Application for the Approval of the Use of Inositol-Stabilized Arginine Silicate as a Source of Arginine in the European Union

Under

Regulation (EC) No 258/97 of the European Parliament and of the Council of 27th January 1997 Concerning Novel Foods and Novel Food Ingredients

Non-Confidential

Submitted by:

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18 May 2016

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EXECUTIVE SUMMARY

Approval is sought under Regulation (EC) No 258/97 of the European Parliament and of the Council of 27th January 1997 concerning novel foods and novel food ingredients, for the approval of Inositol-Stabilized Arginine Silicate (ASI) as a new source of arginine for use in foods for particular nutritional uses (PARNUTS)/foods for specific groups, as an ingredient in food supplements and in nutrition bars for sportsmen.

ASI has high purity (>90%, as indicated by product specifications in Section I.e), and has well-defined specifications and a simple but tightly controlled production process which demonstrates an absence of contaminants. In addition, stability and microbiological analyses performed on the final product support the safety of ASI for use as an ingredient in PARNUTS/foods for specific groups, food supplements and nutrition bars for sportsmen.

The recommended total intake of ASI is 1,500 mg (*i.e.,* providing 675 mg arginine, 435 mg silicon, 390 mg inositol, and 75 mg potassium) per day. On a body weight basis, these doses are approximately equal to 9.6, 6.2, 5.6, and 1.1 mg/kg body weight/day (respectively) for a 70-kg adult. In the case of nutrition bars this daily dose would be delivered typically in 2 to 3 servings.

The safety of ASI is based on a lack of relevant adverse effects reported in studies of ASI (manufactured by Nutrition 21) and its components, arginine, silicate, and inositol. The safety of ASI is supported by product-specific animal toxicology and genotoxicity studies. The results of studies indicate that ASI 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. The results of 2 studies in healthy men demonstrate that ASI dissociates in the acidic environment of the stomach, provides a bioavailable source of L-arginine and silicon, and does not cause adverse effects on blood pressure or heart rate.

Furthermore, as *in vitro* dissociation data demonstrate that the arginine silicate component of ASI dissociates upon ingestion, safety data for each of the individual components of ASI can be used to support its safety. The preclinical and clinical studies on the components of ASI indicate that arginine, silicon, inositol, and potassium have low oral toxicity in experimental animals and humans.

- Results of short-term clinical studies indicate a lack of adverse effects upon administration of up to 21 g arginine/day for 30 days (Adams *et al.*, 1995), while results of longer-term studies support the safety of 14 or 9 g arginine/day, for up to 12 or 24 weeks, respectively (Lerman *et al.*, 1998; Chan *et al.*, 2000).

- The safety of silicon is supported by the permitted use of various silicate salts as food additives in the European Union (EU) and available toxicology data on sodium silicate and sodium metasilicate (European Commission, 2015).

- Inositol is produced by the human kidneys at a rate of approximately 4 g/day, an amount which is considerably greater than the typical dietary intake of inositol (Holub, 1986). Similarly, no adverse effects were observed following administration of up to 12 g inositol/day for 4 weeks (Benjamin *et al.,* 1995; Levine *et al.*, 1995).

- Potassium is the major intracellular cation in the human body, for which the Institute of Medicine (IOM) has established an acceptable intake of 4.5 g/day, based on very low risk of excessive potassium intake in healthy individuals with normal kidney function (IOM, 2004). In the Food Information to Consumers Regulation (European Commission, 2013), a nutrient reference value (NRV; the amount recommended to be consumed daily to maintain a healthy diet) of 2,000 mg/day has been established for potassium (U.K. Department of Health, 2013).

GENERAL INTRODUCTION

Nutrition 21 LLC (hereafter Nutrition 21) wishes to market inositol-stabilized arginine silicate (ASI) as a new source of arginine in the European Union (EU) as a novel ingredient for use as an ingredient in foods for particular nutritional uses (PARNUTS)/foods for specific groups, as an ingredient in food supplements and in nutrition bars for sportsmen (European Parliament and the Council of the European Union, 1997). The use of this ingredient in PARNUTS requires the addition of Inositol-Stabilized Arginine Silicate (ASI) as a new source of arginine to the Union List of Commission Regulation (EC) No 609/2013[1], but until this Regulation is fully adopted (July 2016), it also requires addition to the Annex of Commission Regulation (EC) No 953/2009[2] (Commission of the European Communities, 2009; European Parliament and the Council of the European Union, 2013). In addition to amending these pieces of legislation, based on its chemical composition, ASI is considered a novel food ingredient within the definitions laid down in Commission Regulation (EC) No 258/97[3] (European Parliament and the Council of the European Union, 1997). Novel foods and novel food ingredients are defined as foods or food ingredients that do not have a significant history of use prior to May 1997. Under Article 1 of this regulation, ASI would be classified as a novel food meeting the following definition:

> (c) foods and food ingredients with a new or intentionally modified primary molecular structure;

Article 1(2) of Regulation (EC) No 258/97 states that the regulation *"…shall apply to the placing on the market within the Community of foods and food ingredients which have not hitherto been used for human consumption to a significant degree within the Community and which fall under [one of six defined] categories"* (European Parliament and the Council of the European Union, 1997). The classification of a compound determines the nature of the data that is required for a novel food petition. The 6 defined categories for novel foods are listed below:

> Class 1 - Pure chemicals or simple mixtures from non-GM sources
> Class 2 - Complex NF from non-GM sources
> Class 3 - GM plants and their products
> Class 4 - GM animals and their products
> Class 5 - GM microorganisms and their products
> Class 6 - Foods produced using a novel process

Based on the data provided by Nutrition 21, ASI would be classified as a Class 1 compound as it is a complex synthesised by an electrostatic bond between arginine and silicon (in the

[1] Regulation (EC) No 609/2013 on foods intended for infants and young children, foods for special medical purposes and total diet replacement for weight control.
[2] Commission Regulation (EC) No 953/2009 of 13 October 2009 on substances that may be added for specific nutritional purposes in foods for particular nutritional uses.
[3] Commission Regulation (EC) No 258/97 of the European Parliament and of the Council of 27 January 1997 concerning novel foods and novel food ingredients.

form of potassium silicate), stabilised with inositol. To gain approval for use of ASI in the EU, an application under this specific regulation will be required.

The essential information requirements corresponding with this classification are outlined in a detailed list below, and are expanded upon in separate sections throughout the document, forming the basis of the application (Recommendation 97/618/EC - Commission of the European Communities, 1997).

I Specification of the Novel Food

II Effect of the Production Process Applied to the Novel Food

III Section III is not applicable as it refers to foods produced using organisms, and the source of ASI is not an organism

IV – VIII Sections IV – VIII are not applicable as these refer to foods made from genetically modified organisms, and the source of ASI is not a genetically modified organism

IX Anticipated Intake/Extent of Use of the Novel Food

X Information from Previous Human Exposure to the Novel Food or its Source

XI Nutritional Information on the Novel Food

XII Microbiological Information on the Novel Food

XIII Toxicological Information on the Novel Food

For each category (I through XIII), structured schemes have been developed by the Scientific Committee on Food (SCF), which consist of a decision-tree-like set of questions designed to elicit sufficient data for a comprehensive safety and nutritional evaluation of the novel food. As outlined below in Sections I through XIII, the required questions are identified and subsequently addressed with the appropriate data.

As detailed herein, the safety of ASI is supported by its purity (chemical purity >90%, as indicated by product specifications in Section I.e), the dissociation of its arginine silicate component in the acidic environment of the stomach (yielding arginine, silicon, inositol, and potassium), historical consumption of its components in the diet, safety data provided by Nutrition 21 for the final ASI product, and safety data from additional published and unpublished toxicological and clinical data on the individual components.

I SPECIFICATIONS OF ASI

Based on the SCF guidelines, the following questions must be addressed:

- “Is appropriate analytical information available on potentially toxic inherent constituents, external contaminants and nutrients?”
- “Is the information representative of the novel food when produced on a commercial scale?”
- “Is there an appropriate specification (including species, taxon *etc.* for living organisms) to ensure that the novel food marketed is the same as that evaluated?”

These questions have been addressed collectively in Sections I.a through I.g.

I.a Common Name or Usual Name

ASI; Nitrosigine

I.b Chemical Name

The chemical name for ASI is inositol-stabilized arginine silicate (CAS number not applicable).

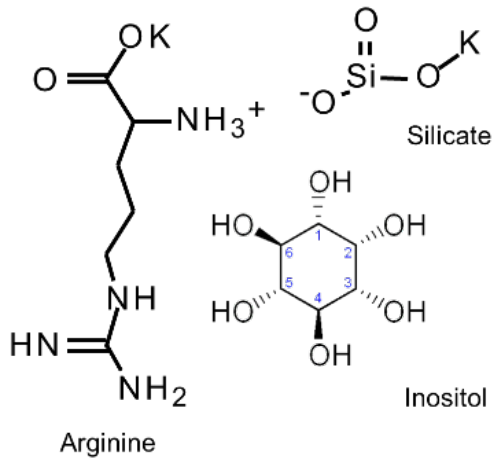
I.c Empirical Formula

ASI has a molecular weight of 508.64 g/mol and empirical formula of $C_{12}H_{26}N_4K_2SiO_{11}$. No structural isomers have been identified.

I.d Structural Formula

The structural formula for ASI is shown in Figure I.d-1 below. *In vitro* dissociation data on ASI (manufactured by Nutrition 21) demonstrates that the arginine silicate component of ASI dissociates upon ingestion in the acidic environment of the stomach, liberating the individual components, arginine, silicate, and inositol.

Figure I.d-1 Structural Formula for Inositol-Stabilized Arginine Silicate (ASI)



ASI is a complex synthesised by an electrostatic bond between the positively charged guanidinyle group of arginine and the negatively charged silicic acid $Si(OH)_3O^-$. This ionic bond is stabilised with inositol, which serves to reduce condensation *via* the formation of hydrogen bonds with $Si(OH)_4$. ASI is a white, free-flowing, odourless powder that is at least 90% soluble in water.

Carbon Spectra and Nuclear Magnetic Resonance (NMR) spectroscopy is used to identify the compound, as demonstrated in Figure I.d-2 and Figure I.d-3, respectively.

Figure I.d-2 Carbon Spectra Analysis of Inositol-Stabilized Arginine Silicate (ASI)

