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Acidulants

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April 6, 2004

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

Re: Notification of GRAS Determination for REGENASURE™ Glucosamine Hydrochloride.

Dear Sir or Madam:

Cargill, Incorporated hereby submits a Notification to the Food and Drug Administration (FDA) that REGENASURE™ Glucosamine Hydrochloride is Generally Recognized As Safe (GRAS). This GRAS determination is based on scientific procedures, including a critical evaluation of scientific information, human and laboratory data, and consensus among a panel of experts qualified by scientific training and experience to evaluate the safety of substances added to food that there is a reasonable certainty that of REGENASURE™ Glucosamine Hydrochloride is not harmful under the intended conditions of use, and therefore meets the requirements of 21 *Code of Federal Regulations* (C.F.R.) 21 § 170.30.

Pursuant to *Federal Register* (FR) 62 F.R. 18938, April 17, 1997, this Notification consists of: (1) this letter, which contains the required GRAS exemption claim; and (2) a document detailing the basis for the GRAS determination. Cargill, Incorporated accepts full responsibility for the contents of this submission. The materials have been enclosed in triplicate.

This GRAS determination for use of REGENASURE™ Glucosamine Hydrochloride in select beverages has been made through scientific procedures. Glucosamine hydrochloride safety is well supported by clinical and laboratory studies.

This Notification includes detailed information regarding the structure and composition of REGENASURE™ Glucosamine Hydrochloride, its intended conditions of use, exposure and method of manufacture. The Notification includes a comprehensive review of the scientific literature and data through December 2003, including clinical studies, absorption, distribution, metabolism and excretion (ADME) data, and toxicological studies.

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Pursuant to proposed 21 C.F.R. § 170.36, Cargill, Incorporated provides the following required information:

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Product Applications Chemist
Acidulants Business Unit

Common Name: Glucosamine Hydrochloride

GRAS Substance: REGENASURE™ Glucosamine Hydrochloride

Intended use: This substance will be used by manufacturers of beverages. REGENASURE™ Glucosamine Hydrochloride is intended for use as a nutrient in food.

Basis for GRAS: Scientific Procedures

The data and information that are the basis for Cargill, Incorporated's REGENASURE™ Glucosamine Hydrochloride GRAS determination are available for FDA's review and copying at the offices of Cargill, Incorporated, 1101 15th Street N.W. Washington, D.C. 20005, (202-785-3060). In addition, Cargill, Incorporated agrees to send the materials to the FDA upon request.

Cargill, Incorporated respectfully requests notice of the receipt of this Notification, and appreciates the input and guidance provided by FDA during meetings held on September 16, 2003 and January 22, 2004. Please contact me at the number above if you have any questions regarding the information and conclusions in this Notification.

Sincerely,

Brent D. Rogers, Product Applications Chemist

000070

GRAS NOTIFICATION

For

REGENASURE™ Glucosamine Hydrochloride

Submitted by:

**Cargill, Incorporated
15407 McGinty Road West
Wayzata, MN 55391**

April 6, 2004

Contact:

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000071

TABLE OF CONTENTS

	Page
1.0 INTRODUCTION.....	1
1.1 Introduction and Summary.....	1
1.2 Glucosamine Background.....	2
1.3 History of Safe Use of Glucosamine.....	2
2.0 BASIS FOR REGULATORY APPROVAL OF GRAS AFFIRMATION.....	4
2.1 GRAS Regulatory Status.....	4
3.0 REGENASURE™-GLUCOSAMINE HYDROCHLORIDE CHEMISTRY AND COMPOSTION.....	4
3.1 Common or Usual Name and Identity.....	4
3.2 Formal Names (IUPAC or CAS Names).....	4
3.3 Trade Name.....	5
3.4 Chemical Formula.....	5
3.5 Structural Formula.....	5
3.6 Quantitative Composition.....	6
3.7 Method of Manufacture.....	9
3.8 Source Information.....	13
3.9 Characteristic Properties.....	14
3.10 Potential Human Toxicants.....	15
3.11 Specifications for Food-Grade Materials.....	17
4.0 APPLICABLE CONDITIONS OF USE IN FOOD.....	19
5.0 INTENDED CONDITIONS OF USE.....	24
6.0 DETAILED SUMMARY OF THE BASIS FOR CONCLUDING THAT REGENASURE™ GLUCOSAMINE HYDROCHLORIDE IS GRAS.....	25
6.1 Introduction.....	25

000072

6.2	Metabolism.....	26
6.3	Absorption, Distribution, Metabolism and Excretion (ADME) Data.....	29
6.4	Animal Toxicity Data.....	30
	6.4.1 Oral Administration.....	30
	6.4.2 Parenteral Administration.....	32
6.5	Mutagenicity Data.....	35
	6.5.1 Mutagenicity Data (<i>in vitro</i>).....	35
	6.5.2 Reverse Mutation In Bacteria.....	35
	6.5.3 Mutagenicity Data (<i>in vivo</i>).....	36
	6.5.4 <i>In Vivo</i> Mouse Micronucleus Assay.....	36
6.6	Human Clinical Studies.....	38
	6.6.1 Materials and Methods.....	38
	6.6.2 Results.....	39
	6.6.3 Effects of Glucosamine On Glucose Metabolism.....	41
	6.6.4 Exposure to Glucosamine	43
	6.6.5 Objective measures Of Safety.....	44
	6.6.6 Common Symptoms with Placebo or Glucosamine.....	44
	6.6.7 Efficacy Assessment.....	45
6.7	Discussion.....	47
6.8	Conclusions.....	51
7.0	EXPERT OPINION ON GRAS STATUS OF REGENASURE™ GLUCOSAMINE HYDROCHLORIDE.....	53
8.0	REFERENCES.....	57
9.0	GLOSSARY.....	68

000073

APPENDICES

- Appendix 1: Kosher Certification for REGENASURE™ Glucosamine Hydrochloride
- Appendix 2: United States Pharmacopeia Monograph for Glucosamine Hydrochloride
- Appendix 3: Ochratoxin A Test Results for REGENASURE™ Glucosamine Hydrochloride
- Appendix 4: Protein Test Results for REGENASURE™ Glucosamine Hydrochloride
- Appendix 5: Expert Allergen Opinion for REGENASURE™ Glucosamine Hydrochloride
- Appendix 6: Pesticide Test Results for REGENASURE™ Glucosamine Hydrochloride
- Appendix 7: Aflatoxin and Pesticide Test Results for REGENASURE™ Glucosamine Hydrochloride
- Appendix 7: Curricula vitae for Expert Panel

TABLES

Table 1:	USP-NF Analysis of REGENASURE™ Glucosamine Hydrochloride.....	8
Table 2:	Microbiological Analysis of REGENASURE™ Glucosamine Hydrochloride.....	9
Table 3:	Nutritional Value for 1.5g (1500mg) of REGENASURE™ Glucosamine Hydrochloride.....	15
Table 4:	Heavy Metals Tests on REGENASURE™ Glucosamine Hydrochloride.....	16
Table 5:	USP-NF Specifications for Glucosamine Hydrochloride.....	18
Table 6:	Intake (mg/kg body weight/day) of Glucosamine Hydrochloride from All Proposed Uses.....	20
Table 7:	List of Food Categories for Proposed Foods.....	21
Table 8:	List of Proposed Foods.....	22
Table 9:	Animal Toxicity Data.....	31
Table 10:	Micronucleus Assay Dosing Scheme.....	37
Table 11:	Human Clinical Studies of Glucosamine Evaluated.....	40
Table 12:	Evaluation of Fasting Plasma Glucose and Safety Parameters.....	42
Table 13:	Overview of Efficacy of Glucosamine for Arthritic Complaints.....	47

000074

FIGURES

Figure 1: Glucosamine Hydrochloride Structural Formula.....	6
Figure 2: Glucosamine Hydrochloride Structural Formula.....	6
Figure 3: Structural Formula of Chitin.....	9
Figure 4: Method of Manufacture for REGENASURE™ Glucosamine Hydrochloride.....	12
Figure 5: Glucosamine Metabolism.....	27

000075

1.0 INTRODUCTION

1.1 Introduction and Summary

This Notification documents the extensive and direct evidence of the “Generally Recognized As Safe” (GRAS) status of Cargill, Incorporated’s glucosamine product, REGENASURE™ Glucosamine Hydrochloride. Within this Notification is an evaluation of the GRAS status of this article of commerce, and the conclusion by a panel of experts qualified by scientific training and experience to evaluate the safety of substances added to food that REGENASURE™ Glucosamine Hydrochloride is GRAS by scientific procedures when used in select beverages in accordance with a use level of 0.75 grams per serving (8 ounces), which is in accordance with the Acceptable Daily Intake (ADI) level (Section 6.6.4).

Cargill, Incorporated’s REGENASURE™ Glucosamine Hydrochloride is a unique, premium quality product that is differentiated from other glucosamine products. Unlike conventional glucosamine, Cargill, Incorporated’s glucosamine is not produced from shellfish. REGENASURE™ Glucosamine Hydrochloride is manufactured from chitin in biomass produced through a fermentation of the common and naturally occurring microorganism *Aspergillus niger*, which is non-pathogenic and non-toxic to humans and other animals. This species has been used safely for food and enzyme production since the 1920’s (Schuster *et al*, 2002). The production process is operated under HACCP and FDA current Food GMP (21 CFR Part 110) guidelines, and is certified to ISO 9001:2000 Standards. REGENASURE™ Glucosamine Hydrochloride has been certified as Kosher Pareve and Kosher for Passover (Appendix 1).

1.2 Glucosamine Background

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Glucosamine is currently a dietary supplement ingredient widely available in the United States and throughout the world, primarily to promote joint health. Glucosamine is a naturally occurring amino monosaccharide that is endogenously formed throughout the human body by cellular glucose metabolism. Glucosamine is a key building block for several structural

polysaccharides found throughout the human body (Institute of Medicine, 2003). These polysaccharides include glycosaminoglycans (GAGs) and proteoglycans, which are components of the structure of cartilage, and hyaluronic acid, keratan sulfate and heparan sulfate, which compose other body tissues (*Physician's Desk Reference for Nutritional Supplements*, 2001). Exogenous sources of glucosamine are typically derived from chitin, a biopolymer which can be found in the exoskeletons of shellfish, insects and the cell walls of certain microorganisms (Ravi Kumar M.N.V., 2000). Cargill, Incorporated produces REGENASURE™ Glucosamine Hydrochloride through a unique process from chitin sourced from a vegetative microorganism, whereas all other known commercial glucosamine products are derived from shellfish.

Cargill, Incorporated plans to market this ingredient for use in beverages. This would provide an alternative to dietary supplement products currently being sold in tablet and capsule form.

Cargill, Incorporated commissioned the services of an independent panel of recognized experts to assess the GRAS status of REGENASURE™ Glucosamine Hydrochloride. The panel is qualified by their scientific training and relevant and ongoing national and international experience to critically evaluate the safety of substances added to foods. This panel of qualified experts determined that REGENASURE™ Glucosamine Hydrochloride is considered GRAS by scientific procedure. A written statement to that effect has been signed and dated by each member of the expert panel, and this statement is included as part of this Notification as Section 7.0.

1.3 History of Safe Use of Glucosamine

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Glucosamine hydrochloride has been widely used as a human dietary supplement for years, both alone and in combination with chondroitin sulfate and/or other products. Before use in humans, glucosamine has been used extensively in animals for therapeutic management of joint pain and stiffness. Research conducted over the last twenty years has indicated that exogenous glucosamine can contribute to alleviation of pain and inflammation in

joints from osteoarthritis, as well as reducing joint space narrowing. Osteoarthritis is the most common type of arthritis, affecting at least half of the 35 million Americans age 65 and older, which is 13% of the U.S. population, according to the National Institutes of Health (NIH) in 2002. Glucosamine has become a safe alternative to non-steroid anti-inflammatory drugs (NSAIDs) that in some people can cause adverse side effects. In the United States the average intake of orally ingested glucosamine by users of the product is approximately 1500mg per day, often taken in 2-3 doses, based on product recommendations. Exogenous glucosamine has been reported to be preferred over endogenous glucosamine production for biosynthesis of cartilage (Roden, 1956). Additionally, glucosamine is readily metabolized throughout the human body in various amounts in several tissues (Wolosker, 1999).

Numerous human studies of glucosamine have been published, and though many were primarily studying efficacy, safety was assessed in some as well. A study by Reginster (Reginster, et al 2001) showed no toxicity reported during a three-year study in which subjects were administered 1500mg of oral glucosamine daily. Two recent meta-analyses of the many clinical studies have been done (McAlindon et al, 2000 and Richy, 2003), and concluded that glucosamine products "are safe" and "safety was excellent", respectively. A recent article published in the *Journal of Arthroplasty* supported the safety of glucosamine, concluding that the clinical evidence shows "no trial has shown significant side effects" (Hungerford, 2003). A Phase III study for glucosamine hydrochloride is currently being conducted by the National Institutes of Health to study glucosamine efficacy.

Glucosamine is typically found as one of three primary forms: D-glucosamine hydrochloride, D-glucosamine sulfate, and N-acetyl-glucosamine (Best Nutrition, 2004). Glucosamine hydrochloride is considered to be more stable than glucosamine sulfate, as it does not require the addition of sodium to stabilize the product, which is typically done with glucosamine sulfate (Institute of Medicine, 2003).

000078

2.0 BASIS FOR REGULATORY APPROVAL OF GRAS AFFIRMATION

2.1 GRAS Regulatory Status

Per Volume 62, Number 74 of the April 17, 1997 Federal Register, pages 18937-18964 (Proposed Rules, 21 CFR Parts 170, *et al.*), Cargill, Incorporated wishes to notify the Food and Drug Administration (FDA) that it has determined that the use of its REGENASURE™ Glucosamine Hydrochloride as a nutritional ingredient in beverages is Generally Recognized As Safe (GRAS). The basis for the GRAS determination is through scientific procedures that were critically reviewed by a panel of experts qualified by scientific training and experience to evaluate the safety of substances added to food, and who concluded that there is a reasonable certainty that REGENASURE™ Glucosamine Hydrochloride is not harmful under the intended conditions of use.

3.0 REGENASURE™ GLUCOSAMINE HYDROCHLORIDE CHEMISTRY AND COMPOSITION

3.1 Common or Usual Name and Identity

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 sulfate, and N-acetyl-glucosamine.

3.2 Formal Names (IUPAC or CAS 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

000079

D-glucose, 2-amino-2-deoxy-hydrochloride
D-glucosamine hydrochloride
D-glucosamine
D(+)-glucosamine
chitosamine

The Chemical Abstracts Service (CAS) Name for glucosamine is glucosamine (9CI), and the CAS Registry Number for glucosamine hydrochloride is [66-84-2]. The European Inventory of Existing Commercial Chemical Substances (EINECS) number for glucosamine is 200-638-1.

3.3 Trade Name

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

3.4 Chemical Formula

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.

3.5 Structural Formula

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

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Figure 1:

Glucosamine HCl Structural Formula

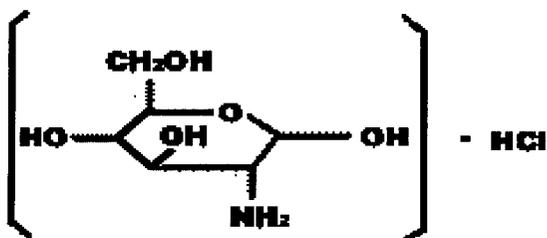
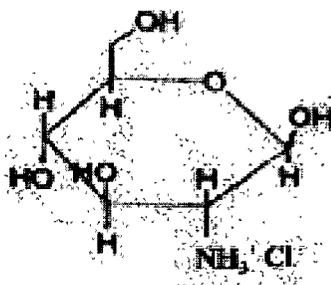


Figure 2:

Glucosamine HCl Structural Formula



3.6 Quantitative Composition

Glucosamine is a single molecule that can be prepared using chitin, a biopolymer found in shellfish carapaces, insect cuticles, and the cell walls of various microorganisms.

REGENASURE™ Glucosamine Hydrochloride is derived from a vegetative microorganism, whereas all other glucosamine products are currently produced using shellfish exoskeletons. There may be slight but significant variations in the composition of preparations containing shellfish-derived glucosamine and REGENASURE™ Glucosamine Hydrochloride. Such variations may be attributed to residual shellfish components in shellfish-derived glucosamine, e.g. potential allergens (i.e. proteins), contaminants (i.e. potential heavy metals from ocean harvested aquatic organisms, or antibiotics like chloramphenicol or nitrofurans from farm-raised crustaceans), which are not in Cargill, Incorporated's product.

The commercial production process for REGENASURE™ Glucosamine Hydrochloride yields high a purity crystalline product that complies with the United States Pharmacopeia-National Formulary (USP-NF) assay specification of 98% to 102%, which results in an extremely low amount of non-glucosamine substances.

000081

Cargill, Incorporated's glucosamine hydrochloride product is comprised of 99.0% dry matter, resulting in a loss on drying amount of less than 1.0%. The product has a low ash content of less than 0.1% ash, and low heavy metals content with less than 0.001%, and less

than 3 $\mu\text{g/g}$ of arsenic. The pH of the product is 3.0 to 5.0 in a 20 mg/mL aqueous solution, and contains less than 0.24% sulfur.

The product is further characterized by identification tests, which specifically identify the product as glucosamine hydrochloride. The specific rotation test of $+70.0^\circ$ to $+73.0^\circ$ helps to measure the purity of glucosamine hydrochloride. Microbiological specifications have been internally set by Cargill, Incorporated to meet food grade specifications, as outlined in Section 3.11 of this Notification.

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 REGENASURE™ Glucosamine Hydrochloride manufactured over an extended period of time were analyzed and compared. Table 1 includes analytical results of the five non-consecutive REGENASURE™ Glucosamine Hydrochloride lots, and Table 2 includes microbiological testing results.

