Application for the Approval of the use REGENASURE® Non-Shellfish Glucosamine Hydrochloride from *Aspergillus niger (RGHAN)*, for use in Certain Foods Products under *Regulation (EC) No 258/97 for the European Parliament and of the Council of 27 January 1997 concerning novel foods and novel food ingredients.*

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FINAL

NON-CONFIDENTIAL

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ADMINISTRATIVE DATA

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SUMMARY AND CONCLUSIONS

Glucosamine is a naturally occurring amino-sugar, found largely in cartilage, which is known to play an important role in the health and resilience of joints. It is a major building block of complex proteins called glycosaminoglycans, which form a component of the structure of cartilage. Glucosamine dietary supplements are widely available in the UK and throughout Europe and the world to support joint health for aging individuals and for people with intensive physical activity, such as sportsmen and women.

Cargill, Incorporated produces REGENASURE® Glucosamine Hydrochloride through a unique process from chitin sourced from a vegetative microorganism, *Aspergillus niger*, whereas all other known commercial glucosamine products are derived from shellfish.

Under *Regulation (EC) No 258/97 of the European Parliament and of the Council of 27th January 1997 concerning novel foods and novel food ingredient*s (hereafter referred to as Regulation 258/97) glucosamine hydrochloride would be regarded as a novel food ingredient. Regulation 258/97 covers a number of classes of foods and food ingredients that have not been exposed to a significant degree to the EU population prior to May 1997. Non-Shellfish Glucosamine (from the fungi *Aspergillus niger*) would be clearly identified as "Novel"[1] under Article 1 (2)(d) of this regulation as "foods and food ingredients consisting of or isolated from microorganisms, fungi, or algae." Although *Aspergillus niger* has been extensively used in the production of many traditional foodstuffs, for example citric acid and soya sauce, it has not been used as a source of chitin for the production of glucosamine before May 1997.

In 2004, Cargill, Incorporated applied to the UK Competent Authority, the Food Standards Agency (FSA), for an expert opinion confirming that REGENASURE® non-shellfish glucosamine hydrochloride derived from *Aspergillus niger* (RGHAN) is substantially equivalent to shellfish glucosamine hydrochloride, when used in food supplement and foods for particular nutritional purposes (Council of the European Communities, 1996). The FSA consulted its expert body, the Advisory Committee for Novel Foods and Processes (ACNFP), and delivered its opinion on the 5th August 2004 that the FSA "..is content that your fungal glucosamine HCl meets the criteria for equivalence, as defined in Article 3(4) of regulation (EC) 258/97." ACNFP, 2004). Notification was then made to the European Commission and no objection was made. Consequently RGHAN is now listed as a notified food on the Commission's web site (European Commission, 2005).

[1] The terms "Novel", "Novelty", and "Substantially Equivalent" as used herein should be interpreted as they relate to food safety standards only. The use of these terms by Cargill is not intended, and should not be used to determine, or interpret, the patentability or validity of Cargill's patent application(s).

Cargill, Incorporated now plans to market RGHAN as a "novel" food ingredient in pasteurised fruit Juices and fruit juice products (including tomato and tomato mixtures and fruit "smoothies"); dehydrated instant drink mixes; fermented milk-based products, yoghurts and fromage frais; sports drinks and iced tea drinks. This would provide an alternative to food supplement products currently being sold in tablet and capsule form. Since such products are not already available in the EU at present with any source of glucosamine and the pattern of consumption would be new, approval is now using the full application procedure. Accordingly, this submission has been prepared pursuant to the *Commission Recommendation of 29 July 1997 concerning the scientific aspects and the presentation of information necessary to support applications for the placing on the market of novel foods and novel food ingredients* (hereafter referred to as the Commission Recommendation of 1997).

Section 4 of the Commission Recommendation of 1997 outlines recommendations made by the Scientific Committee for Food (SCF) pertaining to the "Scientific Classification of Novel Foods for the Assessment of Wholesomeness", which facilitates the safety and nutritional evaluation of a given novel food/food ingredient. Of the six classes identified, RGHAN would be allocated a Class 2.1 designation as "a Complex (non-GM/derived) novel food ingredient; the source of the NF has a history of food use in the community" because *Aspergillus niger* has been extensively used in the production of many traditional foodstuffs, for example citric acid and soya sauce. The recommendation further sets out the headings required for the application dossier and this submission has prepared in accordance with these guidelines.

The specifications for RGHAN are well defined and demonstrate that the manufacturing method for RGHAN is reproducible and produces comparable batches of the end product. The processing method carefully isolates glucosamine produced within the fungal biomass through acid hydrolysis of the fungal biomass, separation of glucosamine from the fungal biomass solids, and precipitation of glucosamine hydrochloride in crystal form. The processing method results in the formation of RGHAN that meets USP-NF specifications for glucosamine hydrochloride.

Due to the pH stability of RGHAN, acidic food systems are most suitable. Consequently Cargill, Incorporated propose to include RGHAN at 750 mg (approximately 623 mg of free-base glucosamine) per daily serving in the following pasteurised food products:

Fruit Juices and fruit juice products including: Tomato, tomato mixtures and fruit "smoothies" (~ 2 – 5 pH)
Dehydrated instant drink mixes (stable in dry form, pH <7 when mixed with liquid and consumed at point of use)
Fermented milk-based products, yoghurts and fromage frais (~ 3 – 5 pH)

Sports Drinks (~ 2 – 5 pH)
Iced Tea Drinks (~ 2 – 6 pH)

It is important to note that foods fortified with RGHAN are intended for population groups that seek nutritional supplementation to maintain joint health. Typically these groups include older people, sportsmen and women. Based on the use levels and serving sizes presented in Table IX.2-1, Cargill, Incorporated has calculated mean and upper level percentile intakes for different population groups, using the comprehensive data contained in the United Kingdom's National Diet and Nutrition Survey Programme.

On a mg/person per day basis the theoretical highest mean and 95th percentile intakes, of approximately 482 mg and 1352 mg RGHAN respectively (*i.e.*, approximately 401 and 1124 mg/day free-base glucosamine), may occur in young people/children between the ages of 4 and 10. However, these calculations are based on such children being specifically marketed for regular consumption of these products, which would not be the case; and indirect consumption could not reasonably be considered to be at a similar level to that of conventional soft drinks, yoghurts *etc.* Of the other population groups, intakes are similar with mean daily intakes for all person consumption ranging from 351 to 393 mg/day of RGHAN (approximately 292 to 327 mg free base glucosamine); and 95th percentile intakes for the same groups ranging from 1162 to 1274 mg/day (*i.e.*, approximately 966 to 1059 mg/day of free base glucosamine).

Glucosamine is a prominent component of the hexosamine pathway, an important branch of glycolysis. Exogenous glucosamine is actively transported from extracellular tissue into cells by glucose transporters; (Uldry *et al.*, 2002) insulin facilitates glucosamine transport into cells (Heart *et al.*, 2000). Once in the cell, glucosamine is phosphorylated by one of the family of hexokinases to glucosamine-6-phosphate (GlucN-6-P). The metabolism of glucosamine is highly regulated by rates of transport into various tissues and by effects of intermediates on key enzymatic steps.

Glucosamine HCl and glucosamine sulphate have demonstrated glucosamine to be well absorbed from the gastrointestinal tract. The approximate bioavailability of glucosamine following oral administration, as determined from the glucosamine sulphate area under the curve (AUC) data, was reported to be approximately 26% of that available after iv or im administration. The low bioavailability of glucosamine following oral administration was attributed to the first pass effect in the liver, which results in the metabolism of glucosamine to smaller molecules and finally to CO_2, water, and urea (Setnikar *et al.*, 1993).

Studies conducted to examine the potential toxicity of glucosamine in various animal species (*e.g.*, rats, dogs, mice, rabbits, and horses) have demonstrated that glucosamine is safe at the doses administered. Acute toxicity studies demonstrated that the oral LD_{50} dose for rats (Sprague-Dawley), mice (CD-1), and rabbits (New Zealand White Albino) were greater than

8000, 8000, and 6000 mg/kg body weight, respectively, for glucosamine sulphate (Setnikar *et al*., 1991a). The acute toxicity study conducted with RGHAN demonstrated that the LD_{50} for in Crl:CD (SD) IGS BR rats was greater than 5000 mg/kg body weight (Glaza, 2002). Similarly, subchronic and chronic oral studies reported no toxicity or occurrence of adverse effects attributable to glucosamine at levels up to 2130 mg/kg body weight/day (free-base). For a 60 kg adult this would be equivalent to up to approximately 127,800 mg/person per day for RGHAN. These compare favourably to predicted intakes of up to 1274 mg/person per day of RGHAN at the 95th percentile for the targeted consumers.

Furthermore, *in vitro* and *in vivo* genotoxicity tests have demonstrated that RGHAN is non-genotoxic. Even though Banerjee and Manna *et al.* (1984) reported a positive result in the mouse chromosomal aberration study, only a single dose was tested. The preclinical studies clearly demonstrated that glucosamine was safe at the administered doses.

The clinical studies using various forms of glucosamine clearly demonstrated that the consumption of glucosamine is well-tolerated and safe at levels comparable with predicted mean and high-level consumption. Volunteers were reported to consume glucosamine supplements over periods ranging from 21 days to 3 years with the majority of the studies providing glucosamine at a dose of approximately 1500 mg/day with glucosamine HCl doses reported as high as 3200 mg/day (approximately 2656 mg/day free-base glucosamine). A range of adverse effects were reported in the clinical trials; however, the majority of the adverse effects were non-specific, mild gastrointestinal symptoms commonly reported in conjunction with glucosamine supplementation (*e.g.*, constipation, diarrhoea, nausea, dyspepsia, excessive gas, abdominal distension, and abdominal cramps), as well as headaches, skin rash, or pruritis. The safety of glucosamine consumption has been supported by various reviews and meta-analyses, as well as by a mutli-center clinical trial conducted by Clegg *et al.* (2006) where 1,583 patients were provided with 1 of 4 treatments including 1500 mg/day of glucosamine HCl and the occurrence of adverse effects were comparable between the glucosamine and placebo groups. Again these levels compare favourably to predicted intakes of RGHAN of up to 1274 mg/person per day at the 95th percentile for the targeted consumers. Our conclusions reflect those of Anderson *et al.* (2005) who reviewed much of the same data and concluded that "Our critical evaluation indicates that glucosamine is safe under current conditions of use and does not affect glucose metabolism".

Based on intakes provided it can be clearly seen that, for the proposed food uses of RGHAN in specific beverages and fermented milk-based products, aimed at the nutritional support of joint health, the safe endpoints of both animal and human safety would clearly not be exceeded by consumption of RGHAN at the recommended maximum use levels.

INTRODUCTION

Glucosamine is a naturally occurring amino-sugar, found largely in cartilage, which is known to play an important role in the health and resilience of joints. It is a major building block of complex proteins called glycosaminoglycans, which form a component of the structure of cartilage. Glucosamine dietary supplements are widely available in the UK and throughout Europe and the world to support joint health for aging individuals and for people with intensive physical activity, *e.g*. sportsmen and women. Exogenous sources of glucosamine are typically derived from chitin, a biopolymer that can be found in the exoskeletons of shellfish, insects and the cell walls of certain microorganisms (Ravi Kumar, 2000). Glucosamine, as currently consumed is available in two main forms:

A. Glucosamine Hydrochloride

   This is the original extract form of glucosamine. It is chemically stable and requires no additives to maintain its activity.

B. Glucosamine Sulphate

   This is a chemically modified form of glucosamine for patent protection. Because the sulphate degrades readily, either sodium or potassium chloride are added to the sulphate. Vitamin C and calcium carbonate are also often added as stabilizers.

Cargill, Incorporated produces REGENASURE® Glucosamine Hydrochloride through a unique process from chitin sourced from a vegetative microorganism, *Aspergillus niger*, whereas all other known commercial glucosamine products are derived from shellfish.

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Article 3 (4) of Regulation 258/97 states "By way of derogation from paragraph 2 (which refers to sections on the full application procedure), the procedure laid down in Article 5 shall apply to foods or food ingredients referred to in Article 1 (2) (b) (d) and (e) which, on the

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basis of the scientific evidence available and generally recognized or on the basis of an opinion delivered by one of the competent bodies (Member State) are substantially equivalent to existing foods as regards their composition, nutritional value, metabolism, intended use and the level of undesirable substances contained within." In 2004, Cargill, Incorporated applied to the UK Competent Authority, the Food Standards Agency (FSA), for an expert opinion confirming that REGENASURE® glucosamine hydrochloride derived from *Aspergillus niger* (RGHAN) is substantially equivalent to shellfish glucosamine hydrochloride, when used in food supplement and foods for particular nutritional purposes (Council of the European Communities, 1989). The FSA consulted its expert body, the Advisory Committee for Novel Foods and Processes (ACNFP), and delivered its opinion on the 5th August 2006 that the FSA "..is content that your fungal glucosamine HCl meets the criteria for equivalence, as defined in Article 3(4) of regulation (EC) 258/97." (ACNFP, 2004) Notification was then made to the European Commission and no objection was made. Consequently RGHAN is now listed as a notified food on the Commission's web site (European Commission, 2005).

Cargill, Incorporated now plans to market RGHAN as a "novel" food ingredient in certain beverages and fermented milk-based products. This would provide an alternative to dietary supplement products currently being sold in tablet, capsule, sachet and liquid concentrate form. Since such products are not already available in the EU at present with any source of glucosamine and the pattern of consumption would be new, approval is now sought using the full application procedure. Accordingly, this submission has been prepared pursuant to the *Commission Recommendation of 29 July 1997 concerning the scientific aspects and the presentation of information necessary to support applications for the placing on the market of novel foods and novel food ingredients* (hereafter referred to as the Commission Recommendation of 1997).

Section 4 of the Commission Recommendation of 1997 outlines recommendations made by the Scientific Committee for Food (SCF) pertaining to the "Scientific Classification of Novel Foods for the Assessment of Wholesomeness", which facilitates the safety and nutritional evaluation of a given novel food/food ingredient. Of the six classes identified, REGENASURE® glucosamine hydrochloride would be allocated a Class 2.1 designation as "a Complex (non-GM/derived) novel food ingredient 2.1 the source of the NF has a history of food use in the community". The recommendation further sets out the headings required for the application dossier as follows:

I. Specification of the novel food

II. Effect of the production process applied to the novel food

III. History of the organism used as the source of the novel food

IV. – VIII. are not applicable to non-GM Foods

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 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. Much of the compositional, undesirable substances and nutritional data presented in this dossier is derived from the original submission dossier from Cargill, Incorporated to the ACNFP as part of their previous substantial equivalence notification.

I. SPECIFICATIONS OF THE NOVEL FOOD

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, taxonomy *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.1 through I.11.

I.1 Common or Usual Name

Glucosamine hydrochloride (HCl) is occasionally referred to as simply "glucosamine." However, "glucosamine" can be used to refer to several common glucosamine forms, including glucosamine hydrochloride, glucosamine sulphate, and N-acetyl-glucosamine.

In it's "Final Opinion on the Substantial Equivalence of Glucosamine HCL derived from *Aspergillus niger"*, in August 2004, the Food Standards Agency/ACNFP noted:

"Additional information- Labelling

12. The applicant intends to label the product as "Non-Shellfish Glucosamine Hydrochloride" with a footnote referring to its source "from the fungus Aspergillus niger".