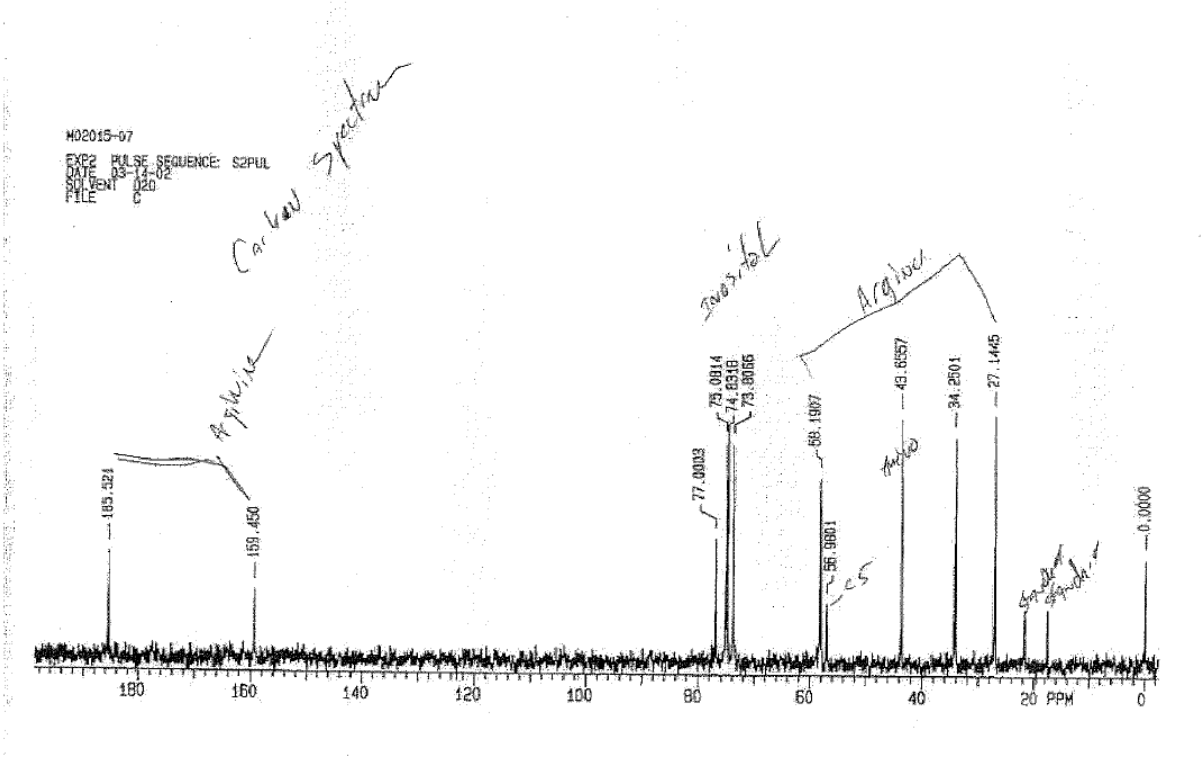
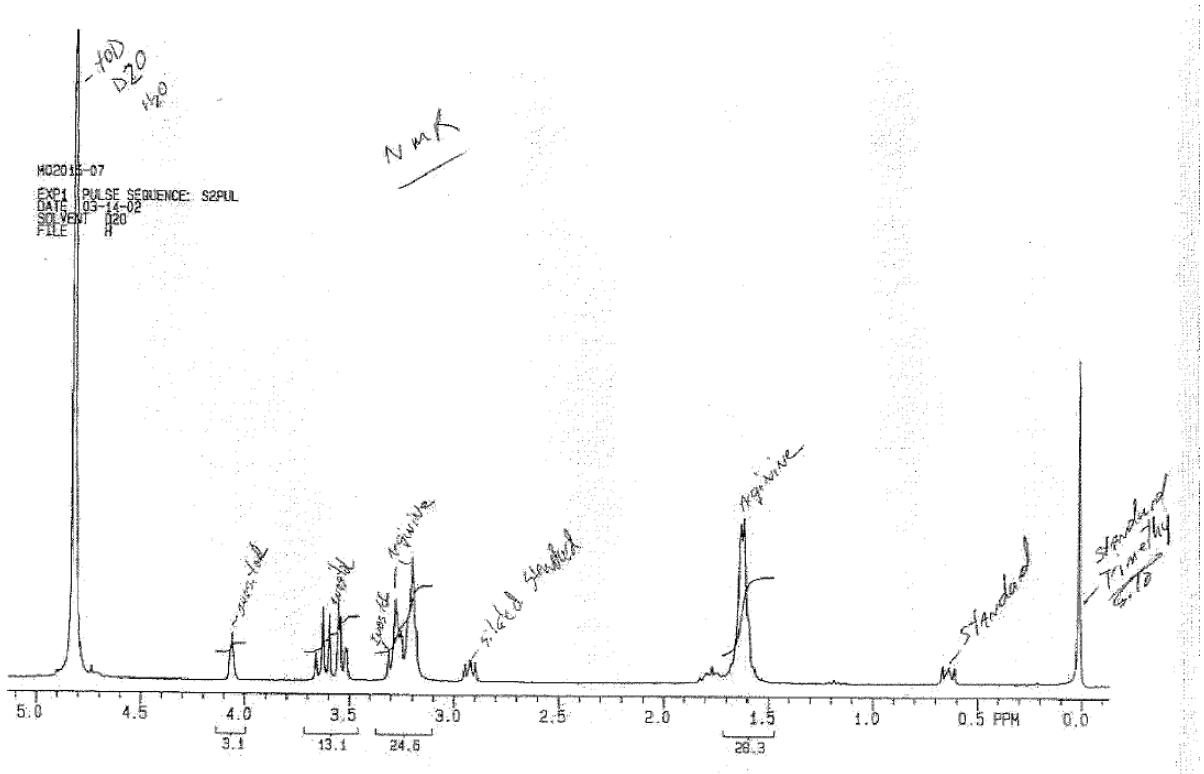


Figure I.d-3 NMR Spectroscopy Analysis of Inositol-Stabilized Arginine Silicate (ASI)



I.e Product Specifications and Analyses for ASI

The chemical and microbiological specifications and analytical methods for ASI are listed in Table I.e-1. The analytical methods listed in Table I.e-1 are internationally recognised, standardised methods for the measurement of the corresponding parameters. Analytical data for 3 representative batches also have been provided, and are summarised in Table I.e-2. Original documentation detailing product specifications and certificates of analysis for batch analyses are provided in Appendix A.

Table I.e-1 ASI Product Specifications and Analytical Methods

Specification Parameter	Specification	Test Methods
Identity	White, free-flowing odourless powder	Organoleptic
Particle Size	≥90% through 80 mesh	Current USP <786>
pH	10.8-11.2	AOAC 18th Edition 943.02
Arginine	380-450 mg/g	Precol. Derivatisation and HPLC
Silicon (as SiO_3)	210-260 mg/g	Perkins-Elmer AA Spectrophotometry
Inositol	230-290 mg/g	Ion chromatography
Potassium	40-60 mg/g	AOAC 17th Edition ICP/AES
Loss on Drying (Moisture)	<4.00%	Current USP <731> LOD
Contaminant Specification		
Arsenic	<1.5 mg/kg	AOAC, ICP Mass Spectrometry
Cadmium	<0.5 mg/kg	AOAC, ICP Mass Spectrometry
Lead	<1.0 mg/kg	AOAC, ICP Mass Spectrometry
Mercury	<1.5 mg/kg	AOAC, ICP Mass Spectrometry
Microbial Specification		
Total Plate Count	<1,000 CFU/g	Current USP
Yeasts and Moulds	<100 CFU/g	Current USP

AOAC = Association of Official Analytical Chemists; ASI = inositol-stabilized arginine silicate; CFU = colony-forming units; HPLC = high-performance liquid chromatography; ICP = inductively coupled plasma; LOD = limit of detection; USP = United States Pharmacopeia.

Example batch analysis results corresponding to the aforementioned specifications are provided in Table I.e-2. All batches were analysed within 2 months of their date of manufacture. The results of these analyses indicate that ASI consistently meets the established specifications for potential metal and microbial contaminants. Specifically, the contents of lead, cadmium, and mercury comply with the limits for food supplements laid out in EC 1881/2006 (*i.e.,* limits of 3, 1, and 0.1 mg/kg, respectively) (Commission of the European Communities, 2006). No limits for the presence of cadmium in food supplements were identified. The average specified contents and the average results of analytical testing for arginine, silicon, and inositol (*i.e.,* the sum of which are 910 and 911mg/g, respectively) demonstrate that the chemical purity of ASI is >90%.

Table I.e-2 Batch Analysis Results for 3 Batches of ASI				
Analysis	**Specification**	**Lot Number and Date of Manufacture[1,2]**		
		NJSD152560 12/19/2013	**NJSD300387 5/17/2014**	**NJSD300631 11/7/2014**
Particle Size (%)	≥90% through 80 mesh	93	96.9	91.9
pH	10.8-11.2	11.0	11.0	10.84
Arginine (mg/g)	380-450	436	446	437
SiO_3 (silicon calculated from total Si) (mg/g)	210-260	229	228	225
Inositol (mg/g)	230-290	249	242	242
Potassium (mg/g)	40-60	52.5	50.0	49.2
Loss on Drying (%)	<4.0	2.9	1.8	2.9
Contaminant Specification				
Arsenic (mg/kg)	<1.5	0.038	0.036	<0.050
Cadmium (mg/kg)	<0.5	0.313	<0.010	<0.025
Lead (mg/kg)	<1.0	0.141	0.116	0.142
Mercury (mg/kg)	<1.5	<0.010	<0.010	<0.025
Microbial Specification				
Total Aerobic Plate Count (CFU/g)	<1,000	<10	<10	<10
Yeast (CFU/g)	<100	<10	<10	<10
Mould (CFU/g)	<100	<10	<10	<10

ASI = inositol-stabilized arginine silicate; CFU = colony-forming units
[1] Values for arginine, SiO_3, inositol, and potassium presented as percentages in certificates of analysis, but were converted to mg/g for this table.
[2] Values for arsenic, cadmium, lead, and mercury presented as ppb in the certificates of analysis but were converted to mg/kg for this table.

I.f Storage

It is recommended that ASI be stored below 25°C, away from moisture.

I.g Stability of ASI

The stability of 2 batches of bulk ASI (Lots NJ280081 and NJ138127) was evaluated up to 4.5 years after the date of manufacture, following storage at ambient temperature (refer to Appendix A for details). No changes were detected with respect to contents of arginine, silicon, inositol, and potassium, and the samples were determined to be stable. The results of stability testing are shown in Table I.g-1 below.

Table I.g-1 Stability of ASI						
Test Parameters[1]	**Specification**	**Lot Number, Date of Manufacture, and Time Since Manufacture**				
		NJ280081 (October, 2008)		**NJ138127 (April, 2012)**		
		0 months	**54 months**	**0 months**	**6 months**	**12 months**
Arginine	380-450 mg/g	446	438	417	446	422
SiO3	210-260 mg/g	253.8	234.9	213.3	216	240.3
Inositol	230-290 mg/g	290	230	238	243	234
Potassium	40-60 mg/g	56	49	53	51	51

ASI = inositol-stabilized arginine silicate
[1] All test results reported as percent (by weight of ASI), and converted to mg/g. SiO_3 reported as Silicon, and converted to SiO_3 using conversion factor of 2.7 (as provided by Nutrition 21).

The results of stability testing conducted on a powdered drink mix containing ASI (packaged in individual serving-size units; designated Lot No. P4156-A-S, manufactured December 2014) indicate that the ASI content in the drink mix remains stable following 12 months of storage under intermediate conditions (*i.e.,* 30°C, 65% humidity) and 9 months of storage under accelerated conditions (*i.e.,* 40°C, 75% humidity) (refer to Appendix A for details). The results of these stability tests are shown in Table I.g-2 below.

Table I.g-2 Stability of ASI Present as a Component of Powdered Drink Mix				
Test Parameters[1]	**Time Since Manufacture and Storage Conditions**			
	Intermediate Storage Conditions (30°C, 65% humidity)		**Accelerated Storage Conditions (40°C, 75% humidity)**	
	0 months	**12 months**	**0 months**	**9 months**
Arginine (mg/g)	193	185	193	178
SiO_3 (mg/g)	94.5	86.4	94.5	83.7
Inositol (mg/g)	109	100	109	104

ASI = inositol-stabilized arginine silicate
[1] All test results reported as percent (by weight of powdered drink mix), and converted to mg/g. SiO_3 reported as Silicon, and converted to SiO_3 using conversion factor of 2.7 (as provided by Nutrition 21).

II EFFECT OF THE PRODUCTION PROCESS APPLIED TO ASI

Based on the SCF guidelines, the following questions must be addressed:

- “Does the novel food undergo a production process?”
- “Is there a history of use of the production process for the food?” If no, “does the process result in a significant change in the composition or structure of the novel food compared to its traditional counterpart?”
- “Is information available to enable identification of the possible toxicological, nutritional and microbiological hazards arising from use of the process?”
- “Are the means identified for controlling the process to ensure that the novel food complies with its specification?”
- “Has the process the potential to alter the levels in the novel food of substances with an adverse effect on public health?”
- “After processing is the novel food likely to contain microorganisms of adverse public health significance?”

These questions have been addressed collectively in Sections II.a and II.b.