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Table 1: USP-NF Analysis of REGENASURE™ Glucosamine Hydrochloride

USP Analysis	Cargill Internal Specification	USP/ NF Specification	Cargill, Incorporated Lot Number					Cargill Average Result
			REALG 3011	REALG 3013	REAGG 3001	RSE 4001A	RSE 4003A	
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%	98.8%	98.1%	98.3%	100.4%	100.3%	99.2%
Loss on drying	1.0% maximum	1.0% maximum	0.2%	0.5%	0.1%	0.2%	0.3%	0.3%
Specific rotation	+70.2° to +72.8°	+70.0° to +73.0°	+71.0°	+71.6°	+71.2°	+71.1°	+70.5°	+71.1°
pH	3.0 to 5.0	3.0 to 5.0	3.4	3.4	3.4	4.3	3.2	3.5
Residue on ignition	0.1% maximum	0.1% maximum	0	0	0	0	0	0
Sulfate	0.24% maximum	0.24% maximum	Pass	Pass	Pass	Pass	Pass	Pass
Arsenic	3 ppm maximum	3 ppm maximum	<0.02 ppm	<0.02 ppm	<0.02 ppm	0.02 ppm	0.05 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

N/A = Not Applicable

* 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.

000083

Table 2: Microbiological Analysis of REGENASURE™ Glucosamine Hydrochloride

Lot Number	Total Plate Count (cfu/g)	Coliform MPN method (MPN/g)	Coliform confirmation (MPN/g)	<i>E. coli</i> MPN method (MPN/g)	Yeast & Molds (cfu/g)	Salmonella (in 25 g)
REALG 3011	<10 cfu/g	<3 MPN/g	<3 MPN/g	<3 MPN/g	<10 cfu/g	Negative
REALG 3013	<10 cfu/g	<3 MPN/g	<3 MPN/g	<3 MPN/g	<10 cfu/g	Negative
REAGG 3001	<10 cfu/g	<3 MPN/g	<3 MPN/g	<3 MPN/g	<10 cfu/g	Negative
RSE 4001A	<10 cfu/g	<3 MPN/g	<3 MPN/g	<3 MPN/g	<10 cfu/g	Negative
RSE 4003A	<10 cfu/g	<3 MPN/g	<3 MPN/g	<3 MPN/g	<10 cfu/g	Negative
Average Results	<10 cfu/g	<3 MPN/g	<3 MPN/g	<3 MPN/g	<10 cfu/g	Negative

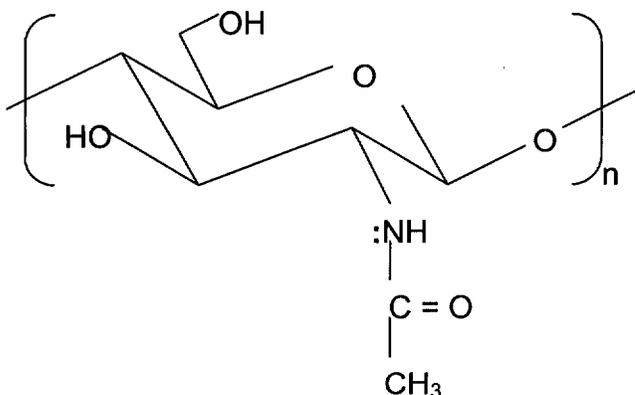
cfu= colony forming units, MPN = most probable number

The results for REGENASURE™ Glucosamine Hydrochloride reported in the preceding tables confirm that the finished product meets the USP-NF specifications and microbiological food standards.

3.7 Method of Manufacture

REGENASURE™ Glucosamine Hydrochloride is derived from the structural polysaccharide chitin, which occurs naturally as a component of the cell walls of the microorganism *Aspergillus niger* (Stagg *et al*, 1973). Chitin consists of unbranched repeating molecular subunits of N-acetyl-D-glucosamine (2-acetamido-2-deoxy-beta-D-glucose). The structural formula of chitin is shown in Figure 3 below.

Figure 3: Structural Formula of Chitin



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The following descriptions explain the process steps in the manufacture of REGENASURE™ Glucosamine Hydrochloride, which is produced in a facility which uses current Good Manufacturing Practices (21 CFR Part 110) for food and Hazard Analysis and Critical Control Point (HACCP) programs, which are updated annually. The facility is also certified as ISO-9001:2000 (International Organization for Standardization).

A) Raw Material Sourcing

The raw materials used in the manufacturing process are monitored at regular intervals to ensure they comply with the food grade specifications. The chitin-containing biomass used in this process is derived from a non-toxic and non-pathogenic strain of *Aspergillus niger*. This strain is derived from a food-grade fermentation process for citric acid production. Therefore the biomass is produced in a carefully controlled manner resulting in biomass that is uniform. Food grade hydrochloric acid is a major raw material for the process, and the quality is monitored to ensure compliance with applicable food grade specifications. Potable water is used in the process. Cargill, Incorporated produces potable water in accordance with food GMP's, and it is monitored to ensure compliance with EPA standards for potable water.

B) Digestion

The basic manufacturing process for this product is to react or "digest" the chitin-containing biomass with hydrochloric acid, both of which are food-grade raw materials, under heated conditions. The typical acid hydrolysis stage of this process is carried out using concentrated hydrochloric acid for several hours at 100°C. This results in the depolymerization and deacetylation of chitin to form glucosamine hydrochloride in one step. The hydrochloric acid catalyzes the hydrolysis of the chitin backbone, the result of which is depolymerization. Essentially, each scission of the backbone involves the addition of one molecule of water across the bond. Depolymerization reduces the chain length of the chitin polymer to oligomers, and ultimately to monomers. The deacetylation involves the loss of the acetyl group (COCH₃) through the consumption of one

000085

molecule each of water and hydrochloric acid and the production of one molecule of acetic acid. The acid hydrolysis is sufficiently strong to depolymerize and deacetylate the chitin to glucosamine, but the process conditions are such that the glucosamine molecules are not significantly degraded.

C) Filtration

Filtration of the digested biomass occurs as a first purification step to remove solid impurities and isolate the filtrate containing glucosamine hydrochloride.

D) Crystallization

Evaporative crystallization is used to concentrate the amount of glucosamine in solution and remove some excess hydrochloric acid. This concentration results in the precipitation of glucosamine as glucosamine hydrochloride.

E) Centrifugation

The separation and purification of glucosamine hydrochloride crystals from the mixture is achieved by using centrifugation. Washing the glucosamine hydrochloride crystals with water constitutes an additional purification step by removing water-soluble impurities during the centrifugation step.

F) Drying

The glucosamine hydrochloride crystals are flash-dried to meet the USP-NF specification of $\leq 1.0\%$ loss on drying.

G) Packaging

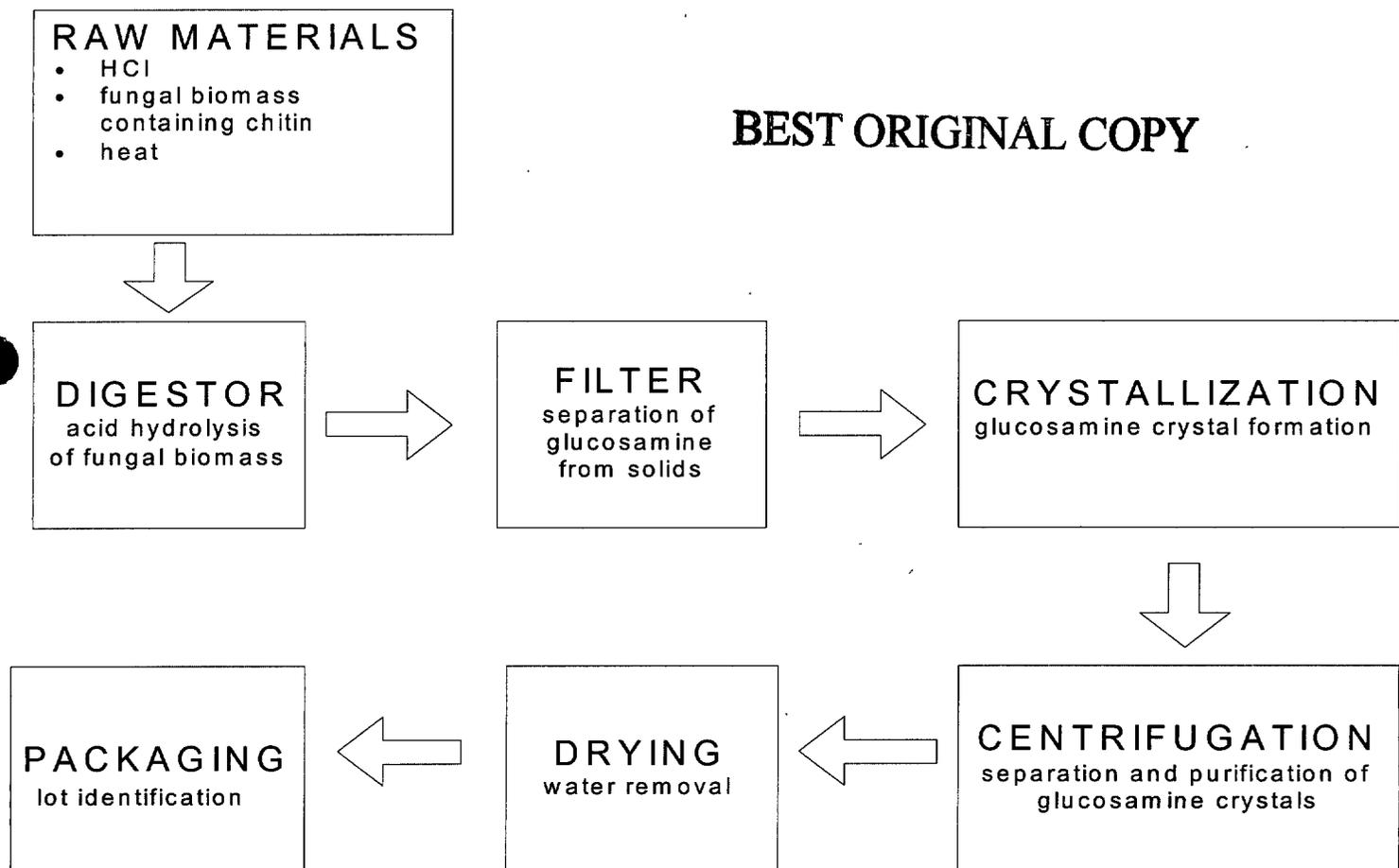
The finished product can be sifted to achieve specific particle sizes and is collected in a final product silo. Alternatively, the product can be granulated to achieve a desirable particle size. The product is packaged in "bag-in-a-box" or supersack containers, with collection of retains for each designated lot. When packaged, boxes of finished product are stored in a food-grade warehouse at the Cargill, Incorporated processing facility to await shipment. A lot identification

000086

system is used for lot numbering, and this could be used in the event of a product recall for product tracking.

This unique production process for glucosamine hydrochloride is specifically covered in U.S. Patent Application 2003/0181419. The progression of production steps is outlined in Figure 4 below.

Figure 4: Method of Manufacture for REGENASURE™ Glucosamine Hydrochloride



000087

3.8 Source Information

The key difference between shellfish-derived and REGENASURE™ Glucosamine Hydrochloride is the source material. For REGENASURE™ Glucosamine Hydrochloride the source is chitin from biomass produced from a fermentation utilizing the microorganism *Aspergillus niger*, which is defined taxonomically as follows:

Class, Deuteromycetes

Order, Monoliales

Family, Moniliaceae

Genus, *Aspergillus*

Species, *niger*

Aspergillus niger is a microorganism which is non-pathogenic and non-toxic for humans and other animals (Godfrey T. *et al*, 1983). *A. niger* is a filamentous and ubiquitous microorganism found in nature, and has been safely and commonly used in food and enzyme production since the 1920's (Schuster *et al*, 2002). The manufacturing process for REGENASURE™ Glucosamine Hydrochloride uses only one strain of *A. niger*, the same strain used to produce food-grade and GRAS citric acid that has been manufactured and sold by Cargill, Incorporated in the United States and abroad for many years. The vegetative state of this microorganism in the process should not be confused with the spore state, which in fungi is sometimes associated with respiratory allergies. The genus *Aspergillus* typically lacks recognized sexual spore formation. Microbiological testing of the finished glucosamine product is carried out routinely to ensure the absence of bacterial and fungal contamination.

A review of the safety of *Aspergillus niger* (Schuster *et al*. in 2002) investigated potential mycotoxins, and stated that "it is only Ochratoxin A that can be regarded as a mycotoxin in the strict sense of the word." The review summarized by stating "It is concluded, with these restrictions, that *A.niger* is a safe production organism". The strain of *Aspergillus niger* used to produce REGENASURE™ Glucosamine Hydrochloride has been selected

000088

because of its safety, and the final product has been tested to verify absence of ochratoxin A (Appendix 3).

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 100°C, is sufficient to destroy proteinaceous material from the source, and subsequent purification steps remove solid impurities. To investigate whether proteinaceous material could withstand the rigorous processing conditions of manufacture, the finished product was tested for presence of proteins (Appendix 4) by Louisa B. Tabatabai, Ph.D., Professor-in-Charge of the Protein Facility at Iowa State University. For the gel electrophoresis that was performed, Sypro Ruby staining followed by Coomassie Brilliant Blue R250 staining was done, with a scan after each stain. The results were interpreted by Dr. Tabatabai as "indicating the absence of protein". An expert opinion (Appendix 5) on the potential allergenicity of REGENASURE™ Glucosamine Hydrochloride derived from *Aspergillus niger* was also obtained from Professor S.L. Taylor, Co-Director of the Food Allergy Research and Resource Program (FARRP) at the University of Nebraska-Lincoln. Dr. Taylor concluded "Food allergens are proteins, and glucosamine is not a protein. When produced via fermentation with *Aspergillus 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".

3.9 Characteristic Properties

Glucosamine hydrochloride is a single molecule amino-sugar with a single chiral center. It is typically manufactured as a white to off-white crystalline product. It has very little odor, and is recognized as having a slightly bitter taste. Glucosamine hydrochloride decomposes at 190.0°C – 194.0°C. The solubility of glucosamine hydrochloride in water is approximately 24% weight per weight in water at 20°C, and it is only slightly soluble in alcohol.

Cargill, Incorporated is not aware of and has no reason to believe there is any evidence to suggest any difference in bioactivity of glucosamine hydrochloride resulting from different

000089

chitin sources. Table 3 contains nutritional information for Cargill, Incorporated's glucosamine product.

Table 3. Nutritional Value for 1.5g (1500mg) of REGENASURE™ Glucosamine Hydrochloride

Nutritional Information per 1.5 grams	
Fat g/1.5 g	0
Protein %	0
Carbohydrates by difference 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
Fiber g/1.5 g	0
Sugar g/1.5 g	0

*The "Carbohydrate" value was calculated by the Atwater method, which is typically used for food substances, and is as follows:

$$100-(\% \text{protein} + \% \text{moist} + \% \text{ash} + \% \text{fat}) = \text{Carbohydrate}$$

3.10 Potential Human Toxicants

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REGENASURE™ Glucosamine Hydrochloride has a very high purity of 98% – 102% glucosamine hydrochloride, and further chemical and microbiological analyses have identified no potential human toxins or toxicants of concern. In addition to the USP-NF monograph, further testing was completed to identify substances greater than 100 ppm in the product, with each substance identified assessed for human toxicity.

Heavy metals in final product lots are qualitatively (pass or fail) tested according to the USP-NF monograph methodology to show a content of less than 0.001%. Additional testing on the product has been done to quantify the actual amount of heavy metals. It was found that

000090

the amount of heavy metals in REGENASURE™ Glucosamine Hydrochloride is less than the quantification levels of the test (analysis by Inductively Coupled Plasma/Mass Spectrometer). Table 4 shows the elements and quantification limits that were assessed.

Table 4: Heavy Metals Tests on REGENASURE™ Glucosamine Hydrochloride

Heavy Metal	Quantification Limit	Result
Arsenic (As)	<0.5 ppm	<0.5 ppm
Cadmium (Cd)	<0.5 ppm	<0.5 ppm
Cobalt (Co)	<0.5 ppm	<0.5 ppm
Mercury (Hg)	<20 ppb	<20 ppb
Lead (Pb)	<0.1 ppm	<0.1 ppm

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Residue on ignition (ash) testing is performed on the final product in accordance with the USP-NF method, and meets the specification of less than or equal to 0.1%. In addition, instrumental analysis by ICP has been done to detect specific elements present in the ash. Silica was present at approximately 145 ppm, and this was determined not to be a health concern. Silicates are commonly used as food-grade anti-caking agents in food. Other elements detected were sodium, calcium and potassium, but all were at levels below 100 ppm. There was a small amount of water insoluble material present, and further testing showed this material to be insoluble in body temperature digestive juice.

The final product is analyzed for loss on drying (moisture), and meets the USP-NF specification of less than or equal to 1.0%. To determine if volatile compounds are present in the product, a volatile organic compounds test by Gas Chromatograph/Mass Spectrometer (GC/MS) was done. There were not any compounds detected at significant levels aside from hydrochloric acid and water, which are food-grade raw materials used in the process to manufacture REGENASURE™ Glucosamine Hydrochloride.

000091

The product has been tested for the presence of soluble organic acids resulting from the manufacturing process. Two organic acids which are used in food were detected at levels below 0.3%. One is a GRAS substance commonly used in a variety of foods, and the other is an "Everything Added to Food in the United States" (EAFUS) substance.

Cargill, Incorporated commissioned an independent analyses of the final product for pesticides, aflatoxin and ochratoxin A. A pesticide screen done by Covance Laboratories, Inc. using USP-NF methodology found no measurable pesticides in the product (Appendix 6). No measurable aflatoxin (aflatoxin B1, B2, G1 and G2) was found in the product when tested by Covance Laboratories, Inc. using Association of Analytical Communities International (AOAC) methodology, with the level of quantitation being 0.5ppb (Appendix 6). Trilogy Analytical Laboratories tested the final product, with no ochratoxin A detected at a quantification limit of 50 ppt (Appendix 3).