Cargill, Incorporated now propose to simplify this labelling to "Non-Shellfish Glucosamine Hydrochloride" with a footnote referring to its source "from *Aspergillus niger*". The term "fungus" is perceived by some consumers as an indirect inference to mould growth and associated hazards due to toxins produced by them. Cargill, Incorporated seeks therefore to remove this reference. The original concern of the ACNFP was to ensure that any consumers with a potential allergy specifically to *Aspergillus* sp., were aware of the source of this glucosamine HCL. However, it must be pointed out that analysis has shown that there is no presence of the organism in the final RGHAN product. Furthermore, such products as citric acid and soya sauce that also use *Aspergillus* sp., as the fermentation organism, have a long and safe history of use without labelling, and allergnicity to this species has typically been associated with respiratory exposure at much higher levels.

I.2 Chemical Names

Formal names and synonyms for glucosamine hydrochloride can include the following:

2-amino-2-deoxy-D-glucose

2-amino-2-deoxy-beta-D-glucopyranose hydrochloride

alpha-D-glucosamine hydrochloride

D-glucose, 2-amino-2-deoxy-hydrochloride

D-glucosamine hydrochloride

I.3 Trade Names

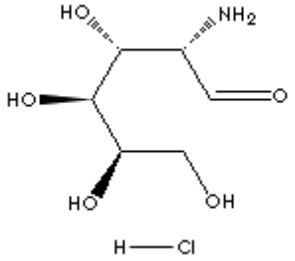
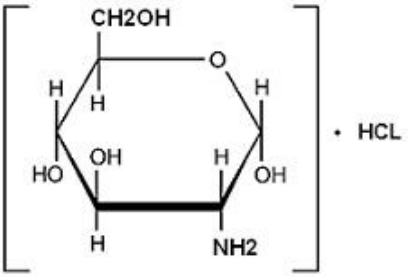
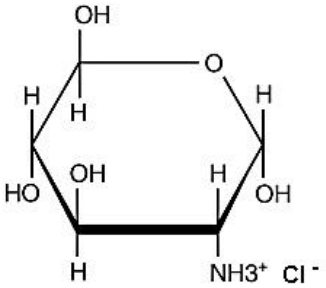
The trade name of Cargill, Incorporated's glucosamine hydrochloride product is REGENASURE® Glucosamine Hydrochloride.

I.4 Chemical Abstract Service (CAS) Number

The Chemical Abstracts Service (CAS) Name for glucosamine is glucosamine (9CI), and the CAS Registry Number for glucosamine hydrochloride is [66-84-2].

I.5 Chemical Structure

The structural formula for glucosamine can be represented using different styles of molecular presentation as shown in Figures I-1, I-2 and I-3.

Figure I-1.	**Figure I-2.**	**Figure I-3.**
		

I.6 Molecular Formula and Weight

Glucosamine hydrochloride is a single molecule. The molecular formula for glucosamine hydrochloride is $C_6H_{13}NO_5 \cdot HCl$. The formula weight for glucosamine hydrochloride is 215.63.

RGHAN contains 83.1% free-base glucosamine. This conversion factor is used throughout this dossier.

I.7 Specification

RGHAN is analysed to confirm that it conforms to specification and purity standards. The current standard that Glucosamine Hydrochloride must conform to is the United States Pharmacopoeia-National Formulary (USP-NF) monograph for Glucosamine Hydrochloride. There are 12 tests outlined in the USP-NF monograph (Appendix 1). These are outlined in Table I-1.

Table I-1 USP-NF Specifications for Glucosamine Hydrochloride

USP-NF Test	*Method*	*USP-NF Specifications*
Identification	197K	A: *Infrared Absorption**
Identification	191	B: It meets the requirements of the tests for *Chloride*
Identification		C: The retention time of the major peak in the chromatogram of the *Assay Preparation* corresponds to that in the chromatogram of the *Standard Preparation*, as obtained in the *Assay*.
Specific Rotation	781S	Between +70.0° to +73.0° (test solution 25 mg per mL)
pH	791	Between 3.0 to 5.0, in a solution containing 20 mg per mL
Loss on Drying	731	Dry it at 105°C for 2 hours: it loses not more than 1.0% of its weight
Residue on Ignition	281	Not more than 0.1%
Sulphate	221	A 0.10 g portion shows no more sulphate than corresponds to 0.25 mL of 0.020 N sulfuric acid: not more than 0.24% is found
Arsenic	Method II (211)	3 µg per g
Heavy Metals	Method II (231)	0.001%
Organic Volatile Impurities	Method I (467)	Meets the requirements
Assay	*	98.0% to 102.0%

USP, 2006
* Glucosamine assay performed using AOAC Official Method 2005.01

In addition Cargill coordinates microbiological testing, the details of which are provided in Section XII.

I.8 Conformance to USP NF Specification – Analysis Results

To correctly characterize the product, testing is performed according to the glucosamine hydrochloride monograph methodology outlined in the USP-NF. Using this methodology, five non-consecutive lots of RGHAN manufactured over an extended period of time were analysed and compared. Table I-2 includes analytical results of the five non-consecutive RGHAN lots.

Table I-2 Analysis Results for RGHAN

			Cargill, Incorporated Lot Number					
USP Analysis	***Cargill Internal Specification***	***USP/ NF Specification***	***RPE5048***	***RSE5053***	***RPE5078***	***RPE6010***	***RSE6013***	***Cargill Average Result***
Identification: chloride	Passes test	Passes test	Pass	Pass	Pass	Pass	Pass	Pass
Identification: HPLC retention time	Passes test	Passes test	Pass	Pass	Pass	Pass	Pass	Pass
+Assay (Purity)	98.0% to 102.0%	98.0% to 102.0%	101.6	99.4%	99.3%	98.2%	99.0%	99.5%
Loss on drying	1.0% maximum	1.0% maximum	0.5%	0.2%	0.4%	0.5%	0.3%	0.4%
Specific rotation	+70.2° to +72.8°	+70.0° to +73.0°	+71.6°	+70.6°	+71.2°	+71.0°	+70.8°	+71.0°
PH	3.0 to 5.0	3.0 to 5.0	3.3	3.3	3.2	3.1	3.3	3.2
Residue on ignition	0.1% maximum	0.1% maximum	0%	0%	0%	0%	0%	0%
Sulphate	0.24% maximum	0.24% maximum	Pass	Pass	Pass	Pass	Pass	Pass
Arsenic	3 ppm maximum	3 ppm maximum	<0.02 ppm	0.03 ppm	0.05 ppm	<0.02 ppm	<0.02 ppm	<0.03 ppm
Heavy Metals	0.001% maximum	0.001% maximum	<0.001%	<0.001%	<0.001%	<0.001%	<0.001%	<0.001%
**Organic volatile impurities*	Passes test	Passes test	N/A	N/A	N/A	N/A	N/A	N/A

* Organic Volatile Impurities is a test that is not applicable to Cargill, Incorporated's process, but this test is periodically performed to verify that REGENASURE® Glucosamine Hydrochloride does pass this test.
USP, 2006
+Glucosamine assay performed using AOAC Official Method 2005.01

I.9 Additional Analyses for Contaminants

Cargill, Incorporated has also had independent analysis conducted by for the following criteria:

- USP/NF Pesticide Screen – This test found no measurable pesticides in the product (Appendix 1).
- Aflatoxin Test – This test found no measurable Aflatoxin B1, B2, G1 and G2 in the product (Appendix 1).
- Ochratoxin A – This test has found no measurable Ochratoxin A in routine samples, with a detection limit of 1 µg/kg (ppb) by testing the glucosamine product (Appendix 2). The production method for RGHAN, as described in Section II, involves a step utilizing concentrated hydrochloric acid at elevated temperatures, which is very

significant, as ochratoxin A has been shown not to be stable in these conditions. Indeed the World Health Organisation Environmental Health Series Report on Ochratoxin (WHO, 1990), states that "On acid hydrolysis, ochratoxin A yields phenylalanine, and the isocoumarin part, ochratoxin alpha". Another part of FAO/WHO has also reviewed ochratoxin. The JECFA 47th Series report on ochratoxin (JECFA, 2001) states, in numerous places, that "ochratoxin alpha is harmless".

Further references regarding the effect of acid hydrolysis on ochratoxin A and the toxicity of ochratoxin-alpha are also available (van der Merwe *et al.*, 1965; Patterson *et al*., 1981; Kiessling *et al.*, 1984; Bredenkamp *et al*., 1989).

I.10 Protein Analysis for Residual Biomass

One could reasonably expect that the digest (acid hydrolysis) stage of the process, which uses concentrated hydrochloric acid to treat the biomass for several hours at 100ºC, is sufficient to destroy proteinaceous material from the source, and subsequent purification steps remove solid impurities. Due to the amine group within glucosamine, the standard Bradford assay for protein as total nitrogen could not be used to measure biomass residues. To investigate whether proteinaceous material could withstand the rigorous processing conditions of manufacture, the finished product was tested for the presence of proteins by the Protein Facility at Iowa State University. Gel electrophoresis followed by Sypro Ruby and Coomassie Brilliant Blue R250 staining was performed. Sypro Ruby has a similar sensitivity to silver staining and is a highly sensitive technique for the qualitative identification of proteins. The results were interpreted as "indicating the absence of protein". The report of this analysis are presented here as Appendix 3. It can be seen from the electrophoresis tests of RGHAN, that no contamination of the sample due to proteins well below the 10 KDa marker could be seen by either the Coomassie or the Sypro-Ruby stain. The controls on the gels show the very high sensitivity of the SyproRuby stain.

II. EFFECT OF THE PRODUCTION PROCESS APPLIED TO THE NOVEL FOOD

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?"
- "Does the process result in a significant change in the composition or structure of the NF compared to its traditional counterpart?"

These questions have been addressed collectively in Sections II.1 and II.2.

II.1 Overview of the Manufacturing Process

The manufacture of RGHAN, is conducted in a facility which uses current Good Manufacturing Practices (21 CFR Part 110 – FDA, 2005) for food and Hazard Analysis and Critical Control Point (HACCP) programs, which are updated annually.

The processing method carefully isolates glucosamine produced within the fungal biomass through acid hydrolysis; separation of glucosamine from the fungal biomass solids, and precipitation of glucosamine hydrochloride in crystal form. This process is essentially the same as that for the production of glucosamine hydrochloride from shellfish sources. The processing method results in the formation of RGHAN that meets USP-NF specifications for glucosamine hydrochloride (see Section II).

III. HISTORY OF THE ORGANISM USED AS THE SOURCE

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

- "Is the novel food obtained from a biological source, *i.e.*, a plant, animal or microorganism?"
- "Has the organism used as the source of the novel food been derived using GM?"
- "Is the source organism characterized?"
- "Is there information to show that the source organism and/or foods obtained from it are not detrimental to human health?"

These questions have been addressed collectively in Sections X.1 and X.2

III.1 Source of RGHAN

For RGHAN, the source is chitin from biomass produced from the fermentation of the fungus *Aspergillus niger* (*A. niger)* which is defined taxonomically as follows:

Class, Deuteromycetes

Order, Monoliales

Family, Moniliaceae

Genus, *Aspergillus*

Species, *niger*

This is a fungal organism that is non-pathogenic and non-toxic for humans and other animals. *A. niger* is a filamentous and ubiquitous fungus found in nature. This species has been used safely for food and enzyme production for many decades (it is not a "Novel" microorganism and has not been genetically modified). The vegetative state of this fungal source is not to be confused with the fungal spore state, which in fungi is sometimes associated with respiratory allergies.

A. niger has been safely and commonly used in food production since the 1920's. The fermentation related to RGHAN uses one strain of *A. niger*, this is a privately developed strain of a proprietary nature, and is internally designated as *'Strain* X'. It was specifically selected for citric acid production. This same strain is used to produce citric acid that has been sold in the US, EU, and abroad since 1993.

A review of the safety of *A. niger* (Schuster *et al,* 2002) summarises that *A. niger* strains "produce a series of secondary metabolites, but it is only ochratoxin A that can be regarded as a mycotoxin in the strict sense of the word. Only 3 to 10% of the strains examined for ochratoxin A production have tested positive under favourable conditions. New and unknown isolates should be checked for ochratoxin A production before they are developed as production organisms. It is concluded, with these restrictions, that *A. niger* is a safe production organism".

The strain of *A. niger* used to produce RGHAN has been selected because of its safety, and that testing has indicated that it is not a ochratoxin A producer.

IX. ANTICIPATED INTAKE/EXTENT OF USE OF NOVEL FOOD

Based on Commission Recommendation 97/618/EC decision trees 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.1 to IX.4

IX.1 Stability of RGHAN

At room temperature, glucosamine HCl (from all sources) is stable at acid pH, and degradation begins when temperatures reach 190°C (374°F). RGHAN is also stable under conditions of pasteurisation providing the aforementioned conditions are maintained. Consequently, the use of glucosamine in food products is most suited to those types of food with lower pH, dry mixes and refrigerated products.

Tables IX.1-1 and IX.1-2 show examples of the acid stability of RGHAN in various beverage products (250 – 750 mg) adjusted to acid pH =3.0, evaluated over storage periods ranging from 9 months to 2 years. The results of the stability study indicate that RGHAN is stable over this time period.

Table IX.1-1 Stability of RGHAN During Pasteurization

Sample	Temperature (°C)	Time	% Recovery
Lemonade	90	0	100
Lemonade	71	20 sec	100
Lemonade	100	20 sec	100
Lemonade	100	5 min	100
100% Juice	100	5 min	100

Table IX.1-2 Stability of RGHAN Over Various Time Points (Stored at room temp)

Sample	PH	Time (months)	TPC (CFU/g)	% Glucosamine Recovery
Isotonic Drink	3.00	9	0	100
Juice Flavour	3.00	24	0	100
Fitness Water	2.93	17	0	100

IX.2 Proposed Use Groups for RGHAN

Due to the pH stability of RGHAN, acidic food systems are most suitable. Consequently Cargill, Incorporated proposes to include RGHAN at 750 mg per daily serving in the following food products:

Fruit Juices and fruit juice products including: Tomato, tomato
mixtures and fruit "smoothies" (~ 2 – 5 pH)
Dehydrated instant drink mixes (stable in dry form, pH <7 when mixed with liquid and consumed at point of use)
Fermented milk-based products, yoghurts and fromage frais (~ 3 – 5 pH)
Sports Drinks (~ 2 – 5 pH)
Iced Tea Drinks (~ 2 – 6 pH)

Table IX.2-1 provides further details of food groups and serving sizes.

Table IX.2-1 Summary of the Individual Proposed Food-Uses and Use-Levels for RGHAN in the U.K.