II.a Raw Materials Used in the Manufacturing Process

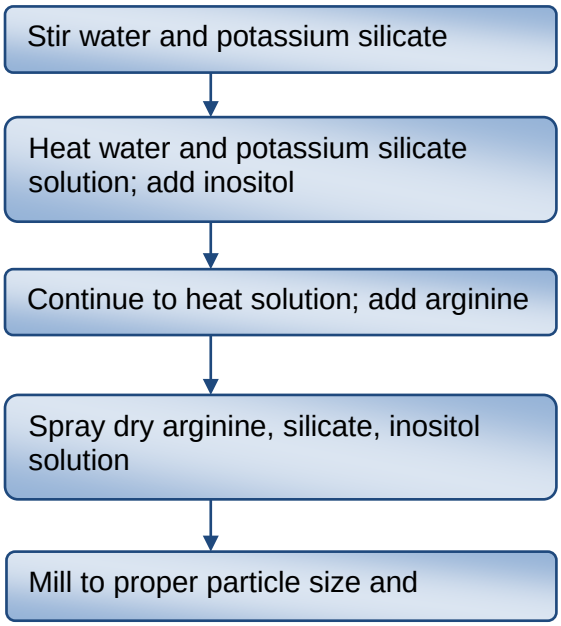
The 3 raw materials used in the production of ASI (*i.e.,* potassium silicate, arginine, and inositol) meet the specifications of the Food and Chemicals Codex and the United States Pharmacopeia, where applicable, and are considered safe and suitable for use in the manufacture of ASI.

II.b Manufacturing Process

As mentioned above, ASI has high purity (>90%, as indicated by product specifications in Section I.e), and has well-defined specifications and a simple but tightly controlled production process which demonstrates an absence of contaminants. ASI is manufactured in accordance with Good Manufacturing Practices (GMP). Since the manufacturing process for ASI is a combination of the unaltered starting materials, the starting materials are present in the final product.

A schematic of the manufacturing process is provided in Figure II.b-1 below.

Figure II.b-1 Schematic of the Manufacturing Process for Inositol-Stabilized Arginine Silicate (ASI)



No additional purification, extraction, or concentration steps are undertaken. Carbon spectrum and NMR spectral data (see Figure I.d-2 and Figure I.d-3 above) demonstrate that there are no by-products produced during the production process.

IX INTAKE/EXTENT OF USE OF ASI

Based on the SCF guidelines, the following questions must be addressed:

- “Is there information on the anticipated uses of the novel food based on its properties?”
- “Is there information to show anticipated intakes for groups predicted to be at risk?”
- “Will introduction of the novel food be restricted geographically?”
- “Will the novel food replace other foods in the diet?”

These questions have been addressed collectively in Sections IX.a through IX.b.

IX.a Estimated Consumption of ASI as Indicated for Food Supplement Use

ASI is intended to be used as an ingredient in nutrition bars for sportsmen either in normal foods or in PARNUTS/food for specific groups (European Parliament and the Council of the European Union, 1997), as an ingredient in food supplements intended for consumption by adults engaged in athletic endeavours (*i.e.,* foods for intense muscular effort and foods for special medical purposes). The proposed uses of ASI are intended to replace the current uses of arginine-HCl in the specified food categories. The recommended intake of ASI is 1,500 mg/day; this dose corresponds with the dose of ASI permitted for use as a New Dietary Ingredient in the United States (U.S.). ASI is therefore intended to be included at a level of 500 mg/serving in nutrition bars and in PARNUTS and food supplements with intended intakes of 2 to 3 servings/day, thus providing a maximum target intake of 1,500 mg ASI/day. Due to a lack of safety data on the composite products in which ASI is to be included, products containing ASI are not intended for consumption by children or pregnant or lactating women[4]. The expected daily exposure to ASI and its components under the recommended conditions of use is presented in Table IX.a-1.

Table IX.a-1 Expected Consumption of ASI

Ingredient	Maximum per Serving (mg)	Maximum per Day	
		mg/day[1]	mg/kg bw/day[2]
ASI	**500 (nutrition bars)**	**1,500**	**21.4**
	500 (PARNUTS and food supplements)	**1,500**	**21.4**
	Other meal replacements	**1,500**	**21.4**
L-Arginine	225	675	9.6
Silicon	145	435	6.2
Inositol	130	390	5.6
Potassium	20 to 30	75	1.1

ASI = inositol-stabilized arginine silicate; bw = body weight
[1] Based on intake of 3 servings/day.
[2] Based on average adult body weight of 70 kg.

IX.b Risk Management Controls

Control of intake of ASI is achieved through 3 basic principles:

1. Since the food use will be in meal replacement products then the maximum intake of these products is assumed to be 3 servings per day.

2. In the specialist PARNUTS/FSG category of total diet replacements specific labelling requirements are laid down. This is discussed in more detail below in Section IX.b.1.

[4] The estimated daily intakes of the components of ASI alone (shown in Table IX.a-1) are below estimates of dietary intake and/or endogenous production, and are not expected to be of safety concern to pregnant or lactating women.

3. There are strict labelling requirements for food supplements to control maximum dose. This is discussed in more detail below in Section IX.b.2.

IX.b.1 PARNUTS (Foods for Weight Reduction/ Foods for Special Medical Purposes)/ Foods for Specific Groups (Total Diet Replacement) Products

Commission Directive 96/8/EC on foods intended for use in energy-restricted diets for weight reduction lays down labelling requirements for dietary replacement products (Commission of the European Communities, 1996). The name under which the product is sold shall be "Total diet replacement for weight control" (for products presented as a replacement for the whole of the daily diet), or "Meal replacement for weight control" (for products presented as a replacement for one or more meals of the daily diet).

The labelling of the products shall bear the following mandatory particulars:

- The available energy value expressed in kJ and kcal, and the content of proteins, carbohydrates, and fat per specified quantity of the product ready for use as proposed for consumption;
- The average quantity of each mineral and each vitamin for which mandatory requirements are stipulated in paragraph 5 of Annex 1 (Commission Directive 96/8/EC), per specified quantity of the product ready for use as proposed for consumption;
- Instructions for appropriate preparation, when necessary and a statement as to the importance of following those instructions;
- If a product, when used as instructed, provides a daily intake of polyols in excess of 20 g, there shall be a statement to the effect that the food may have a laxative effect;
- A statement on the importance of maintaining an adequate daily fluid intake;
- For total diet replacement products, a statement that the product provides adequate amounts of all essential nutrients for the day; and a statement that the product should not be used for more than 3 weeks without medical advice;
- For meal replacement products, a statement to the effect that the products are useful for the intended use only as part of an energy-restricted diet and that other foodstuffs should be a necessary part of such diet.

The labelling, advertising, and presentation of the products concerned shall not make any reference to the rate or amount of weight loss which may result from their use.

Commission Directive 1999/21/EC on dietary foods for special medical purposes (FSMPs) lays down safety requirements for FSMPs (Commission of the European Communities, 1999). The formulation of FSMPs shall be based on sound medical and nutritional

principles. Their use, in accordance with the manufacturer's instructions, shall be safe and beneficial and effective in meeting the particular nutritional requirements of the persons for whom they are intended, as demonstrated by generally accepted scientific data. They must comply with the compositional criteria specified in the Annex to 1999/21/EC (Essential Composition of FSMPs).

IX.b.2 Food Supplements

As per Directive 2002/46/EC of the European Parliament and Council, Article 1 "concerns food supplements marketed as foodstuffs and presented as such. These products shall be delivered to the ultimate consumer only in a pre-packaged form" (European Parliament and the Council of the European Union, 2002).

Article 6, Section 3 indicates that the labelling instruction should contain the following particulars:

a) the names of the categories of nutrients or substances that characterise the product or an indication of the nature of those nutrients or substances;

b) the portion of the product recommended for daily consumption;

c) a warning not to exceed the stated recommended daily dose;

d) a statement to the effect that food supplements should not be used as a substitute for a varied diet; and,

e) a statement to the effect that the products should be stored out of reach of young children.

X INFORMATION FROM PREVIOUS HUMAN EXPOSURE TO ASI

Based on the SCF guidelines, the following questions must be addressed:

- “Is there information from previous direct, indirect, intended or unintended human exposure to the novel food or its source which is relevant to the EU situation with respect to production, preparation, population, lifestyles and intakes?”

- “Is there information to demonstrate that exposure to the novel food is unlikely to give rise to mitochondrial, toxicological and/or allergenicity problems?”

These questions have been addressed in Sections X.a through X.c.

X.a Natural Occurrence of ASI or its Components in the Diet

Although the arginine silicate of ASI is manufactured by chemical synthesis and is not present in the diet in its complexed form, data from an *in vitro* dissociation study and a pharmacokinetic study in men demonstrate that the arginine silicate dissociates upon ingestion in the acidic environment of the stomach, liberating its components (*i.e.,* arginine, silicon, inositol, and potassium).

Arginine is a normal constituent of a healthy diet, with an acceptable daily intake (ADI) of "acceptable" established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (JECFA, 2006). The expected intake of arginine under the intended conditions of use of ASI is 675 mg/day. L-Arginine is supplied *via* the diet and also can be synthesised endogenously from citrulline, a urea cycle intermediate, in the liver and kidney (FASEB, 1992). Consumption of arginine as a food additive is insignificant compared to the amount consumed as a component of food proteins (Newberne *et al.*, 1998; U.S. FDA, 2015). 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, 2005).

Since arginine can be produced endogenously, there is no set recommended daily allowance or upper limit for consumption (IOM, 2005). As the average consumption of arginine in the human diet is 5.4 g/100 g dietary protein (*i.e.,* not including supplement use) (FASEB, 1992), and the mean and 90th percentile daily dietary protein intakes for individuals (in the U.S.) are 75.2 and 114.0 g, respectively, the mean and 90th percentile daily intakes of arginine from food are estimated to be 4.06 g and 6.16 g, respectively (IOM, 2005). Additionally, doses of arginine of up to 21 g/day in dietary supplement form have been reported for treatment of cardiovascular dysfunction (PDRNS, 2008). The addition of 675 mg arginine/day from the consumption of ASI would increase the mean dietary exposure from 4.06 g/day to 4.735 g/day (*i.e.*, an increase of approximately 17%). This level of exposure to arginine is considerably lower than doses reported to be well tolerated in clinical studies, and is not expected to pose a safety concern.

Silicon also is a natural constituent of a typical human diet, with a typical dietary intake of 20 to 50 mg/day [Scientific Committee on Food (SCF)/European Food Safety Authority (EFSA), 2006], and a safe upper limit of 700 mg/day established by the Expert group on Vitamins and Minerals (EVM) (EVM, 2003). Silicon is the second most abundant element on earth, and 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). Additionally, refined and processed foods contain large amounts of silicon, and silicate additives are used as anti-caking agents (T.J. Clark & Company, 2015).

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 dietary 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 AI or tolerable upper intake level in humans; however, the IOM reported that there was no evidence that consumption of silicon that occurs naturally in food or water would lead to adverse health effects (IOM, 2001).

Calcium silicate and silicon dioxide/silicic acid gel were given a positive opinion by EFSA for addition to food supplements for nutritional purposes following a review by the Panel on Food Additives and Nutrient Sources added to Food (ANS) in 2009 (EFSA, 2009b). Taking into account the UK Expert group on Vitamins and Minerals (EVM) Safe Upper Limit for silicon consumption (700 mg silicon/day) for adults over a lifetime (equivalent to 10 mg silicon/kg body weight/day for a 70 kg adult) (EVM, 2003), the Panel concluded that it had no safety concerns regarding the use of calcium silicate and silicon dioxide/silicic acid gel in food supplements providing up to 700 mg silicon/day (EFSA, 2009b). The EFSA Panel on Dietetic products, Nutrition and Allergies (NDA) has not set a Tolerable Upper Intake Level (UL) for silicon but has estimated that the typically dietary intake of silicon is between 20 and 50 mg/day (equivalent to 0.3 to 0.7 mg/kg body weight/day for a 70 kg adult), which is unlikely to cause adverse health effects (SCF/EFSA, 2006). The safety of silicates is supported by the permitted use of various silicate salts as food additives in the European Food Additives Database (European Commission, 2015). The addition of 435 mg silicon/day from the consumption of ASI would increase the mean dietary exposure from 20 to 50 mg/day to 455 to 485 mg/day. This level of exposure to silicon is within the safe upper limit of 700 mg/day established by the EVM (EVM, 2003).

