

**Proposal for making a “Substantial Equivalence” notification for Non-Shellfish
Glucosamine Hydrochloride 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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INDEX

	Page
1. Background to “Substantial Equivalence”	4
2. Introduction	4
2.1. What is Glucosamine?	4
3. Objective	5
4. A Comparison of Composition	6
4.1 Molecular Structure of Glucosamine Hydrochloride	6
4.2 Process Techniques	6
4.2.1 Source Material	6
4.2.2 Extraction Process	7
4.3 Specification	11
4.4 Certificates of Analysis	11
5. Nutritional Value	12
6. Proposed Labelling	12
7. Intended Use	13
8. Levels of Undesirable Substances	14

LIST OF TABLES

Table 1	The Nutritional Value for 1.5 g (1500 mg) of Non-Shellfish Glucosamine Hydrochloride	12
Table 2	Examples of Glucosamine Food Supplement Products Currently on UK Market	13

LIST OF FIGURES

Figures 1, 2, 3	Molecular Structure of Glucosamine Hydrochloride	6
Figure 4	Shellfish Glucosamine Hydrochloride Process Flow Diagram	9
Figure 5	Non-Shellfish Glucosamine Hydrochloride Process Flow Diagram	10

LIST OF APPENDICES

- Appendix 1. Expert Opinion on Allergenicity
- Appendix 2. USP Monograph
- Appendix 3. Ochratoxin A Results
- Appendix 4. Protein Analysis – by Louisa B. Tabatabai, PhD, Professor-in-Charge of the Protein Facility at Iowa State University
- Appendix 5. Cargill Certificates of Analysis for Non-Shellfish Glucosamine HCL and Chinese Shellfish Glucosamine Hydrochloride -summary
- Appendix 6. FTIR Comparison Non-Shellfish Glucosamine HCL and Chinese Shellfish Glucosamine Hydrochloride
- Appendix 7. Covance Aflatoxins Analysis and Pesticide Screen
- Appendix 8. Examples of Labels Glucosamine Hydrochloride Currently on the Market
- Appendix 9. List of Efficacy References

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1. Background to “Substantial Equivalence”

Regulation No 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.”

However, Article 3 (4) 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 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.”

Article 5 states “...the applicant shall notify the Commission of the placing on the market when he does so. Such notification shall be accompanied by the relevant details provided for in Article 3 (4). The Commission shall forward to Member States a copy of that notification within 60 days and, at the request of a Member State, a copy of the relevant details.”

2. Introduction

2.1. What is Glucosamine?

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. sport nutrition. It is typically extracted from chitin, which can be found in shellfish waste, primarily of Asian (typically Chinese) origin. Glucosamine 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.

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).

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.

3. Objective

The objective of this submission is to show the, "Substantial Equivalence" of "Non-Shellfish Glucosamine Hydrochloride" made from biomass (which also contains chitin) from the fungus *Aspergillus niger* (*A. niger*) to Shellfish Glucosamine Hydrochloride 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. Furthermore we propose that "Non-Shellfish Glucosamine Hydrochloride" is classified in accordance with Section 1 of this dossier.

Consequently this proposed notification, in accordance with Articles 3(4), we will provide a comparison to existing Shellfish Glucosamine Hydrochloride for the following parameters:

- Section 4. Composition
- Section 5. Nutritional Value
- Section 6. Metabolism
- Section 7. Intended Use
- Section 8. Level of Undesirable Substances

4. A Comparison of Composition

4.1 Molecular Structure of Glucosamine Hydrochloride

Figure 1

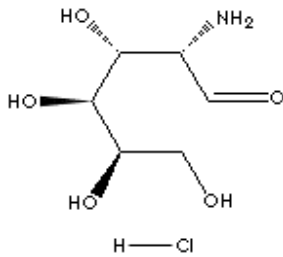


Figure 2

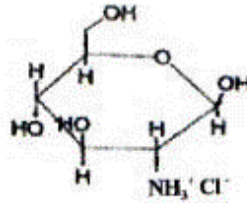
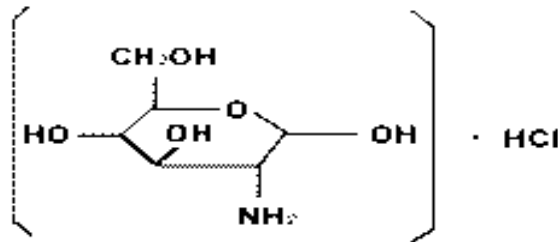