Food Category	Proposed Food-Uses	Serving size	Use-Levels (mg/serving size)	Use-Levels (%)
Fruit Juices and Fruit Juice Products	Fruit Juice	250g (not canned) 330 g (canned)	750	0.3 0.227
	Fruit Smoothies	200g	750	0.375
Soft Drinks (including ready to drink varieties of iced tea)	Soft Drinks, not low-calorie	250g (not canned) 330 g (canned)	750	0.3 0.227
	Soft Drinks, low-calorie	250g (not canned) 330 g (canned)	750	0.3 0.277
Dry Beverages	Hot Chocolate mixes, Cocoa, Malted Drinks, *etc*.	250g	750	0.3
Fermented milk-based products, yoghurts and fromage frais	Fromage Frais	100g	750	0.75
	Yogurt	125g	750	0.6
Sports Drinks	Sports Drinks	250g (not canned) 330 g (canned)	750	0.3 0.227

It is important to note that foods fortified with RGHAN are intended for population groups that seek nutritional supplementation to maintain joint health. Typically these groups include older people and sportsmen and women. It is also important to note that such food groups would be consumed as an alternative to food supplement or PARNUTS products, rather than in combination with them. By definition food supplements and PARNUTS products must be clearly distinguishable from normal food products.

IX.3 Predicted Intakes

Based on the use levels and serving sizes presented in Table X.2-1, Cargill, Incorporated has calculated mean and upper level percentile intakes for different population groups, using the comprehensive data contained in the United Kingdom's National Diet and Nutrition Survey Programme (NDNS), a joint initiative between the United Kingdom (U.K.) Food Standards Agency and the Department of Health. Calculations for the mean and high-level (90^{th}, 95^{th} and 97.5^{th} percentile) all-person (*i.e.*, across the population) and all-user intakes (*i.e.*, across actual consumers of the specific food groups), and percent consuming were performed for each of the individual typical food-uses for RGHAN. Details of the specific food-codes selected from the surveys are provided in Appendix 4. Similar calculations were used to determine the estimated total intake of RGHAN from all typical food-uses combined. In both cases, the per-person and per-kilogram body weight intakes were reported for the following population groups:

young people/children ages 4 to 10;
female teenagers, ages 11 to 18;
male teenagers, ages 11 to 18;
female adults, ages 16 to 64;
male adults, ages 16 to 64.

Consumption data from individual dietary records, detailing food items ingested by each survey participant on each of the survey days, were collated by computer and used to generate estimates for the intakes of RGHAN by the U.K. population. Estimates for the daily intakes of RGHAN represent projected 7-day averages for each individual from Days 1 to 7 of NDNS data; these average amounts comprised the distribution from which mean and percentile intake estimates were produced. Mean and percentile estimates were generated using ratio estimation and nonparametric techniques, incorporating survey weights where appropriate, in order to provide representative intakes for specific U.K. population groups. All-person intake refers to the estimated intake of RGHAN averaged over all individuals surveyed regardless of whether they consumed food products in which RGHAN is currently typical for use, and therefore includes "zero" consumers (those who reported no intake of food products containing RGHAN during the 7 survey days). All-user intake refers to the estimated intakes of RGHAN by those individuals consuming food products in which the use of RGHAN is under consideration, hence the 'all-user' designation. Individuals were considered users if they consumed 1 or more food products in which RGHAN is typical for use on 1 of the 7 survey days.

The intended population groups for RGHAN-fortified products are older people as well as extremely active people such as sportsmen and women, in most cases seeking nutritional maintenance healthy joints as an alternative to food supplements/PARNUTS products. However, we have also included data for young people (children) between the ages of 4 and 10 for risk assessment purposes.

It can be seen from Table IX.3-1, that on a mg/person per day basis the theoretical highest mean and 95th percentile intakes, of approximately 482 mg/day and 1352 mg/day of RGHAN, respectively (equivalent to approximately 401 and 1124 mg/day for free-base glucosamine) may occur in young people/children between the ages of 4 and 10. However, these calculations are based on such children being specifically marketed for regular consumption of these products, which would not be the case; and indirect consumption could not reasonably be considered to be at a similar level to that of conventional soft drinks, yoghurts *etc.* Of the other population groups, intakes are similar with mean daily intakes for all person consumption ranging from 351 to 393 mg/day of RGHAN (*i.e.*, approximately 292 to 327 mg/day of free-base glucosamine); and 95th percentile intakes for the same groups ranging from 1160 to 1274 mg/day of RGHAN (*i.e.*, approximately 964 to 1059 mg/day of free-base glucosamine).

Table IX.3-1 Summary of the Estimated Daily Intake of RGHAN from All Proposed Food Categories in the U.K. by Population Group (NDNS Data)

Population Group	Age Group (Years)	% User	Actual # of Total Users	All-Person Consumption				All-User Consumption			
				Mean (mg)	Percentile (mg)			Mean (mg)	Percentile (mg)		
					90	95	97.5		90	95	97.5
Children/ Young People	4-10	88.8	743	481.55	1080.00	1351.56	1593.00	543.34	1121.29	1382.60	1615.29
Female Teenagers	11-18	80.5	359	382.10	925.62	1161.76	1472.06	473.75	979.57	1360.67	1684.32
Male Teenagers	11-18	76.7	319	392.50	979.94	1273.71	1761.43	519.59	1118.51	1542.36	1794.86
Female Adults	16-64	69.1	662	377.97	952.12	1179.64	1436.14	517.49	1064.61	1270.29	1477.75
Male Adults	16-64	64.0	490	350.89	900.00	1188.86	1520.10	534.35	1089.70	1404.43	1812.00

On a mg/kg body weight per day basis, Table IX.2-4, the same disproportionate effect is seen for the young people/children group. Again, more realistic figures come from the targeted consumer groups. Here, as before, intakes are similar for the different age groups, with the greatest potential consumption coming from male teenagers (sportsmen *etc.*) with a mean for all consumers of nearly 7.5 mg/kg body weight/day and a 95th percentile intake for the same group of nearly 32 mg/kg body weight per day.

Table IX.3-2 Summary of the Estimated Daily Per Kilogram Body Weight Intake of RGHAN from All Proposed Food Categories in the U.K. by Population Group (NDNS Data)

Population Group	Age Group (Years)	% User	Actual # of Total Users	All-Person Consumption				All-User Consumption			
				Mean (mg)	Percentile (mg)			Mean (mg)	Percentile (mg)		
					90	95	97.5		90	95	97.5
Children/Young People	4-10	88.8	743	19.05	43.80	52.87	68.06	21.72	45.11	56.57	71.44
Female Teenagers	11-18	80.5	359	7.30	18.07	22.79	32.96	9.30	19.52	26.22	33.80
Male Teenagers	11-18	76.7	319	7.46	19.85	26.14	31.37	9.95	22.11	28.97	32.46
Female Adults	16-64	69.1	662	5.38	13.82	17.07	20.90	7.78	15.85	19.35	23.58
Male Adults	16-64	64.0	490	4.15	11.00	14.19	19.18	6.48	12.72	17.87	22.49

Both sets of intakes compare favourably with toxicology and clinical study endpoints, as described in Section XIII. The single acute toxicity study conducted with RGHAN demonstrated that the LD_{50} for in Crl:CD (SD) IGS BR rats was greater than 5000 mg/kg body weight (Glaza, 2002). Similarly, subchronic and chronic oral studies reported no toxicity or occurrence of adverse effects attributable to glucosamine at levels up to

2130 mg/kg body weight/day (free-base). For a 60 kg adult this would be equivalent to up to approximately 127800 mg/person per day for RGHAN.

Volunteers were reported to consume glucosamine supplements over periods ranging from 21 days to 3 years with the majority of the studies providing glucosamine at a dose of approximately 1500 mg/day with glucosamine HCl doses reported as high as 3200 mg/day (*i.e.*, approximately 2656 mg/day free-base glucosamine).

Tables IX.3-2 to IX.3-7 provide breakdowns of the contributions of the various food groups to the total intakes described above. The pattern of consumption is consistent with fruit juices followed by yoghurts representing the highest consumption for each population group.

Table IX.3-3 Estimated Daily Intake of RGHAN from Individual Proposed Beverage-Uses of RGHAN in the United Kingdom by Children/Young People Using Representative Data

Food Category	% Users	Actual # of Users	All-Person Consumption				All-Users Consumption			
			Mean (mg/d)	Percentile (mg/d)			Mean (mg/d)	Percentile (mg/d)		
				90	95	97.5		90	95	97.5
Fruit Juices and Fruit Juice Products										
Fruit Juices	51.5	431	153.61	464.57	703.71	908.57	295.46	681.00	838.29	1,164.00
Fruit Smoothies	0.5	4	0.23	na	na	na	68.54	133.93	133.93	133.93
Soft Drinks										
Ready-to-Drink Soft Drinks (not low-calorie)	43.8	367	86.98	322.90	483.96	574.18	245.56	514.43	604.65	715.45
Ready-to-Drink Soft Drinks (low-calorie)	Na	na	20.89	na	178.07	299.16	na	na	Na	na
Dry Beverages										
Dry Drink Mixes	24.5	205	49.63	156.00	299.14	453.60	207.79	456.00	734.83	750.86
Fermented Milk-based Products, Yoghurts and Fromage Frais										
Fromage Frais	28.0	234	42.58	160.71	225.00	311.79	160.57	291.43	410.36	544.29
Yoghurt	47.4	397	124.99	374.57	549.43	691.71	261.80	578.57	711.43	830.57
Sports Drinks										
Energy, Sport, and Isotonic Drinks	1.3	11	2.64	na	na	na	223.92	512.57	681.86	681.86

Table IX.3-4 Estimated Daily Intake of RGHAN from Individual Proposed Beverage-Uses of RGHAN in the United Kingdom by Female Teenagers Using Representative Data

Food Category	% Users	Actual # of Users	All-Person Consumption				All-Users Consumption			
			Mean (mg/d)	Percentile (mg/d)			Mean (mg/d)	Percentile (mg/d)		
				90	95	97.5		90	95	97.5
Fruit Juices and Fruit Juice Products										
Fruit Juices	51.8	231	174.23	518.57	828.00	1008.00	347.89	827.57	1008.00	1132.71
Fruit Smoothies	Na	na	na	na	na	na	na	na	Na	na
Soft Drinks										
Ready-to-Drink Soft Drinks (not low-calorie)	24.4	109	55.92	216.06	349.42	462.19	215.24	462.19	595.95	680.23
Ready-to-Drink Soft Drinks (low-calorie)	3.8	17	6.28	na	na	98.93	166.61	306.28	416.29	494.64
Dry Beverages										
Dry Drink Mixes	24.0	107	37.07	107.14	205.71	324.00	167.16	374.57	606.86	795.00
Fermented Milk-based Products Yoghurts and Fromage Frais										
Fromage Frais	7.2	32	9.07	na	60.00	133.93	140.00	233.57	463.93	623.57
Yoghurt	39.7	177	95.77	321.43	450.00	553.71	232.15	508.29	564.00	642.86
Sports Drinks										
Energy Sport and Isotonic Drinks	2.0	9	3.76	na	na	na	168.85	276.00	455.57	455.57

Table IX.3-5 Estimated Daily Intake of RGHAN from Individual Proposed Beverage-Uses of RGHAN in the United Kingdom by Male Teenagers Using Representative Data

Food Category	% Users	Actual # of Users	All-Person Consumption				All-Users Consumption			
			Mean (mg/d)	Percentile (mg/d)			Mean (mg/d)	Percentile (mg/d)		
				90	95	97.5		90	95	97.5
Fruit Juices and Fruit Juice Products										
Fruit Juices	44.5	185	175.34	561.86	807.86	1152.00	397.53	898.29	1187.57	1472.57
Fruit Smoothies	0.2	1	0.45	na	Na	na	227.14	227.14	227.14	227.14
Soft Drinks										
Ready-to-Drink Soft Drinks (not low-calorie)	23.6	98	48.96	161.45	286.50	439.24	213.28	468.53	580.29	835.75
Ready-to-Drink Soft Drinks (low-calorie)	5.8	24	12.05	na	61.34	146.41	220.98	643.43	643.43	774.41
Dry Beverages										
Dry Drink Mixes	20.7	86	45.18	128.57	282.86	421.71	236.31	591.43	721.67	828.00
Fermented Milk-based Products Yoghurts and Fromage Frais										
Fromage Frais	7.0	29	9.19	na	66.43	115.71	141.63	300.00	334.29	334.29
Yoghurt	34.6	144	91.57	283.71	506.57	732.86	272.72	662.57	737.14	853.71
Sports Drinks										
Energy Sport and Isotonic Drinks	5.3	22	9.75	na	45.00	138.00	197.95	455.30	686.84	686.84

Table IX.3-6 Estimated Daily Intake of RGHAN from Individual Proposed Beverage-Uses of RGHAN in the United Kingdom by Female Adults Using Representative Data

Food Category	% Users	Actual # of Users	All-Person Consumption				All-Users Consumption			
			Mean (mg/d)	Percentile (mg/d)			Mean (mg/d)	Percentile (mg/d)		
				90	95	97.5		90	95	97.5
Fruit Juices and Fruit Juice Products										
Fruit Juices	44.7	428	145.02	483.43	648.00	810.43	308.63	649.29	823.29	1083.00
Fruit Smoothies	0.2	2	0.68	na	Na	na	273.21	369.64	369.64	369.64
Soft Drinks										
Ready-to-Drink Soft Drinks (not low-calorie)	10.5	101	28.47	75.58	205.77	364.45	237.00	498.60	628.79	797.76
Ready-to-Drink Soft Drinks (low-calorie)	2.2	21	2.70	na	Na	na	123.01	264.34	342.29	375.93
Dry Beverages										
Dry Drink Mixes	19.4	186	49.69	162.86	326.57	473.14	252.91	567.00	689.57	1006.63
Fermented Milk-based Products Yoghurts and Fromage Frais										
Fromage Frais	4.8	46	8.12	na	36.43	108.21	148.59	321.43	321.43	593.57
Yoghurt	37.6	360	137.21	479.14	642.86	822.86	343.67	698.57	857.14	1025.96
Sports Drinks										
Energy Sport and Isotonic Drinks	2.9	28	6.08	na	Na	81.07	207.97	447.51	496.48	1163.19

Table IX.3-7 Estimated Daily Intake of RGHAN from Individual Proposed Beverage-Uses of RGHAN in the United Kingdom by Male Adults Using Representative Data

Food Category	% Users	Actual # of Users	All-Person Consumption				All-Users Consumption			
			Mean (mg/d)	Percentile (mg/d)			Mean (mg/d)	Percentile (mg/d)		
				90	95	97.5		90	95	97.5
Fruit Juices and Fruit Juice Products										
Fruit Juices	43.5	333	163.18	511.71	739.29	1009.29	364.16	771.86	1030.71	1320.00
Fruit Smoothies	0.4	3	0.84	na	Na	na	213.57	321.43	321.43	321.43
Soft Drinks										
Ready-to-Drink Soft Drinks (not low-calorie)	8.2	63	20.37	na	128.61	289.27	240.00	522.34	613.36	1092.96
Ready-to-Drink Soft Drinks (low-calorie)	0.9	7	1.90	na	Na	na	207.41	741.96	741.96	741.96
Dry Beverages										
Dry Drink Mixes	12.5	96	34.29	72.86	250.71	364.71	271.66	640.71	803.14	1060.29
Fermented Milk-based Products Yoghurts and Fromage Frais										
Fromage Frais	2.3	18	4.47	na	Na	na	190.30	428.57	535.71	535.71
Yoghurt	30.8	236	111.73	396.00	558.86	752.57	352.16	678.86	878.57	995.14
Sports Drinks										
Energy Sport and Isotonic Drinks	5.4	41	14.13	na	81.07	162.14	261.94	428.06	977.14	1213.29

IX.4 At Risk Groups

As glucosamine is a common metabolite in most tissues of the body, the metabolism of which shares common enzymes involved in glucose metabolism as well as insulin activity and secretion has interested investigators examining the possible effects on susceptible groups such as diabetics. This aspect is reviewed in detail in Sections XIII.2.3.4 and XIII.3.