3.11 Specifications for Food-Grade Materials

The current quality specification standard for glucosamine hydrochloride is the United States Pharmacopeia-National Formulary (USP-NF) monograph for glucosamine hydrochloride (*United States Pharmacopeia*, 2003). REGENASURE™ Glucosamine Hydrochloride lots meet all of the USP-NF specifications, which are listed in Table 5 as follows.

000092

Table 5: USP-NF Specifications for Glucosamine Hydrochloride

USP-NF Test	Method	USP-NF Specifications
Identification	197K	A: <i>Infrared Absorption</i> *
Identification	191	B: It meets the requirements of the tests for <i>Chloride</i>
Identification		C: The retention time of the major peak in the chromatogram of the <i>Assay Preparation</i> corresponds to that in the chromatogram of the <i>Standard Preparation</i> , as obtained in the <i>Assay</i> .
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%
Sulfate	221	A 0.10 g portion shows no more sulfate than corresponds to 0.25 mL of 0.020 N sulfuric acid: not more than 0.24% is found
Arsenic	Method II (211)	3 ug per g
Heavy Metals	Method II (231)	0.001%
Organic Volatile Impurities	Method I (467)	Meets the requirements
Assay		98.0% to 102.0%

*Infrared Absorption has been run on Cargill, Incorporated's product confirming that REGENASURE™ Glucosamine Hydrochloride passes this Identification test.

In addition Cargill, Incorporated coordinates microbiological testing in accordance with food-grade standards as follows:

Total Plate Count colony forming units/gram - cfu/g <10

Yeast and Mold cfu/g <10

Coliform cfu/g <3

Coliform confirmation cfu/g <3

E.coli cfu/g <3

Salmonella Negative in 25g initial sample

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4.0 APPLICABLE CONDITIONS OF USE IN FOOD

Estimated Daily Intake (EDI) values for REGENASURE™ Glucosamine Hydrochloride used in beverages were prepared by Exponent, Inc. Exponent, Inc.'s intake assessment uses their proprietary Food and Residue Evaluation (FARE™) software, and food consumption data from the USDA Continuing Survey of Food Intakes by Individuals (CSFII) conducted between 1994 and 1996, and from the 1998 supplemental CSFII survey of children up to age 9. These surveys were conducted by the Agricultural Research Service of the U.S. Department of Agriculture. For the CSFII 1994-96 survey, 21,700 individuals of all ages were asked to provide food intakes on 2 nonconsecutive days. For CSFII 1998, 11,800 children from 0-19 yrs were surveyed. The US FDA 21 Code of Federal Regulations section 170.3 was also referenced to help identify the specific food categories included in the assessment.

For each population group listed in the assessment, the mean, median and the 90th percentile estimates of daily intake on both a *per capita* and "per user" basis are provided. (The *per capita* analysis includes consumption data for everyone who participated in the CSFII survey; the "per user" analysis only includes consumption data for respondents who reported eating the food of interest). Table 6 contains information on the intake values as well as the percent of that population who consumed any of the designated beverages.

000094

Table 6. Intake (mg/kg bw/day) of Glucosamine Hydrochloride From All Proposed Uses

Population	Per capita	Per user	% of Population consuming any/all foods
US population			46%
Mean	9.195	19.887	
Median	0	12.504	
90th percentile	26.294	43.174	
Children 1-6			68%
Mean	32.698	48.428	
Median	21.083	36.242	
90th percentile	83.107	98.431	
Children 7-12			55%
Mean	12.534	22.696	
Median	6.754	18.319	
90th percentile	33.321	43.183	
Males 13-19			50%
Mean	9.511	19.145	
Median	0	14.234	
90th percentile	29.308	41.058	
Males 20+			43%
Mean	6.067	14.090	
Median	0	9.896	
90th percentile	17.476	29.404	
Females 20+			42%
Mean	5.263	12.671	
Median	0	9.518	
90th percentile	15.871	25.440	
Males and Females 40+			42%
Mean	5.030	11.837	
Median	0	8.695	
90th percentile	15.133	24.384	

000095

The data was calculated assuming that glucosamine hydrochloride was in all proposed beverages at a level of 0.75 g/8 oz serving.

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The values in Table 6 indicate that the estimated exposure to REGENASURE™ Glucosamine Hydrochloride from the proposed products would be well under the Acceptable Daily Intake (ADI) of 184 mg/kg bw/day (Section 6.6.4). Based on the EDI for the US population, using the "per user", 90th percentile value (the highly exposed consumer) and an average person's weight of 70kg, the daily intake would be about 3,022 mg of

REGENASURE™ Glucosamine Hydrochloride. Additional exposure to glucosamine hydrochloride as a dietary supplement might occur, with a typical recommended dose of 1,500 mg/day. If you add this additional exposure of 1,500 mg to 3,022 mg the total would be 4,522 mg/day of product for an average 70 kg adult. This calculates out (4,522 mg/day divided by 70 kg bw) to be 65 mg/kg bw/day, which is well below the recommended ADI of 184 mg/kg bw/day (Section 6.6.4). These calculated intake values are conservative because they assume that 100% of the products ingested by the consumer contain REGENASURE™ Glucosamine Hydrochloride at the proposed level, which is not likely to occur. Also, from the EDI analysis, it appears that the highest exposure occurs in the age group of 1-6 year olds, which is an age group that is not a target for product marketing, nor would normally be considered as having an interest in the benefits of glucosamine hydrochloride consumption. The exposure for this age group for the “per user,” 90th percentile category is 98.431 mg/kg bw/day, which would be well under the recommended ADI of 184 mg/kg bw/day (Section 6.6.4).

The food categories for the beverages selected for this study can be found in the USDA’s Continuing Survey of Food Intakes by Individuals and the US FDA 21 Code of Federal Regulations section 170.3. These food categories are listed in Table 7. The list of proposed foods for REGENASURE™ Glucosamine Hydrochloride can be found in Table 8.

Table 7. List of Food Categories for Proposed Foods

<i>21 CFR § 170.3 broad food categories</i>	<i>USDA’s CSFII Food categories</i>
(16) Fresh fruits and fruit juices	Citrus Fruit Juices
(3) Beverages and bases, non alcoholic	Fruit juices and nectars excluding citrus
(7) Coffee and tea	Non-alcoholic beverages
(31) Milk products*	Flavored milk & milk drinks, fluid
(36) Vegetable and vegetable juices	Tomato and tomato mixtures

*only one product from this category

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000096

Table 8. List of Proposed Foods

Flavored Milk & Milk Drinks, fluid

11553100FRUIT SMOOTHIE DRINK, NFS

Citrus Fruit Juices

61200500ACEROLA JUICE
61201000GRAPEFRUIT JUICE, NFS
61201020GRAPEFRUIT JUICE, UNSWEETENED, NS AS TO FORM
61201220GRAPEFRUIT JUICE, CANNED, BOTTLED, CARTON, UNSWEET
61201230GRAPEFRUIT JUICE, CANNED, BOTTLED, CARTON, W/ SUGAR
61201240GRAPEFRUIT JUICE, CANNED/BOTTLE/CARTON, W/ LOW CAL SWEETENER
61204000LEMON JUICE, NS AS TO FORM
61204200LEMON JUICE, CANNED OR BOTTLED
61207000LIME JUICE, NS AS TO FORM
61207200LIME JUICE, CANNED OR BOTTLED
61210000ORANGE JUICE, NFS
61210220ORANGE JUICE, CANNED/BOTTLED/CARTON, UNSWEETENED
61210230ORANGE JUICE, CANNED/BOTTLED/CARTON, W/ SUGAR
61210250ORANGE JUICE, W/ CALCIUM, CAN/BOTTLE/CARTON, UNSWEETENED
61213000TANGERINE JUICE, NFS
61213220TANGERINE JUICE, CANNED, UNSWEETENED
61213230TANGERINE JUICE, CANNED, W/ SUGAR
61214000GRAPE-TANGERINE-LEMON JUICE
61216000GRAPEFRUIT & ORANGE JUICE, NFS
61216220GRAPEFRUIT & ORANGE JUICE, CANNED, UNSWEETENED
61216230GRAPEFRUIT & ORANGE JUICE, CANNED, W/ SUGAR
61219000ORANGE & BANANA JUICE
61219100PINEAPPLE-ORANGE-BANANA JUICE
61219150ORANGE-WHITE GRAPE-PEACH JUICE
61219650APRICOT-ORANGE JUICE
61222000PINEAPPLE-GRAPEFRUIT JUICE, NFS
61222200PINEAPPLE-GRAPEFRUIT JUICE, CANNED, NS SWEETENED
61222220PINEAPPLE-GRAPEFRUIT JUICE, CANNED, UNSWEETENED
61222230PINEAPPLE-GRAPEFRUIT JUICE, CANNED, W/ SUGAR
61225000PINEAPPLE-ORANGE JUICE, NFS
61225200PINEAPPLE-ORANGE JUICE, CANNED, NS AS TO SWEETENER
61225220PINEAPPLE-ORANGE JUICE, CANNED, UNSWEETENED
61225230PINEAPPLE-ORANGE JUICE, CANNED, W/ SUGAR
61226000STRAWBERRY-BANANA-ORANGE JUICE

Fruit Juices and Nectars Excluding Citrus

64100100FRUIT JUICE, NFS (INCLUDE MIXED FRUIT JUICES)
64100110FRUIT JUICE BLEND, 100% JUICE, W/ VITAMIN C
64100120AMBROSIA JUICE (INCL KNUDSEN'S)
64101010APPLE CIDER (INCLUDE CIDER, NFS)
64104010APPLE JUICE
64104050APPLE JUICE, W/ ADDED VITAMIN C
64104090APPLE JUICE WITH ADDED VITAMIN C AND CALCIUM
64104150APPLE-CHERRY JUICE

000097

64104200APPLE-PEAR JUICE
 64104450APPLE-RASPBERRY JUICE
 64104500APPLE-GRAPE JUICE
 64104550APPLE-GRAPE-RASPBERRY JUICE
 64104600BLACKBERRY JUICE (INCL BOYSENBERRY JUICE)
 64105400CRANBERRY JUICE, UNSWEETENED
 64105500CRANBERRY-WHITE GRAPE JUICE MIXTURE, UNSWEETENED
 64116010GRAPE JUICE, NS AS TO ADDED SWEETENER
 64116020GRAPE JUICE, UNSWEETENED
 64116030GRAPE JUICE, W/ SUGAR
 64116040GRAPE JUICE, LOW CALORIE SWEETENER
 64116050GRAPE JUICE, NS AS TO SWEETENED, W/ ADDED VITAMIN C
 64116100GRAPE JUICE, UNSWEETENED, W/ ADDED VITAMIN C
 64116150GRAPE JUICE, W/ SUGAR, W/ ADDED VITAMIN C
 64120010PAPAYA JUICE
 64121000PASSION FRUIT JUICE
 64122030PEACH JUICE, W/ SUGAR
 64123000PEAR-WHITE-GRAPE-PASSION FRUIT JUICE,W/ADDED VIT C
 64124010PINEAPPLE JUICE, NS AS TO SWEETENED
 64124020PINEAPPLE JUICE, UNSWEETENED
 64124030PINEAPPLE JUICE, W/ SUGAR
 64124060PINEAPPLE JUICE, UNSWEETENED, W/ VIT C
 64124200PINEAPPLE-APPLE-GUAVA JUICE, W/ ADDED VITAMIN C
 64125000PINEAPPLE JUICE-NON-CITRUS JUICE BLEND, UNSWEETENED
 64132010PRUNE JUICE, NS AS TO ADDED SWEETENER
 64132020PRUNE JUICE, UNSWEETENED
 64132030PRUNE JUICE, W/ SUGAR
 64132500STRAWBERRY JUICE
 64133100WATERMELON JUICE
 64134000FRUIT SMOOTHIE DRINK, W/ FRUIT ONLY

Tomatoes and Tomato Mixtures

74302000TOMATO JUICE COCKTAIL
 74303000TOMATO & VEGETABLE JUICE, MOSTLY TOMATO (INCL V-8)
 74303100TOMATO & VEGETABLE JUICE, MOSTLY TOMATO, LOW SODIUM

Non-alcoholic Beverages

92301000TEA, NS AS TO TYPE, UNSWEETENED
 92301060TEA, NS AS TO TYPE, PRESWEETENED W/ SUGAR
 92301080TEA, PRESWEETENED W/ LOW CALORIE SWEETENER
 92301100TEA, NS AS TO TYPE, DECAFFEINATED, UNSWEETENED
 92301130TEA, NS AS TO TYPE, PRESWEETENED, NS AS TO SWEETNER
 92301160TEA, DECAFFEINATED, W/ SUGAR, NFS
 92301180TEA, DECAFFEINATED, LOW CALORIE SWEETENER, NFS
 92301190TEA, PRESWEETENED, NS SWEETENER, DECAFFEINATED
 92304000TEA, MADE FROM FROZEN CONCENTRATE, UNSWEETENED
 92304700TEA, FROM FROZ CONC, DECAF, PRESWEETEND, LOW CALORIE
 92305000TEA, MADE FROM POWDERED INSTANT, PRESWEETENED
 92305010TEA, MADE FROM POWDERED INSTANT, UNSWEETENED
 92305040TEA, MADE FROM POWDERED INSTANT,PRESWEETEND W/SUGAR

000098

92305050 TEA, FROM POWDER, DECAFFEINATED, PRESWEET W/ SUGAR
 92305090 TEA, MADE FROM POWDERED INSTANT, W/LO CAL SWEETENER
 92305110 TEA, FROM INSTANT, DECAF, PRESWEETENED, LOW CALORIE
 92305180 TEA, MADE FROM POWDERED INSTANT, DECAF, UNSWEET
 92305800 TEA, FROM POWDER, DECAFFEINATED, PRESWEETENED
 92307000 TEA, POWDERED INSTANT, UNSWEETENED, DRY
 92307400 TEA, POWDERED INSTANT, SWEETENED, NS SWEETENER, DRY
 92400000 SOFT DRINK, NFS
 92400100 SOFT DRINK, NFS, SUGAR-FREE
 92410110 CARBONATED WATER, SWEETEND (INCL TONIC, QUININE WATER)
 92410210 CARBONATED WATER, UNSWEETENED (INCL CLUB SODA)
 92410250 CARBONATED WATER, SUGAR-FREE
 92431000 CARBONATED JUICE DRINK, NS AS TO TYPE OF JUICE
 92432000 CARBONATED CITRUS JUICE DRINK
 92433000 CARBONATED NONCITRUS JUICE DRINK
 92510610 FRUIT DRINK (INCLUDE FRUIT PUNCH & FRUIT ADE)
 92510810 GRAPEADE & GRAPE DRINK
 92511220 ORANGE DRINK (INCLUDE ORANGE ADE, YABA DABA DEW)
 92511250 CITRUS FRUIT JUICE DRINK (INCL 5-ALIVE)
 92530950 VEGETABLE & FRUIT JUICE DRINK, W/ VIT C
 92531010 ORANGE DRINK & ORANGEADE W/ VITAMIN C ADDED
 92553000 FRUIT-FLAVORED THIRST QUENCHER BEVERAGE, LOW CAL
 92560000 FRUIT-FLAVORED THIRST QUENCHER BEVERAGE
 92570500 FLUID REPLACEMENT, 5% GLUCOSE IN WATER
 92582000 FRUIT-FLAVORED DRINK, LOW CALORIE, CALCIUM-FORTIFD
 92582050 FRUIT-FLAVORED DRINK, VITAMIN & MINERAL FORTIFIED
 92582100 CITRUS JUICE DRINK, CALCUIM FORTIFIED
 92582110 ORANGE BREAKFAST DRINK, CALCIUM FORTIFIED
 92900100 TANG, DRY CONCENTRATE
 92900110 FRUIT-FLAVORED CONCENTRATE, DRY, W/ SUGAR & VIT C
 92900200 FRUIT-FLAV BEV, DRY CONC, LO CAL (INCL CRYSTAL LIGHT)
 92900300 FRUIT-FLAV THIRST QUENCH BEV, DRY CONC (GATORADE)

It is expected that REGENASURE™ Glucosamine Hydrochloride may only be used in select beverages on this list, and in some cases perhaps not at the intended level of use of 0.75 g/8 oz. In all likelihood, beverage formulations with glucosamine will be done for specific products for specific target groups, rather than for very broad beverage categories.

5.0 INTENDED CONDITIONS OF USE

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Cargill, Incorporated currently markets REGENASURE™ Glucosamine Hydrochloride as a dietary supplement. Cargill, Incorporated plans to market this ingredient in beverages for

nutritional value. This ingredient is not intended as an alternative (replacement) source of nutrition, or to impart physiochemical characteristic effects on the food. The addition of REGENASURE™ Glucosamine Hydrochloride to foods would provide a source of exogenous glucosamine to support joint health.

6.0 DETAILED SUMMARY OF THE BASIS FOR CONCLUDING THAT REGENASURE™ GLUCOSAMINE HYDROCHLORIDE IS GRAS

6.1 Introduction

Glucosamine, 2-amino-2-deoxy-D-glucose, is an amino monosaccharide that is an essential component of mucopolysaccharides and chitin. Glycosaminoglycans, or mucopolysaccharides, are large complexes of negatively charged carbohydrate chains that are incorporated into mucous secretions, connective tissue, skin, tendons, ligaments and cartilage. Glucosamine and its acetylated derivative, N-acetylglucosamine, are readily synthesized in the body from glucose. Because of its high concentration in joint tissues, the hypothesis that glucosamine supplements would provide symptomatic relief for osteoarthritis was developed more than 30 years ago (D'Ambrosio, 1981). Many clinical trials have tested this hypothesis (Institute of Medicine, 2003) and glucosamine supplements are widely used to relieve arthritic complaints (Haupt, 1999).

To meet the demand for glucosamine nutritional supplements, three forms of glucosamine are commonly available: glucosamine hydrochloride, glucosamine sulfate, and N-acetyl-glucosamine. These glucosamine compounds are generally derived from chitin, a biopolymer present in the exoskeleton of marine invertebrate animals. The glucosamine derived from chitin in the cell walls of many microorganisms appears to be chemically identical to that found in marine invertebrates (Institute of Medicine, 2003).