Figure 3



Molecular Structure of Glucosamine Hydrochloride

Figures 1, 2, and 3 above represent different styles of molecular presentation of Glucosamine Hydrochloride (Glucosamine HCL). To check for correct molecular structure the specification for Glucosamine Hydrochloride, 3 analytical tests are performed:

- A. Infrared absorption
- B. High Performance Liquid Chromatography
- C. Specific Rotation.

The specification for these and comparison to Shellfish Glucosamine Hydrochloride is detailed in Sections 4.3 and 4.4 below.

Glucosamine Hydrochloride from *A. niger* is chemically identical to Glucosamine Hydrochloride derived from shellfish. It is a single molecule, independent of source with only one chiral centre. Glucosamine Hydrochloride from either *A. niger* or shellfish is produced as a crystalline product.

4.2 Process Techniques

4.2.1 Source Material

Obviously the key difference between Shellfish and Non-Shellfish Glucosamine Hydrochloride is the source material. For Non-Shellfish Glucosamine Hydrochloride the source is chitin from

biomass produced from the fermentation of the fungus *A. niger*, which is defined taxonomically as follows:

Class, Deuteromycetes
Order, Moniliales
Family, Moniliaceae
Genus, *Aspergillus*
Species, *niger*

This is a fungal organism which 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. This process is operated under HACCP and Food GMP guidelines.

Microbiological testing is carried out routinely to ensure the absence of bacterial and fungal contamination (including spores). The specifics of this testing are outlined in Section 4.3 below.

A review of the safety of *A. niger* (Schuster *et al.*² in 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 Non-Shellfish Glucosamine Hydrochloride 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.

An expert opinion on the potential allergenicity of Non-Shellfish Glucosamine Hydrochloride 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 1. 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”.