X. INFORMATION FROM PREVIOUS HUMAN EXPOSURE TO THE NOVEL FOOD OR ITS SOURCE

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 nutritional, microbiological, toxicological and/or allergenicity problems?"

These questions have been addressed collectively in Section X.1 through X.4.

X.1 Previous Glucosamine Intake

Widespread and uncontrolled glucosamine supplementation in a variety of forms, quantities and levels is currently utilised by a substantial and heterogeneous adult population group throughout the world; this is reflected by the fact that glucosamine sales are estimated to be in excess of 300 million dollars a year world-wide (Biggee *et al*., 2004).

There is no established formal Recommended Daily Intake (RDI) for Glucosamine and actual recommendations vary. The most widely recommended daily intake is up to 1500 mg of Glucosamine (Sulphate or Hydrochloride) and this is largely based on the advice of Theodosakis (1997). Table X.1-1 lists some products currently on the UK Market.

RGHAN, and indeed glucosamine from all sources is currently used only in Food Supplements (as defined by Article 2 of EU Directive 2002/46/EC) and PARNUTS products as defined by Directive 89/398/EEC.

Fortified food products containing RGHAN would provide an extension of choice for targeted population groups, *as an alternative*, to food supplements.

Table X.1-1 Examples of Glucosamine Food Supplement Products Currently on UK Market

Brand	Bought from	Recommended Daily Intake of Glucosamine	Product Form
"Glucosamine Hydrochloride" (Higher Nature, Burwash Common, East Sussex)	Tesco NutriCentre Online	Up to 1200 mg (as 400 mg tablets)	Tablet
"Glucosamine Hydrochloride with Vitamin C" (Biocare Ltd, Kings Lynn, Birmingham)	Tesco NutriCentre Online	Up to 1600 mg (as 800 mg tablets)	Tablet
"Advanced Glucosamine Complex" Solgar Vitamin and Herb UK, HP23 5PT	Boots Herbal Stores	Up to 1000 mg (as tablets with 200 mg HCL, 200 mg sulphate and 100 mg N-Acetyl)	Tablet
"Neways Glucosamine Plus" Neways, Glasgow	www.max-health.co.uk	Up to 1500 mg per day (as 500 mg Glucosamine HCL tablets)	Tablet
"Glucosamine" Ardern Healthcare, Tenbury Wells, Worcestershire	On-line	Up to 1500 mg per day (as tablets) Glucosamine HCL	Tablet
Seven Seas Joint Care Cod Liver Oil	On-line	Up to 100 mg per day Glucosamine sulphate (as one capsule)	Capsule
Bio Care Glucosamine HCl	On-line	800 mg per tablet	Tablet
"Logic Glucosamine and Chondroitin" The Health Company (Europe) Ltd	On-line	1000 mg Glucosamine sulphate KCl in 30 ml per day	Liquid
"Collagen Drink mix Glucosamine Sulphate plus Vitamins & Minerals" Avesta Ltd	On-line	1000 mg Glucosamine Sulphate per serving	Drink Mix

Table X.1-1 Examples of Glucosamine Food Supplement Products Currently on UK Market

Brand	Bought from	Recommended Daily Intake of Glucosamine	Product Form
"LookFit Sports Nutrition Glucosamine Drink Mix"	On-line	510 mg Glucosamine Sulphate	Drink Mix
"Joint Care High Potency Glucosamine plus Chondroitin (plus fish oil omega-3)" Seven Seas	Boots	500 mg (as 250 mg in capsules) as Glucosamine sulphate	Capsule

XI. NUTRITIONAL INFORMATION ON THE NOVEL FOOD

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 Sections XI.1 to XI.4.

XI.1 Nutritional Value

The nutritional value of RGHAN is provided in Table XI-1. Glucosamine is a single molecule, independent of source. It has only one chiral centre. We are not aware of any evidence to suggest any difference in bioactivity resulting from different chitin sources.

Table XI.1-1 The Nutritional Value for 0.75 g (750 mg) of Non-Shellfish Glucosamine Hydrochloride

Nutritional Information per 0.75 grams	
Fat g/0.75 g	0
Protein %	0
Carbohydrates g/.075g	0.745
Cholesterol mg/.075g	0
Calories /.075g	2.67
Calcium mg/0.75 g	0
Iron mg/0.75 g	0
Sodium mg/0.75 g	0
Potassium mg/0.75 g	0
Vitamin A I.U./0.75 g	0
Vitamin C mg/0.75 g	0
Fibre g/0.75 g	0
Sugar g/0.75 g	0

XI.2 Nutritional Support for Joint Care

Glucosamine is a natural component of cartilage and is taken as food supplements by many people to help maintain healthy joints and as nutritional support for specific nutritional purposes, in particular by the elderly and the active (for example sportsmen and women).

XI.3 Proposed Labelling

Cargill does not typically manufacture food products as presented to the final consumer, and in this case Cargill is providing RGHAN as the raw material to food product manufacturers. On the product itself Glucosamine Hydrochloride should be identified as:

"Non-Shellfish Glucosamine Hydrochloride*" or "Non-Shellfish Glucosamine HCL*" and as a footnote to the ingredients list "* from *Aspergillus niger*".

This would correctly inform the consumer seeking to avoid potential shellfish allergens.

The product may also carry a Kosher label, as RGHAN has been certified as Kosher Pareve and Kosher for Passover since it is not derived from shellfish (which can be forbidden in a Kosher diet).

In addition to carrying a Kosher label, the product may also carry a Halal label.

XII. MICROBIOLOGICAL INFORMATION ON THE NOVEL FOOD

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?"

- "Is there information to show that the NF is unlikely to contain microorganisms and/or their metabolites of adverse public health significance?"

This question has been addressed in Section XII

XII.1 Microbial Contamination of Final Product

The results for RGHAN reported in the following table confirms that the finished product meets the USP-NF specifications and microbiological food standards.

Table XII.1-1 Microbiological Analysis of REGENASURE® Glucosamine Hydrochloride

Lot Number	Total Plate Count (cfu/g)	Yeast & Molds (cfu/g)	Coliform MPN method (MPN/g)	Coliform confirmation (MPN/g)	*E. coli* MPN method (MPN/g)	*Staphylococcus aureus*(cfu/g)	*Salmonella* (in 25 g)
RPE5048	<10 cfu/g	<10 cfu/g	<3 MPN/g	<3 MPN/g	<3 MPN/g	<10 cfu/g	Negative
RSE5053	<10 cfu/g	<10 cfu/g	<3 MPN/g	<3 MPN/g	<3 MPN/g	<10 cfu/g	Negative
RPE5078	<10 cfu/g	<10 cfu/g	<3 MPN/g	<3 MPN/g	<3 MPN/g	<10 cfu/g	Negative
RPE6010	<10 cfu/g	<10 cfu/g	<3 MPN/g	<3 MPN/g	<3 MPN/g	<10 cfu/g	Negative
RSE6013	<10 cfu/g	<10 cfu/g	<3 MPN/g	<3 MPN/g	<3 MPN/g	<10 cfu/g	Negative
Average Results	<10 cfu/g	<10 cfu/g	<3 MPN/g	<3 MPN/g	<3 MPN/g	<10 cfu/g	Negative

cfu= colony forming units, MPN = most probable number, "<" signifies limit of detection for method. No growth was observed on all testing conducted.

Cargill Acidulants commissioned an independent analyses of the final product for pesticides, aflatoxin and ochratoxin A. Further details of this analysis are provided in Section I.9.

XIII. TOXICOLOGICAL ASSESSMENT OF THE NOVEL FOOD

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?"
- "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 Sections XIII.1 through XIII.3

XIII.1 Toxicological Evaluation of *Aspergillus niger*

A review of the safety of *A. niger* (Schuster *et al*., 2002) summarises that *A. niger* strains "produce a series of secondary metabolites, but it is only ochratoxin A that can be regarded as a mycotoxin in the strict sense of the word. Only 3 to 10% of the strains examined for ochratoxin A production have tested positive under favourable conditions. New and unknown isolates should be checked for ochratoxin A production before they are developed as production organisms. It is concluded, with these restrictions, that *A. niger* is a safe production organism".

The strain of *A. niger* used to produce RGHAN has been selected because of its safety, and it is not a Ochratoxin A producer. Typical analysis results of the final product as verification are attached as Appendix 3.

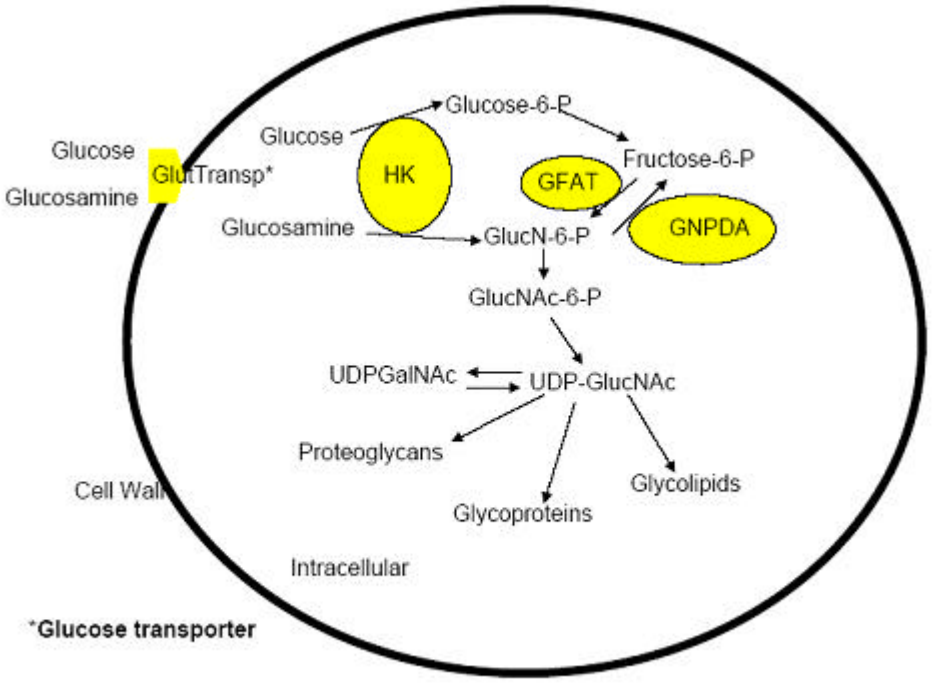
XIII.2 Toxicological Evaluation of Glucosamine

An excellent review of the toxicology of glucosamine and its safety in humans has been published by Anderson and his colleagues from the University of Kentucky (Anderson *et al.*, 2005). Their critical evaluation indicated that glucosamine is safe under current conditions of use and does not affect glucose metabolism. Much of the data presented below is from this review, which we have updated in light of recent significant publications, including that of perhaps the most important human study to date, the Glucosamine/chondroitin Arthritis Intervention Trial (GAIT) study (Clegg *et al.* 2006).

XIII.2.1 Metabolism

Glucosamine is a prominent component of the hexosamine pathway, an important branch of glycolysis. Exogenous glucosamine is actively transported from extracellular tissue into cells by glucose transporters (Figure XIII.2.1-1); (Uldry *et al.*, 2002) insulin facilitates glucosamine transport into cells (Heart *et al.*, 2000). Once in the cell, glucosamine is phosphorylated by one of the family of hexokinases to glucosamine-6-phosphate (GlucN-6-P). GlucN-6-P can also be produced endogenously from fructose-6-phosphate and glutamine by GlucN-6-P synthetase, commonly called glucosamine:fructose-6-P aminotransferase (GFAT) (Wu *et al.*, 2001). GFAT irreversibly catalyses the first and rate-controlling step in the synthesis of uridine diphosphate-N-acetylglucosamine (UDP-GlucNAc), a precursor of all macromolecules containing amino sugars. GlucN-6-P is readily converted back to fructose-6-phosphate by glucosamine-6-phosphate deaminase (GNPDA) (Wolosker and Kline, 1998). GlucN-6-P is acetylated to N-acetyl-glucosamine-6-P (glucNAc-6-P) by glucosamine-phosphate N acetyltransferase and subsequently converted to UDP-GlucNAc by UDP-N-acetyl-glucosamine pyrophosphorylase. In some tissues, glucNAc-6-P is converted to glucNAc-1-P by phosphoacetylglucosamine mutase before being converted to UDP-GlucNAc (Milewski, 2002).

Figure XIII.2.1-1 Glucosamine Metabolism



The metabolism of glucosamine is highly regulated by rates of transport into various tissues and by effects of intermediates on key enzymatic steps. For example, in many tissues the affinity of glucosamine for glucose transporters is several-fold lower than for glucose but in some mammalian tissues, the affinity of glucosamine for GLUT2 transporters is higher than for glucose (Uldry *et al.*, 2002). The affinity of the family of hexokinases in different tissues for glucosamine compared to glucose may also regulate utilization of glucosamine in various tissues. GFAT is unique among the subfamily of aminotransferase enzymes because it is not display any ammonia-dependent activity and requires glutamine as amino donor (Milewski, 2002). GFAT is strongly inhibited by the end product of this synthetic pathway, UDP-GlucNAc (Milewski, 2002).

Between 2-5% of fructose-6-P or of the flux through the glycolytic pathway enters the hexosamine pathway *via* glucosamine (Milewski, 2002). In humans the endogenous production of glucosamine is in the range of 4-20 g/day (median values of ~14 g/day or 200 mg/kg/day) (Wells *et al.*, 2001; Vosseller *et al.*, 2002; Hart, 2003; Wells *et al.*, 2003).

Some, but not all, studies in animals suggest that glucosamine administration may produce insulin resistance and hyperglycaemia by affecting insulin secretion and action (Echard *et al.*, 2001; IOM, 2003). However, most *in vitro* and animal studies have achieved blood and tissue levels 100 to 2000 times higher than would be expected with glucosamine doses used in humans (Heart *et al.*, 2000; Monauni *et al.*, 2000; Nelson *et al.*, 2000; Echard *et al.*, 2001). Thus, it is important to rigorously review available data in humans to assess the effects of

glucosamine intake on glucose homeostasis. Glucosamine is usually taken orally, as opposed to intraarterially (ia) or intramuscularly (im), and in humans 90% is absorbed (Setnikar, 2001). Orally administered glucosamine has only 26% of the bioavailability of intravenously administered glucosamine (Barclay *et al.*, 1998). A significant fraction of orally administered glucosamine undergoes first-pass metabolism in the liver (Barclay *et al.*, 1998). Blood levels achieved after oral glucosamine are only 20% of those achieved with intravenous glucosamine (Setnikar, 2001; IOM, 2003). Recent data on pharmacokinetics, bioavailability, and metabolism of glucosamine in rats (Aghazadeh-Habashi and Sattari, 2002) are similar to those reported for humans (Setnikar *et al.*, 1993; Setnikar and Rovati, 2001).