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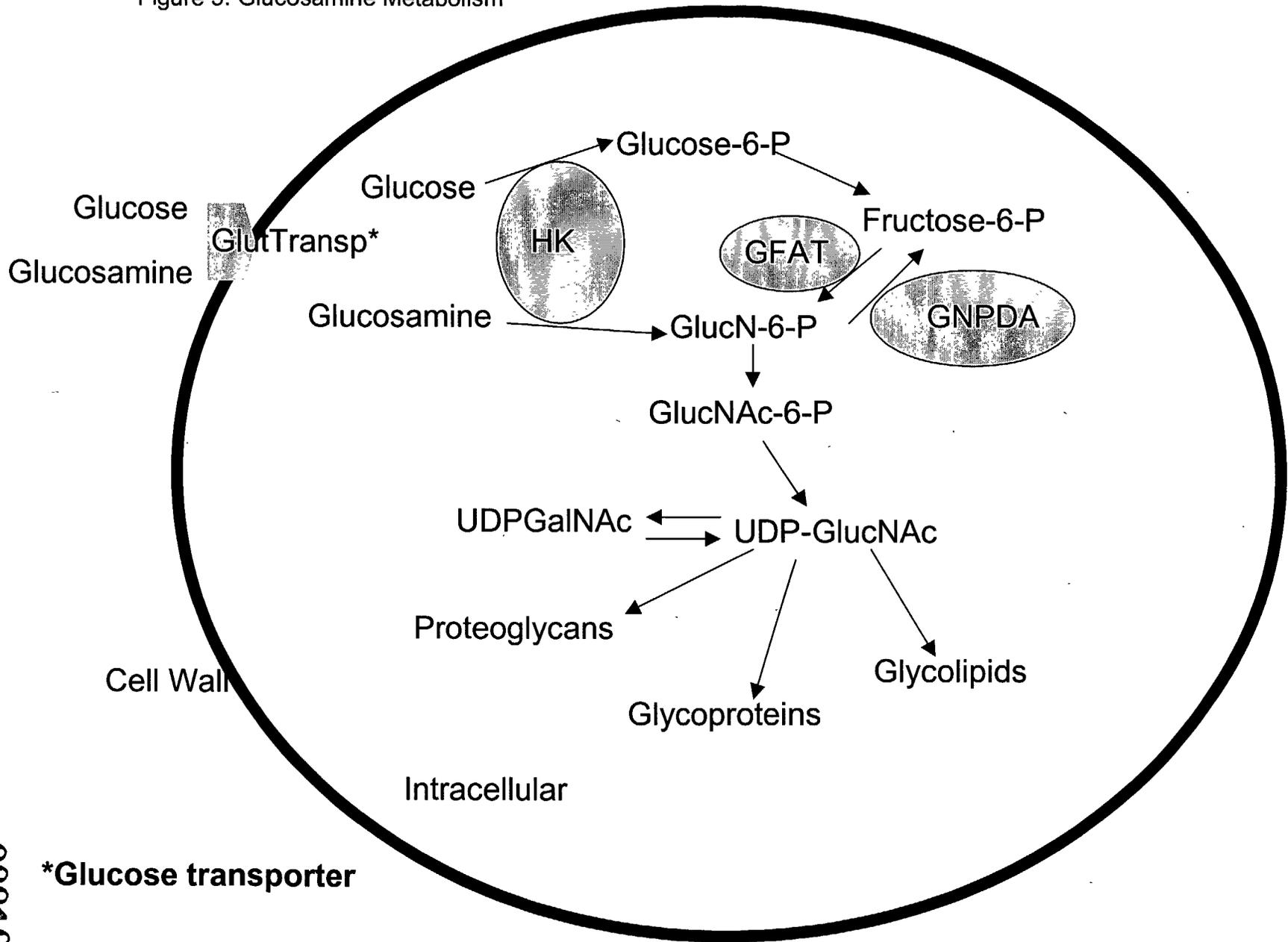
This review summarizes the metabolism and animal toxicity data of glucosamine. The available data from humans was examined to assess the effects of glucosamine on glucose metabolism. The effects of chronic glucosamine intake on blood chemistries, hematological parameters, urinalysis, occult blood in the feces, blood pressure and pulse rate was tabulated. Side effects reported with glucosamine compared to placebo from placebo-controlled trials was compared. Finally, an overview of the efficacy of glucosamine for arthritic complaints is provided.

6.2 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 5); (Uldry, 2002) insulin facilitates glucosamine transport into cells (Heart, 2000). Glucosamine is phosphorylated by one of the family of hexokinases to glucosamine-6-phosphate (GlucN-6-P). Endogenous GlucN-6-P is formed from fructose-6-phosphate and glutamine by GlucN-6-P synthetase, commonly called glucosamine:fructose-6-P amidotransferase (GFAT), (Wu, 2001). GFAT irreversibly catalyzes 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, 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 formation of UDP-GlucNAc (Milewski, 2002). UDP-GlucNAc can be converted to UDP-N-acetylgalactosamine (UDP-GalNAc) by UDP-N- acetylglucosamine 4-epimerase (Wu, 2001).

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Figure 5: Glucosamine Metabolism



*Glucose transporter

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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, 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 amidotransferase enzymes because it does 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-GlcNAc (Milewski, 2002). Ambient testosterone or estrogen levels may affect tissue GFAT activity (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 grams/day (median values of ~ 14 g/d or 200 mg/kg/d) (Hart, 2003; Wells, 2001; Vosseller, 2002; Wells, 2003). With intakes of 200-350 grams of carbohydrate, humans would produce an estimated 4-17.5 grams of glucosamine per day. With the intake of ~1.5 grams of oral glucosamine daily, blood levels would be equivalent to infusing 20% of this amount (Institute of Medicine, 2003; Setnikar, 2001), or 0.3 grams per day (4 mg/kg/day). Furthermore, when large amounts of carbohydrate are ingested for short or intermediate periods of time, insulin sensitivity improves in healthy subjects (Anderson, 1973) and in diabetic subjects (Anderson, 1977). For example, when 80% of energy as carbohydrate (~400 grams of carbohydrate) was fed to healthy volunteers for 11-13 weeks, they had improved glucose tolerance and insulin sensitivity (Anderson, 1973) despite estimated endogenous production of 8-20 grams of glucosamine per day (median production of 200 mg/kg/day).

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Some, but not all, studies in animals suggest that glucosamine administration may produce insulin resistance and hyperglycemia by affecting insulin secretion and action (Institute of Medicine, 2003; Echard, 2001). 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, 2000; Echard, 2001; Nelson, 2000; Monauni, 2000). 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, 1998). A significant fraction of orally administered glucosamine undergoes first-pass metabolism in the liver (Barclay, 1998). Blood levels achieved after oral glucosamine are only 20% of those achieved with intravenous glucosamine (Institute of Medicine, 2003; Setnikar, 2001). Recent data on pharmacokinetics, bioavailability, and metabolism of glucosamine in rats (Aghazadeh-Habashi, 2002) are similar to those reported for humans (Setnikar, 2001; Setnikar, 1993).

6.3 Absorption, Distribution, Metabolism and Excretion (ADME) Data

Setnikar and colleagues (Setnikar, 1986) administered uniformly labeled [^{14}C] glucosamine HCl diluted with unlabeled glucosamine sulfate by intravenous and oral routes of administration to 8 male and 8 female Beagle dogs for 144 hours. Samples of plasma feces, urine, CO_2 and all organs were analyzed. Immediately after iv administration into dogs of radiolabeled glucosamine, 10% of the labeled glucosamine was found as free glucosamine in plasma; this was quickly cleared by the liver and kidney and excreted into urine. The remaining 90% of radioactivity in plasma was either bound to plasma proteins or incorporated into plasma proteins. Plasma activity quickly increased, reaching a peak at 8 hours. During this phase, radioactivity diffused rapidly into the liver and kidney and subsequently was found in skeletal tissues and articular cartilage. After oral administration of radiolabeled glucosamine to dog, 87% was absorbed. In the dog, there were no gender effects on any parameters.

Setnikar and colleagues (Setnikar, 1984) also administered uniformly labeled [^{14}C] glucosamine HCl diluted with unlabeled glucosamine sulfate intravenously and orally to 44 male and 44 female rats for 144 hours. Samples of plasma feces, urine, CO_2 , all organs and whole carcass were analyzed. At 1-2 hours after intravenous or oral administration, glucosamine radioactivity in plasma was bound to and/or incorporated into plasma proteins.

000104

After peaking at 2- 4 hrs, radioactivity declined from plasma at a slower rate ($t_{1/2} = 28$ and 46hrs, after IV or oral administration, respectively). Analyses of radioactivity in urine, feces and CO_2 revealed this (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 feces indicating a high degree of glucosamine absorption. Analyses of radioactivity in tissues and organs showed that [^{14}C]-glucosamine quickly entered into all tissues including the cartilages reaching a maximum at 8hrs. The conclusion from these studies in rats and dogs suggest that they are similar to the ADME data in humans, and therefore both animal models are appropriate for establishing safety of glucosamine in humans (Setnikar, 2001).

6.4 Animal Toxicity Data

6.4.1 Oral Administration

Oral administration of glucosamine at very large doses (5000-8000 mg/kg body weight) is well tolerated without documented toxicity. The LD50 for glucosamine for rats, mice, and rabbits exceeds 5000 mg/kg with a median value of >6000 mg/kg (Table 9). Glaza et al. (Glaza, 2002) performed acute oral toxicity tests by administering glucosamine hydrochloride to rats. During these studies, 5000 mg/kg/day of unlabelled glucosamine HCl (Lot No. GP-11, Cargill, Incorporated) was administered orally to 5 male and 5 female rats for 15 days. All animals were observed clinically, 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 glucosamine HCl was 5000 mg/kg.

000105

Table 9. Animal Toxicity Data

Study	Species	Duration, days	Description	Dose, mg/kg
Leuschner q. by Meninger	rats	acute	LD50 for oral administration; no toxicity observed, no necropsy abnormalities	>8000
Leuschner q. by Meninger	rats	365	No histopathology; 7 vs 2 premature deaths (not clinically significant)	2700
Echard	rats	60	Added to diet:GTT unchanged, no toxicity,serum triglycerides increased	5000
Echard	rats	60	Added to diet: SHR, decrease in blood pressure	5000
Glaza	rats	15	GCI : no test material-related effects	5000
Beren	rats	52	GCI added to diet: no adverse effects	2500
Sugimura	rats	12	Included in diet: normal growth rate, low doses; no toxicity; decreased growth rate at high doses in weanlings	960
Leuschner	mice	acute	LD50 for oral administration; no toxicity, no necropsy abnormalities	>8000
Sigma-Aldrich	mice	acute	LD50 estimation	>5000
Senin	mice	acute	LD50 estimation, no mortality at this dose	5000
Stender	rabbits	acute	single oral dose, no glucose changes	1000
Leuschner	rabbits	acute	LD50 for oral administration; no toxicity, no necropsy abn.	>6000
Stender	rabbits	84	GCI added to diet; no significant cholesterol effects	1500
Neumann	dogs	183	Added to diet; no clinical, laboratory or histopathology abnormalities	2149
McNamara	dogs	30	2 capsules twice daily; no histopathology	200
Caron	horses	336	GCI added to diet; no adverse effects	21
Fenton	horses	56	Added to diet; no adverse effects reported	18
Hanson	horses	42	Added to diet: no adverse effects reported	22

Glucosamine HCl indicated by **GCI**. GTT is glucose tolerance test.

000106

Echard et al (Echard, 2001) examined the effects of oral administration of glucosamine alone and in combination with chondroitin sulfate administered at a dose of 5000 mg/kg body weight were examined in 32 male spontaneously hypertensive rats (SHR) and 32 male Sprague-Dawley rats for 9 weeks. 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 conclusion of this study was that there were no consistent effects on blood chemical parameters and organ histology suggesting no overall toxicity of glucosamine in this 9-week study in these 2 species of rats.

In studies cited by Setnikar et al (Setnikar, 1991) ingestion of glucosamine sulfate at 2700 mg/kg in rats for 52 weeks and 2149 mg/kg in dogs for 26 weeks was not associated with any toxicity in either species. Since the available data indicate that the effects, metabolism and disposal of glucosamine are similar in rat, dog and human, the animal data appears relevant and appropriate (Setnikar, 2001).

As summarized in Table 9, fourteen studies in rats, mice, rabbits and dogs have administered glucosamine orally in doses of approximate 1000-8000 mg/kg/day (median dose, 2600 mg/kg/day) for 1-365 days. The median dose given was 216.5-fold higher than the usual dose given in humans and 20.9-fold higher than the recommended ADI. Oral glucosamine appears to be well tolerated by mice (Setnikar I, Pacini MA, 1991) rats (Echard, 2001; Setnikar I, Pacini MA, 1991; Beren, 2001; Sugimura, 1959), rabbits (Setnikar I, Pacini MA, 1991; Stender, 1977), and dogs (Setnikar I, Cereda R, 1991; McNamara TE, 1996). In three studies horses tolerated doses of 18-22 mg/kg (Caron, 2002; Fenton, 1999; Hanson, 1967).

6.4.2 Parenteral Administration

000107

To frame the *in vitro* and *in vivo* data, these comparisons to plasma and tissue levels resulting from glucosamine administration to humans appear useful. Healthy men have serum glucosamine concentrations of ~0.04 mmol/L when they are not consuming

supplemental glucosamine (Monauni, 2000; Pouwels, 2001). Intravenous infusion of ~9.7 g of glucosamine produces steady state serum glucosamine concentrations of ~0.65 mmol/L (Monauni, 2000; Pouwels, 2001). Infusion of 30.45 grams of glucosamine produces steady state serum glucosamine concentrations of ~1.42 mmol/L (Monauni, 2000). From these concentrations, regression analyses were used to estimate serum glucosamine concentrations for humans with daily intakes of usual doses (23.1 mg/kg body weight) and the recommended ADI. Intake of usual oral doses of glucosamine in humans would achieve serum levels of approximately 0.06 mmol/L representing an increase of 0.02 mmol/L or about 50%. For the ADI (Section 6.6.4), intake of 184 mg/kg body weight would achieve serum levels of ~0.17 mmol/L representing an increase of ~0.13 mmol/L. Many of the *in vitro* studies used glucosamine concentrations of 10 to 200 mmol/L or 218 to 3333-fold higher than expected with usual oral doses in humans. Because oral glucosamine administration achieves serum concentrations on only 20% those achieved with parental administration (Setnikar I, 1993), the doses tested of 240 to 8000 mg/kg body weight given parenterally would achieve serum levels of glucosamine that would be 4000 to 133,333-fold higher than usual oral doses of glucosamine for humans.

The effects of intravenous or intraperitoneal administration of glucosamine have also been examined. The LD₅₀ for intraperitoneal injection of glucosamine in rats is >5200 mg/kg body weight. Based on the pharmacokinetics (Setnikar I, 1986) this achieves and sustains venous glucosamine concentrations equivalent to an oral dose of glucosamine of ~ 22,000 mg/kg. The LD₅₀ for intravenous injection of glucosamine in rats is >1700 mg/kg body weight or equivalent to an oral dose of ~ 7400 mg/kg (Setnikar I, 1986). In mice the LD₅₀ for intraperitoneal injection of glucosamine is >6600 mg/kg body weight, while the LD₅₀ for intravenous injection is >1600 mg/kg. The intravenous 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. These studies are difficult to apply to toxicity concerns in humans because the amounts of glucosamine are equivalent to achieving and sustaining venous glucosamine levels anticipated with oral doses of 1000 to 17,000 mg/kg body weight (Setnikar I, 1993; Setnikar I, 1986).

000108

The rat model often has been selected for study because it is unusually sensitive to the effects of parenteral glucosamine administration on glucose metabolism (Institute of Medicine, 2003). The Institute of Medicine draft report (Institute of Medicine, 2003) reviews 17 reports of administration of glucosamine to rats by intravenous or intraperitoneal routes. The doses ranged from 312 to 9937 mg/kg body weight with a median of 1700 mg/kg. Because oral administration of glucosamine achieves only 20% of the serum concentrations seen with parenteral administration (Setnikar I, 2001) these doses are equivalent to a median oral dose of ~8500 mg/kg body weight. With these enormous doses and using different experimental approaches, alterations in serum glucose levels and glucose metabolism were commonly observed. However, oral administration of glucosamine at very high doses (1000 to 2149 mg/kg body weight) does not affect blood glucose levels in rats (Echard, 2001), rabbits (Stender, 1977), or dogs (Setnikar I, Cereda R, 1991).

The Institute of Medicine report (Institute of Medicine, 2003) summarized 30 *in vitro* studies using a variety of isolated, cultured and homogenized cell systems. The effects on glucose metabolism, insulin secretion, lipid metabolism, cytokine action, and cartilage function were studied. Concentrations of glucosamine ranged from 0.1 to 100 mmol/L for the low concentrations used (average, 14.7 and median, 5 mmol/L) and from 1 to 125 mmol/L for the high concentrations used (average, 31.8 and median, 10 mmol/L). All studies used doses slightly to enormously higher than expected tissue concentrations of 0.06 mmol/L for humans on usual doses of glucosamine. While the toxicity effects in these systems are difficult to interpret, these models do support other physiologic observations. Glucosamine inhibits GFAT, the enzyme responsible for glucosamine synthesis, with an effective dose (ED_{50}) of 0.21 (Traxinger, 1991). In certain tissues glucosamine has a higher affinity for glucose transporters than glucose (Uldry, 2002) and is incorporated into glycoproteins faster than glucose (Ajiboye, 1989). Glucosamine also stimulates proteoglycan synthesis (Bassleer, 1992). This supports the suggestion that exogenous glucosamine acts mainly as a substrate for biosynthesis of mucopolysaccharides and biopolymers of joints and bones (Setnikar I, 2001).