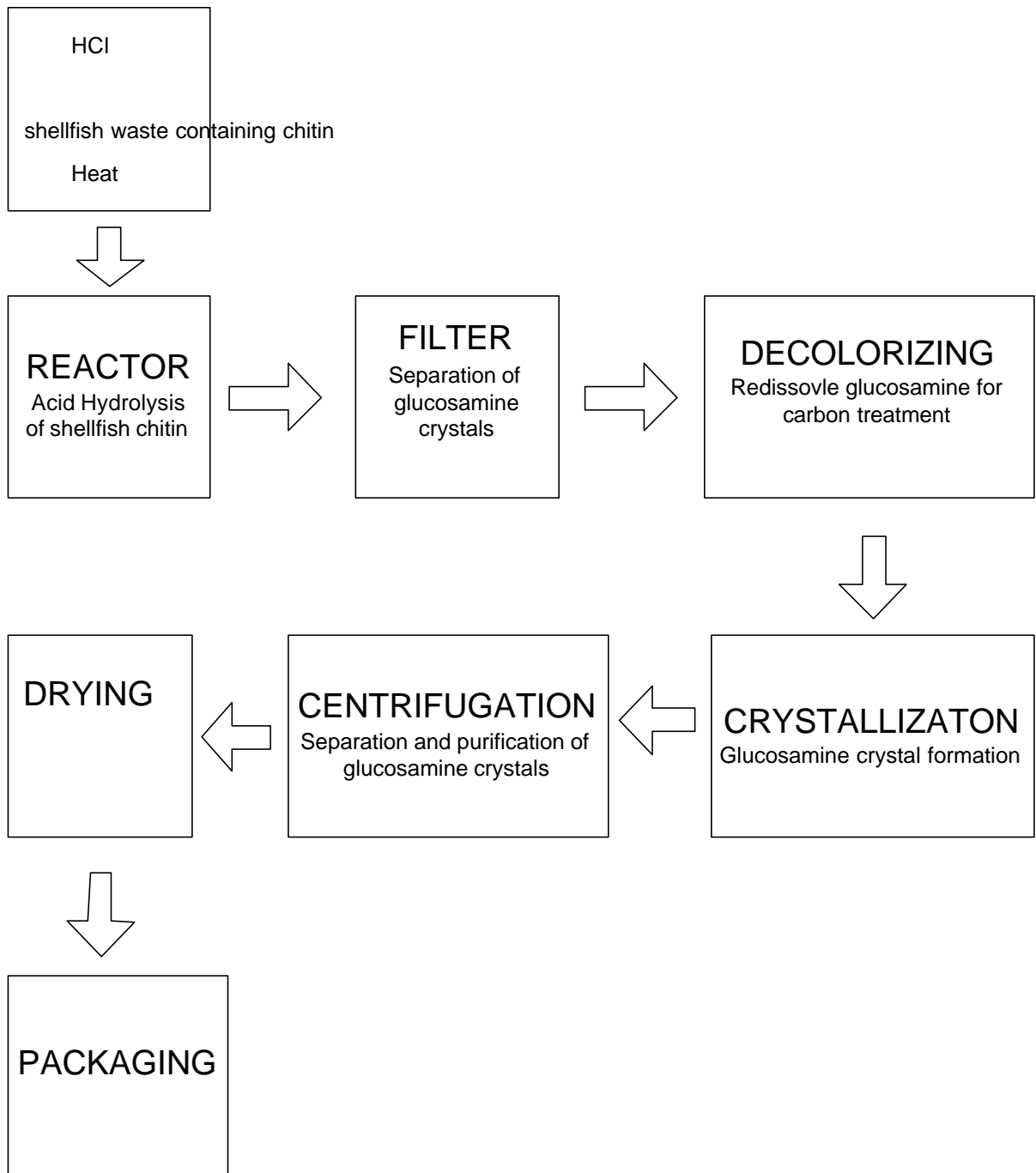
4.2.2 Extraction Process

Figures 4 and 5 give overviews of the glucosamine process for Shellfish and Non-Shellfish Glucosamine Hydrochloride. Other than the raw materials, the extraction process is similar for both glucosamine sources. In both processes the chitin from the source undergoes hydrolysis to produce glucosamine.

² Schuster E, Dunn-Coleman N, Frisvad JC, Van Dijck PW. On the safety of *Aspergillus niger*—a review. *Appl Microbiol Biotechnol.* 2002 Aug;59(4-5):426-35. Epub 2002 Jun 25.

Non-Shellfish Glucosamine Hydrochloride was tested for presence of proteins by Louisa B. Tabatabai, PhD, Professor-in-Charge of the Protein Facility at Iowa State University. Sypro Ruby staining followed by Coomassie Brilliant Blue R250 staining was the methodology used, with a scan after each stain. Dr. Tabatbai interpreted the results as "indicating the absence of protein" (see Appendix 4).

Figure 4 Shellfish Glucosamine Hydrochloride Process Flow Diagram



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Figure 5 Non-Shellfish Glucosamine Hydrochloride Process Flow Diagram

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4.3 Specification

Cargill Non-Shellfish Glucosamine Hydrochloride 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 2). These are as follows:

- Identification
 - Infrared absorption
 - Titration
 - High Performance Liquid Chromatography
- Specific Rotation
- Assay
- pH
- Loss on Drying
- Residue on Ignition
- Sulphate
- Arsenic
- Heavy Metals
- Organic volatile impurities

In addition Cargill coordinates microbiological testing as follows:

- Total Plate count cfu/g <10
- Yeast and Mould cfu/g <10
- Coliform cfu/g <3
- Coliform confirmation cfu/g <3
- E. coli* cfu/g <3
- Salmonella Negative in 25g initial sample

4.4 Certificates of Analysis

Cargill samples pass all USP-NF tests, as the Cargill Certificates of Analysis provided in Appendix 5 show.

Certificates of analysis for Chinese-sourced Shellfish Glucosamine Hydrochloride are also provided as Appendix 5 to show that Cargill Non-Shellfish Glucosamine Hydrochloride meets or exceeds the quality of these products based on the USP-NF criteria.