XIII.2.1.1 Absorption, Distribution, Metabolism, and Excretion (ADME) Studies

Setnikar *et al.* (1984) administered uniformly labelled [^{14}C] glucosamine HCl diluted with unlabeled glucosamine sodium sulphate intravenously and *via* oral gavage to 44 male and 44 female Sprague-Dawley rats (*i.e.*, 22 rats per sex/route), which were sacrificed after 144 hours. Samples of plasma faeces, urine, CO_2, and tissues (*i.e.*, all organs and whole carcass) were analysed. At 1-2 hours after intravenous (iv) or oral administration, glucosamine radioactivity in plasma was bound to and/or incorporated into plasma proteins. After peaking at 2-4 hrs, radioactivity declined from plasma at a slower rate (t½ = 28 and 46 hrs, after iv or oral administration, respectively). Plasma sample measurements demonstrated that radioactivity from glucosamine was rapidly absorbed from the gastrointestinal tract. Analyses of radioactivity in urine, faeces and CO_2 revealed: (a) there were no gender effects, (b) about half of the radioactivity was excreted as CO_2, (c) 40% of the radioactivity was excreted in the urine, (d) only 2% of the administered dose ended up in faeces indicating a high degree of glucosamine absorption. Analyses of radioactivity in tissues and organs showed that [^{14}C]-glucosamine quickly entered into all tissues, especially the liver and kidneys during the first 2 hours post-administration, as well as in cartilage and other tissues with the maximum concentration of radioactivity in the different organs attained within a maximum at 8 hours.

In an unpublished study, Neuteboom (1995) administered ascending single oral doses of glucosamine sulphate (*i.e.*, 125.6, 1256, 3392 mg crystalline glucosamine sulphate or 100, 1000, and 2700 mg glucosamine sulphate) to male and female Sprague-Dawley rats (2 rats/sex/dose) *via* gavage. In addition, radiolabelled crystalline glucosamine sulphate was administered to an additional 3 groups of rats (2 rats/sex/group) *via* oral gavage at the same dose levels and kept in separate metabolic chambers. The pattern of radioactivity excretion indicated that the quantity of radioactivity excreted was proportional to dose and that the primary route of excretion of the radiolabel was *via* expired CO_2 (~69 to 74% of the initial dose), followed by faecal (16 to 21% after 168 hours) and urinary excretion (3.9 to 5.2% after 168 hours). The distribution pattern of radioactivity was comparable to the pattern reported

by Setnikar *et al.* (1984) with the greatest amount of radioactivity present in the liver, followed by the kidneys, and then the cartilage of the femoral head. The amount of radioactivity in the tissues was reported to be proportional to the administered dose.

The distribution and excretion of radiolabel crystalline glucosamine sulphate was further examined in Sprague-Dawley rats (2/sex/dose) administered 12.6 mg/kg body weight crystalline glucosamine sulphate (Zanolo and Fumero, 1988). Rats were administered a single oral dose, as well as single doses on 6 consecutive days. The glucosamine sulphate radiolabel was demonstrated to be mainly associated with the plasma proteins (*i.e.*, less than 10% associated with the deproteinized plasma). The radioactivity in the plasma was reported to reach a steady state after the third dose on the third day, while urinary excretion of the radiolabel was reported to range from 5 to 8%.

Setnikar *et al.* (1986) also administered 10 mg/kg body weight of uniformly labelled [^{14}C]-glucosamine HCl diluted with unlabeled glucosamine sulphate *via* iv and oral (*i.e.*, gastric tube) routes to Beagle dogs (8/sex). The dogs were monitored up to 144 hours post-dose to permit the collection of plasma, urine, faecal, and breath CO_2 samples for the determination of the pharmacokinetics of glucosamine. Following the completion of the study period the dogs were sacrificed and the organs collected for analysis of the distribution of radioactivity. Immediately after iv administration of radiolabelled glucosamine to the dogs (~30 minutes), 90% of the labelled glucosamine was present as free glucosamine in the plasma, while the remaining 10% of radioactivity in plasma was either bound to plasma proteins (*e.g.*, a and ß-globulins) or incorporated into various tissues and organs. Glucosamine and thus the radioactivity was rapidly cleared from the plasma either through rapid excretion in the urine or through incorporation into various organs and tissues throughout the body. The liver and kidneys were reported to contain the greatest amount at 2 and 4 hours post-dose, as well as lesser amounts in the articular cartilage. Radioactivity also was detected in expired [^{14}C]-CO_2; however, the amount could not be determined quantitatively. The total plasma radioactivity was reported to display "unusual pharmacokinetic behaviour" as demonstrated by the total plasma radioactivity declining rapidly from 5 minutes post-injection until 54 minutes post-injection and then subsequently increasing until 8 hours post-injection where the total plasma radioactivity levels are equivalent to 5 minutes post-injection. The total plasma radioactivity levels were then reported to decrease slowing with a $t_{1/2}$ = 70 h (disappearance). After oral administration of radiolabel led glucosamine to the dogs, approximately 87% was absorbed. The non-precipitable radioactivity (as a percent of total radioactivity) in the plasma after oral dosing was reported to be equal to the non-percipitable plasma radioactivity (as a percent of total radioactivity) following iv dosing. In the dog, there were no gender effects on any parameters.

In addition to iv and oral pharmacokinetic studies conducted with Beagle dogs, Setnikar *et al.* (1986) examined the pharmacokinetics of glucosamine with 6 healthy human volunteers

(3/sex) provided with 800 mg (iv) or 6 g (oral) of glucosamine sulphate. Plasma, urine, and faecal samples were collected for up to 24 hours following iv or oral doses of glucosamine. Following iv administration, the pharmacokinetics of glucosamine were reported to be comparable to that of the non-precipitable fraction in dogs. The majority of the glucosamine removed from the plasma and excreted *via* the urine occurred during the initial 2 hours following the iv dose. The cumulative fraction of glucosamine excreted *via* the urine in humans (~38% of the administered dose) was reported to be comparable to the fraction of radioactivity excreted *via* the urine of dogs (~30% of the administered dose). Furthermore, quantifiable amounts of glucosamine sulphate were detected in the urine of human volunteers (~1.2% of the administered dose) over 24 hours, even though glucosamine was below the detection limit (~ 10 µg/mL) in the plasma.

The metabolism of glucosamine sulphate was further examined in 6 healthy human volunteers (2 subjects/administration route) provided either with 400 mg intravenously or intramuscularly, or 250 mg orally (Setnikar *et al.*, 1993). Radioactivity was detected in the plasma immediately after iv administration and was reported to be rapidly eliminated with an initial $t_{1/2}$ equal to 0.28 hours (during the initial 2 hours) and a $t_{1/2}$ equal to 70 hours after the concentration of radioactivity reaches its peak (*i.e.*, between 8 to 10 hours post-administration). Similar pharmacokinetics was reported for individuals provided with an iv or im injection. Glucosamine sulphate was reported to be well absorbed from the gastrointestinal tract of the volunteers provided glucosamine sulphate orally with approximately 90% absorbed. The approximately bioavailability following oral administration, as determined from the glucosamine sulphate area under the curve (AUC) data, was reported to be approximately 26% of that available after iv or im administration. The low bioavailability of glucosamine sulphate following oral administration was attributed to the first pass effect in the liver, which results in the metabolism of glucosamine sulphate to smaller molecules and finally to CO_2, water, and urea. The excretion of the radioactivity from the subjects occurred through both faecal and urinary routes with approximately 10% and 11.3% measured in the urine and faeces, respectively, of the subjects that consumed glucosamine sulphate orally. Miniscule amounts of radioactivity were measured in the faeces of subjects provided glucosamine sulphate *via* iv or im, while urinary excretion was much higher compared to orally dosed subjects. The remainder of the radioactivity was likely removed as $^{14}CO_2$; however, breath CO measurements were not performed.

The pharmacokinetics and bioavailability of glucosamine hydrochloride (HCl) was examined in 5 male Sprague-Dawley rats administered 350 mg/kg body weight in iv and oral crossover studies with a 2-day washout period between doses (Aghazadeh-Habashi and Sattari, 2002). No significant difference was reported between the mean glucosamine AUC values following the intraperitoneal (ip) and iv administration; however, the mean oral AUC was significantly lower compared to iv and ip routes. The absolute oral bioavailability was reported to be 0.19 ± 0.21 (*i.e.*, 19 to 21%). Aghazadeh-Habashi and Sattari (2002) concluded from the

pharmacokinetic data that glucosamine HCl is rapidly absorbed, highly distributed, has low bioavailability, and is efficiently cleared. The low bioavailability was attributed to the first-pass effect of the gut rather than the liver, as the low bioavailability was evident after oral dosing, but not ip. Sekitar *et al.* (1993) reported a similar profile in human volunteers provided with glucosamine sulphate *via* oral, iv, and ip routes.

The results of the animal studies are comparable to the results reported in the human pharmacokinetic studies. The comparable results of the animal and human studies suggests that glucosamine is metabolized *via* analogous pathways and therefore both dogs and rats represent appropriate models for establishing safety of glucosamine in humans (Setnikar, 2001).

XIII.2.2 Animal Toxicity Data

XIII.2.2.1 Acute Studies

Oral administration of glucosamine at very large doses (5000 to 8000 mg/kg body weight) is well tolerated without documented toxicity. The LD_{50} for glucosamine for rats and mice exceeds 5000 mg/kg body weight, while the LD_{50} for rabbits has been reported to be greater than 6000 mg/kg body weight (Setnikar *et al.*, 1991a).

In addition to the acute toxicity studies conducted by Setnikar *et al.* (1991a), Glaza (2002) performed acute oral toxicity tests by administering glucosamine hydrochloride to rats. During these studies, a single 5000 mg/kg body weight/day dose of unlabelled RGHAN (Lot No. GP-11, Cargill, Incorporated) was administered orally to 5 male and 5 female rats on Day 1 and the animals were monitored for an additional 14 days for signs of toxicity. All animals were observed clinically (*i.e.*, twice daily) for body weight changes, mortality, and morbidity. After 15 days, all animals were euthanized by overexposure to carbon dioxide and subjected to macroscopic necropsy examination. The necropsy included examination of the external surface of the carcass and all organs and tissues in the thoracic, abdominal, pelvic and oral cavities. Results of the clinical observations revealed no test material-related effects. Anatomical examination also revealed no test material-related effects on the animals. Based on these results, the no-observable-effect level (NOEL) for this preparation of RGHAN was 5000 mg/kg body weight.

A summary of the acute toxicity studies performed with glucosamine is presented in Table XIII.2.2.1-1.

Table XIII.2.2.1-1 Summary of Single Dose Acute Oral Toxicity Studies of Glucosamine

Species[1]	Strain	No.	Dose (mg/kg bw)	Glucosamine Form	Adjusted Dose (free glucosamine)	LD_{50} (mg/kg bw)	Reference
Rat	Sprague-Dawley	10/sex/dose	Up to 8000	Glucosamine sulphate	6312	>8000	Setnikar *et al*. (1991a)
Rat	Crl:CD® (SD)IGS BR	10 (5/sex)	5000	Glucosamine HCl	4155	>5000	Glaza (2002)
Rat	Sprague-Dawley (albino)	10 (5/sex/dose)	5000	Glucosamine sulphate	3945	>5000	Senin *et al*. (1987)
		10 (5/sex/dose)	6250[2]	Glucosamine-SP[3]	3945	>6250	
Mouse	CD-1	10/sex/dose	Up to 8000	Glucosamine sulphate	6312	>8000	Setnikar *et al*. (1991a)
Mouse	Swiss NMRI	10 (5/sex/dose)	Up to 5000	Glucosamine sulphate	3945	>5000	Senin *et al*. (1987)
		10 (5/sex/dose)	6250[2]	Glucosamine-SP	3945	>6250	
Rabbit	New Zealand (White) Albino	4/sex/dose	Up to 6000	Glucosamine sulphate	4734	>6000	Setnikar *et al*. (1991a)

[1] Male and female animals
[2] 6250 mg/kg body weight of glucosamine-SP is equivalent to 5000 mg/kg body weight of glucosamine sulphate
[3] Refers to a mixed salt preparation of glucosamine sulphate and sodium chloride
bw = body weight

XIII.2.2.2 Subchronic and Chronic Toxicity Studies

In addition to the studies examining the potential toxicity of single doses of glucosamine, a number of studies were conducted in rats (Sugimura *et al.*, 1959; Leuschner and Neumann, 1987; Beren *et al.*, 2001; Echard *et al.*, 2001; Lee *et al.*, 2004), dogs (Neumann and Leuschner, 1985; McNamara *et al.*, 1996), rabbits (Stender and Astrup, 1977), and horses (Hanson *et al.*, 1967; Fenton *et al.*, 1999; Caron *et al.*, 2002) to determine the potential effects of glucosamine administration over an extended period of time. Echard *et al*. (2001) compared the effects of oral glucosamine HCl administration *vs*. the consumption of a baseline diet in 8 male spontaneously hypertensive rats (SHR) and 8 male Sprague-Dawley rats over a period of 9 weeks. Rats were fed glucosamine hydrochloride in the diet at a concentration of 0.5% w/w, which equates to approximately 300 mg/kg body weight. Samples taken included blood, heart, liver, and kidneys for analytical and histological analyses. The analytical measurements included serum alanine aminotransferase, aspartate aminotransferase, and blood urea nitrogen. The authors reported that no consistent effects on blood chemical parameters and organ histology were observed, and concluded that glucosamine was non-toxic under the given study conditions.

The potential effects of chronic administration of glucosamine sulphate on rats and dogs were examined in a pair of unpublished dietary studies (Neumann and Leuschner, 1985; Leuschner and Neumann, 1987) cited by Setnikar *et al.* (1991b). The rats were administered 300, 900, or 2700 mg/kg body weight of glucosamine sulphate for 52 weeks, while dogs were provided with 159, 478, or 2149 mg/kg body weight of glucosamine sulphate in their diet over 26 weeks. As no treatment related adverse effects were observed in either species, the NOAEL is at least 2130 mg/kg body weight/day in rats and 1696 mg/kg body weight/day in dogs for free-base glucosamine.

Table 2.2.2-1 provides a summary of the available sub-chronic oral toxicity study data for all forms of glucosamine.