000109

6.5 Mutagenicity Data

6.5.1 *In vitro* Data

Glucosamine yielded negative results in the *E. coli* reverse mutation studies of Brusick et al (Brusick, 1980). However, in studies by Nanjou et al, (Nanjou, 1984), glucosamine was found to induce strand breakage in the DNA of bacteriophage Φ X174 RF1, which the authors believed to be associated with the presence of an amino group. Studies by Watanabe et al. (Watanabe, 1985), also reported PL-1 phage inactivation and pBR322 plasmid DNA breakage. Using plasmid pBR322 to study structure-activity relationships in the induction of strand breakage by amino sugars, Kashige et al. (Kashige, 1994) reported that the addition of 100 mmol D-glucosamine in Tris-HCl buffer resulted in a decrease in the amount of circular duplex plasmid DNA (ccc-DNA) and an equivalent increase in the amount of nicked open-circular plasmid DNA (oc-DNA). The authors suggest that introduction of acidic groups as sulfate and phosphate at the 6 position of the molecule is responsible for the DNA-breaking activity of D-glucosamine. They also suggested that an active oxygen species and/or D-glucosamine radicals generated in the process of autoxidation of the amino sugar were involved in the DNA strand breakage (Kashige, 1991), a possibility supported by the electron spin resonance analyses of glucosamine by Yamaguchi et al. (Yamaguchi, 1998).

6.5.2 Reverse Mutation In Bacteria

000110

In unpublished studies performed by Cargill, Incorporated, the mutagenic activity of REGENASURE™ Glucosamine Hydrochloride 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 *Salmonella typhimurium* and at the tryptophan locus of *Escherichia coli*. Tester strains used in the mutagenicity assay were *Salmonella typhimurium* tester strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* strain WP2uvrA. The assay was

conducted with five doses of REGENASURE™ Glucosamine Hydrochloride 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 ug 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, REGENASURE™ Glucosamine Hydrochloride did not cause a positive increase in the mean number of revertants per plate with any of the tester strains. These test results did not reveal any mutagenic activity (Mecchi, 2003).

6.5.3 *In vivo* Data

The effect of D-glucosamine on the bone marrow chromosomes was examined in mice by Banerjee and Manna (Banerjee, 1984). Glucosamine HCl (10 mg/kg body weight) was administered to Swiss albino mice via intraperitoneal (ip) injection. Bone marrow chromosome aberrations were assessed at 12 different intervals between 10 minutes and 30 days and compared to mice injected with distilled water as controls. Whereas chromosome aberrations in control bone marrow samples were negligible, there was a significant increase in chromosome aberration frequency in samples from mice treated with glucosamine. The nonrandom distribution of chromatid breaks within the chromosomes led the authors to speculate these might be due to some physicochemical stress at inherent weaker regions in the chromosomes. Manna and colleagues (Manna, 2004) also 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.

6.5.4 *In Vivo* Mouse Micronucleus Assay

000111

This assay was carried out by Cargill, Incorporated to evaluate REGENASURE™ Glucosamine Hydrochloride for *in vivo* clastogenic activity and/or disruption of the mitotic apparatus by detecting micronuclei in polychromatic erythrocyte (PCE) cells in Crl: CD-1®

(ICR) BR mouse bone marrow (mouse strain from Charles Rivers Laboratories). 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. For the micronucleus assay, REGENASURE™ Glucosamine Hydrochloride (test article) 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 (Table 10). 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. REGENASURE™ Glucosamine HCl did not induce any signs of clinical toxicity in any of the treated animals at up to 2000 mg/kg (the maximum allowable dose based on regulatory guidelines), nor did it induce any statistically significant increases in micronucleated PCEs at any dose level examined. 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). Glucosamine HCl was evaluated as negative in the mouse bone marrow micronucleus assay under the conditions of this assay.

Table 10: Micronucleus Assay Dosing Scheme

Target Treatment (mg/kg)	Stock Concentration (mg/mL)	Route of Administration	Dosing Volume (mL/kg)	Animals harvested at 24 hours	Animals harvested at 48 hours
500	50	Oral gavage	10	6 Male	-
1000	100	Oral gavage	10	6 Male	-
2000	200	Oral gavage	10	6 Male	6 Male
Vehicle control, cell culture grade water	0	Oral gavage	10	6 Male	6 Male
Positive control, cyclophosphamide, 80	8	Oral gavage	10	Male	-

000112

6.6 Human Clinical Studies

6.6.1 Materials and Methods

This section focuses on clinical studies performed with human subjects. The relevant articles were identified by Medline search and by review of articles referenced in primary reports and review articles. A previous review (Institute of Medicine, 2003) and two meta-analyses (McAlindon, 2000; Richey, 2003) included detailed literature searches. For this current review, the articles from these three previous reports were reviewed, and a Medline search was performed for the years 2000-2003 using these key words: glucosamine and humans. A review of the references of all relevant articles for additional references was done. Articles included in this review relate to glucosamine administration to humans for investigational or therapeutic purposes. Appropriate articles were tabulated and the relevant data was extracted and tabulated. Semiquantitative and statistical analyses of data were performed.

The total number of patients represents the sum of all patients studied or the sum of all patients who had the specific measure described. Patient-years were calculated as follows: Number of patients multiplied by number of study days divided by 365. The ratio of side effects from glucosamine or placebo was calculated as follows: Number of patients treated with glucosamine with side effects divided by number of patients treated with placebo with side effects.

The average ratio of side effects in each study for glucosamine and placebo was averaged, the standard error of these values calculated, and the 95% upper and lower confidence interval were calculated.

Significant differences were reported for 22 studies from patients with osteoarthritis. Since two meta-analyses (McAlindon, 2000; Richey, 2003) have carefully evaluated efficacy, reported outcomes were tabulated and simple arithmetic means and median values were used to characterize these reports. The P value reported represents the median of P

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values reported for each individual study since many studies had multiple P values reported. When significant difference was reported but the P value was not provided, a value of 0.05 was assigned. When values were not clinically significant, a value of 0.1 was assigned; this is justified because these studies reported favorable trends in efficacy or significant values for some outcome measures. The average P value is simply the average of reported P values. Five studies included comparisons of glucosamine to ibuprofen. These values are reported as percentages of patients who developed side effects in these two groups.

6.6.2 Results

Thirty-five studies, including 32 studies of chronic glucosamine administration, were included in this analysis (Table 11). This includes data on 3073 patients treated with glucosamine for a total of 979 patient-years. Twenty-six chronic studies use a randomized, controlled trial (RCT) design, two were controlled studies and five studies were observational. Of the chronic studies, 29 used glucosamine alone, five included chondroitin sulfate and one included other supplements in the test preparation. Seven studies were comparator trials in which glucosamine was compared to other agents (ibuprofen in five studies, phenylbutazone in one study and piroxicam in one study). Of the 32 chronic studies, 28 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, 2001) and three on temporomandibular joint complaints (Nguyen, 2003; Shankland, 1998; Thie, 2001) were included to make the safety assessment as comprehensive as possible.

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Table 11: Human Clinical Studies of Glucosamine Evaluated

Study	Type Study	Glucosamine form	Other Treatment	Route*	Dose mg/d	No.of subjects	Duration days	Patient Years
Almada	RCT	SO4	None	Oral	1500	6	84	1.4
Braham	RCT	HCl	None	Oral	2000	25	84	5.8
D'Ambrosio	RCT	SO4	None	oral/iv/im	1500	15	21	0.9
Das	RCT	HCl	CHS	Oral	2000	46	192	24.2
Drovanti	RCT	SO4	None	Oral	1500	40	30	3.3
Forster	RCT	SO4	None	Oral	1500	78	90	19.2
Giordano	Observational	SO4	None	Oral	1500	20	365	20.0
Houpt	RCT	HCl	None	Oral	1500	45	147	18.1
Hughes	RCT	SO4	None	Oral	1500	39	168	18.0
Leffler	RCT	HCl	CHS, Mn	Oral	1500	31	112	9.5
Monauni: First	Controlled	uncertain	None	iv	9.7g	10	300	0.0
Second study	Controlled	uncertain	None	iv	30.5g	5	300	0.0
Muller-Fassbender	RCT-C	SO4	Vs. ibuprofen	Oral	1500	100	28	7.7
Mund-Hoym	Controlled	SO4	Vs phenylbutazone	oral/im	1000	40	32	3.5
Murad	Controlled	SO4	Supplement	Oral	uncert	57	35	5.5
Nguyen	RCT	HCl	CHS	Oral	1500	19	84	4.4
Noack	RCT	SO4	None	Oral	1500	120	28	9.2
Pavelka	RCT	SO4	None	Oral	1500	84	1095	252.0
Pouwels	Controlled	SO4	None	iv	~7.2g	6	300	0.0
Salte	RCT	SO4	None	Oral	1500	11	49	1.5
Qiu	RCT-C	SO4	vs ibuprofen	Oral	1500	88	28	6.8
Reichelt	RCT	SO4	None	Im	114	73	42	8.4
Reginster	RCT	SO4	None	Oral	1500	87	1095	261.0
Rindone	RCT	SO4	None	Oral	1500	49	60	8.1
Rovati	RCT-P-C	SO4	vs piroxicam	Oral	1500	80	150	32.9
Rovati First	RCT	SO4	None	Oral	1500	123	28	9.4
Second study	RCT	SO4	None	Oral	1500	76	42	8.7
Third study	RCT-C	SO4	vs ibuprofen	Oral	1500	100	28	7.7
Scroggie	RCT	HCl	CHS	Oral	1500	22	90	5.4
Shankland	Observational	HCl	CHS	Oral	3200	50	35	4.8
Tapadinhas	Observational	SO4	None	oral	1500	1367	50	187.3
Thie	RCT-C	SO4	vs ibuprofen	oral	1500	22	90	5.4
Yu	Observational	SO4	None	oral	1500	12	28	0.9
Vajranetra	Observational	SO4	None	oral/ia	1500	108	84	24.9
Vas	RCT-C	SO4	vs ibuprofen	oral	1500	19	56	2.9
Sum						3073		979

* Abbreviations: RCT- randomized controlled trial; C, comparator; P, placebo; CHS, chondroitin sulfate; iv, intravenous; im, intramuscular; ia, intraarticular; uncert, uncertain.

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6.6.3 Effects Of Glucosamine On Glucose Metabolism In Humans

The reviewed studies are listed in Table 12. Four clinical trials reported fasting blood glucose values and mean values decreased nonsignificantly from 95.6 to 92.6 mg/dL. The Reginster study enrolled 108 subjects, followed them for 3 years, and they reported that blood glucose values were slightly lower (Reginster, 2001). Four other clinical trials indicated that there were no significant changes in clinical chemistry values implying no change in blood glucose values. Scroggie and colleagues (Scroggie, 2003) measured glycosylated hemoglobin (HbA1c) in 22 diabetic and 12 control subjects over 90 days. There were no significant changes in these values for diabetic or control subjects. In total, 9 studies assessed fasting glucose values and none reported deterioration of the blood glucose values. These 9 studies included 336 subjects treated for a total of 567 patient-years. For the entire group of 32 chronic studies of older subjects, three developed diabetes with placebo treatment and two developed diabetes with glucosamine treatment.

In two other studies (Monauni, 2000; Pouwels, 2001) performed on metabolic research wards, large amounts of glucosamine (~7.2 grams or 9.7 grams of the glucosamine free base) were infused over 5 hours with no change in blood glucose values. These studies indicate that intake of glucosamine at recommended doses of 1500 mg or greater daily has essentially no effect on fasting blood glucose values in humans. These observations were reinforced by the recent report of Yu et al (Yu, 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.

000116

Table 12: Evaluation of Fasting Plasma Glucose and Safety Parameters

Study	Glucose before	mg/dl after	Summary	Blood chem	CBC	UA	Occult blood	BP P GluN/P	Side Effects
Almada	94	94		NA	NA	NA	NA	NA	NA
Braham	NA			NA	NA	NA	NA	NA	1.10
D'Ambrosio	109	97		NSC	NSC	NSC	NA	NSC	1.00
Das	NA			NA	NA	NA	NA	NA	0.89
Drovanti	82	82		NSC	NSC	NA	NSC	NA	0.83
Forster	NA			NA	NA	NA	NA	NA	0.20
Giordano	NSC			NSC	NSC	NSC	NA	NA	1.00
Houpt	NA			NA	NA	NA	NA	NA	1.00
Hughes	NSC			NSC	NSC	NSC	NA	NA	0.90
Leffler	NA			NA	NSC	NA	NSC	NSC	0.97
Monauri 1	NSC			NA	NA	NA	NA	NSC**	NA
Second study	minimal effect			NA	NA	NA	NA	NSC**	NA
Muller-Fassbender	NA			NA	NA	NA	NA	NSC	NA
Mund-Hoym	NA			NA	NA	NA	NA	NA	NA
Murad	NA			NA	NA	NA	NA	NA	NA
Nguyen	NA			NA	NA	NA	NA	NA	1.43
Noack	NSC			NSC	NSC	NSC	NA	NSC	0.62
Pavelka	NSC			NSC	NSC	NSC	NA	NA	0.56
Pouwels	NSC			NA	NA	NA	NA	NSC**	NA
Rujalte	NA			NSC	NSC	NSC	NA	NA	0.00
Shiu	NA			NSC	NSC	NSC	NA	NA	NA
Reichelt	NA			NA	NA	NA	NA	NA	NA
Reginster	slightly lower			NSC	NSC	NSC	NA	NSC	0.82
Rindone	NA			NA	NA	NA	NA	NA	0.50
Rovati	NA			NA	NA	NA	NA	NA	0.62
Rovati First	NA			NSC	NSC	NSC	NA	NA	0.62
Second study	NA			NSC	NSC	NSC	NA	NA	0.71
Third study	NA			NSC	NSC	NSC	NA	NA	NA
Scroggie	HbA1c	NSC		NA	NA	NA	NA	NA	NA
Shankland	NA			NA	NA	NA	NA	NA	NA
Tapadinhas	NA			NA	NA	NA	NA	NA	NA
Thie	NA			NA	NA	NA	NA	NA	NA
Yu	97.2	97.2		NA	NA	NA	NA	NA	NA
Vajranetra	NA			NA	NA	NA	NA	NA	NA
Vas	NA			NA	NSC	NA	NSC	NSC	NA
Average	95.6	92.6							
No. with reports	4	4	9	11	13	10	3	6	18
Total patients			336	703	753	663	90	372	988
Total patient years			567	591	603	587	16	290	706
Side Effects									0.76

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

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6.6.4 Exposure To Glucosamine In Humans

Research volunteers or patients with arthritic complaints or skin conditions have received glucosamine for periods of 21-1095 days. The most common dose was 500 mg three times daily or 1500 mg/day. One group of 50 subjects received glucosamine hydrochloride at a dose of 3200 mg/day for 35 days. In terms of patient years (number of patients multiplied by duration of treatment), 3073 human volunteers or patients have received glucosamine for 979 patient-years. There have been no serious or life-threatening effects reported.

In two metabolic ward studies, volunteers have received large doses of glucosamine intravenously over 300 minutes. Pouwels and colleagues (Pouwels, 2001) intravenously infused ~7.2 grams of glucosamine as the sulfate salt over a 300 minute period into 10 healthy volunteers. This was well tolerated and not associated with reported side effects. Monauni and colleagues (Monauni, 2000) intravenously infused 9.7 grams of glucosamine over a 300 minute period into 10 healthy volunteers. Again this was well tolerated with no reported side effects. When they subsequently intravenously infused 30.5 grams of glucosamine (436 mg/kg/day or more than 20 times the usual daily dose) into 5 healthy volunteers, this dose was well tolerated by 4 subjects and only one had symptoms—he developed a headache. These amounts (7.2 grams, 9.7 grams, and 30.5 grams) were of the free-base glucosamine.

000118

These studies indicate that glucosamine is well tolerated by healthy volunteer subjects at very high doses. Individuals with degenerative joint disease also tolerate 1500-3200 mg/day for periods of 3 years. 3200 mg/day or 49 mg/kg/day has been tolerated by older subjects for periods of 35 days. Because the blood level achieved with intravenous glucosamine is approximately five-fold higher than with oral administration (Setnikar I, 2001) it appears that humans can easily tolerate more than 9.7 grams/day. In calculating the acceptable daily intake (ADI) of glucosamine, these calculations were used. Humans tolerate more than 9.7 grams of free-base glucosamine. These people have average weights of ~70 kg. The calculation of mg/kg is as follows: 9700 mg divided by 70 kg equals

more than 138 mg/kg/day of the free base glucosamine. Because glucosamine hydrochloride provides 83% free base, humans tolerate more than 166 mg/kg/day (138 divided by 0.83) of glucosamine hydrochloride. Furthermore, since only 90% of glucosamine is absorbed (Setnikar I, 1993) humans tolerate more than 184 mg/kg/day (166 divided by 0.9) of the glucosamine hydrochloride. **The conservative recommendation is that an acceptable daily intake of glucosamine is 184 mg/kg/day.**

6.6.5 Objective Measures Of Safety

Thirteen studies reported specific safety measures including some of these assessments: chemistry panel including liver and kidney safety assessments, hematologic parameters (white blood count, red blood count, hemoglobin, and platelet count), urinalyses, occult blood measurements of stool, and cardiovascular parameters including blood pressure and pulse rate (Table 12). None of the studies reported adverse effects on these measurements from glucosamine administration. In general these safety reports included about 700 subjects representing approximately 600 patient-years. Specifically the number of studies assessing various parameters were as follows: chemistry panel, 11; hematologic parameters, 13; urinalyses, 10; occult blood, 3; and cardiovascular parameters, 6. Blood pressure and pulse rate were monitored continuously for the 21 subjects who had large amounts of glucosamine infused intravenously with no reported adverse effects (Monauni, 2000; Pouwels, 2001). None of the studies reported significant changes in these parameters.

6.6.6 Common Symptoms with Placebo or Glucosamine

Nonspecific symptoms are commonly reported in clinical trials. In a 3-year study, 93% of subjects receiving placebo reported symptoms (Reginster, 2001). The most common symptoms reported with placebo or glucosamine were these: mild gastrointestinal symptoms including constipation, diarrhea, nausea, dyspepsia, excessive gas, abdominal distension, and abdominal cramps; headache; and skin rash or pruritis. Eighteen chronic studies that provided side effect data comparing glucosamine to placebo were analyzed.

