice at doses of up to 1% inositol in the diet [providing approximately 1,500 mg/kg body weight (FDA, 1993)] (Einat *et al.*, 1999b; Hecht *et al.*, 2001). Clinical studies investigating the potential effects of inositol in psychiatric disorders have demonstrated that inositol is well tolerated in humans at doses of up to 12 g/day for a period of 4 weeks, with no report of adverse effects (Benjamin *et al.*, 1995; Levine *et al.*, 1995). A few reported side effects have included insomnia, nausea, and flatus (Benjamin *et al.*, 1995; Levine *et al.*, 1995; Barak *et al.*, 1996; Levine, 1997; PDRNS, 2001). Inositol has been reported to result in beneficial behavioral effects in animals and in humans with psychiatric disorders. Although there are no traditional safety studies available for inositol, the high tolerability and lack of adverse effects in its clinical uses support the safety of this dietary ingredient.

Inositol may have additive effects with specific serotonin reuptake inhibitors (SSRIs), and reportedly should be avoided by pregnant and nursing women due to a lack of relevant long-term safety data (PDRNS, 2001).

ASSESSMENT OF SAFETY

ASI Complex contains arginine, silicon, and inositol. Under the current intended conditions of use, the recommended daily intake of ASI Complex is 1,500 mg/day (or approximately 21.4 mg/kg body weight/day assuming a body weight of 70 kg). Using the levels outlined in the product specifications, this will provide a maximum daily intake of 360, 60, and 187.5 mg of arginine, silicon, and inositol, respectively. The recommended daily intake of ASI Complex provides exposures to the individual components of ASI Complex that are similar or less than exposures from the diet. As humans are already exposed to these components either directly or indirectly through the diet, ASI Complex is not expected to present unknown risks to human health.

The safety of the new dietary ingredient, ASI Complex, is supported by existing dietary exposures to the components of the NDI through the consumption of a variety of foods, and by product-specific animal toxicology and genotoxicity studies. These studies indicate that ASI Complex does not cause adverse effects when administered orally for up to 8 weeks in rats, and also lacks mutagenic potential as demonstrated in 3 separate genotoxicity studies.

Additionally, preclinical and clinical studies on the components of ASI Complex indicate that arginine, silicon, and inositol have low oral toxicity in experimental animals and humans. Short-term clinical studies have demonstrated that no adverse effects are observed following administration of doses of up to 21 g arginine/day for 30 days (Adams *et al.*, 1995), while longer-term studies of 12 and 24 weeks support the safety of supplementation of 14 and 9 g arginine/day, respectively (Lerman *et al.*, 1998; Chan *et al.*, 2000). Similarly, no adverse effects were observed following administration of doses of 12 g inositol/day for 4 weeks (Benjamin *et al.*, 1995; Levine *et al.*, 1995) or 45 mg silicon/day for 31 days (Van Dyck *et al.*, 1999). The safety of silicon is further supported by a 21-month study in mice and a 2-year study in rats in which the animals were fed levels of silicon providing doses of up to 7.5 and 5 g silicon/kg body weight/day, respectively, which, on a body weight basis, are greater than 1,000 times the level of silicon provided under the recommended conditions of use of ASI Complex.

Based on the available data, we have concluded that Arginine Silicate Complex is safe for use as a New Dietary Ingredient under the current intended conditions of use and as an ingredient in other potential dietary supplement formulations.

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