Table XIII.2.2.2-1 Summary of Sub-chronic Oral Toxicity Studies of Glucosamine Administered *via* Gavage

Species[1]	Strain	No.[(1)]	Sex	Dose (mg/kg bw/day)	Glucosamine Form	Adjusted Dose (mg/kg bw free glucosamine)	Duration (days)	Significant Findings	Reference
Rat	F344	40[(2)]	M, F	0 to 2,834[(3)]	N-acetylglucosamine	0 to 2,294.9	91	No obvious toxicity. NOAEL of 2,476 and 2,834 mg/kg bw/day determined for male and female rats, respectively	Lee *et al*. (2004)
Rat	Sprague-Dawley (albino)	6	M	0 to 20% in the diet (~0 to 5,000)	D-glucosamine	0 to 5,000	12	No toxicity, decreased growth rate at high doses in weanlings	Sugimura *et al*. (1959)
Rat	D/A (RTI[avI])	12	F	312.5	Glucosamine HCl	259.7	52	No adverse effects attributable to compound reported[(4)].	Beren *et al*. (2001)
Rat	Sprague-Dawley	8	M	~500 (0.5% w/w)[(5)]	Glucosamine HCl	415.5	63	No adverse effects reported*	Echard *et al*. (2001)
Rat	Spontaneously Hypertensive	8	M	0.5% w/w (~500)[(5)]	Glucosamine HCl	415.5	63	No adverse effects reported*	Echard *et al*. (2001)
Rat	NR	NR	NR	300, 900, and 2,700	Glucosamine sulphate	236.7, 710.1, and 2,130.3	365	No adverse effects reported, NOAEL 2,700 mg/kg bw/day of glucosamine sulphate	Leuschner and Neumann (1987)
Dog	Beagle	10	M, F	1,000	Glucosamine HCl	831	30	No adverse effects reports; however, minor changes in haematologic and haemostatic variables within laboratory reference range	McNamara *et al*. (1996)
Dog	NR	NR	NR	159, 478, and 2,149	Glucosamine sulphate	125.4, 377.1, and 1,695.6	183	No adverse effects reported, NOAEL 2,149 mg/kg bw glucosamine sulphate in dogs	Neumann and Leuschner (1985)
Rabbit	White Danish Country	12	M	~ 233.6 and 892.8 (0.5 and 2.0% in diet)[(6)]	Glucosamine HCl	194.2 and 742.0	84	No adverse effects reported	Stender and Astrup (1977)

Table XIII.2.2.2-1 Summary of Sub-chronic Oral Toxicity Studies of Glucosamine Administered *via* Gavage

Species[1]	Strain	No.[(1)]	Sex	Dose (mg/kg bw/day)	Glucosamine Form	Adjusted Dose (mg/kg bw free glucosamine)	Duration (days)	Significant Findings	Reference
Horse	NR	25	M, F	10,800 and 14,400	Glucosamine HCl	8,974.8 and 11,966.4	42	No adverse effects reported	Hanson *et al*. (1997)
Horse	Yearling Quarterhorses	10	M, F	10, 18, and 29[(7)]	Glucosamine salt not specified	ND	56	No adverse effects reported	Fenton *et al*. (1999)
Horse	Standardbred	9[(8)]	NS	20 (8 g/day)	Glucosamine HCl	16.62	336	No adverse effects reported*	Caron *et al*. (2002)

[1] The number of animals receiving the treatment
[2] 10 rats/sex/dose
[3] Rats administered 0, 0.625, 1.25, 2.5, or 5% corresponding to 0, 301.7, 587.8, 1,218.0, 2,475.6 or 0, 350.9, 694.9, 1,412.1, and 2,833.6 for male and female rats, respectively
[4] The authors reported 3 deaths; however, post-mortem examination demonstrated that these deaths were anaesthetic-related
[5] Dose obtained from diet based on FDA (1993) diet conversion table
[6] Calculated based on the average weight of the rabbits at baseline (*i.e.*, 3.21±0.10 kg and 3.36±0.10 kg in the 0.5 and 2.0% groups, respectively
[7] Horses were fed glucosamine at doses of 29, 18, and 10 mg/kg bw/day during weeks 1 to 4, 5 to 6, and 7 to 8, respectively
[8] There were initially 10; however, 1 was removed from the study due to issues related to athletic training (*i.e.*, the horse was too immature)
* Study protocol did not explicitly mention any scheduled monitoring for signs of ill health
NS = Not Specified
ND = Not Determined

XIII.2.2.3 Parenteral Administration

The effects of intravenous or intraperitoneal administration of glucosamine have also been examined. The LD_{50} for ip injection of glucosamine in rats is >5200 mg/kg body weight, and the LD_{50} for iv injection is ~1700 mg/kg body weight or equivalent to an oral dose of ~7400 mg/kg (Setnikar *et al*, 1991a). In mice the LD_{50} for intraperitoneal injection of glucosamine is >6600 mg/kg body weight, while the LD_{50} for intravenous injection is ~1600 mg/kg. The iv infusion of large amounts of glucosamine, from 240 to 4000 mg/kg body weight, has variable effects on blood glucose and glucose metabolism in rats.

The rat model often has been selected for study because it is unusually sensitive to the effects of parenteral glucosamine administration on glucose metabolism (IOM, 2003). The Institute of Medicine (IOM) draft report (IOM, 2003) reviews 14 reports of the potential effect of glucosamine administration on glucose metabolism in rats by intravenous or intraperitoneal routes, with doses ranging from 240 to 9937 mg/kg body weight. Of these studies Meninger *et al*. (2000) reported that infusion of 564 mg/kg did not affect blood glucose levels and Fushimi *et al.* (1974) reported that ip infusion of 250 mg/kg body weight of glucosamine did not induce hyperglycaemia; however, glucose metabolism was demonstrated to be altered (*i.e.*, higher blood glucose levels, reduced uptake of glucose, decreased disposal of glucose) in 12 other studies with infused doses ranging from 240 to 9937 mg/kg. Since orally administered glucosamine has only a 20% bioavailability relative to parenteral administration (Aghazadeh-Habashi and Sattari, 2002) it would appear that a large safety margin exists for orally administered glucosamine with respect to potential toxicity arising from alterations in glucose metabolism. For example, oral administration of glucosamine at very high doses (1000 to 2149 mg/kg body weight) does not affect blood glucose levels in rats (Echard *et al.*, 2001), rabbits (Stender and Astrup, 1977), or dogs (Setnikar *et al.*, 1991a).

XIII.2.2.4 Mutagenicity and Genotoxicity Studies

In vitro Studies

In unpublished studies performed by Cargill, Incorporated, the mutagenic activity RGHAN was evaluated in the *Salmonella-Escherichia coli*/Mammalian –Microsome Reverse Mutation Assay (Mecchi, 2003). This assay examines the ability of the test substance to induce reverse mutations both in the presence or absence of mammalian microsomal enzymes at the histidine locus in the genome of several strains of *S. typhimurium* and at the tryptophan locus of *E. coli*. Tester strains used in the mutagenicity assay were *S. typhimurium* tester strains TA98, TA100, TA1535, and TA1537 and *E. coli* strain WP2uvrA. The assay was conducted with five doses of RGHAN in both the presence and absence of microsomal enzymes prepared from Aroclor™-induced rat liver (S9 mix), along with vehicle and positive controls using three plates per dose. Doses tested with all tester strains were 100, 333, 1000, 3330, and 5000 µg per plate or concentrations of ~0.53 to 26.5 mmol/L. Results from this

Salmonella-Escherichia coli/Mammalian –Microsome Reverse Mutation Assay indicate that under the conditions of this study, RGHAN did not cause a positive increase in the mean number of revertants per plate with any of the tester strains. The results of the above study are in accordance with previous work reviewed by Bruswick *et al.* (1980) who state that glucosamine was not mutagenic in an *E. coli* WP_2 reverse mutation system. It should be noted however, that some studies suggest that glucosamine and other amino sugars can have clastogenic effects *in vitro*. For example, Nanjou *et al.* (1984) and Watanabe *et al.* (1985) have observed that glucosamine can inactivate various bacteriophages, and break DNA strands. The mechanism by which glucosamine induces DNA strand breakage is thought to be indirectly mediated through destabilization of the amine group at neutral pH; this in turn leads to the generation of active oxygen molecules which can damage DNA in systems without significant detoxification systems (Kashige *et al.*, 1991, 1994; Yamaguchi *et al.*, 1998).

***In vivo* Studies**

An *in vivo* micronucleus assay was conducted by Cargill, Incorporated using Crl: CD-1® (ICR) BR mice. For the micronucleus assay, RGHAN was mixed with cell culture grade water and dosed by oral gavage to six males per dose level (500, 1000, or 2000 mg/kg) for each scheduled harvest timepoint. The high dose of 2000 mg/kg selected for this study was based on relevant acute toxicity information (Glaza, 2002), and is the maximum allowable dose based on regulatory guidelines. Five animals per harvest timepoint dosed with the test article and with the vehicle control article were euthanized approximately 24 or 48 hours after dosing for extraction of the bone marrow. At least 2000 PCE's per animal were analyzed for the frequency of micronuclei. Cytotoxicity was assessed by scoring the number of PCE's and normochromatic erythrocytes (NCEs) in at least the first 500 erythrocytes for each animal. RGHAN did not induce any signs of clinical toxicity in any of the treated animals at up to 2000 mg/kg, nor were any statistically significant increases in micronucleated PCEs detected. Additionally, glucosamine HCl was not cytotoxic to the bone marrow at any dose level tested (*i.e.*, no statistically significant decrease in the PCE:NCE ratios were observed).

Banerjee and Manna *et al.* (1984) investigated the genotoxicity of glucosamine HCl using a mouse bone marrow chromosome aberration assay. Glucosamine HCl (10 mg/kg body weight) was administered to Swiss albino mice *via* ip injection, and bone marrow chromosome aberrations were assessed at 12 different intervals between 10 minutes and 30 days post injection. Compared to mice injected with distilled water, glucosamine significantly increased the chromosome aberration frequency by an average of 18%; the authors could not determine a potential mechanism for the observed effect. Finally, Manna *et al.* (2004) examined the micronuclei of five exotic fish injected intraperitoneally with glucosamine HCl at 10 mg/kg body weight. The percentage of micronuclei was slightly but not significantly higher in glucosamine injected fish than in controls.

In summary, based on negative findings of genotoxicity in both in vitro and in vivo tests it can be concluded that RGHAN is non-genotoxic. Although Banerjee and Manna *et al.* (1984) have shown that glucosamine was positive in a mouse chromosomal aberration study, only a single dose was used and the implications of the study are thus unclear in light of the overwhelming evidence of safety in animals and humans. It is not clear why some agents test negative in the rat bone marrow micronucleus assay yet induce clastogenic responses in aberration tests, however it has been suggested that the mechanism may be indirectly mediated by oxidative damage in cells that contain different buffering capacities, in which case the effects would only be an issue at very high concentrations; previous experiments suggest this is the case for glucosamine, as it has been shown that the clastogenic effects of glucosamine depend upon the production of reactive oxygen species (Kashige *et al*., 1991, 1994; Yamaguchi *et al.*, 1998).

XIII.2.3 Human Studies

XIII.2.3.1 Clinical Study Summaries

A comprehensive literature search of Medline and references from previously published reviews and meta-analyses, revealed 37 studies. The studies include data on 3783 patients treated with glucosamine for a total of 1191 patient-years. Twenty-nine chronic studies used a randomized, controlled trial (RCT) design, two were controlled studies and five studies were observational. Of the chronic studies, 25 used glucosamine alone, six included chondroitin sulphate and one included other supplements in the test preparation. Nine studies were comparator trials in which glucosamine was compared to other agents. Of the chronic studies, 26 used oral therapy exclusively, one used intramuscular administration alone, and three used oral administration in conjunction with intravenous, intramuscular, or intra-articular administration. The short-term studies were included to assess glucose metabolism. Four studies, one on skin wrinkles (Murad and Tabibian, 2001) and three on temporomandibular joint complaints (Shankland, 1998; Thie *et al.*, 2001; Nguyen *et al.*, 2001) were included to make the safety assessment as comprehensive as possible. Information relating to study design, glucosamine dose, type and duration of treatment are tabulated below (Table XIII.3.1-1).

Table XIII.2.3.1-1 Human Clinical Studies Evaluating the Supplemental use of Glucosamine

Study	Type of Study	Glucosamine Form	Other Treatment	Route	Dose mg/d	Free-base Form mg/d	No. of Subjects*	Duration Days	Subject Years
Almada *et al.* (2000)	RCT	SO4	None	Oral	1500	1184	6	84	1.4
Braham *et al*. (2003)	RCT	HCl	None	Oral	2000	1660	25	84	5.8
Cibere *et al*. (2004)	RCT-P	SO4	None	Oral	1500	1184	71	180	35.0
Clegg *et al*. (2006)	RCT-P-C	HCL	Celecoxib CHS	Oral	1500	1245	313	168	144.1
Crolle and D'Este (1980)	RCT-P	SO4	Piperazine-bisiodomethylate	Oral/im	1500	1184	15	21	0.9
D'Ambrosio *et al*. (1981)	RCT	SO4	None	oral/iv/im	1500	1184	15	21	0.9
Das and Hammad (2000)	RCT	HCl	CHS	Oral	2000	1660	46	192	24.2
Drovanti *et al*. (1980)	RCT	SO4	None	Oral	1500	1184	40	30	3.3
Forster *et al*. (1996)	RCT	SO4	None	Oral	1500	1184	78	90	19.2
Giordano *et al*. (1996)	Observational	SO_4	None	Oral	1500	1184	20	365	20.0
Houpt *et al*. (1999)	RCT	HCl	None	Oral	1500	1245	45	147	18.1
Hughes and Carr (2002)	RCT	SO_4	None	Oral	1500	1184	39	168	18.0
Leffler *et al*. (1999)	RCT	HCl	CHS, Mn	Oral	1500	1245	31	112	9.5
McAlindon *et al*. (2000)	RCT-P	SO_4	None	Oral	1500	1184	186	42	21.4
Monauni: (2000)	Controlled	Uncertain	None	iv	9.7g	-	10	--	--
	Controlled	Uncertain	None	iv	30.5g	-	5	--	--
Muller-Faßbender *et al* (1994)	RCT-C	SO_4	Ibuprofen	Oral	1500	1184	100	28	7.7
Mund-Hoym (1980)	Controlled	SO_4	Phenylbutazone	oral/im	1000	1184	40	32	3.5
Murad and Tabibian (2001)	Controlled	SO_4	Supplement	Oral	uncert	1184	57	35	5.5

Table XIII.2.3.1-1 Human Clinical Studies Evaluating the Supplemental use of Glucosamine

Study	Type of Study	Glucosamine Form	Other Treatment	Route	Dose mg/d	Free-base Form mg/d	No. of Subjects*	Duration Days	Subject Years
Nguyen *et al*. (2001)	RCT	HCl	CHS	Oral	1500	1245	19	84	4.4
Noack *et al*. (1994)	RCT	SO_4	None	Oral	1500	1184	120	28	9.2
Pavelka *et al*. (2002)	RCT	SO_4	None	Oral	1500	1184	84	1095	252.0
Pouwels *et al*. (2001)	Controlled	SO_4	None	iv	~7.2g	1184	6	--	--
Pujalte *et al*. (1980)	RCT	SO_4	None	Oral	1500	1184	11	49	1.5
Qiu *et al*. (1998)	RCT-C	SO_4	Ibuprofen	Oral	1500	1184	142	28	10.9
Reichelt *et al*. (1994)	RCT	SO_4	None	Im	114	1184	73	42	8.4
Reginster *et al*. (2001)	RCT	SO_4	None	Oral	1500	1184	87	1095	261.1
Rindone *et al*. (2000)	RCT	SO_4	None	Oral	1500	1184	49	60	8.1
Rovati (1992)	RCT-P-C	SO_4	Piroxicam	Oral	1500	1184	80	150	32.9
	RCT	SO_4	None	Oral	1500	1184	123	28	9.4
	RCT	SO_4	None	Oral	1500	1184	76	42	8.7
	RCT-C	SO_4	Ibuprofen	Oral	1500	1184	100	28	7.7
Scroggie *et al*. (2003)	RCT	HCl	CHS	Oral	1500	1245	22	90	5.4
Shankland (1998)	Observational	HCl	CHS	Oral	3200	2656	50	35	4.8
Tannis *et al*. (2004)	RCT	SO_4	None	Oral	1500	1184	11	84	2.5
Tapadinhas *et al*. (1982)	Observational	SO_4	None	oral	1500	1184	1367	50	187.3
Thie *et al*. (2001)	RCT-C	SO_4	Ibuprofen	oral	1500	1184	22	90	5.4
Usha and Naidu (2004)	RCT-P-C	Uncertain	Methylsulfonly-methane	oral	1500	-	60	84	4.9
Vajranetra (1984)	Observational	SO_4	None	oral/ia	1500	1184	108	84	24.9

Table XIII.2.3.1-1 Human Clinical Studies Evaluating the Supplemental use of Glucosamine

Study	Type of Study	Glucosamine Form	Other Treatment	Route	Dose mg/d	Free-base Form mg/d	No. of Subjects*	Duration Days	Subject Years
Vaz (1982)	RCT-C	SO_4	Ibuprofen	oral	1500	1184	19	56	2.9
Yu *et al*. (2003)	Observational	SO_4	None	oral	1500	1184	12	28	0.9
Sum							**3783**		**1191**

* Abbreviations: RCT- randomized controlled trial; C, comparator; P, placebo; CHS, chondroitin sulphate; iv, intravenous; im, intramuscular; ia, intra-articular; Mn, manganese

As seen in Table XIII.2.3.1-1, glucosamine administration under controlled settings has a long-established and well-documented history of use. Daily administration of glucosamine to research volunteers or subjects has been reported for periods ranging from 21 days to a long as three years. Although, the most common glucosamine intake in human studies is 1500 mg/day, glucosamine HCl intakes as high as 3200 mg/day (2656 mg/day free-base glucosamine) over 35-day period have been reported in the literature (Shankland, 1998). In terms of subject years (number of subject multiplied by duration of treatment), 3783 human volunteers or patients have received glucosamine for 1191 patient-years. In studies that span a historical time-scale of 26 years, glucosamine use is consistently well tolerated and there have been no serious or life-threatening effects reported.