The contents of the placebo capsules used in these studies were: not stated, in 9 studies; lactose, in 3; excipients, in 3; inert material, in 1; calcium carbonate, in 1; and 50% maltodextrin and 50% whey protein, in 1. These studies, as summarized in Table 11, included 988 subjects and 706 patient-years of observation. In 13 of the 18 studies, symptoms were reported less commonly in glucosamine-treated subjects than in placebo-treated subjects. The ratio of symptoms for glucosamine compared to those for placebo is presented for each study. The placebo has a score of 1.0 and the frequency of symptoms with glucosamine is a fraction of this. When the frequency of symptoms is the same the ratio for glucosamine is 1.0. When less symptoms are reported for glucosamine than placebo, the ratio is less than 1.0. Only two studies reported that symptoms were more common with glucosamine than placebo. The frequency of symptoms with glucosamine ranged from none (0.0) to 143% (1.43) of those reported for placebo. The average for the ratio of symptoms for glucosamine compared to placebo was 0.76 (95% confidence interval, 0.61 to 0.92). This suggests that symptoms were 24% less common with glucosamine than placebo and that this was statistically significant. Richey and colleagues (Richey, 2003), in their meta-analysis, indicated that the adverse effect rate with glucosamine was 80% of that for placebo.

Five studies compared side effects of glucosamine with ibuprofen, the most commonly used non-steroidal anti-inflammatory agent for arthritis. The prevalence of side effects in patients using glucosamine was 10.0% compared to 32.5% for patients using ibuprofen. The Institute of Medicine report also concluded that side effects were less common with glucosamine than with ibuprofen (Institute of Medicine, 2003).

6.6.7 Efficacy Assessment

The efficacy of glucosamine for arthritic complaints has been extensively studied and two recent meta-analyses (McAlindon, 2000; Richey, 2003) are available. McAlindon and colleagues (McAlindon, 2000) conclude that glucosamine was moderately efficacious for relief of arthritic complaints. Richey and colleagues (Richey, 2003) conclude that glucosamine had highly significant efficacy on all aspects of knee osteoarthritis including

joint space narrowing, pain, and mobility scores. Twenty-two clinical studies of patients with osteoporosis were reviewed (Table 13); this does not include the three studies of TMJ symptoms. Twelve studies reported significant differences and included P values (from 0.05 to 0.001). Seven indicated that significant improvement was seen but did not provide P values; a P value of 0.05 was assigned to these studies. Only three studies indicated that no significant difference was seen and two noted a slight improvement with glucosamine administration; a P value of 0.1 was assigned to these studies since they reported favorable but not quite statistically significant results. The average of all reported and imputed P values for the 22 studies was 0.040 and the median P value was 0.05. While a detailed analysis of efficacy was not undertaken, this survey indicates that glucosamine administration, at a dose of 1500 mg/day, is moderately effective in decreasing arthritic complaints.

000121

Table 13: Overview of Efficacy of Glucosamine for Arthritic Complaints

Study	Joints Evaluated	Arthritis Symptoms Significant difference
Braham	knees	0.038
D'Ambrosio	generalized OA	0.01
Das	knees	0.04
Drovanti	generalized OA	0.005
Forster	knees	sign diff (0.05)
Giordano	generalized OA	0.001
Haupt	Knees	NCS (0.1)
Hughes	knees	NCS (0.1)
Leffler	knees or back	0.02
Muller-Fassbender	knees	sign diff (0.05)
Mund-Hoym	back	sign diff (0.05)
Noack.	knees	0.05
Pavelka	knees	0.01
Pujalte	generalized OA	0.01
Qiu	knees	sign diff (0.05)
Reichelt	Knees	Sign diff (0.05)
Reginster	Knees	sign diff (0.05)
Rindone	knees	NCS (0.1)
Rovati	knees	sign diff (0.05)
Rovati: First study	knees	0.014
Second study	knees	0.012
Tapadinhas	generalized OA	0.001
Vajranetra	knees	sign diff (0.05)
Average		0.040
Median		0.050
No. with reports		23
Total patients		2645
Total patient years		933

Abbreviations: NA= not applicable; OA, osteoarthritis; NA, not available; NCS, not clinically significant

6.7 Discussion

000122

Glucosamine has been extensively studied in animals and humans. Studies in animals indicate that enormous amounts (5000-8000 mg/kg) can be administered orally without evidence of toxicity. Studies using rats, mice, rabbits and dogs have administered glucosamine orally in doses of approximate 1000-8000 mg/kg/day (median dose, 2600

mg/kg body weight) for 1-365 days. The usual dose used for humans is ~23 mg/kg body weight and the recommended ADI is 184 mg/kg body weight. Thus, the median dose given in these animal studies was 113-fold higher than the usual dose given in humans and 14-fold higher than the recommended ADI.

To evaluate safety for humans, data was reviewed from 32 clinical trials including 3073 individuals consuming glucosamine for periods of 21-1095 days (979 patient-years). Like the Institute of Medicine review (Institute of Medicine, 2003), we conclude that mild, transient side effects are seen in placebo and glucosamine treated individuals. The analysis of side effects suggests that side effects are about 24% less frequent in glucosamine-treated individuals than in placebo-treated individuals; Richy et al (Richy, 2003) calculated that side effects are 20% less common in glucosamine-treated subjects than in the placebo groups. This analysis and that of the Institute of Medicine (Institute of Medicine, 2003) indicate that side effects from glucosamine are substantially lower than from ibuprofen, a widely used non-steroidal anti-inflammatory drug.

The effects of glucosamine on glucose metabolism have interested laboratory investigators for many years because pharmacologic concentrations of glucosamine affect insulin action and secretion. Glucosamine is a common metabolic product in most tissues of the body and is incorporated into glycosaminoglycans (Setnikar I, 2001). Setnikar and Rovati (Setnikar I, 2001) have reviewed the metabolism of glucosamine in humans and these data can be summarized. Glucosamine sulfate or hydrochloride salts are dissociated in the stomach and free glucosamine enters the small intestine where 90% is absorbed. Much of the glucosamine is metabolized in the first pass through the liver. The blood level of glucosamine after oral administration approximates 20% of that observed with intravenous administration. Glucosamine is taken up by cells by glucose transporter proteins but the affinity of glucosamine for these transporters is substantially lower than that of glucose (Nelson, 2000). Thus, it seems likely that the concentration of glucosamine in most cells would be substantially lower than that in plasma. With intravenous administration of 9.7 grams over 5 hours, serum glucosamine concentrations of 0.7 mmol/l were achieved (Monauni, 2000). With administration of 500 mg in three divided doses it

000123

seems unlikely that serum concentrations above 0.06 mmol/l would be achieved. *In vitro* studies that show effects of glucosamine on glucose metabolism have used concentrations of 2.5 to 50 mmol/l (Heart, 2000; Nelson, 2000; Sakai, 2003). The effective dose for a 50% change (ED₅₀) in insulin-stimulated glucose uptake in isolated fat cells is 25-30 mmol/L (Heart, 2000) or ~420 times the tissue level likely to be achieved with oral administration of usual doses of glucosamine in humans. Thus, it seems very unlikely that oral administration of 1500 mg/d (the commonly used amount) to 3200 mg/d (an amount used in one chronic study) of glucosamine would have a discernable effect on metabolic pathways involved in glucose metabolism in humans.

From the estimates of endogenous glucosamine production, 2-5% of ingested carbohydrate (Hart, 2003), it appears that endogenous production would far exceed the effects of ingested glucosamine. With intakes of 200-350 grams of carbohydrate, humans would produce an estimated 4-17.5 grams of glucosamine per day. With the intake of ~1.5 grams of oral glucosamine daily, blood levels would be equivalent to infusing 20% of this amount (Institute of Medicine, 2003; Setnikar, I 2001) or 0.3 grams per day. Furthermore, when large amounts of carbohydrate are ingested for short or intermediate periods of time, insulin sensitivity improves in healthy subjects (Anderson, 1973) and in diabetic subjects (Anderson, 1977). For example, when 80% of energy as carbohydrate (~400 grams of carbohydrate) was fed to healthy volunteers for 11-13 weeks, they had improved glucose tolerance and insulin sensitivity (Anderson, 1973) despite estimated endogenous production of 8-20 grams of glucosamine per day. Thus, in humans, it seems unlikely that providing 1.5 grams of exogenous glucosamine --with anticipated metabolic effects on liver and peripheral tissues of only 20-26% (0.3-0.4 grams) (Institute of Medicine, 2003; Setnikar I, 2001) of this-- that adverse effects on glucose metabolism or insulin sensitivity in humans will be seen.

Because of the effects of large concentrations of glucosamine on glucose metabolism in animal and *in vitro* models, the available data related to this question in humans was rigorously examined. In clinical trials there is no evidence that glucosamine in usual doses affects fasting plasma glucose concentrations. In one clinical trial (Yu, 2003)

000124

glucosamine administration had no effect on estimates of insulin sensitivity. Finally, when large amounts of glucosamine (7.2 or 9.7 grams) was infused into healthy volunteers, no adverse effects on blood glucose concentrations were observed over the 5-hour period of study (Monauni, 2000; Pouwels, 2001).

The potential toxicity of intermediary metabolites resulting from glucosamine administration can also be evaluated. Glucosamine is a basic component of the disaccharide units of articular cartilage glycosaminoglycans. Exogenous glucosamine is a preferred substrate for the biosynthesis of these components since, *in vitro*, it is preferentially taken up, in comparison with glucose, and stimulates glycosaminoglycan synthesis by articular chondrocytes (Reichelt, 1994). Because of its chemico-physical properties, glucosamine is rapidly distributed throughout the body and selectively incorporated into articular cartilage (Setnikar I, 2001). Exogenous glucosamine stimulates the chondrocytes to synthesize glycosaminoglycans and proteoglycans including the protein moiety. This is seen in animal and human chondrocyte cultures (Reichelt, 1994; Bassler, 1992).

The entry of glucosamine into cells is stimulated by insulin and involves the glucose-transporter system (Heart, 2000; Pouwels, 2001). Glucosamine is then phosphorylated to glucosamine-6-phosphate by tissue hexokinases (Monauni, 2000; Pouwels, 2001). These intermediary metabolism pathways are finely regulated as indicated by the following observations:

1. glucose-6-phosphate is a potent inhibitor of most hexokinases and glucosamine-6-phosphate is a weak inhibitor of this family of enzymes (Nelson, 2000);
2. glucosamine inhibits the glucose transporter system (e.g., GLUT-4) further limiting its entry into cells (Nelson, 2000);
3. glucosamine inhibits GFAT activity, limiting formation of glucosamine-6-phosphate from fructose-6-phosphate;

000125

4. glucoseamine-6-phosphate is converted to fructose-6-phosphate by glucosamine-6-phosphate deaminase, preventing excessive flux through this relatively minor metabolic pathway (Wolosker, 1998); and
5. when free glucosamine enters cells, its downstream metabolism is significantly limited by one or two steps distal to its phosphorylation. The conversion of glucosamine-6-phosphate to N-acetyl-glucosamine-6-phosphate and then further conversion to UDP-N-acetylglucosamine-6-phosphate, specifically, are limited (Nelson, 2000). Thus, it appears very unlikely that cellular metabolites of free glucosamine would exceed physiologic levels after oral glucosamine intake.

In reviewing these clinical trials, data on the efficacy of glucosamine administration on symptoms of osteoarthritis was tabulated. The observations made are consistent with the rigorous meta-analyses of McAlindon et al (Wolosker, 1998), and Richy et al (McAlindon, 2000; Richy, 2003). Individuals with osteoarthritis of the knee or spine have significantly less symptoms while taking glucosamine than those taking placebo. McAlindon et al (McAlindon, 2000) conclude that glucosamine is moderately efficacious for relief of symptoms of osteoarthritis. Richy et al (Richy, 2003) conclude that glucosamine has highly significant effects on all aspects of knee osteoarthritis. The effects of glucosamine sulfate compared to glucosamine hydrochloride have not been examined in a comparator trial. Because of the disassociation of the salt in the stomach, it seems unlikely that the two preparations would have differing effects. Comparing side effects and reports of efficacy across trials suggests that the two glucosamine salts have similar effects.

6.8 Conclusions

Oral glucosamine administration is well tolerated by animals and humans. Glucosamine had been administered to rats, mice, rabbits, dogs and horses in more than 19 reported studies. In 14 studies, daily doses of 1000-8000 mg/kg body weight were used. These studies documented no glucosamine-related adverse effects. Many *in vitro* studies have used enormous quantities of glucosamine,

000126

usually ranging from 2.5-50 mmol/L (median concentration, 7.5 mmol/L). One ED₅₀ calculation for glucosamine effects *in vitro* was 30 mmol/L. Since the estimated cellular concentration of glucosamine with usual doses are estimated at 0.06 mmol/L, the median amounts used *in vitro* are ~125-fold higher than expected tissue levels with usual doses. To examine possible toxic effects in humans, results from 32 clinical trials with glucosamine were reviewed. These trials included 3073 subjects studied for 979 patient-years. While there have been concerns originating from some *in vitro* animal studies that glucosamine might adversely affect glucose metabolism, careful studies in humans show not adverse effects on glucose homeostasis. Overall, 9 studies including 336 subjects for 567 patient-years reported no adverse effects on glucose metabolism. Glucosamine is well tolerated by humans for periods of up to three years. While the usual dose is 1500 mg/day in three doses, doses of up to 3200 mg/day were well tolerated. Healthy young subjects had no adverse effects from infusion of 9.7 grams and only one of five developed a headache when 30.5 grams was infused. This suggests that humans tolerate intake of at least 184 mg/kg/day of glucosamine daily. In 13 clinical trials reporting safety information there were no adverse effects of glucosamine on blood chemistries, hematologic parameters, urinalysis, occult blood in feces, or cardiovascular parameters. Symptoms or side effects were reported significantly less frequently with glucosamine than with placebo. Reported side effects were 24% less common in subjects treated with glucosamine than with placebo. Finally, glucosamine appears to be moderately to highly effective in decreasing symptoms resulting from osteoarthritis.

000127

THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF GLUCOSAMINE: OPINION OF AN EXPERT PANEL

11 March 2004

Introduction

Cargill, Incorporated, convened a panel of independent biomedical scientists, qualified by their scientific and/or medical training and experience to evaluate the safety of food ingredients and food and recognized nationally and internationally for this expertise (the "Expert Panel"), to independently and collectively critically evaluate available published and unpublished information on glucosamine and on REGENASURE™ Glucosamine Hydrochloride to determine its safety and generally recognized as safe (GRAS) status under the proposed conditions of use based on scientific procedures.

The members of the Expert Panel included Professors Joseph F. Borzelleca of the Medical College of Virginia, Robert J. Nicolosi of the University of Massachusetts-Lowell and James W. Anderson, University of Kentucky School of Medicine. The curricula vitae are provided in Appendix 7.

A thorough search of the scientific literature was conducted by Cantox (to 2003) and updated through December 2003 by Keller & Heckman. The results of these searches were made available to the Expert Panel. Cargill, Incorporated also provided a comprehensive package of appropriate published information and other relevant materials to the Expert Panel. Included was information on the method of manufacture, specifications, batch analyses, proposed uses, exposure estimates, and safety and efficacy studies. The Expert Panel independently and collectively critically evaluated these materials and other materials deemed appropriate or necessary, consulted by telephone, and then met in Chicago and in Washington, D.C. with scientists from Cargill, Incorporated. Following an executive session, the Expert Panel unanimously concluded that glucosamine (REGENASURE™ Glucosamine Hydrochloride), meeting appropriate food grade specifications and manufactured in accordance with current food Good Manufacturing Practice, and used as described herein, is safe and generally recognized as safe (GRAS) based on scientific procedures.

A summary of the basis for this GRAS determination follows.

000128

Identity and Characterization

Glucosamine, 2-amino-2-deoxy-D-glucose; the CAS No. for glucosamine hydrochloride is 66-84-2 and the EINECS No. is 200-638-1, the formula is $C_6H_{13}NO_5 \cdot HCl$ and the molecular weight is 215.63. Glucosamine can be prepared from chitin sourced from shellfish but the glucosamine hydrochloride that is the subject of this GRAS determination (REGENASURE™ Glucosamine Hydrochloride), is prepared from a vegetative microorganism source using a proprietary process.

Manufacturing, Specifications, Batch Analyses

REGENASURE™ Glucosamine Hydrochloride is derived from chitin, which occurs naturally in the cell walls of the microorganism *Aspergillus niger*, a non-toxic and non-pathogenic organism. This strain is derived from a food-grade fermentation process for the production of citric acid. The biomass is digested with hydrochloric acid, which depolymerizes and deacetylates the polysaccharide, chitin, to form glucosamine. The digested biomass is filtered, evaporatively crystallized, centrifuged, dried, and packaged. Only food grade materials are used in the manufacture of glucosamine.

The glucosamine hydrochloride produced is 98-102% pure. Sensitive chemical and microbiological analyses have failed to identify potential human toxins. It meets or exceeds USP-NF specifications for glucosamine hydrochloride. Batch analyses demonstrate that the product can be consistently produced.

REGENASURE™ Glucosamine Hydrochloride is manufactured in accordance with food Good Manufacturing Practices.

Exposure, Proposed Uses, and Estimated Daily Intake (EDI)

Estimates on the background exposure of dietary glucosamine could not be found. Glucosamine hydrochloride is used as a dietary supplement at doses of 500- 2000 mg/day but estimates of average intakes could not be determined. The proposed uses include fruit juices and drinks, vegetable juices and drinks, carbonated beverages, and instant teas. The proposed level of use is 0.75 g glucosamine hydrochloride/8 oz. For the US population, the per capita and per user intake means are estimated to be 9.195 and 19.887 mg/kg bw/day; and, at the 90th percentile, the intakes are estimated to be 26.294 and 43.174 mg/kg bw/day respectively.