EFSA has estimated that dietary exposure to inositol is approximately 335 to 1,500 mg/day (EFSA, 2009a), and doses up to 12 g inositol/day were reported to be well tolerated in clinical studies (Benjamin *et al.*, 1995; Levine *et al.*, 1995). Inositol also is produced by the human kidneys at a rate of approximately 4 g/day, an amount which is considerably greater than dietary intake of inositol (Holub, 1986). The addition of 390 mg inositol/day from the consumption of ASI would increase the mean dietary exposure from 335 to 1,500 mg/day to 725 to 1,890 mg/day. This level of exposure to inositol is considerably lower than the intakes of inositol reported to be well tolerated in clinical studies (12 g/day) (Benjamin *et al.*, 1995; Levine *et al.*, 1995).

A nutrient reference value (NRV) of 2,000 mg has been set for potassium by EFSA (European Parliament and the Council of the European Union, 2011), and an acceptable intake (AI) of 4.5 g potassium/day for adults (>18 years of age) has been established by the Institute of Medicine (IOM) (IOM, 2004). Considering the specification for potassium listed in Table I.e-2 (40 to 60 mg/g ASI), a daily intake of 1,500 mg ASI would provide 75 mg potassium. As this dose is approximately 60 times lower than the AI established by the IOM, and approximately 30 times lower than the NRV set by EFSA, adverse effects due to intake of potassium as a component of ASI are not expected to occur.

X.b Current Reviews of ASI and its Components

The U.S. Food and Drug Administration (FDA) "acknowledged with no objections" Nutrition 21's New Dietary Ingredient (NDI) Notification for ASI in the U.S. Intended uses in this notification include energy and meal replacement bars, and milk-based and non-milk-based meal replacement beverages, providing up to 750 mg ASI/serving (providing up to 333.5 mg arginine, 192.9 mg silicon, and 215.0 mg inositol per serving), with a recommended 2 servings per day. Arginine silicate inositol also is listed as a Natural Health Product in Canada (Health Canada, 2015).

The safety of long-term consumption of arginine and silicon/silicates is reasonably characterised in published literature, and has been reviewed by JECFA, the IOM, and the Cosmetic Ingredient Review (CIR) (IOM, 2001, 2005; CIR, 2003, 2005; JECFA, 2006). Arginine is a normal macronutrient constituent of a healthy diet, and is essential for growing children (although not for adults) (JECFA, 2006). JECFA (2006) has established an ADI of "acceptable" for arginine, indicating a lack of safety concerns at current levels of intake. Due to a lack of identified adverse effects attributable to consumption of arginine, Shao and Hathcock (2008) were unable to set an upper limit for arginine. Instead, they derived an observed safe level of 20 g arginine/day based on a lack of adverse effects reported in a study in which 20 subjects with heart failure consumed 20 g arginine/day for 4 weeks (Chin-Dusting *et al.*, 1996).

Calcium silicate and silicon dioxide/silicic acid gel were given positive scientific opinions for addition to food supplements for nutritional purposes following a review by the Panel on Food Additives and Nutrient Sources added to Food (ANS) in 2009 (EFSA, 2009b). This review took into account the United Kingdom (UK) EVM Safe Upper Limit for silicon consumption (700 mg silicon/day) for adults over a lifetime (equivalent to 10 mg silicon/kg body weight/day for a 70 kg adult) (EVM, 2003). Various silicate complexes also are permitted for use as food additives in the EU (E 551 through E 559) (European Commission, 2015). In addition, JECFA has listed potassium aluminium silicate and aluminium silicate for use as anticaking agents, with no ADIs allocated (JECFA, 2014).

Inositol is a ubiquitous constituent of the diet, with an estimated dietary intake of 1 g/day (PDRNS, 2008), and is well-tolerated in clinical studies at doses of up to 12 g/day, consumed for 4 weeks (Benjamin *et al.*, 1995; Levine *et al.*, 1995). 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. Furthermore, inositol is produced by the human kidneys, with endogenous synthesis approaching 4 g/day (Holub, 1986). The EFSA ANS panel reviewed the safety of inositol hexanicotinate (inositol hexaniacinate) as a source of niacin (vitamin B_2) added for nutritional purposes in food supplements in a Scientific Opinion (EFSA, 2009a). The Panel concluded that a daily dose of 11 mg inositol hexanicotinate would result in the release of 2.4 mg inositol upon hydrolysis. On the basis that the estimated normal dietary intake of inositol amounts to 335 to 1,500 mg myo-inositol (the most important form of naturally occurring inositol)/day, the Panel concluded that this level of intake would present no safety

concerns. Furthermore, inositol is considered Generally Recognized as Safe (GRAS) by the FDA (21 CFR §184.1370), for addition to food as a nutrient supplement without limitation other than current GMP (U.S. FDA, 2015). An NRV of 2,000 mg has been set for potassium by EFSA (European Parliament and the Council of the European Union, 2011), and an AI of 4.5 g potassium/day for adults (>18 years of age) has been established by the IOM. A UL was not set for potassium since intake from foods poses no risk to healthy individuals with normal kidney function due to ready excretion of excess potassium in the urine. The IOM noted that in individuals with impaired urinary excretion of potassium, intake above the AI may result in hyperkalaemia associated with adverse cardiac effects (*i.e.,* arrhythmias), but that such individuals are typically under medical supervision (IOM, 2004).

Considering the results of the published reviews on arginine, silicon/silicates, inositol, and potassium, their ubiquity in the diet, and that the arginine silicate component of ASI dissociates upon ingestion in the acidic environment of the stomach, the safety of ASI can be supported by that of its components alone.

X.c Potential Allergenicity Concerns

No reports of allergic-type reactions to ASI or its components have been identified in the published literature.

XI NUTRITIONAL INFORMATION ON ASI

Based on the SCF guidelines, the following question must be addressed:

- “Is there information to show that the novel food is nutritionally equivalent to existing foods that it might replace in the diet?”

This question has been addressed in Section XI.a.

XI.a Nutritional Benefits of ASI

ASI is intended to be used to provide a new dietary source of arginine, replacing arginine-HCl used in similar food categories. Arginine plays essential roles in the formation of nitric oxide (a vasodilator), creatine (used for storage of high-energy phosphates used for adenosine triphosphate-dependent processes), proteins, and growth hormone release (Stipanuk and Watford, 2006). Endogenous production of arginine in adults is usually sufficient to meet metabolic requirements, although during periods of rapid growth, exogenous sources of arginine may be required to meet physiological demands (Appleton, 2002; May *et al.*, 2002; Wilmore, 2004). Supplemental arginine has been reported to help maintain lean body mass (*i.e.,* muscle mass), exert ergogenic effects, enhance athletic performance, and increase synthesis of muscle tissue (Lind, 2004; Wilmore, 2004). Accordingly, ASI is marketed as an ingredient in a variety of athletic supplement products (see Appendix B for details).

The benefit of using inositol-stabilised arginine silicate, compared to using Arginine-HCl, as an ingredient in food supplements is that the arginine silicate complex provides a bioavailable source of arginine and silicon.

XII MICROBIOLOGICAL INFORMATION ON ASI

Based on the SCF guidelines, the following question must be addressed:

- "Is the presence of any microorganisms or their metabolites due to the novelty of the product/process?"

This question has been addressed below in Table I.e-2 (Batch Analysis Results for 3 batches of ASI).

XII.a Microbiological Specifications and Analyses for ASI

The results of analysis of microbiological specifications for 3 sample lots of ASI are listed in Table XII.a-1. These results confirm the absence of microbiological contamination in the final product. Certificates of analysis are provided in Appendix A.

Table XII.a-1 Specifications for ASI

Specification Parameter	Specification	Lot Number and Date of Manufacture		
		NJSD152450 12/19/2013	NJSD300387 5/17/2014	NJSD300631 11/7/2014
Total Aerobic Plate Count (CFU/g)	<1,000	<10	<10	<10
Yeast (CFU/g)	<100	<10	<10	<10
Mould (CFU/g)	<100	<10	<10	<10

ASI = inositol-stabilized arginine silicate; CFU = colony-forming units

XIII TOXICOLOGICAL INFORMATION ON ASI

Based on the SCF guidelines, the following questions must be addressed:

- "Is there a traditional counterpart to the novel food that can be used as a baseline to facilitate the toxicological assessment?"
- "Compared to the traditional counterpart, does the novel food contain any new toxicants or changed levels of existing toxicants?"

or

- "Is there information from a range of toxicological studies appropriate to the novel food to show that the novel food is safe under anticipated conditions of preparation and use?"
- "Is there information which suggests that the novel food might pose an allergenic risk to humans?"

These questions have been addressed collectively in Section XIII.

Introduction

The safety of ASI, as described herein, is based on a lack of relevant adverse effects reported in studies of ASI and its components, arginine, silicate, inositol, and potassium. The safety of ASI is supported by the results of product-specific animal toxicology and genotoxicity studies, which demonstrate that ASI does not cause adverse effects upon oral administration to rats for up to 8 weeks (at a dose of 1.81 g/kg body weight/day), and also lacks mutagenic potential. The results of 2 studies in healthy men demonstrate that ASI dissociates in the acidic environment of the stomach, provides a bioavailable source of L-arginine and silicon, and does not cause adverse cardiovascular effects at doses up to 1,500 mg/day.

The preclinical and clinical studies on the individual components of ASI demonstrate that arginine, silicon, inositol, and potassium have low oral toxicity in experimental animals and humans. The recommended consumption of ASI would provide 675 mg L-arginine, 435 mg silicon, 390 mg inositol, and 75 mg potassium per day. These intakes are well below established upper limit doses and doses reported to be well tolerated in human studies [*i.e.,* 20 g arginine/day (Shao and Hathcock, 2008), 700 mg silicon/day (EVM, 2003), 12 g inositol/day (Benjamin *et al.*, 1995; Levine *et al.*, 1995), and 4.5 g potassium/day (IOM, 2004)], even when combined with background dietary intakes. An analysis of the toxicological, toxicokinetic, and human data on ASI supports the conclusion that it can be safely used as an ingredient in in (PARNUTS)/foods for specific groups, as an ingredient in food supplements and in nutrition bars for sportsmen, at levels providing up to 1,500 mg/day.

XIII.a Absorption, Metabolism, Distribution, and Excretion

In vitro dissociation data indicate that the arginine silicate component of ASI dissociates in the acidic environment of the stomach. Thus, the safety of Nitrosigine can be based on the individual safety profiles of its components (*i.e.,* arginine, silicate, inositol, and potassium). The metabolic fates of ASI and its components are detailed herein. *In vitro* dissociation data on ASI demonstrates that the arginine silicate component of ASI dissociates upon ingestion in the acidic environment of the stomach. The identity of arginine in a sample of ASI (Sample 1460045) was compared to an arginine standard using high performance liquid chromatography-mass spectrometry. Both ASI (Sample 1460045) and the arginine standard were extracted in 0.1 N HCl with stirring and sonication, and were then diluted into a final solution of 50:50 acetonitrile: purified water. As shown in Appendix A, the retention times and spectral profile were consistent between ASI (Sample 1460045) and the arginine standard. On this basis, it can be concluded that the arginine silicate component of ASI dissociates upon ingestion in the acidic environment of the stomach. Thus, the safety of the Nitrosigine can be based on the individual safety profiles of its components (*i.e.,* arginine, silicate, inositol, and potassium). The metabolic fates of ASI and its individual components are detailed herein.

XIII.a.1 ASI

Nutrition 21 conducted an uncontrolled clinical study to assess the bioavailability and safety of ASI and its effects on systemic nitric oxide levels (unpublished study report). In this study, 10 men (18 to 40 years of age, non-smokers, with BMI ≥18 to <30 kg/m^2, blood pressure <140/90 mm Hg, and normal serum creatinine concentrations) were assigned to consume 1,500 mg ASI/day (provided as 3 caplets to be taken together on an empty stomach, each providing 190 to 225 mg arginine, 115 to 245 mg inositol, 40 to 50 mg silicon, and 20 to 30 mg potassium) for 14 days. Plasma arginine, serum and urinary silicon, and salivary nitrite levels (the latter as a measure of systemic nitric oxide levels) were assessed for 6 hours after consumption of daily doses of ASI on Days 1 and 14 of the study period. Plasma arginine and serum silicon levels were significantly increased from baseline (*i.e.,* before dosing on the testing days) for up to 3 hours after consumption on Days 1 and 14. No significant changes in the measured pharmacokinetic parameters (*i.e.,* maximal concentration, time to maximal concentration, area under the curve, terminal elimination rate constant, and half-life) for plasma arginine or serum silicon were reported between Days 1 and 14.