XIII.2.3.2 Glucosamine and Adverse Events

A number of non-specific symptoms are commonly reported in glucosamine supplementation trials: mild gastrointestinal symptoms including constipation, diarrhoea, nausea, dyspepsia, excessive gas, abdominal distension, and abdominal cramps; headache; and skin rash or pruritis.

Of the studies obtained from the literature, nineteen report specific side-effect data comparing glucosamine to placebo, these studies, summarized in Table XIII.2.3.2-1 included 3976 subjects and 850 patient-years of observation. In 12 of the 19 studies, symptoms were less common in glucosamine-treated subjects than in placebo-treated subjects. Only two studies reported that symptoms were more common with glucosamine than placebo; Braham *et al.* (2003) and Nguyen *et al.* (2001). In the Braham study the ratio of adverse events in the glucosamine groups relative to controls was 11/10. In the Nguyen study, treatment group subjects received 1500 mg of glucosamine HCl + 1200 mg of chondroitin sulphate for 12 weeks. Although the occurrence of mild transient symptoms was equal in the treatment group and control groups, a higher dropout rate was experienced in the glucosamine group (39% *vs*. 9%); the authors were unable to explain the apparent discrepancy. As the Nguyen study utilised a small population group (n=23 glucosamine / 22 placebo) and involved the co-administration of chondroitin sulphate, the relevance of the higher dropout rate in this study is difficult to interpret, particularly in light of the large number of studies supporting its safe use.

The incidence of glucosamine related side-effects has been summarized statistically in two comprehensive efficacy meta-analyses conducted by Richy *et al.* (2003) and Towheed *et al.* (2006). Richy *et al.* (2003) investigated the efficacy of glucosamine and chondroitin sulphate in randomized controlled trials for knee osteoarthritis and identified 15 studies from the literature meeting their specific inclusion criteria. Of those 15 studies, 11 studies contained sufficient adverse event reporting (7 glucosamine sulphate and 5 chondroitin sulphate) to be included in the adverse event analysis. Richy *et al.* (2003) concluded that the safety profile of both chondroitin sulphate and glucosamine sulphate were excellent, as the global relative

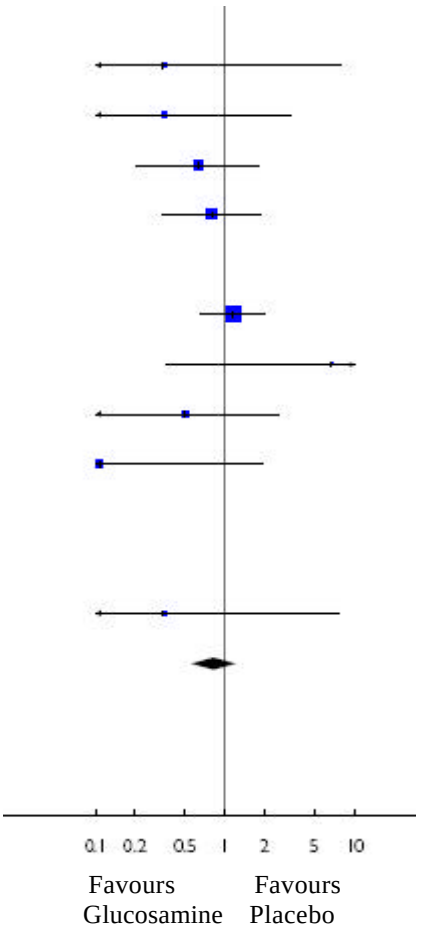
risk of an adverse event for patients given either supplement was 0.8 (95% CI = 0.59 -1.08; P = 0.15).

Furthermore, Towheed *et al.* (2006) conducted an extensive literature search of all randomized controlled trials evaluating the effectiveness and toxicity of glucosamine supplementation for osteoarthritis. The authors identified 20 studies of sufficient quality, based on the following inclusion criteria: clinical trials with a randomized controlled design evaluating both effectiveness and safety of glucosamine in osteoarthritis; studies with placebo or comparative controls, and both single- and double-blinded in nature. The authors concluded that the safety profile of glucosamine was excellent. Out of 1160 subjects randomized to glucosamine supplementation only 4% were withdrawn due to toxicity. The total number of subjects reporting an adverse reaction was 275 (26%) based on 17 RCTs (n=1045). For the placebo controls, the incidence of withdrawals and the reporting of adverse events was 5% and 32% respectively. Data summarizing these studies are shown in Tables XIII.2.3.2-1 and -2.

Finally, in addition to the above, the results of the study by Clegg *et al.* (2006) who very recently completed the largest and perhaps most well designed clinical trial to date, is an excellent example that glucosamine supplementation is safe. The study was a multi-centre, double-blind, placebo- and celecoxib- controlled GAIT evaluating both efficacy and safety. 1583 Patients were randomized to one of four treatment groups receiving 1500 mg of glucosamine HCl (1245 mg of free-base glucosamine) daily, 1200 mg of chondroitin sulphate daily, glucosamine and chondroitin sulphate daily, 200 mg of celecoxib daily or placebo for 24 weeks. The study was conducted according to good clinical practice with adverse events and serious adverse events assessed by the investigator at each study visit. Safety monitoring included complete blood counts, measurements of serum aspartate aminotransferase, alanine aminotransferase, glucose, creatinine, partial thromboplastin time, and urine analysis at each study visit. A test for faecal occult blood was also performed at the end of the study. Throughout the study there were no serious gastrointestinal adverse events or deaths. The total number of withdrawals in the glucosamine group due to adverse events was 9 (3.7%) *versus* 11 (4.4%) in the placebo group. The results of this study are in accordance with the previously discussed large database of earlier studies supporting the safety of chronic glucosamine supplementation.

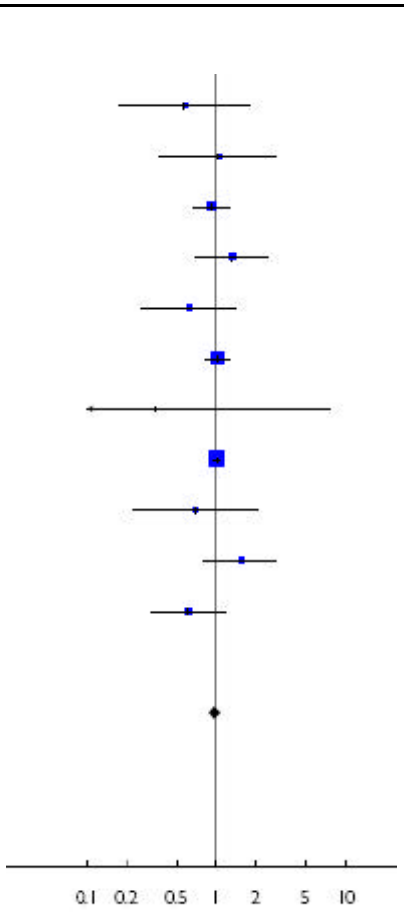
Table XIII.2.3.2-1 Glucosamine *versus* Placebo, Toxicity (Number of Withdrawals due to Adverse Events) Towheed *et al.*, 2006

Study	Glucosamine n/N	Placebo n/N	Relative Risk (Fixed) 95% CI	Weight (%)	Relative Risk (Fixed) 95% CI
Cibere *et al.* (2004)	0/71	0/66		0.0	Not estimable
Crolle and D'Este (1980)	0/15	0/15		0.0	Not estimable
D'ambrosio *et al.* (1981)	0/15	0/15		0.0	Not estimable
Drovanti *et al.* (1980)	0/40	0/40		0.0	Not estimable
Houpt *et al.* (1999)	0/46	0/55		0.0	Not estimable
Hughes and Carr (2002)	0/40	1/40		2.9	0.33 [0.01, 7.95]
McAlindon *et al.* (2004)	1/01	¾		5.8	0.34 [0.04, 3.25}
Noack *et al.* (1994)	5/126	8/126		15.7	0.63 [0.21, 1.86]
Pavelka *et al.* (2002)	8/101	10/101		19.6	0.80 [0.33, 1.94]
Pujalte *et al.* (1980)	0/12	0/12		0.0	Not estimable
Reginster *et al.* (2001)	21/106	18/106		35.3	1.17 [0.66, 2.06]
Reichelt *et al.* (1994)	3/79	0/76		1.0	6.74 [0.35, 128.29]
Rindone *et al.* (2000)	2/49	4/49		7.8	0.50 [0.10, 2.60]
Rovati (1997)	0/79	4/77		8.9	0.11 [0.01, 1.98]
Usha and Naidu (2004)	0/30	0/28		0.0	Not estimable
Vajaradul (1981)	0/30	0/30		0.0	Not estimable
Zenk *et al.* (2002)	0/14	1/14		2.9	0.33 [0.01, 7.55]
Total (95% Cl)	954	954		100.0	0.82 [0.56, 1.21]



Total events: 40 (Glucosamine), 49 (Placebo)
Test for heterogenicity chi-square = 7.09, df = 8, p = 0.53
Test for overall effect z = 1.00, p = 0.3

Table XIII.2.3.2-2 Glucosamine *versus* Placebo, Toxicity (Number of Patients Reporting Adverse Events) Towheed *et al.*, 2006.

Study	Glucosamine n/N	Placebo N/N	Relative Risk (Fixed) 95% CI	Weight (%)	Relative Risk (Fixed) 95% CI
Crolle and D'Este, (1980)	0/15	0/15		0.0	Not estimable
D'ambrosio *et al*. (1981)	0/15	0/15		0.0	Not estimable
Drovanti *et al*. (1980)	0/40	7/40		2.6	0.57 [0.18, 1.80]
Houpt *et al*. (1999)	6/46	7/55		2.4	1.02 [0.37, 2.84]
Hughes and Carr (2002)	25/40	27/40		10.0	0.93 [0.67, 1.28]
McAlindon *et al*. (2004)	18/101	14/104		5.1	1.32, [0.70, 2.52]
Noack *et al*. (1994)	8/126	13/126		4.8	0.62, [0.26, 1.43]
Pavelka *et al*. (2002)	67/101	65/101		24.1	1.03, [0.84, 1.26]
Pujalte *et al*. (1980)	0/12	1/12		0.6	0.33, [0.01, 7.45]
Reginster *et al*. (2001)	100/106	99/106		36.7	1.01, [0.94, 1.08]
Reichelt *et al*. (1994)	5/79	7/76		2.6	0.69, [0.23, 2.07]
Rindone *et al*. (2000)	17/49	11/49		4.1	1.55, [0.81, 2.95]
Rovati (1997)	12/79	19/77		7.1	0.62, [0.32, 1.18]
Vajaradul (1981)	0/30	0/30		0.0	Not estimable
Total (95% CI)	839	846		100.0	0.97 [0.88, 1.08]
Total events: 262 (Glucosamine), 270 (Placebo)					
Test for heterogenicity chi-square = 9.03, df = 10, p = 0.53					
Test for overall effect z = 0.49, p = 0.6					

XIII.2.3.3 Studies Including Objective Safety Endpoints

As shown in Table XIII.2.3.2-1, a total of 16 studies reporting specific safety measures were identified in the literature, and included the following toxicity assessments: chemistry data including liver and kidney safety assessments, haematological parameters (white blood count, red blood count, haemoglobin, and platelet count), urinalyses, occult blood measurements of stool samples, and cardiovascular parameters including blood pressure and pulse rate. None of the studies summarized in table 12 reported adverse effects in any objective measurement

of clinical safety following glucosamine administration. In general the previous studies included about 4000 subjects representing approximately 800 patient-years. Specifically, the number of studies assessing various safety parameters are as follows: chemistry panel, 14; haematological parameters, 16; urinalyses, 12; occult blood, 4; and cardiovascular parameters, 10. In addition, blood pressure and pulse rate were monitored continuously in 21 subjects who had large amounts of glucosamine infused intravenously with no reported adverse effects (Monauni *et al.*, 2000; Pouwels *et al.*, 2001).