Biological Data Relating to Safety

Glucosamine is an essential component of mucopolysaccharides, which are large complexes of negatively charged carbohydrate chains that are incorporated into mucous secretions, connective tissue, skin, tendons, ligaments, and cartilage. Glucosamine and its acetylated derivative, N-acetylglucosamine, are readily synthesized in the body from glucose.

Glucosamine directly enters the hexosamine biosynthetic pathway. In humans, the endogenous production of glucosamine is in the range of 4-10 grams per day, or 2.5% of ingested carbohydrate.

Animal ADME.

Glucosamine is well absorbed from the gastrointestinal tract and distributes into several tissues including cartilage. Following absorption, glucosamine may be “incorporated into plasma proteins, degraded into smaller molecules (carbon dioxide, water, and urea) or utilized for other biosynthetic processes.”

000129

Acute Toxicity

The acute oral LD₅₀ of orally administered glucosamine hydrochloride to male and female rats is greater than 5000 mg/kg bw, the limit dose. It may be considered "practically non-toxic".

Sub chronic Toxicity

Glucosamine hydrochloride was fed to dogs as a dietary admixture for 26 weeks and no adverse effects were reported on any parameter evaluated. The highest dose fed was 2149 mg/kg bw/day and this was the NOAEL.

Long-term Toxicity

Glucosamine hydrochloride was fed to rats as a dietary admixture for 52 weeks and no adverse effects were reported on any parameter evaluated. The highest dose fed was 2700 mg/kg bw/day and this was the NOAEL.

Mutagenicity

Glucosamine hydrochloride was evaluated in the *Salmonella typhimurium* assay ('Ames test') using tester strains TA98, TA 100, TA 1535, and TA 1537 and in the *Eschericia coli* assay using strain WP2uvrA, in the presence and absence of S9 fractions (microsomal activation).

Glucosamine hydrochloride was negative in all assays.

Glucosamine hydrochloride was also negative in the mouse micronucleus assay.

The animal data indicate that glucosamine hydrochloride is non-toxic at the doses tested.

Human

The results from 35 studies, including 32 published clinical trials with chronic glucosamine administration that included 3073 subjects studied for 979 patient-years, were critically evaluated. The usual dose was 1500 mg/day, 500 mg three times daily although patients tolerated divided doses up to 3200 mg/day. Infusion studies indicate that humans can tolerate at least 9.7 grams of free base glucosamine or 138 mg/kg bw/day of the free base. In the clinical trials that reported safety information, there were no adverse effects reported on blood chemistries, hematological parameters, urinalysis, occult blood in feces, or cardiovascular parameters. Reported side effects (usually gastrointestinal) were 24% less common in subjects treated with glucosamine than with placebo.

Discussion, Summary, and Acceptable Daily Intake (ADI)

A critical evaluation of available information on the safety and efficacy of glucosamine hydrochloride indicates that it is a non-toxic amino monosaccharide at the doses tested. Glucosamine is produced endogenously. Exogenously administered glucosamine is well absorbed from the gastrointestinal tract and is incorporated into biosynthetic pathways. The acute, sub chronic, and long-term toxicity studies indicate that it is of low-order toxicity; it was non-toxic at the doses tested. Glucosamine hydrochloride is not mutagenic. Human studies support the safety of glucosamine hydrochloride at doses up to 3200 mg/kg bw/day (higher oral doses were not reported). The conservative recommendation is that an acceptable daily intake of glucosamine is 184 mg/kg/day.

000130

Conclusion

Based on its independent and collective critical evaluation of the data and information currently available, the Panel concludes that glucosamine hydrochloride (REGENASURE™ Glucosamine Hydrochloride), produced in accordance with current food Good Manufacturing Practice and meeting appropriate food grade and USP-NF specifications, is safe for the intended uses described herein.

The Panel further concludes that other experts qualified by scientific training and experience, and evaluating the same data and information, would generally recognize that glucosamine (REGENASURE™ Glucosamine Hydrochloride), is safe for the uses described herein.

The Panel concludes that glucosamine hydrochloride (REGENASURE™ Glucosamine Hydrochloride) is GRAS based on scientific procedures for the uses described herein.

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000131

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9.0 GLOSSARY

ADI	acceptable daily intake
ADME	absorption, distribution, metabolism, and excretion
ALT	alanine aminotransferase
AOAC	Association of Analytical Communities International
AST	aspartate aminotransferase
BP	blood pressure
BUN	blood urea nitrogen
CAS	Chemical Abstracts Service
CFR	<i>Code of Federal Regulations</i>
CHS	chondroitin sulfate
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

000143

EINECS	European Inventory of Existing Commercial Chemical Substances
EPA	Environmental Protection Agency
FDA	Food and Drug Administration
FR	<i>Federal Register</i>
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 hemoglobin
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 b.w.	kilograms of body weight
LD ₅₀	lethal dose for 50% of the population

000144

mg	milligrams
mg/dL	milligrams per deciliter
mg/kg	milligrams per kilogram
mg/mL	milligrams per milliliter
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	randomized controlled trial
RCT-P	randomized controlled trial, placebo
RCT-P-C	randomized controlled trial, placebo, comparator
SD	Sprague-Dawley rats
SHR	spontaneously hypertensive rats
TMJ	temporomandibular joint

000145

UA urinalysis
USDA United States Department of Agriculture
USP-NF United States Pharmacopeia-National Formulary

000146

10.0 APPENDICES

APPENDIX 1

Kosher Certification for REGENASURE™ Glucosamine Hydrochloride

000147



Orthodox Union

Union of Orthodox Jewish Congregations of America • איחוד קהילות האורתודוקסים באמריקה

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Chairman

DR. CHAIM WASSERMAN

Vice Chairman

DAVID FUND

Rabbinic Administrator

RABBI MENACHEM GENACK

Rabbinic Administrator (1950-1972)

RABBI ALEXANDER S. ROSENBERG

August 20, 2002

TO WHOM IT MAY CONCERN:

Please be advised that Regenasure Glucosamine HCl produced by Cargill Acidulants BU, Minneapolis, MN is manufactured under the supervision of the Orthodox Union and is certified kosher for Passover and year-round use. This glucosamine product is certified kosher for all food applications, as the glucosamine is derived from a non-shellfish source and thereby not limited in its certification for only medical applications.

Sincerely yours,
UNION OF ORTHODOX JEWISH
CONGREGATIONS OF AMERICA

Rabbi Elyahu Safran
Senior Rabbinic Coprdinator

ES:sb

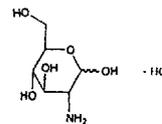
000148

APPENDIX 2

United States Pharmacopeia Monograph for Glucosamine Hydrochloride

000149

Glucosamine Hydrochloride



$C_6H_{13}NO_5 \cdot HCl$ 215.63

D-Glucose, 2-amino-2-deoxy-, hydrochloride.

2-Amino-2-deoxy- β -D-glucopyranoside hydrochloride [66-84-2].

» Glucosamine Hydrochloride contains not less than 98.0 percent and not more than 102.0 percent of $C_6H_{13}NO_5 \cdot HCl$, calculated on the dried basis.

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards (11)—*USP Glucosamine Hydrochloride RS*.

Identification—

A: *Infrared Absorption* (197K).

B: It meets the requirements of the tests for *Chloride* (191).

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 $+70.0^\circ$ and $+73.0^\circ$.

Test solution: 25 mg per mL.

pH (791): between 3.0 and 5.0, in a solution containing 20 mg per mL.

Loss on drying (731)—Dry it at 105° for 2 hours; it loses not more than 1.0% of its weight.

Residue on ignition (281): not more than 0.1%.

Sulfate (221)—A 0.10-g portion shows no more sulfate than corresponds to 0.25 mL of 0.020N sulfuric acid; not more than 0.24% is found.

Arsenic, Method II (211): 3 μ g per g.

Heavy metals, Method II (231): 0.001%.

Organic volatile impurities, Method I (467): meets the requirements.

000150

Assay—

Phosphate buffer—Mix 1.0 mL of phosphoric acid with 2 liters of water, and adjust with potassium hydroxide to a pH of 3.0.

Mobile phase—Prepare a mixture of *Phosphate buffer* and acetonitrile (3:2). Sonicate for 15 minutes, and pass through a filter having a 0.5- μ m or finer porosity. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Standard preparation—Dissolve an accurately weighed quantity of USP Glucosamine Hydrochloride RS in water to obtain a solution having a known concentration of about 1.0 mg per mL.

Assay preparation—Transfer about 100 mg of Glucosamine Hydrochloride, accurately weighed, to a 100-mL volumetric flask. Dissolve in 30 mL of water, shake by mechanical means, dilute with water to volume, and mix.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 195-nm detector and a 4.6-mm \times 25-cm column that contains packing L7. The flow rate is

about 0.6 mL per minute. Chromatograph the *Standard preparation* and record the responses as directed for *Procedure*: the tailing for the glucosamine peak is not more than 2.0; and the standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 μ L) of *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the glucosamine peaks. Calculate the percentage of $C_6H_{13}NO_5 \cdot HCl$ in the portion of Glucosamine Hydrochloride by the formula:

$$10,000(C/W)(r_U/r_S)$$

in which *C* is the concentration, in mg per mL, of USP Glucosamine Hydrochloride RS in the *Standard preparation*; *W* is the weight, in mg, of Glucosamine Hydrochloride used to prepare the *Assay preparation*; and *r_U* and *r_S* are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

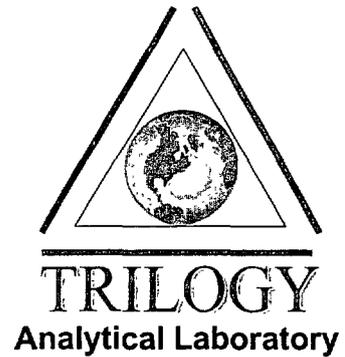
BEST ORIGINAL COPY

Appendix 3

Ochratoxin A Test Results for REGENASURE™ Glucosamine Hydrochloride

000151

ANALYTICAL RESULTS CERTIFICATE



Sample Receipt Date November 25, 2003

Client Address:
Cargill, Inc/Acidulants
#1 Cargill Drive
Eddyville, IA 52553

Trilogy Invoice Number: 7835

1 = REALG 3012 glucosamine hydrochloride

Trilogy Sample ID: 7265

<u>Analysis Compound</u>	<u>Detection Limit</u>	Sample Number:	7265
Ochratoxin A	1 ppb	1	ND

BEST ORIGINAL COPY

ND = None Detected
All samples analyzed by HPLC methodology

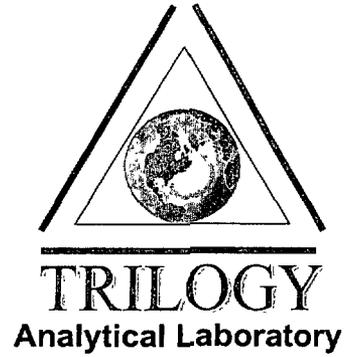
Results Approved by

If you have any questions regarding these sample results please do not hesitate to contact us. We will be happy to review methodology and assist with result interpretation. Sample results reported above are for the sample submitted only. They are not a guarantee of the condition of the larger sample from which these samples were purportedly taken.

Trilogy Analytical Laboratory, Inc.
111 West Fourth St. ♦ Washington, MO 63090
Phone: 636-239-1521 ♦ Fax: 636-239-1531

000152

TRILOGY ANALYTICAL RESULTS CERTIFICATE



Sample Receipt Date: March 10, 2004

Client Address:
Cargill, Inc
Acidulants R&D
#1 Cargill Dr.
Eddyville, IA 52553

Trilogy Invoice Number: 8582

Sample Description:

Trilogy Sample ID: 8043

- | | | |
|---|-------------------|-----------------|
| 1 | Lot # REASG 3002A | Glucosamine HCl |
| 2 | Lot # RFE 3011B | Glucosamine HCl |
| 3 | Lot # RSE 4001A | Glucosamine HCl |
| 4 | Lot # RSE 4003A | Glucosamine HCl |

Analysis Compound	Detection Limits	Sample Number: 8043			
		<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>
Ochratoxin A	50ppt	ND	ND	ND	ND

BEST ORIGINAL COPY

ND = No Compound Detected

Results Approved by:

If you have any questions regarding these sample results please do not hesitate to contact us. We will be happy to review methodology and assist with result interpretation.

Sample results reported above are for the sample submitted only. They are not a guarantee of the condition of the larger sample from which these samples were purportedly taken.

000153

Appendix 4
Protein test results for REGENASURE™ Glucosamine Hydrochloride

000154

Protein Facility

1182 Molecular Biology Building, Ames, Iowa 50010

515-294-3267

Fax: 515-294-9968

Email: protein@iastate.edu

Date sample submitted: November 21, 2003

Sample ID: G82A and G82B

Sample amount submitted: G82A, approx 5-10 g; G82B, approx 5-10 g

Sample description:

Sample G82A: Control Ferro/Phanstiehl Pharmaceutical Quality Glucosamine Hydrochloride, from shellfish, Lot#27126A; Purity: Assay: 99.9%

Sample G82B: REGENASURE TM Glucosamine, Lot#REALG 3012

Description of work to be performed:

- 1) SDS-PAGE, Sypro Ruby staining, followed by Coomassie Brilliant Blue R250 staining, and scan after each stain.
- 2) Spike control and test samples with protein at different concentrations-discuss with Dr. Tabatabai

Protocol to be followed:

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Stock solutions

- 1) Preparation of control sample G82A- dissolve 498.3 mg of sample A in 25 ml of dH₂O in a 25 ml volumetric flask
- 2) Preparation of sample G82B- dissolve 501.75 mg of sample B in 25 ml of dH₂O in a 25 ml volumetric flask
- 3) Preparation of bovine serum albumin (Pentex, Fraction V, from Miles Lot 81-003-3 #380- dissolve 100.4 g of bovine serum albumin (BSA) in 5.0 ml of dH₂O in a 5 ml volumetric flask

Working solutions, Sample A

Sample Number	Sample G82A (19.93 mg/ml)	BSA (20.08 mg/ml)	dH ₂ O	Conc*
A-1	0.500 ml	0 ml	0.500 ml	9.97/0
A-2	0.500 ml	0.005 ml	0.495 ml	9.97/0.100
A-3	0.500 ml	0.050 ml	0.450 ml	9.97/1.00
A-4	0.500 ml	0.500 ml	0.500 ml	9.97/10.0

*Concentration of Sample G82A/BSA in mg/ml

000155

Working solutions, Sample B

Sample Number	Sample G82B (20.07 mg/ml)	BSA (20.08 mg/ml)	dH2O	Conc*.
B-1	0.500 ml	0 ml	0.500 ml	10.03/0
B-2	0.500 ml	0.005 ml	0.495 ml	10.03/0.100
B-3	0.500 ml	0.050 ml	0.450 ml	10.03/1.00
B-4	0.500 ml	0.500 ml	0.500 ml	10.03/10.0

*Concentration of Sample G82B/BSA in mg/ml

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SDS-PAGE Protocol:

- 1) Gel: Resolving gel, 15% acrylamide; Stacking gel, 4% acrylamide, Running buffer, 25 mM Tris-HCl, 192 mM glycine, 0.1% SDS, pH 8.3
- 2) Mix 20 μ L sample and 20 μ L 2X sample buffer, heat at 100°C for 5 min and load 10 μ L of the mixture in duplicate lanes lanes 2 through 9
- 3) Load 20 μ L of BioRad Molecular Weight Markers Cat. No. 161-0362 in lane 1
- 4) Running conditions: 180 V for 45 min
- 5) Stain overnight with Sypro Ruby (Molecular Probes), destain 30 min; stain 45 min in 0.1% Coomassie Blue, followed by two changes in destain solutions, one hour each
- 6) Scan gels after Sypro Ruby staining and after Coomassie Blue staining, print images.

General comments

The methodology for visualizing only proteins and not polysaccharides or nucleic acids, is to use either Sypro Ruby (a proprietary ruthenium-containing fluorescent dye from Molecular Probes) or Coomassie Brilliant Blue, R250. Alternatively, both Sypro Ruby and Coomassie Brilliant Blue can be used consecutively. Sypro Ruby has the same sensitivity as the commonly used alkaline silver stain reagent (according to information supplied by Molecular Probes, www.probes.com). In our facility, as little as 0.5 microgram of protein can be visualized with Coomassie Brilliant Blue. The Sypro Ruby stain is 100-fold more sensitive compared to Coomassie Brilliant Blue, and therefore as little as 5 nanogram of protein can be visualized. According to information supplied by Molecular Probes, as little as 75 femtomol (or 1.5 nanogram of protein with a molecular weight of 20,000 dalton) was visualized by using Sypro Ruby.

The alkaline silver stain reagent will detect protein as well as polysaccharides and we therefore do not use this stain in the Protein Facility for detecting proteins.

Sensitivity relates to both quantity of protein detected and the size of the protein/polypeptide detected. Proteins are those macromolecules that do not pass through a dialysis tubing which has a molecular weight cut-off from 12,000 to 14,000 dalton. Any proteinaceous macromolecule that passes through the dialysis membrane is considered a peptide (or polypeptide).

000156

The gel system used, a 15% homogeneous acrylamide gel, will detect peptides of 6,500-10,000 daltons and proteins as large as 200,000 daltons (BioRad Lab information). Smaller peptides will appear in the solvent front at the bottom of the gel.

Specific results and conclusions

- 1) Sample G82A, the control glucosamine without BSA and spiked with BSA are shown in Fig. 1 (Sypro Ruby-stained) and Fig. 2 (Coomassie Blue-stained). The control lanes A-1 and A-2 do not show the presence of a stained band, indicating the absence of protein in these lanes.
- 2) Sample G82B, the test sample containing REGENASURE™ Glucosamine without BSA and spiked with BSA are shown in Fig. 3 (Sypro Ruby-stained) and in Fig. 4 (Coomassie Blue-stained). The control lanes A-1 and A-2 do not show the presence of a stained band, indicating the absence of protein in these lanes.