Urinary silicon levels (measured in 3-hour increments) increased significantly from baseline on Days 1 and 14, with significantly higher levels measured on Day 14 between 0 and 3 hours after dosing (compared to the same time interval on Day 1).

Although salivary nitrite levels did not increase significantly from baseline on Days 1 or 14, the average baseline salivary nitrite level increased significantly between Day 1 and Day 14. The results of this study indicate that ASI provides a bioavailable source of arginine and silicon, and contributes to improved nitric oxide production.

Nutrition 21 conducted a prospective, randomised, double-blind, active-comparator crossover study to compare the pharmacokinetics of ASI to Arginine-HCl in healthy men (unpublished study report). Safety parameters were evaluated as well; these are discussed in Section XIII.b.4 below. In this study, 10 men (18 to 40 years of age, non-smokers, with BMI ≥18.5 to <25 kg/m^2, blood pressure <140/90 mm Hg, and normal serum creatinine concentrations) were assigned to consume approximately 500 mg arginine from ASI or Arginine-HCl per day (each daily serving provided in 2 capsules to be taken with 240 mL water, on an empty stomach) for periods of 15 days, in random order. Study subjects were instructed to consume their assigned product each morning during the study period (except for Study Days 1 and 15, on which the products were given at the test centre prior to blood sampling for pharmacokinetic analysis), and not to eat for 30 minutes after consumption of the study products. On sampling days, the products were consumed following a 6- to 10-hour fast, and blood samples were collected at baseline and 0.5, 1, 1.5, 2, 3, 4, 5, and 6 hours after consumption of the assigned study product.

The blood plasma levels of L-arginine following consumption of ASI or Arginine-HCl, show a rapid rate of absorption peaking (t_{max}) approximately 1 hour after consumption. However, the rise in plasma levels were seen to be negligible in relation to background plasma levels of arginine. After peaking, plasma arginine levels then declined rapidly, with baseline level being attained 4 to 6 hours after consumption.

On Day 1 of the supplementation period, the plasma concentration-time curves were very similar for ASI and Arginine-HCl, with the increases above baseline being superimposable to a relative degree. Day 1 data therefore indicate that the arginine component of the ASI formulation dissociates and is bioavailable, and that the release and absorption rates of arginine from both formulations are similar. The rate of clearance of the arginine from the plasma indicates that there is no potential for accumulation following a daily dosing regimen using products providing approximately 500 mg arginine/day.

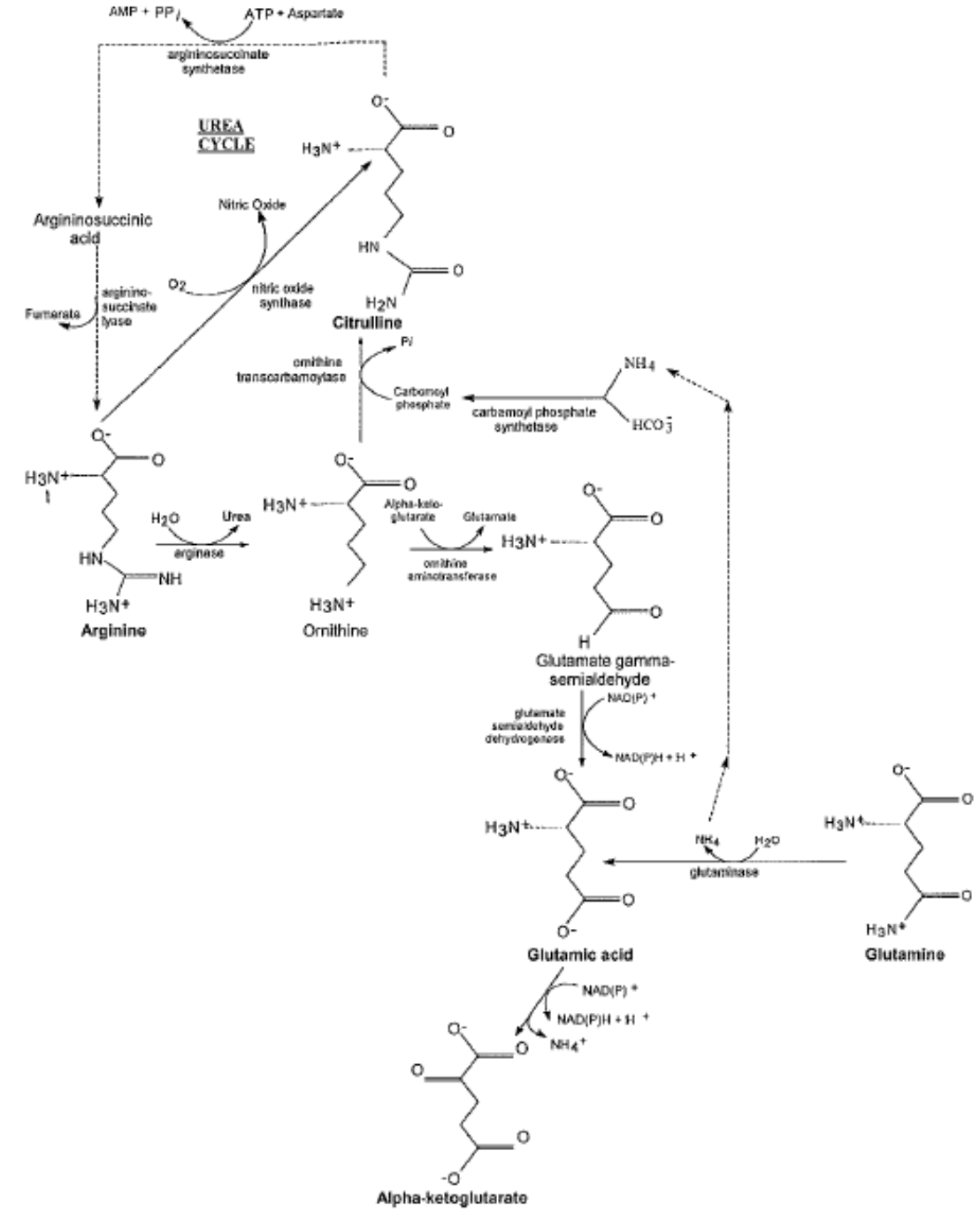
On Day 15, the plasma concentration-time curve also was very similar between subjects consuming ASI or Arginine-HCl. However, due to a higher baseline concentration of arginine in the ASI group in comparison to the Arginine-HCl group, the results indicate a potential difference between formulations when the baseline values were factored into the overall analysis. Upon evaluation of the data, it would appear that the baseline plasma arginine concentration of the ASI group is greater than would be expected following a direct comparison with the Arginine-HCl group and the fact that at 4, 5, and 6 hours after consumption the plasma levels of arginine are lower than baseline.

Overall, the results demonstrate a very similar kinetic profile on Days 1 and 15, with Day 1 results showing remarkable similarity in plasma arginine concentration curves between groups. In summary, the data support the dissociation and bioavailability of arginine from the ASI complex, and indicates that there appears to be no difference in overall arginine bioavailability between groups.

XIII.a.2 Arginine

Arginine 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 catalyse 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). The high arginase activity of the liver results in very low levels of arginine available in the systemic circulation (Edmonds *et al.*, 1987). The catabolic pathway for arginine is outlined in Figure XIII.a.2-1.

Figure XIII.a.2-1 Catabolic Pathway for Arginine, Including the Urea Cycle



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

XIII.a.3 Silicon

The bioavailability of dietary silicon is dependent on the compound in question. Some forms, such as silicic acid, are well absorbed, as demonstrated by high levels of urinary excretion (*i.e.,* typically between 41 and 75%). Silicon is widely distributed in all tissues (including brain, lungs, and liver) at varying levels. It is present in the blood primarily as silicic acid (not

bound to proteins) at levels of approximately 20 to 44 mg/kg. Higher levels are present in other tissues such as bone, nails, tendons and walls of the aorta, reaching as high as 1,500 mg/kg.

XIII.a.4 Inositol

Dietary inositol is readily absorbed from the small intestine *via* active transport, and is used in a wide variety of metabolic processes in tissues throughout the body where it performs important biological functions. Myo-inositol typically predominates in mammalian cells and tissues and is typically the form of primary nutritional and metabolic interest (Holub, 1986). Inositol catabolism primarily occurs in the kidney, where it is converted to D-glucose and D-glucuronolactone, or is completely oxidised to carbon dioxide and water (Howard and Anderson, 1967; Clements and Diethelm, 1979; Holub, 1986).

XIII.a.5 Potassium

In healthy individuals, approximately 85 to 90% of ingested potassium is absorbed, primarily in the small intestine (Holbrook, 1984; NRC, 1989; Sheng, 2000). The high intracellular concentration of potassium is maintained *via* the activity of the Na^+/K^+-ATPase pump. Plasma potassium concentrations appear to be independent of dietary intake due to the renal regulation of potassium balance (NRC, 1989). Essentially, urinary excretion of potassium is regulated by the hormone aldosterone, and is increased or decreased in situations of excess or deficiency (respectively) (Rodriguez-Soriano, 1995; Sheng, 2000). The majority of dietary potassium (approximately 77 to 90%) is excreted in the urine, while the remainder is excreted mainly in the faeces, with much smaller amounts being lost in sweat (Pietinen, 1982; Holbrook, 1984; Agarwal, 1994). In the generally healthy population (with normal kidney function), excess potassium is readily excreted in the urine. However, in individuals in whom urinary excretion of potassium is impaired, a lower potassium intake is appropriate due to potential adverse cardiac effects (arrhythmias) resulting from hyperkalaemia. Such individuals are typically under medical supervision (IOM, 2004).

XIII.b Safety Studies on ASI

XIII.b.1 Acute Toxicity

Sprague-Dawley rats (6/group; sex not reported) were administered a single dose of 2,000 mg ASI/kg body weight by oral gavage (Devine, 2003 [unpublished]). No mortalities were observed, and clinical signs of toxicity (including salivation, respiratory depression, diarrhoea, and tremors) resolved within 24 hours of dosing. As such, a median lethal dose of >2,000 mg/kg body weight was determined for ASI.

The administered dose of 2,000 mg/kg body weight is approximately equal to a dose of 140,000 mg for a 70-kg human. As this single bolus dose is 93.3 times greater than the maximum daily dose of ASI under its recommended conditions of use (*i.e.,* 1,500 mg/day), these results do not pose safety concerns.

XIII.b.2 Sub-Chronic Toxicity

Three repeated-dose animal studies on ASI (manufactured by Nutrition 21) were identified in the literature. These studies were conducted primarily to assess the efficacy of ASI (at a dose of 1.81 g/kg body weight/day) with respect to metabolism, vascular function, and biomarkers of metabolic syndrome in rats. Although safety-related endpoints were measured in all 3 studies, these studies are not traditional safety studies. However, given that the dose employed in these studies is approximately 85 times greater than the intake of ASI under the intended conditions of use, the lack of compound-related adverse effects reported in all 3 studies is supportive of the safety of ASI at the intended intake of 1,500 mg/day.

To determine the effects of ASI on metabolism and vascular function, Russel conducted 2 studies in which male and female JCR:LA-cp rats (8 to 10/group; proportion of males and females not reported) were given control diets or diets providing 1.81 g ASI/kg diet or 1 g arginine-HCl/kg diet (such that both treatment groups received 1 g arginine/kg body weight/day) for 4- and 8-week study periods. An additional group of lean JCR:LA-cp rats was also used as a control (lean control group). No adverse effects were reported with respect to clinical signs of toxicity, serum lipid levels, markers of bone metabolism and vascular function, or renal and hepatic histology.