Table 2.3.3-1 Summary of Studies with Safety Information

Study	Glucose (mg/dl)		Blood Chem	Haematology	UA	Occult Blood	BP P	Side-Effects Gluc/Placeb
	Before	After						
Almada *et al*. (2000)	94	94	--	--	--	--	--	--
Braham *et al*. (2003)	--		--	--	--	--	--	1.10
Clegg	NSC	NSC	NSC	NSC	NSC	NSC	NSC	0.81
Crolle and D'Este, 1980	95	103 (NS)	NSC	NSC	--	--	--	--
D'Ambrosio *et al*. (1981)	109	97 (NS)	NSC	NSC	NSC	--	NSC	1.00
Das and Hammond (2000)	--		--	--	--	--	--	0.89
Drovanti *et al*. (1980)	82	82	NSC	NSC	--	NSC	--	0.83
Forster *et al*. (1996)	--		--	--	--	--	--	0.20
Giordano *et al*. (1996)	NSC		NSC	NSC	NSC	--	--	1.00
Houpt *et al*. (1999)	--		--	--	--	--	--	1.00
Hughes and Carr (2002)	NSC		NSC	NSC	NSC	--	--	0.90
Leffler *et al*. (1999)	--		--	NSC	--	NSC	NSC	0.97
Monauni (2000)	NSC		--	--	--	--	NSC	--
	minimal effect		--	--	--	--	NSC	--
Muller-Faßbender *et al*. (1994)	--		--	--	--	--	NSC	--
Mund-Hoym (1980)	--		--	--	--	--	--	--
Murad and Tabibian (2001)	--		--	--	--	--	--	--
Nguyen *et al*. (2001)	--		--	--	--	--	--	1.43

Table 2.3.3-1 Summary of Studies with Safety Information

Study	Glucose (mg/dl)		Blood Chem	Haematology	UA	Occult Blood	BP P	Side-Effects Gluc/Placeb
	Before	After						
Noack *et al*. (1994)	NSC		NSC	NSC	NSC	--	NSC	0.62
Pavelka *et al*. (2002)	NSC		NSC	NSC	NSC	--	--	0.56
Pouwels *et al*. (2001)	NSC		--	--	--	--	NSC	--
Pujalte *et al*. (1980)	--		NSC	NSC	NSC	--	--	0.00
Qiu *et al*. (1998)	--		NSC	NSC	NSC	--	--	--
Reichelt *et al*. (1994)	--		--	--	--	--	--	--
Reginster *et al*. (1994)	slightly lower		NSC	NSC	NSC	--	NSC	0.82
Rindone *et al*. (2000)	--		--	--	--	--	--	0.50
Rovati (1992)	--		--	--	--	--	--	0.62
	--		NSC	NSC	NSC	--	--	0.62
	--		NSC	NSC	NSC	--	--	0.71
	--		NSC	NSC	NSC	--	--	--
Scroggie *et al*. (2003)	HbA1c	NSC	--	--	--	--	--	--
Tannis *et al*. (2004)	82.3	79.2	--	--	--	--	--	--
Tapadinhas *et al*. (1982)	--		--	--	--	--	--	--
Thie *et al*. (2001)	--		--	--	--	--	--	--
Vajranetra (1984)	--		--	--	--	--	--	--
Vaz (1982)	--		--	NSC	--	NSC	NSC	--
Yu *et al*. 2003)	97.2	97.2	--	--	--	--	--	--
No. with reports	6	6	14	16	12	4	10	19
Total patients								

Abbreviations: NA, not available; NSC, not clinically significant; HbA1c, glycosylated haemoglobin; chem., chemistry; Haemat, haematology; UA, urinalysis; occult blood, stool measurement; BP, blood pressure; P, pulse; GlucN/P, ratio of side effects from glucosamine divided by those from placebo.

XIII.2.3.4 Effects of Glucosamine on Glucose Metabolism

Clinical trials reporting fasting blood glucose values in subjects receiving glucosamine supplementation are summarized in Table 2.3.3-1. Reginster *et al.* (2001) reported that blood glucose values were slightly lower in patients receiving glucosamine (106 subjects) over a 3-year period, although the difference was not significant. Other clinical trials summarized in

this table indicated no significant changes in clinical chemistry values, implying no change in blood glucose values. Scroggie *et al.* (2003) measured glycosylated haemoglobin (HbA1c) in 22 diabetic and 12 control subjects over 90 days. There were no significant changes in HBA1c observed between diabetic and control subjects. In two other studies (Monauni *et al.*, 2000; Pouwels *et al.*, 2001) performed in metabolic research wards, large amounts of glucosamine (~7.2 grams or 9.7 grams of the glucosamine free-base) infused over 5 hours produced no change in blood glucose values. Tannis *et al.* (2004) reported that daily administration of glucosamine sulphate (1500 mg) over 12 weeks was associated with no significant changes in fasting plasma glucose, insulin values or changes resulting from an oral glucose tolerance test. These results are in accordance with a study by Yu *et al.* (2003), indicating that administration of 1500 mg glucosamine for 28 days had no effect on glucose tolerance or insulin sensitivity of 10 non-diabetic subjects. In total 18 studies either directly or indirectly report that daily glucosamine salt administration at levels of 1500 mg or higher has essentially no effect on fasting blood glucose values in humans.

XIII.2.3.5 Glucosamine Use: High Intakes and Long-Term Use

The intravenous administration of glucosamine in very high quantities has been reported in the literature. Pouwels *et al.* (2001) intravenously infused ~7.2 grams of glucosamine as the sulphate salt over a 300 minute period in 10 healthy volunteers. The authors observed that glucosamine administration was well tolerated and not associated with side-effects. Monauni *et al.* (2000) intravenously infused 9.7 grams of glucosamine over a 300-minute period in 10 healthy volunteers; again this was well tolerated with no reported side effects. When the authors subsequently infused 30.5 grams of glucosamine (436 mg/kg/day or more than 20 times the usual daily dose) into 5 healthy volunteers, this quantity was well tolerated by 4 subjects and one subject developed mild symptoms (headache). These studies indicate that systemic exposure of glucosamine at very high levels is well tolerated by healthy volunteer subjects. As these reports were studies involving the intravenous administration of glucosamine, it should be noted that glucosamine was in its free-base form.

As mentioned, oral administration of glucosamine is generally administered in amounts of 1500 mg/day for use in osteoarthritis therapy, however the use of higher quantities have been reported in the literature. Shankland (1998) administered 3200 mg/day of glucosamine HCl (2656 mg of free-base glucosamine) to 50 individuals over a 35-day period. Each individual also received 2400 mg of a chondroitin sulphate mixture and 1000 mg of calcium ascorbate daily. The only reported side-effects were gastrointestinal discomfort in two individuals that subsided following discontinuation of supplement use. Das *et al.* (2000) performed a randomized controlled trial evaluating the efficacy of daily administration of 2000mg/day of glucosamine HCl, 1600 mg of chondroitin sulphate, and 304 mg of manganese ascorbate in 93 individuals over 6 months. The incidence of adverse events was 19% in control subjects and 17% in treated subjects. Finally, Braham *et al.* (2003) also reported that daily

administration of glucosamine in quantities of 2000 mg/day was well tolerated. The results of these studies suggest that high intakes of glucosamine, and high intakes of glycosaminoglycans in general are well tolerated.

The long-term use of glucosamine was addressed by Poolsup *et al.* (2005) in a meta-analysis of two long-term studies, one by Reginster *et al.* (2001) and the other by Pavelka *et al.* (2002). Both studies investigated the efficacy and safety of glucosamine sulphate in ~200 patients randomized to daily administration of 1500 mg of glucosamine or placebo for three years. The pooled frequency of adverse events was not significantly different between glucosamine treatment and placebo (RR=1.02; 95% CI=0.93-1.11). Common problems of abdominal pain, dyspepsia, diarrhoea, increased blood pressure, fatigue, and rash were equal in glucosamine supplemented groups and placebo controls.

XIII.2.3.6 Glucosamine Hydrochloride *vs*. Glucosamine Sulphate

As sulphate is a component of cartilage, a majority of the osteoarthritis efficacy studies have used glucosamine sulphate rather than the HCl salt. It should be noted however, that justification for use of the sulphate salt over HCl as the counter-ion is purely theoretical and no clinical evidence suggests that one is "better" than the other. As the association of glucose with its counter ion is a weak interaction, disassociation of the salt occurs in the stomach and it seems unlikely that the two preparations would have differing effects. A comparison of the two glucosamine forms has recently been performed by Qiu *et al.* (2005) in 142 subjects randomized to receive glucosamine sulphate (1500 mg) or glucosamine HCl (1440 mg) daily over a period of four weeks. Extensive safety monitoring was performed in each subject both before and upon completion of the study and included a number of clinical parameters: the monitoring of adverse event occurrence, abnormal changes in blood pressures, biochemical indices, blood routines, urine routines, EGGs, faecal occult blood tests and a number of additional parameters not detailed in the study. Patients experiencing adverse reactions suffered from mild to moderate gastrointestinal discomfort or constipation. The adverse reaction rates were 4.2% and 7.0% for the HCl and sulphate groups respectively. The results of this study and comparisons of the side effects and reports of efficacy across numerous trials suggest that both glucosamine salts have similar effects. The only difference between glucosamine forms that may need consideration regarding safety is in the quantity of glucosamine free-base in each preparation (PDRNS, 2001). Thus, subjects receiving the HCl form of glucosamine would receive slightly higher levels of glucosamine free-base on a per weight basis relative to subjects receiving the sulphate form. Given the overwhelming evidence of safety for glucosamine use in clinical trials, it is unlikely that such differences are of clinical relevance from a safety perspective.

XIII.2.3.7 Potential Allergenicity Concerns

An expert opinion on the potential allergenicity of RGHAN derived from fermentation of *A. niger* from Professor S.L. Taylor from the Institute of Agriculture and Natural Resources – Food Allergy Research and Resource Program, University of Nebraska is submitted here as Appendix 5. He concludes, "Food allergens are proteins, and glucosamine is not a protein. When produced *via* fermentation with *A. niger*, there should be little, if any, concern about the introduction of proteinaceous allergens from the fermenting organism or the fermentation substrate. Thus I can find no reason to be concerned about the possible allergenicity of glucosamine when produced in this manner". Further detail on the analyses of RGHAN for the presence of potentially allergenic proteins/peptides is provided in Section I.2.

XIII.3 Discussion and Conclusions on the Toxicology of RGHAN

Glucosamine is a prominent component of the hexosamine pathway, an important branch of glycolysis. Exogenous glucosamine is actively transported from extracellular tissue into cells by glucose transporters; (Uldry *et al.*, 2002) insulin facilitates glucosamine transport into cells (Heart *et al.*, 2000). Once in the cell, glucosamine is phosphorylated by one of the family of hexokinases to glucosamine-6-phosphate (GlucN-6-P). The metabolism of glucosamine is highly regulated by rates of transport into various tissues and by effects of intermediates on key enzymatic steps.

Glucosamine HCl and glucosamine sulphate have demonstrated glucosamine to be well absorbed from the gastrointestinal tract. The bioavailability of glucosamine following oral administration, as determined from the glucosamine sulphate AUC data, was reported to be approximately 26% of that available after iv or im administration. The low bioavailability of glucosamine sulphate following oral administration was attributed to the first pass effect in the liver, which results in the metabolism of glucosamine sulphate to smaller molecules and finally to CO_2, water, and urea (Setnikar *et al.*, 1993)

Studies conducted to examine the potential toxicity of glucosamine in various animal species (*e.g.*, rats, dogs, mice, rabbits, and horses) demonstrated that glucosamine is safe at the doses administered. Acute toxicity studies demonstrated that the oral LD_{50} dose for rats (Sprague-Dawley), mice (CD-1), and rabbits (New Zealand White Albino) were greater than 8000, 8000, and 6000 mg/kg body weight, respectively, for glucosamine sulphate (Setnikar *et al.*, 1991a). The single acute toxicity study conducted with RGHAN demonstrated that the LD_{50} for in Crl:CD (SD) IGS BR rats was greater than 5000 mg/kg body weight (Glaza, 2002). Similarly, subchronic and chronic oral studies reported no toxicity or occurrence of adverse effects attributable to glucosamine at levels up to 2130 mg/kg body weight/day (free-base). For a 60 kg adult this would be equivalent to up to approximately 127800 mg/person per day for RGHAN. These compare favourably to predicted intakes of up to 1274 mg/person per day at the 95^{th} percentile for the targeted consumers.

Furthermore, *in vitro* and *in vivo* genotoxicity tests have demonstrated that RGHAN was non-genotoxic. Even though Banerjee and Manna *et al.* (1984) reported a positive result in the mouse chromosomal aberration study, only a single dose was tested. The preclinical studies clearly demonstrated that glucosamine was safe at the administered doses.

The clinical studies using various forms of glucosamine have clearly demonstrated that the consumption of glucosamine is well tolerated and safe at levels comparable with predicted mean and high-level consumption. Volunteers were reported to consume glucosamine supplements over periods ranging from 21 days to 3 years with the majority of the studies providing glucosamine at a dose of approximately 1500 mg/day with glucosamine HCl doses reported as high as 3200 mg/day (*i.e.*, approximately 2656 mg/day free-base glucosamine). A range of adverse effects were reported in the clinical trials; however, the majority of the adverse effects were non-specific, mild gastrointestinal symptoms commonly reported in conjunction with glucosamine supplementation (*e.g.*, constipation, diarrhoea, nausea, dyspepsia, excessive gas, abdominal distension, and abdominal cramps), as well as headaches, skin rash, or pruritis. The safety of glucosamine consumption has been supported by various reviews and meta-analyses, as well as by a multi-centre clinical trial conducted by Clegg *et al.* (2006) where 1583 patients were provided with 1 of 4 treatments including 1500 mg/day of glucosamine HCl and the occurrence of adverse effects were comparable between the glucosamine and placebo groups. Again these levels compare favourably to predicted intakes of RGHAN of up to 1274 mg/person per day at the 95th percentile for the targeted consumers. Our conclusions reflect those of Anderson *et al.* (2005) who reviewed much of the same data and concluded that "Our critical evaluation indicates that glucosamine is safe under current conditions of use and does not affect glucose metabolism".

Based on intakes provided it can be clearly seen that, for the proposed food uses of RGHAN in specific beverages and fermented milk-based products, aimed at the nutritional support of joint health, the safe endpoints of both animal and human safety would clearly not be exceeded by consumption of RGHAN at the recommended maximum levels.

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GLOSSARY

ADI	acceptable daily intake
ADME	absorption, distribution, metabolism, and excretion
ALT	alanine aminotransferase
AOAC	Association of Analytical Communities International
AST	aspartate aminsotransferase
BP	blood pressure
BUN	blood urea nitrogen
CAS	Chemical Abstracts Service
CFR	*Code of Federal Regulations*
CHS	chondroitin sulphate
CSFII	Continuing Survey of Food Intakes by Individuals for the years 1989-1992, United States Department of Agriculture
cfu/g	colony forming units per gram
cGMP	current Good Manufacturing Practices
chem	chemistry
DNA	deoxyribonucleic acid
EAFUS	Everything Added to Food in the United States
ED_{50}	effective dose for 50% of the population
EDI	estimated daily intake
EINECS	European Inventory of Existing Commercial Chemical Substances
EPA	Environmental Protection Agency
FDA	Food and Drug Administration
FR	*Federal Register*
g	grams
GAG	glycosaminoglycan
GC/MS	gas chromatograph/mass spectrometer
GlucN/P	ratio of side effects from glucosamine divided by those from placebo
GRAS	Generally Recognized as Safe
HACCP	Hazard Analysis and Critical Control Points
HbA1c	glycosylated haemoglobin
HCl	hydrochloride
HPLC	high performance liquid chromatography
ISO 9001:2000	quality management system
IUPAC	International Union of Pure and Applied Chemistry
ia	intraarterial
im	intramuscular
IOM	Institute of Medicine
ip	intraperitoneal
iv	intravenous
kg bw	kilograms of body weight
LD_{50}	lethal dose for 50% of the population
mg	milligrams
mg/dL	milligrams per decilitre
mg/kg	milligrams per kilogram
mg/mL	milligrams per millilitre
mmol	millimolar

MPN	most probable number
NA	not available
NCE	normochromatic erythrocytes
NOAEL	no observable adverse effect level
NOEL	no observable effect level
NSAID	non-steroidal anti-inflammatory drug
NSC	not clinically significant
OA	osteoarthritis
P	pulse
P value	probability value
PCE	polychromatic erythrocyte
ppm	parts per million
RCT	randomised controlled trial
RCT-P	randomised controlled trial, placebo
RCT-P-C	randomised controlled trial, placebo, comparator
SD	Sprague-Dawley rats
SHR	spontaneously hypertensive rats
TMJ	temporomandibular joint
UA	urinalysis
USDA	United States Department of Agriculture
USP-NF	United States Pharmacopeia-National Formulary