Appendix 6 shows specifically the FTIR scans of both the Chinese and Cargill materials described above.

Also Cargill has had independent analysis conducted by Covance Laboratories for the following criteria:

USP/NF Pesticide Screen – This test found no measurable pesticides in the product (Appendix 7).

Aflatoxin Test – No measurable Aflatoxin B1, B2, G1 and G2 was found in the product (Appendix 7).

Additionally the production organism has been assessed for the ability to produce Ochratoxin A by testing the glucosamine product, as described in Section 4.2.1 above (Appendix 3).

5. Nutritional Value

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 1 The Nutritional Value for 1.5 g (1500 mg) of Non-Shellfish Glucosamine Hydrochloride

Nutritional Information per 1.5 grams	
Fat g/1.5 g	0
Protein %	0
Carbohydrates g/1.5g	1.49
Cholesterol mg/1.5g	0
Calories / 1.5g	5.33
Calcium mg/1.5 g	0
Iron mg/1.5 g	0
Sodium mg/1.5 g	0
Potassium mg/1.5 g	0
Vitamin A I.U./1.5 g	0
Vitamin C mg/1.5 g	0
Fibre g/1.5 g	0
Sugar g/1.5 g	0

6. Proposed Labelling

Dietary supplements containing glucosamine are regulated in the EU by *Directive 2002/46/EC of the European Parliament and of the Council of 10 June 2002 on the approximation of the laws of the Member States relating to food supplements*. Articles 6 to 9 of this directive deal specifically with labelling. In addition to this Glucosamine Hydrochloride is also used in “PARNUTS” products as defined by *Council Directive 89/398/EEC of 3 May 1989 on the approximation of the laws of the Member States relating to foodstuffs intended for particular nutritional uses. Article 1(2)*. This directive includes within Annex I:

5. Dietary foods for special medical purposes

8. Foods intended to meet the expenditure of intense muscular effort, especially for sportsmen.

Labelling criteria for these products are defined within specific legislation and draft legislation (in the case of Sports Nutrition products) for these products.

Cargill does not typically manufacture dietary food supplement products as presented to the consumer, and in this case Cargill is providing Glucosamine Hydrochloride as the raw material to food supplement manufacturers. Examples of labels for Glucosamine Hydrochloride currently on the market are provided here as Appendix 8. Only one change is proposed. 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 the fungus *Aspergillus niger*”.

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

The product might also carry a kosher label, as Cargill’s Non-Shellfish Glucosamine Hydrochloride product “REGENASURE™” 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).

7. Intended Use

There is no established formal Recommended Daily Intake (RDI) for Glucosamine and actual recommendations vary. Whilst we seek in this petition not to discuss efficacy, or claims as such (this being outside the scope of the Novel Foods Regulations), we do submit as Appendix 9 a list of references discussing this issue. 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 (1998) (Appendix 9.). Table 2 lists some products currently on the UK Market.

Glucosamine Hydrochloride from *A. niger* is proposed to be 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 Article 1 (2) and Annex I Number 5 – Dietary Foods for Special Medical Purposes and Number 8 – Sports Nutrition products) of products in which Shellfish Glucosamine already appears in the EU.

Table 2 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

Table 2 Examples of Glucosamine Food Supplement Products Currently on UK Market			
Brand	Bought from	Recommended Daily Intake of Glucosamine	Product Form
"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
"Dona 200-S"	On-line	250 mg per tablet	Tablet
"Flexeze Glucosamine Gel" Goldshield	On-line		Cream
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
Bovine-Max	On-line	250 mg in 5 ml	Liquid
"Collagen Drink mix Glucosamine Sulphate plus Vitamins & Minerals" Avesta Ltd	On-line	1000 mg G lucosamine Sulphate per serving	Drink Mix
"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

8. Levels of Undesirable Substances

These are extensively covered by USP-NF requirements and the additional analyses conducted by Cargill, as discussed in Section 4.

The purity of the Glucosamine Hydrochloride from either source is 98 to 102%, resulting in an extremely low amount of non-glucosamine substances, which in the case of Non-Shellfish Glucosamine Hydrochloride have been characterized to show product safety.