Proctor *et al.* (2005) conducted a study to evaluate the effects of increased availability of arginine on vascular function in insulin-resistant male JCR:LA-cp rats. These rats, which are homozygous for the autosomal recessive *cp* gene, have impaired nitric oxide metabolism and vascular function, and are prone to developing metabolic syndrome. Beginning at 8 weeks of age, JCR:LA-cp rats were given control diet, diet supplemented with arginine-HCl (providing 1 g arginine/kg body weight/day), or diet supplemented with ASI (1.81 g/kg body weight/day, providing 1 g arginine/kg body weight/day) for 6 weeks. An additional group of male rats heterozygous for the *cp* gene (*cp*/+) were given control diet. Blood samples were collected after Study Week 5, and urine and tissue samples (*i.e.,* heart, aorta, and kidneys) were collected at termination at the end of Study Week 6. No adverse effects attributable to consumption of ASI were reported with respect to food intake or body weight; aortic contractility, coronary blood flow, or post-ischemia reactive hyperaemia (measured *ex vivo*); or urinary albumin, urinary albumin:creatinine, or glomerular histology. Elevated fasting plasma insulin levels in ASI-supplemented rats were attributed to arginine's insulin-secretogogue properties, and were not associated with any adverse effects on vascular function. No additional toxicologically relevant effects were reported.

Proctor *et al.* (2007) conducted a follow-up study to evaluate the effects of ASI or arginine-HCl on metabolic biomarkers of metabolic syndrome (*i.e.,* plasma insulin and glucose levels, and serum lipids levels) in male and female JCR:LA-cp rats. Animals (10/sex/group) were given the same diets as described above, from ages 8 to 12 weeks in males and from ages 12 to 18 weeks in females. Similar to the previous study (Proctor *et al.*, 2005), no adverse effects attributable to ASI were reported with respect to body weight or food consumption. Significantly elevated fasting plasma insulin levels were reported in ASI-treated males,

although no significant difference in post-prandial plasma insulin levels between ASI-treated males and control males was reported. In ASI-treated females, significant reductions in fasting and post-prandial insulin levels were reported compared to female controls. Significantly increased cholesterol ester and total cholesterol levels in ASI-treated females were considered by the study authors to be small. The study authors noted also that increased total cholesterol levels may have been indicative of a change in lipoprotein composition, with increasing high-density lipoprotein levels, which would have favourable physiological effects. The study authors provided no additional toxicologically relevant data, but concluded that their results support the use of ASI in the dietary management of metabolic syndrome.

XIII.b.3 Genotoxicity

The potential genotoxicity of ASI (manufactured by Nutrition 21) was evaluated in 2 *in vitro* tests and 1 *in vivo* test, the results of which are summarised in Table XIII.b.3-1. No mutagenic or genotoxic effects were reported in a reverse mutation assay, a chromosomal aberration assay, or a micronucleus assay (unpublished study reports). ASI's lack of genotoxic potential is corroborated by the long histories of consumption of its components, their natural occurrence in the human diet, and a lack of structural alerts for genotoxicity.

Table XIII.b.3-1 Genotoxicity Studies Conducted with ASI

Type of Study	System	Route of Administration, Dose, and Duration	Parameters Evaluated	Results	Reference
***In vitro* Studies**					
Bacterial reverse mutation assay	*Salmonella typhimurium* strains TA97a, TA98, TA100, TA02, and TA1535	0, 1,000, 1,500, 2,000, 2,500, or 3,000 µg/plate (-S9) 0, 500, 1,000, 2,000, 3,000, or 4,000 µg/plate (+S9)	• Numbers of revertant colonies	• The authors concluded that ASI was non-mutagenic	[unpublished study report]
Chromosomal aberration assay	Chinese hamster ovary cells	325 to 4,000 µg/mL (+/-S9)	• Number of structural or numerical chromosome aberrations	• No significant differences in structural or numerical aberrations compared to controls	[unpublished study report]
***In vivo* Studies**					
Micronucleus assay	ICR mice	Intraperitoneal injection 0 (control), 50, 100, or 200 mg/kg bw Single dose	• Mortality • Clinical signs of toxicity • Incidence of micronucleated polychromatic erythrocytes	• No mortalities • Clinical signs of toxicity: piloerection (all doses) and lethargy (200 mg/kg bw) • Mean ratio of polychromatic erythrocytes to total erythrocytes reduced (most	[unpublished study report]

Table XIII.b.3-1 Genotoxicity Studies Conducted with ASI					
Type of Study	**System**	**Route of Administration, Dose, and Duration**	**Parameters Evaluated**	**Results**	**Reference**
				doses) • No significant increase in the number of micronucleated polychromatic erythrocytes in treated groups compared to controls	

-S9 = without metabolic activation; +S9 = with metabolic activation; ASI = inositol-stabilized arginine silicate

XIII.b.4 Human Studies

The results of 2 clinical studies of ASI support the safety of ASI at doses up to 1,500 mg/day, consumed over 2 weeks by healthy men. As mentioned in Section XIII.a.1 above, Nutrition 21 conducted an uncontrolled clinical study to assess the bioavailability and safety of ASI and its effects on systemic nitric oxide levels (unpublished study report). In this study, 10 men (18 to 40 years of age, non-smokers, with BMI ≥18 to <30 kg/m^2, blood pressure <140/90 mm Hg, and normal serum creatinine concentrations) were assigned to consume 1,500 mg ASI/day (provided as 3 caplets to be taken together on an empty stomach, each providing 190 to 225 mg arginine, 115 to 245 mg inositol, 40 to 50 mg silicon, and 20 to 30 mg potassium) for 14 days. Adverse events were monitored throughout the study, and vital signs (*i.e.,* blood pressure and heart rate) were assessed on Days 1 and 14 of the study period. No significant changes were reported with respect to systolic or diastolic blood pressure or resting heart rate. Several adverse events were reported (increased hunger, vaso-vagal symptoms, shortness of breath, and sore throat); all were reported to be mild and "not related" or "probably not related" to consumption of ASI, and the study authors concluded that "None of these adverse events were of clinical concern." The results of this study support the safety of ASI, at a dose of 1,500 mg/day, consumed over 2 weeks by healthy men.

Nutrition 21 also conducted a prospective, randomised, double-blind, active-comparator crossover study to compare the pharmacokinetics of ASI to Arginine-HCl in healthy men (unpublished study report; described in Section XIII.a.1). Briefly, 10 healthy men (18 to 40 years of age, non-smokers, with BMI ≥18.5 to <25 kg/m^2, blood pressure <140/90 mm Hg, and normal serum creatinine concentration) were assigned to consume approximately 500 mg arginine from 2 capsules of Arginine-HCl or ASI per day for periods of 15 days, in random order. In addition to plasma L-arginine concentrations, several safety parameters were evaluated at baseline and on Study Days 1 and 15. No significant differences between treatments were reported with respect to systolic or diastolic blood pressure, although a trend toward decreased diastolic blood pressure (compared to baseline; P=0.068) was reported after consumption of ASI. The study authors noted that the decrease in diastolic

blood pressure (-3.0 ± 4.57 mm Hg) was not likely to be clinically significant. No significant differences between treatments were reported with respect to heart rate, and no adverse events or subjective comments were recorded. This study supports the safety of ASI, at a dose of approximately 1,100mg/day (850 mg arginine, silicon, and inositol/day as the ASI complex), consumed over 2 weeks by healthy men.

XIII.c Safety Studies on Components of ASI

As mentioned above, *in vitro* data (provided in Appendix A) indicate that the arginine silicate component of ASI dissociates in the acidic environment of the stomach, liberating arginine, silicon, inositol, and potassium. Thus, the safety of these components is relevant to the safety of ASI under the intended conditions of use. Safety data pertaining to potassium also are included due to the presence of the mineral at low levels in ASI.

XIII.c.1 Arginine

The expected intake of arginine under the intended conditions of use of ASI is 675 mg/day.

L-Arginine (2-amino-5-guanidinovaleric acid) serves a variety of important metabolic and physiological functions. Arginine is an intermediate in the urea cycle, which is involved in eliminating 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 synthesised endogenously from citrulline, a urea cycle intermediate, in 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, 2005). Arginine is consumed regularly in the diet as a constituent of plant and animal protein. Consumption of arginine as a food additive is insignificant compared to the amount consumed as a component of food proteins (Newberne *et al.*, 1998; U.S. FDA, 2015). Arginine is considered GRAS by the Flavor and Extract Manufacturers' Association.

Since arginine can be produced endogenously, there is no set recommended daily allowance (IOM, 2005). In 2005, the IOM stated that there were insufficient data to establish a UL for any of the amino acids (IOM, 2005). As the average consumption of arginine in the human diet is 5.4 g/100 g dietary protein (*i.e.,* not including supplement use) (FASEB, 1992), and the mean and 90th percentile daily dietary protein intakes for individuals (in the U.S.) are 75.2 and 114.0 g, respectively, the mean and 90th percentile daily intakes of arginine from food are estimated to be 4.06 g and 6.16 g, respectively (IOM, 2005). Additionally, doses of arginine of up to 21 g/day in dietary supplement form have been reported for treatment of cardiovascular dysfunction (PDRNS, 2008). The addition of 675 mg arginine/day from the consumption of ASI would increase the mean dietary exposure from 4.06 g/day to 4.735 g/day (*i.e.,* an increase of approximately 17%). This level of exposure to arginine is considerably lower than doses reported to be well tolerated in clinical studies, and is not expected to pose a safety concern.

XIII.c.1.1 Acute Toxicity Studies

The oral median lethal dose (LD_{50}) of arginine was reported to be 16 g/kg body weight in rats (Kyowa Hakko Koyo Co., Ltd., 2005), indicating a low order of acute toxicity.

XIII.c.1.2 Subchronic Toxicity Studies

Sprague-Dawley rats (6/sex/group) were given a standard diet with 0, 1.25, 2.5, or 5% L-arginine *ad libitum* for 13 weeks, beginning at 6 weeks of age, and were observed for 5 weeks following dosing to examine recoverability from any potential effects (Tsubuku *et al.*, 2004). The study 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. Ophthalmology, haematology, blood chemistry, pathology, histopathology, and urinalysis evaluations were conducted at various time points during and after the dosing period. The rats were examined for clinical signs of toxicity twice daily during the dosing period and daily for 5 weeks after dosing. No compound-related changes were observed, and the study authors determined the no-observed-adverse-effect level (NOAEL) for both sexes to be 5% L-arginine in the diet.

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 hypercholesterolaemic rabbits. Rabbits administered L-arginine *ad libitum* in drinking water at doses up to 2.25% [providing approximately 3,937 mg L-arginine or arginine/kg body weight (U.S. 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 hypercholesterolaemic knockout mice administered 2.25% L-arginine *ad libitum* in drinking water [providing approximately 5,625 mg L-arginine or arginine/kg body weight (U.S. 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 L-arginine or arginine/kg body weight (U.S. FDA, 1993)] for up to 8 weeks (Maxwell *et al.*, 2001).

XIII.c.1.3 Genotoxicity/Mutagenicity Studies

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

XIII.c.1.4 Supporting Data

Due to the limited availability of preclinical safety studies on L-arginine, studies investigating L-arginine salts are included herein to support the safety of L-arginine.

L-arginine hydrochloride is of low acute oral toxicity in rats (LD_{50} = 12,000 mg/kg body weight; RTECS, 2000). Significantly increased thymic weight (22% greater than controls), thymic lymphocyte content (45% greater than controls), and *in vitro* reactivity of thymic lymphocytes were reported in CBA/J mice given diets containing 0.5, 1.2, or 3% L-arginine hydrochloride [*i.e.,* providing approximately 750, 1,800, or 4,500 mg/kg body weight/day (U.S. FDA, 1993)] for 6 days (Barbul, 1986). However, no adverse effects were reported in rats orally administered 1,000 mg L-arginine hydrochloride/kg body weight/day for 7 days (Drago *et al.*, 1984), or in rats given diets providing up to 4,500 mg L-arginine hydrochloride/kg body weight/day for 15 days (Ronnenberg *et al.,* 1991).

XIII.c.1.5 Clinical Studies on Arginine

Clinical studies on arginine are discussed below and summarised in Table XIII.c.1.5-1.

Numerous studies have been published in which the safety and efficacy of L-arginine were examined in subjects with various health conditions. These data have been evaluated in detail by Shao and Hathcock (2008), who noted that an absence of any consistent pattern of adverse effects related to oral arginine supplementation provides support for a substantial level of confidence in its safety. In none of the human studies reviewed was any systemic and credible hazard related to arginine supplementation reported; therefore Shao and Hathcock (2008) found no basis for identifying a NOAEL or lowest-observed-adverse-effect level, and thus it was not possible to set a UL. Instead, Shao and Hathcock (2008) derived an observed safe level (OSL; described as an estimate of safe exposure determined from the highest intake with convincing evidence of safety without the application of additional safety factors) of 20 g arginine/day based on a lack of adverse effects reported in a study in which 20 subjects with heart failure consumed 20 g arginine/day for 4 weeks (Chin-Dusting *et al.*, 1996).

In a study conducted by Schulman *et al.* (2006), in which 6 deaths were reported among myocardial infarction patients receiving 9 g L-arginine/day, 5 of which were reported in patients 60 years of age or older. In comparison, no deaths occurred in the control group. The causes of the deaths in the L-arginine-treated group included a case of myocardial rupture following a recurrent anterior infarction, and 2 cases of presumed sepsis. Causes of death were not reported for 2 additional patients who were found dead at home without prior symptoms, or for a sixth patient who had been in the L-arginine group who died suddenly 3 weeks after the end of the treatment period and 4 months following his acute anterior myocardial infarction. Schulman *et al.* (2006) intended to increase nitric oxide (NO) production *via* arginine supplementation for the possible beneficial effects of nitric oxide on cardiovascular parameters. The role of NO in septic shock and the effects of arginine supplementation on inducible nitric oxide synthase activity and NO production in septic patients have not been fully elucidated. As a result, it is not possible to determine the potential role of L-arginine-induced NO production in the septic patients of the Schulman *et al.* (2006) study. Shao and Hathcock (2008) reviewed the results reported by Schulman *et al.* (2006) and concluded that the deaths were not treatment-related and identified several limitations with the study. Although Schulman *et al.* (2006) attributed the deaths to

consumption of arginine, Shao and Hathcock (2008) assert that the study was not powered to detect mortality. Furthermore, the results reported by Schulman *et al.* (2006) are not supported by other data, and the lack of detail regarding patient deaths precluded any conclusions about the possible mode of action of arginine (Abumrad and Barbul, 2006).

Appleton (2002) reviewed the clinical uses of arginine and reported that caution should be exercised in recommending arginine to any patient with a history of genital or oral herpes, asthma, or cancer. In the case of herpes, it has been postulated that high doses of arginine might stimulate replication of the virus and/or provoke an outbreak, although this correlation has not been validated by controlled clinical trials. As it is possible that NO may induce bronchoconstriction, the use of high-dose arginine in asthmatics is not generally recommended. Finally, as polyamines act as growth factors for cancer and arginine may stimulate polyamine synthesis, chronic, high-dose administration of arginine in cancer patients is contraindicated. Appleton (2002) noted that supplementation with arginine is otherwise generally safe at typically recommended doses of 1 to 15 g/day.

Böger (2007) reviewed the pharmacodynamics of L-arginine and noted that the effects of L-arginine supplementation on human physiology appear to be multicausal and dose-related. The author reported that supplemental doses of 3 to 8 g/day appear to be safe and not to cause acute pharmacological effects in humans. Similarly, based on their evaluation of the pharmacokinetics and safety of arginine supplementation in animals, Wu *et al.* (2007) reported that a 70 kg human should be able to tolerate long-term enteral supplemental doses of 15 g arginine/day in addition to basal intake (4 to 6 g/day) from regular diets.

Grimble (2007) reported that L-arginine induces water and electrolyte secretion that is mediated by NO, which acts as an absorbagogue at low levels and as a secretagogue at high levels. The action of many laxatives is NO mediated and there are reports of diarrhoea following oral administration of arginine. On this basis, Grimble (2007) reviewed adverse gastrointestinal effects of subjects consuming arginine supplements. The clinical data cover a wide span of arginine intakes (*i.e.,* 3 to >100 g/day), but the standard of reporting adverse effects (*e.g.* nausea, vomiting, and diarrhoea) was variable. When daily doses were consumed as a single dose of 3 to 6 g, adverse effects were rarely reported. Healthy athletes appeared to be more susceptible than diabetic patients to gastrointestinal symptoms when daily doses were consumed as a single dose of >9 g. Most adverse effects occurred at single doses of >9 g (*i.e.,* doses >128.6 mg/kg for a 70 kg adult), often when part of a daily regime of >30 g/day (>174 mmol/day). Grimble (2007) reported that adverse effects seemed dependent on the dosage regime, and were not observed when divided doses were ingested.

Adams *et al.* (1995) conducted a randomised, double-blind crossover study in which 12 healthy men (27 to 37 years of age) were given 21 g L-arginine/day (taken as 3 separate doses) or placebo for 3 days. No adverse effects with respect to blood lipid levels, blood biochemistry, hemodynamic parameters, heart rate, or blood pressure were reported. Although 2 subjects reported mild adverse effects (abdominal bloating or mild headaches)

while consuming L-arginine, and 1 subject reported mild gastrointestinal symptoms while consuming placebo, the study authors reported that the study products were well-tolerated.

Lerman *et al.* (1998) conducted a randomised, double-blind study to investigate the effects of L-arginine (9 g/day for 6 months) *versus* placebo on coronary endothelial function in 26 subjects without significant coronary artery disease. Subjects in the L-arginine group were given 3 g L-arginine/day for the first week, 6 g/day during the second week, and were given the target dose of 9 g/day from the third week until the end of the study period. Two subjects withdrew from the study due to minor adverse gastrointestinal effects (1 from each group). No other adverse effects were reported.

Chan *et al.* (2000) conducted an intervention study in which hypercholesterolaemic (n=10) subjects were given 14 or 21 g L-arginine/day for 12 weeks. No adverse effects on plasma insulin or lipid concentrations, or any adverse events were reported.

Bednarz *et al.* (2005) evaluated the safety and efficacy of L-arginine (3.0 g/day for 30 days) or placebo in addition to routine therapy on the clinical course of myocardial infarction (MI) in 792 patients (551 men, mean age 64 years) with ST segment elevation MI admitted within 24 hours after the onset of symptoms. In the 30 days following MI, cardiovascular death, reinfarction, successful resuscitation, shock/pulmonary oedema, or recurrent myocardial ischemia was reported in 24% patients treated with L-arginine (n=394) and 27% with placebo (n=398) (OR 0.63, 95% CI 0.39-1.02, p=0.06). No serious, compound-related adverse effects (*e.g.,* death, MI) were reported. Nine patients in the L-arginine group and 10 in the placebo group were withdrawn from the study due to suspected compound-related adverse effects. These effects included hypotension in 2 L-arginine subjects and 1 placebo subject (0.5 and 0.3%, respectively), and gastrointestinal disorders in 7 (1.8%) and 9 (2.3%) subjects, respectively. This study, which is the first attempt to use L-arginine in MI, showed that oral L-arginine supplementation was well tolerated.

Evans *et al.* (2004) conducted a study in which 12 healthy subjects were given increasing doses of 3, 9, 21, and 30 g L-arginine/day at 1-week intervals for 1 month. Of the 12 subjects, 4 experienced diarrhoea and 1 vomited at upon consumption of 21 g/day. At 30 g/day, 9 subjects experienced diarrhoea, and only 5 subjects completed the daily doses for the week. However, no adverse events were reported at the lower doses of 3 or 9 g/day.

Oka *et al.* (2005) conducted a pilot study to determine the lowest effective oral dose of L-arginine required to improve walking distance in patients with peripheral arterial disease and intermittent claudication. This randomised double-blind study included 72 patients who were given L-arginine at doses ranging from 0 to 9 g/day for 12 weeks. Although this was not a typical safety study, the study authors concluded L-arginine was well-tolerated and reported no significant adverse effects. In light of these results, Wilson *et al.* (2007) conducted a randomised, double-blind, placebo controlled clinical trial in 133 patients with intermittent claudication due to peripheral arterial disease. The subjects were given 3 g L-arginine/day for 6 months, and several safety parameters were assessed, including clinical chemistry, haematology, blood lipids, urinary nitrogen oxides, and plasma nitrogen oxides

and urea. No differences were reported with respect to serious or total adverse events between the two groups. However, the study authors suggested a possible adverse effect on functional capacity due to the limited improvement of absolute claudication distance in patients given L-arginine compared to placebo. Wilson *et al.* (2007) further stated this might be attributable to counter-regulatory mechanisms increasing nitrate tolerance in response to chronic exposure of L-arginine, which functions as an NO donor.

Ast *et al.* (2010) evaluated the anti-hypertensive efficacy and safety of L-arginine in 19 healthy subjects and 35 mildly hypertensive patients in a randomised double-blind trial. Subjects were given L-arginine (6 or 12 g/day) or placebo for 4 weeks. Researchers assessed 24-hour ambulatory blood pressure, blood cell count, lipids, and glucose. No adverse events were reported and L-arginine was well-tolerated at both doses.

Park *et al.* (1992) reported that consumption of 30 g L-arginine/day for 3 days by patients with breast cancer (number not specified) significantly increased their rate of tumour protein synthesis. These results have not been replicated in subsequent studies, and a number of preclinical studies have reported that L-arginine supplementation actually suppresses tumour growth (Milner and Stepanovich, 1979; Tachibana *et al.*, 1985; Barbul, 1986).

Table XIII.c.1.5-1 Clinical Studies on Arginine

Reference	Study population	Dose and Duration	Results and Comment
Park *et al.* (1992)	Breast cancer patients (number NR)	30 g/day or standard diet 3 days	Increased rate of tumour protein synthesis Results not corroborated by other clinical and preclinical studies (Milner and Stepanovich, 1979; Tachibana *et al.*, 1985; Barbul, 1986)
Adams *et al.* (1995)	12 healthy men	21 g/day or placebo 30 days	No compound-related adverse effects reported
Lerman *et al.* (1998)	26 subjects without significant coronary artery disease	9 g/day or placebo 6 months	No compound-related adverse effects reported
Chan *et al.* (2000)	10 hypercholesterolaemic subjects	14 or 21 g/day 12 weeks	No adverse effects reported (with respect to blood lipid and insulin levels)
Schulman *et al.* (2006)	153 subjects with recent first MI	9 g/day or placebo 6 months	6 deaths in arginine group (*vs*. none in placebo group; P=0.01) Arginine was otherwise well-tolerated; adverse effects generally mild (gastrointestinal symptoms or dizziness) and not compound-related
Bednarz *et al.* (2005)	792 subjects having recently (within 24 hours of enrolment) presented with symptoms of acute MI	3 g/day or placebo 30 days	Supplementation was well-tolerated No serious, compound-related adverse effects No adverse effects on cardiac complications
Evans *et al.* (2004)	12 healthy subjects	Doses increasing weekly, from 3 g/day to 9, 21, and 30 g/day 1 month	21 g/day: diarrhoea (4 cases); vomiting (1 case) 30 g/day: diarrhoea (9 cases); 5/12 subjects completed this phase of study 3 or 9 g/day: no compound-related adverse effects

Table XIII.c.1.5-1 Clinical Studies on Arginine			
Reference	**Study population**	**Dose and Duration**	**Results and Comment**
Oka *et al.* (2005)	72 subjects with peripheral arterial disease and intermittent claudication	3, 6, or 9 g/day or placebo 12 weeks	Treatment was well-tolerated; no significant adverse effects reported
Wilson *et al.* (2007)	133 subjects with peripheral arterial disease and intermittent claudication	3 g/day or placebo 6 months	No compound-related adverse effects
Ast *et al.* (2010)	19 healthy subjects and 35 mildly hypertensive subjects	6 or 12 g/day or placebo 4 weeks	No compound-related adverse effects; no adverse effects on blood pressure, cell counts, lipids, or glucose levels

MI = myocardial infarction; NR = not reported

The mean estimated dietary consumption of arginine (4.06 g/day) in combination with consumption of arginine under the recommended conditions of use of ASI (675 mg/day) is well below the OSL of 20 g arginine/day established by Shao and Hathcock (2008), and is therefore not expected to result in adverse health effects.

XIII.c.2 Silicates and Silicon

The silicon in ASI is supplied by potassium silicate. The expected intake of silicon under the intended conditions of use of ASI is 435 mg/day.

Silicon is the second most abundant element on earth, however, it is rarely found in the free form; it is generally found as silicon dioxide (*i.e.,* silica) or in silicates (Merck, 2006). 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). Additionally, refined and processed foods contain large amounts of silicon, and silicate additives have been increasingly used in prepared foods and confections such as anti-caking agents (T.J. Clark & Company, 2015). Although most dietary forms of silicon are poorly absorbed, silicon that is absorbed is mainly excreted in the urine (Jugdaohsingh *et al.*, 2002; PDRNS, 2008).

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 dietary 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 AI or tolerable upper intake level (UL) in humans; however, the IOM reported that there was no evidence that consumption of silicon that occurs naturally in food or water would lead to adverse health effects (IOM, 2001).

Calcium silicate and silicon dioxide/silicic acid gel were given a positive opinion by EFSA for addition to food supplements for nutritional purposes following a review by the Panel on

Food Additives and Nutrient Sources added to Food (ANS) in 2009 (EFSA, 2009b). Taking into account the UK Expert group on Vitamins and Minerals (EVM) Safe Upper Limit for silicon consumption (700 mg silicon/day) for adults over a lifetime (equivalent to 10 mg silicon/kg body weight/day for a 70 kg adult) (EVM, 2003), the Panel concluded that it had no safety concerns regarding the use of calcium silicate and silicon dioxide/silicic acid gel in food supplements providing up to 700 mg silicon/day (EFSA, 2009b). The European Food Safety Authority (EFSA) Panel on Dietetic products, Nutrition and Allergies (NDA) has not set a UL for silicon but has estimated that the typically dietary intake of silicon is between 20 and 50 mg/day (equivalent to 0.3 to 0.7 mg/kg body weight/day for a 70 kg adult), which is unlikely to cause adverse health effects (SCF/EFSA, 2006). The safety of silicates is supported by the permitted use of various silicate salts as food additives in the European Food Additives Database (European Commission, 2015).

The available safety data pertaining to potassium silicate are limited. However, due to their chemical similarities, it is appropriate to utilise data available for sodium silicate to assess the safety of potassium silicate. In a letter to the PQ Corporation, the FDA stated that it views potassium silicate to be interchangeable with sodium silicate (U.S. FDA, 1978), particularly when they have the same molar ratio (Schleyer and Blumberg 1982; Falcone 1997; Kuhr 1998). Potassium and sodium silicate are both highly soluble in water, and ingested silica derived from sodium silicate is indistinguishable from ingested silica derived from natural waters (FASEB, 1979).

Soluble silicates vary in toxicity based on their molar ratio. Evidence of an inverse relationship exists between molar ratio and toxicity, with a higher molar ratio eliciting lower toxicity. For instance, a higher molar ratio of 3.38 of sodium silicate has an LD_{50} value of 8,650 mg/kg/body weight compared to a lower molar ratio of 0.5 of sodium silicate exhibiting an LD_{50} value of 500 mg/kg/body weight. The molar ratio of the potassium silicate used in the manufacturing of ASI is 2.5. In an acute oral study, potassium silicate with a molar ratio of 2.25 demonstrated an LD_{50} value of 5,700 mg/kg/body weight. All treatment-related effects were reversible and no gross alterations were observed during autopsy (OECD SIDS, 2004).

As mentioned above, calcium silicate and silicon dioxide/silicic acid gel have been approved for addition to food supplements for nutritional purposes following a review by the ANS (EFSA, 2009b). The UK Expert group on Vitamins and Minerals established an UL of 700 mg silicon/day for adults (EVM, 2003). The EFSA NDA Panel has estimated that typical dietary intake of silicon is 20 to 50 mg/day, which is well below the established UL (SCF/EFSA, 2006). In addition, JECFA has approved potassium aluminium silicate and aluminium silicate for use as anticaking agents (JECFA, 2014). For calcium silicate and magnesium silicate, JECFA has established ADIs of “not specified”, indicating that the total dietary intake does not represent a health hazard. JECFA (2014) concluded that the available data support the “biological inertness” of orally administered silicates, and noted that any absorbed silicates are excreted by the kidneys without evidence of accumulation. Under the recommended conditions of use, ASI provides 435 mg silicon/day. This level of consumption, in addition to the typical dietary intake of silicon (*i.e.,* 20 to 50 mg/day), is

below the UL of 700 mg silicon/day determined by the EVM (2003), and is therefore not expected to result in adverse health effects.

XIII.c.3 Inositol

The expected intake of inositol under the intended conditions of use of ASI is 390 mg/day.

Inositol is produced by the human kidneys at a rate of approximately 4 g/day, an amount which is considerably greater than dietary intake of inositol (Holub, 1986). Dietary inositol is readily absorbed from the small intestine, is widely distributed, and is used in a wide variety of metabolic processes. 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).

Results of pre-clinical behavioural and anticarcinogenicity studies have demonstrated that inositol is of 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 inositol/kg body weight/day (U.S. FDA, 1993)], or in mice at doses of up to 1% inositol in the diet [providing approximately 1,500 mg inositol/kg body weight/day (U.S. FDA, 1993)] (Einat *et al.*, 1999b; Hecht *et al.*, 2001).

Results of clinical studies investigating the potential effects of inositol in psychiatric disorders have demonstrated that inositol is well-tolerated in humans at doses up to 12 g/day for 4 weeks (Benjamin *et al.*, 1995; Levine *et al.*, 1995). A few reported adverse effects in have included insomnia, nausea, and flatulence (Benjamin *et al.*, 1995; Levine *et al.*, 1995; Barak *et al.*, 1996; Levine, 1997; PDRNS, 2008). In a more recent review, Larzelere *et al.* (2010) noted that inositol was fairly well-tolerated, but at high doses (not further specified) was associated with mild gastrointestinal distress, and at very high doses (not further specified), it has been reported to cause sleepiness, headache, and dizziness.

In an open-label, dose-escalation clinical study, 16 smokers with bronchial dysplasia were given myo-inositol at increasing doses of 12, 18, 24, and finally, 30 g/day, with doses increased monthly (Lam *et al.*, 2006). Based on reports of primarily gastrointestinal symptoms, the maximum tolerated dose was determined to be 18 g/day. In a separate group of 10 subjects given 18 g myo-inositol/day for 3 months, gastrointestinal symptoms were reported only during the first month of the study.

High-dose supplementary inositol may theoretically have additive effects with selective serotonin reuptake inhibitors (SSRIs), although this has not been demonstrated in clinical studies in subjects receiving 12 g inositol/day and SSRIs (PDRNS, 2008). High-dose inositol also should be avoided by pregnant and nursing women due to a lack of relevant long-term safety data (PDRNS, 2008). However, considering the endogenous production of approximately 4 g inositol/day, dietary consumption of up to 1,500 mg/day (EFSA, 2009a), and a maximum tolerated dose of 12 g inositol/day in several clinical studies, the

consumption of 390 mg inositol/day under the intended conditions of use of ASI is not expected to result in adverse health effects.

XIII.c.4 Potassium

Potassium is the major intracellular cation in the human body. Its major functions include its effects on transmembrane potential and its role as the determinant of intracellular ionic strength (NRC, 1989; IOM, 2004). An NRV of 2,000 mg has been set for potassium by EFSA (European Parliament and the Council of the European Union, 2011), and an AI of 4.5 g potassium/day for adults, based on very low risk of excessive potassium intake in healthy individuals with normal kidney function, has been established by the IOM (2004). Considering the specification for potassium listed in Table I.e-2 (40 to 60 mg/g ASI), a daily intake of 1,500 mg ASI would provide 75 mg potassium. As this dose is approximately 60 times lower than the AI established by the IOM, and approximately 30 times lower than the NRV set by EFSA, adverse effects due to intake of potassium as a component of ASI are not expected to occur.

EVALUATION AND CONCLUSION

Approval is sought under Regulation (EC) No 258/97 of the European Parliament and of the Council of 27th January 1997 concerning novel foods and novel food ingredients, for the approval of ASI as a new source of arginine for use in PARNUTS/foods for specific groups and as an ingredient in food supplements and in nutrition bars for sportsmen. As demonstrated in Sections I through XIII, ASI is safe to consume within the parameters described herein.

ASI is intended to be used in PARNUTS/foods for specific groups, as an ingredient in food supplements and in nutrition bars for sportsmen, at levels providing up to 500 mg/serving. The recommended total intake of ASI is 1,500 mg (*i.e.,* providing 675 mg arginine, 435 mg silicon, 390 mg inositol, and 75 mg potassium) per day. On a body weight basis, these doses are approximately equal to 9.6, 6.2, 5.6, and 1.1 mg/kg body weight/day (respectively) for a 70-kg adult.

The safety of ASI is based on its chemical purity (>90%, as indicated by product specifications in Section I.e), as well as clearly defined product specifications and manufacturing details demonstrating the stability of the final product and an absence of chemical and microbiological contaminants. The dissociation of the arginine silicate component of ASI upon ingestion in the acidic environment of the stomach (yielding arginine, silicon, inositol, and potassium) is supported by the results of an *in vitro* dissociation study and 2 pharmacokinetic studies in men (unpublished study reports). Thus, the safety of ASI is largely supported by knowledge of the metabolism, history of consumption, and safety of its dissociated components due to their natural presence in foods.

The safety of ASI is based on a lack of relevant adverse effects reported in studies of ASI (manufactured by Nutrition 21) and its components, arginine, silicate, and inositol. The safety of ASI is supported by the results of product-specific animal toxicology and genotoxicity studies, which demonstrate that ASI does not cause adverse effects upon oral administration to rats for up to 8 weeks (at a dose of 1.81 g/kg body weight/day), and also lacks mutagenic potential. The results of 2 studies in healthy men demonstrate that ASI dissociates in the acidic environment of the stomach, provides a bioavailable source of L-arginine and silicon, and does not cause adverse cardiovascular effects at doses up to 1,500 mg/day.

The preclinical and clinical studies on the individual components of ASI demonstrate that arginine, silicon, inositol, and potassium have low oral toxicity in experimental animals and humans. As mentioned above, the recommended consumption of ASI would provide 675 mg L-arginine, 435 mg silicon, 390 mg inositol, and 75 mg potassium per day. These intakes are well below established upper limit doses and doses reported to be well tolerated in human studies [*i.e.,* 20 g arginine/day (Shao and Hathcock, 2008), 700 mg silicon/day (EVM, 2003), 12 g inositol/day (Benjamin *et al.*, 1995; Levine *et al.*, 1995), and 4.5 g potassium/day (IOM, 2004)], even when combined with background dietary intakes. Collectively, a comparative analysis of the toxicological, toxicokinetic, and human

bioanalytical data on ASI supports the conclusion that it can be safely used as an ingredient in in PARNUTS/foods for specific groups, as an ingredient in food supplements and in nutrition bars for sportsmen, at levels providing a maximum recommended dose of 1,500 mg/day.

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Table of CFR Sections Referenced (Title 21—Food and Drugs)

Part	Section §	Section Title
160—Eggs and egg products	160.105	Dried eggs
169—Food dressings and flavorings	169.179	Vanilla powder
172—Food additives permitted for direct addition to food for human consumption	172.320	Amino acids
173—Secondary direct food additives permitted in food for human consumption	173.310	Boiler water additives
182—Substances generally recognized as safe	182.1045	Glutamic acid
	182.1711	Silica aerogel
	182.2729	Sodium calcium aluminosilicate, hydrated
184—Direct food substances affirmed as generally recognized as safe	184.1	Substances added directly to human food affirmed as generally recognized as safe (GRAS)
	184.1370	Inositol
	184.1069	Malic acid

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