000157

Appendix 5
Expert Allergen Opinion for REGENASURE™ Glucosamine Hydrochloride

000158

June 25, 2002

John Bohlmann
Cargill, Inc.
#1 Cargill Drive
Eddyville, IA 52553

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Dear Mr. Bohlmann:

As requested some time ago, I wish to provide my expert opinion on the possible allergenicity of glucosamine derived by fermentation with *Aspergillus niger*. In my opinion, this ingredient when derived by fermentation would not be allergenic. Food allergens are proteins, and glucosamine is not a protein. When produced via fermentation with *Aspergillus 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.

In reaching my expert opinion, I searched the medical literature for publications on the allergenicity of glucosamine and *Aspergillus niger*. There are a few reports of allergic reactions to glucosamine but these reports relate to glucosamine produced from shellfish waste. As you know, glucosamine can be manufactured from crustacean shells. Since crustacea are commonly allergenic foods and the shells may contain adhering muscle tissue that includes the shellfish allergens, shellfish-derived glucosamine might be allergenic under some circumstances. In fact, some glucosamine in the marketplace contains a label statement describing the shellfish origin of the product.

I hope that my expert opinion will be useful to you and appreciate your patience with me with respect to the long delay in fulfilling this request.

Sincerely,

Steve L. Taylor, Ph.D.
Professor and Co-Director

Cc: S. Hefle

000159

Appendix 6
Aflatoxin and Pesticide Results for REGENASURE™ Glucosamine Hydrochloride

000160

SAMPLE NUMBER: 20500644

GLUCOSAMINE HCL: LOT #GP-11

AFLATOXIN

<u>ASSAY</u>	<u>ANALYSIS</u>	<u>UNITS</u>
B1	< .5	PPB
B2	< .5	PPB
G1	< .5	PPB
G2	< .5	PPB

USP PESTICIDE SCREEN

USP PESTICIDE SCREEN
COMPOUND NAME

<u>COMPOUND NAME</u>	<u>MG/KG</u>
ALACHLOR	< .02
ALDRIN AND DIELDRIN (SUM OF)	< .05
AZINPHOS-METHYL	< 1.0
BROMOPROPYLATE	< 3.0
CHLORDANE (SUM OF CIS- AND TRANS- ISOMERS AND OXYCHLORDANE)	< .05
CHLORFENVINPHOS	< .5
CHLORPYRIFOS	< .2
CHLORPYRIFOS-METHYL	< .1
CYPERMETHRIN (AND ISOMERS)	< 1.0
DDT (SUM OF ISOMERS)	< 1.0
DELTAMETHRIN	< .5
DIAZINON	< .5
DICHLORVOS	< 1.0
DITHIOCARBAMATES (AS CS2)	< 2.0
ENDOSULFAN (SUM OF ENDOSULFAN ISOMERS AND ENDOSULFAN SULFATE)	< 3.0
ENDRIN	< .05
ETHION	< 2.0
FENITROTHION	< .5
FENVALERATE	< 1.5
FONOFOS	< .05
HEPTACHLOR (SUM OF HEPTACHLOR AND HEPTACHLOR EPOXIDE)	< .05
HEXACHLOROBENZENE	< .1
HEXACHLOROCYCLOHEXANE ISOMERS (OTHER THAN GAMMA)	< .3
LINDANE (GAMMA-HEXACHLOROCYCLOHEXANE)	< .6
MALATHION	< 1.0
METHIDATHION	< .2
PARATHION	< .5
PARATHION METHYL	< .2
PERMETHRIN	< 1.0
PHOSALONE	< .1

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000161

SAMPLE NUMBER: 20500644

PAGE 3

GLUCOSAMINE HCL: LOT #GP-11

USP PESTICIDE SCREEN

(CONTINUED)

PIPERONYL BUTOXIDE	<	3.0
PRIMIPHOS-METHYL	<	4.0
PYRETHRINS (SUM OF)	<	3.0
QUINTOZENE (SUM OF QUINTOZENE, PENTACHLOROANILINE AND METHYL PENTACHLOROPHENYL SULFIDE)	<	1.0

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000162

Appendix 7
Curricula vitae for Expert Panel

000163

Curriculum Vitae
JAMES W. ANDERSON, MD
University of Kentucky
Metabolic Research Group

PLACE AND DATE
OF BIRTH:

FAMILY:

EDUCATION:

TRAINING:

MILITARY:

FACULTY APPOINTMENTS:

1978-present	Professor of Medicine University of Kentucky College of Medicine
1978-present	Professor of Clinical Nutrition University of Kentucky, Lexington, Kentucky
1985-present	Medical Director, HMR Fasting Program, University of Kentucky, Lexington, Kentucky
1973-03	Chief, Endocrine-Metabolic Section VA Medical Center, Lexington, Kentucky
1983-84	Visiting Scientist, Massachusetts Institute of Technology Cambridge, Massachusetts
1980-83	Chief, Medical Service VA Medical Center, Lexington, Kentucky
1973-78	Associate Professor of Medicine University of Kentucky College of Medicine Lexington Kentucky
1968-73	Assistant Professor of Medicine University of California Medical School San Francisco, California

000164

AWARDS: 2002 Vahouny Medal for Excellence in Dietary
Fiber Research

CERTIFICATION AND LICENSE:

1975 American Board Internal Medicine
1973 Kentucky

PROFESSIONAL ORGANIZATIONS:

Alpha Omega Alpha
American College of Nutrition - Fellow
American College of Physicians - Fellow
American Diabetes Association
American Federation for Clinical Research
American Society for Clinical Nutrition
Endocrine Society
Obesity Research Network
Society for Experimental Biology and Medicine

EDITORIAL BOARD:

1998 PRIME Health & Fitness
1997 Vegetarian Nutrition
1991-97 Medicine, Exercise, Nutrition and Health
1990-96 Diabetes in the News
1987-present Diabetes: Self-Management
1989-present Prevention
1974-77 American Journal of Clinical Nutrition

REVIEWER:

American Journal of Clinical Nutrition
American Journal of Medicine
Annals of Internal Medicine
Archives of Internal Medicine
Diabetes
Diabetes Care
Diabetologia
Journal of the American Medical Association
Journal of Laboratory and Clinical Medicine
Journal of Nutrition
Journal of American College of Nutrition
Journal of American Dietetic Association
Journal of Clinical Endocrinology and Metabolism
Metabolism
New England Journal of Medicine

EDITOR: 1979-96 HCF Nutrition Newsletter (Circulation 10,000)

PROFESSIONAL SERVICE:

2000-present Obesity Research Network,

000165

2000-present Chairman of the Board and President
American College of Nutrition, Secretary

PROFESSIONAL SERVICE, continued:

1996-00 Obesity Research Network; Co-Founder & Coordinator
1997-00 American College of Nutrition, Board of Directors
1994 Special Study Section: NIDDK for "NIDDM"
Primary Prevention Trial
1988-00 Diabetes Advisory Committee
Kentucky Diabetes Control Program
1986 LSRO-FDA Expert Panel on Dietary Fiber
1985-86 NIH Diabetes and Hypertension Treatment Panel
1982-86 Scientific Advisory Committee,
Wheat Industry Council
1975-76 Kentucky Diabetes Association President
1974-present Director, Metabolic Research Group

GRANTS AS PRINCIPAL INVESTIGATOR:

2000-present VA Cooperative Study #465, Glycemic Control and
Complications in Diabetes Mellitus Type 2 VADT (VA
Diabetes Trial) 85 to be enrolled \$1,182,850
2000-present NIH - R21 Program Project, "Soy protein in early
diabetic nephropathy" - \$100,000 (Co-PI)
2000-02 Bupropion SR Obesity Study. PI for 6 Site Study
\$1.86 million; Sponsor: Glaxo-Wellcome
1991-98 VA Cooperative Study #363, The VA HDL Intervention
Trial (HIT): Secondary prevention of Coronary Heart
Disease in Men with Low HDL-Cholesterol and
Desirable LDL-Cholesterol - \$484,500
1991-93 VA Cooperative Study #344A, Implantable Insulin
Pump Study - \$83,691
1990-93 NIH Program Project, "Diabetes Diet, Glycemia and
Microcirculation in Humans" - \$133,706 for Third Year
1986-89 NIH - HL 37902 Program Project, "Dietary Fiber and
Cardiovascular Risk Factors" \$1.6 million
1970-88 Veterans Administration Medical Center Merit Review
Grants for 18 consecutive years
1977-80 National Institute of Arthritis, Metabolism and Digestive
Diseases Grant, "High Carbohydrate, High Fiber Diets
for Diabetes"
1974-present University of Kentucky Diabetes Research Fund Grants
from Foundations, Corporations and Individuals,
\$500,000 per annum average (1995-2002)

COMMITTEES:

University of Kentucky
2001 Task Force on Coordination of Clinical
Research & Patient Care

000166

1996-01 Executive Committee-Nutrition Graduate Program
 1994-95 Medical Center Clinical Sciences Area, Advisory
 Committee

COMMITTEES, continued:

University of Kentucky

1991-93 University of Kentucky/Veterans Affairs Research
 Advisory Committee
 1990-93 University of Kentucky Press Committee

VA Medical Center

1993-98 SDTU Ad-Hoc Committee, Chairman

UK Medical Center

1993-00 Nutrition Committee

UK College of Medicine

1992-95 Problem Based Learning Committee
 1986-96 Faculty Promotions Committee

TEACHING:

Undergraduate

1973-present Ward teaching attending
 20 weeks per year (average)
 Four formal lectures per year

Graduate

1973-present Ward teaching attending
 20 weeks per year (average)
 Four to six conferences per year
 1980-83 VA Morning Report for Residents
 Supervise 6 days/week

Postgraduate

1974-present Annual Endocrine Conference (UK) (postgraduate)
 1975-present Chair, yearly, of one or two national or international
 workshops on nutrition, diabetes, dyslipidemia or
 obesity
 1981-90 Annual High Fiber Diet Workshop - Director (UKMC)

PUBLIC SERVICE:

1996-present Downtown Christian Unity Taskforce
 1994-96 Chairman, Board of Trustees, Georgetown College,
 Georgetown, Kentucky
 1990-96 Executive Committee, Board of Trustees,
 Georgetown College, Georgetown, Kentucky
 1989-96 Board of Trustees, Georgetown College,
 Georgetown, Kentucky
 1974-present Deacon, Sunday School Teacher, Calvary Baptist
 Church

000167

PUBLICATIONS, REFEREED JOURNALS:

1. Anderson JW, Kilbourn KG, Robinson J, and Wright PH. Diabetic acidosis in rats treated with anti-insulin serum. *Clin Sci* 24:417-430, 1963.
2. Anderson JW, Sawyer KC Jr, Sheridan DP. Cystic fibrosis of the pancreas with diabetes mellitus. *Rocky Mountain Med J* 60:32-35, 1963.
3. Anderson JW, McConahey WM, Alarcon-Segovia D, Emslander RF, Wakim KG. Diagnostic value of thyroid antibodies. *J Clin Endocrinol Metab* 27:937-944, 1967.
4. Anderson JW. Hyperglycemic diabetic stupor: A spectrum of disorders. *Mil Med* 133:538-542, 1968.
5. Anderson JW, Herman RH. Classification of reactive hypoglycemia. *Am J Clin Nutr* 22:646-650, 1969.
6. Gorman CA, Anderson JW, Flock EV, Owen CA Jr, Wakim KG. Effect of experimentally induced thyroiditis on biosynthesis of thyroxine in rats. *Acta Endocrinol* 62:11-20, 1969.
7. Pastore RA, Anderson JW, Herman RH. Anterior and posterior hypopituitarism associated with sickle cell trait. *Ann Intern Med* 71:593-598, 1969.
8. Anderson JW, Wakim KG, McConahey WM. The influence of experimental thyroiditis on thyroid function. *Mayo Clinic Proc* 44:711-724, 1969.
9. Anderson JW, Herman RH, Newcomer KL. Improvement of glucose tolerance of fasting obese patients given oral potassium. *Am J Clin Nutr* 22:1589-1596, 1969.
10. Anderson JW, Zakim D. The influence of alloxan-diabetes and fasting on glycolytic and gluconeogenic enzyme activities of rat intestinal mucosa and liver. *Biochem Biophys Acta* 201:236-241, 1970.
11. Anderson JW. Pyruvate carboxylase and phosphoenolpyruvate carboxykinase in rat intestinal mucosa. *Biochem Biophys Acta* 208:165-167, 1970.
12. Everett ED, Newcomer KL, Anderson JW, Bergin J, Overholdt EL. Goodpasture's syndrome. Response to mercaptopurine and prednisone. *JAMA* 213:1849-1852, 1970.
13. Anderson JW, Herman RH. Treatment of reactive hypoglycemia and sulfonyleureas. *Am J Med Sci* 261:16-23, 1971.
14. Ho W, Anderson JW. Phosphofructokinase in rat jejunal mucosa: Subcellular distribution, isolation, and characterization. *Biochem Biophys Acta* 227:354-363, 1971.
15. Anderson JW, Herman RH, Tyrrell JB, Cohen RB. Hexokinase: A compartmented enzyme. *Am J Clin Nutr* 24:642-650, 1971.

000168

16. Tyrrell JB, Anderson JW. Glycolytic and pentose phosphate pathway enzymes in jejunal mucosa, adaptive responses to alloxan diabetes and fasting in the rat. *Endocrinology* 89:1178-1185, 1971.
17. Anderson JW, Herman RH. Effect of fasting, caloric restriction and refeeding on glucose tolerance of normal men. *Am J Clin Nutr* 25:41-52, 1972.
18. Anderson JW, Stowring L. Glycolytic and gluconeogenic enzyme activities in renal cortex of diabetic rats. *Am J Physiol* 224:930-936, 1973.
19. Anderson JW, Rosendall AF. Gluconeogenesis in jejunal mucosa of guinea pig. *Biochem Biophys Acta* 304:384-388, 1973.
20. Anderson JW, Herman RH, Zakim D. Effect of high glucose and high sucrose diets on glucose tolerance of normal men. *Am J Clin Nutr* 26:600-607, 1973.
21. Anderson JW, Tyrrell JB. Hexokinase activity of rat intestinal mucosa: Demonstration of four isozymes and changes in subcellular distribution with fasting and refeeding. *Gastroenterology* 65:69-76, 1973.
22. Murphy ED, Anderson JW. Tissue glycolytic and gluconeogenic enzyme activities in mildly and moderately diabetic rats, influence of tolbutamide administration. *Endocrinology* 94:27-34, 1974.
23. Anderson JW. Glucose metabolism in jejunal mucosa of fed, fasted, and streptozotocin-diabetic rats. *Am J Physiol* 226:226-229, 1974.
24. Anderson JW, Jones AL. Biochemical and ultrastructural study of glycogen in jejunal mucosa of diabetic rats. *Proc Soc Exp Biol Med* 145:268-272, 1974.
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29. Anderson JW, Herman RH. Effects of carbohydrate restriction on glucose tolerance of normal men and reactive hypoglycemic patients. *Am J Clin Nutr* 28:748-755, 1975.
30. Anderson JW. Hyperglycemic nonketotic coma. *J Ky Med Assoc* 73:211-213, 1975.
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000171

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70. Anderson JW, Tietyen-Clark J. Dietary fiber: Hyperlipidemia, hypertension and coronary heart disease. *Am J Gastroenterol* 81:907-919, 1986.
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74. Anderson JW, Gustafson NJ. Hypocholesterolemic effects of oat and bean products. *Am J Clin Nutr* 48:749-753, 1988.
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78. Anderson JW, Geil PB. New perspectives in nutrition management of diabetes mellitus. *Am J Med* 85:159-165, 1988.

000172

79. Anderson JW, Gustafson NJ. Hypocholesterolemic effects of oat and bean products. *Michigan Dry Bean Digest* 13:2-5, 1989.
80. Anderson JW, Bridges SR, Tietyen J, Gustafson NJ. Dietary fiber content of a simulated American diet and selected research diets. *Am J Clin Nutr* 49:352-357, 1989.
81. Anderson JW, Story L, Zettwoch N, Gustafson NJ, Jefferson BS. Metabolic effects of fructose supplementation in diabetic individuals. *Diabetes Care* 12:337-344, 1989.
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83. Anderson JW, Gustafson NJ. Adherence to high-carbohydrate, high-fiber diets. *Diabetes Educ* 15:429-434, 1989.
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86. Anderson JW, Gustafson NJ, Spencer DB, Tietyen J. Serum lipid response of hypercholesterolemic men to single and divided doses of canned beans. *Am J Clin Nutr* 51:1013-1019, 1990.
87. Anderson JW, Wood CL. Oat bran and serum cholesterol. (Letter). *N Eng J Med* 322:1747-1748, 1990.
88. Anderson JW, Smith BM, Geil PB. High-fiber diets for diabetes. Safe and effective treatment. *Post-grad Med* 88:157-168, 1990.
89. Fukagawa NK, Anderson JW, Hagemen G, Young VR, Minaker KL. High-carbohydrate, high-fiber diets increase peripheral insulin sensitivity in healthy young and old adults. *Am J Clin Nutr* 52:524, 528, 1990.
90. Anderson JW, Spencer DB, Hamilton CC, Smith SF, Tietyen J, Bryant CA, Oeltgen P. Oat-bran cereal lowers serum total and LDL cholesterol in hypercholesterolemic men. *Am J Clin Nutr* 52:495-499, 1990.
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93. Anderson JW, Floore TL, Geil PB, O'Neal DS, Balm TK. Hypercholesterolemic effects of different bulk-forming hydrophilic fibers as adjuncts to dietary therapy in mild to moderate hypercholesterolemia. *Arch Intern Med* 151:1597-1602, 1991.

000173

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95. Anderson JW, Zeigler JA, Deakins DA, Floore TL, Dillon DW, Wood CL, Oeltgen PR, Whitley RJ. Metabolic effects of high-carbohydrate, high-fiber diets for insulin-dependent diabetic individuals. *Am J Clin Nutr* 54:936-943, 1991.
96. Anderson JW, Riddell-Lawrence S, Floore TL, Dillon DW, Oeltgen PR. Bakery products lower serum cholesterol concentration in hypercholesterolemic men. *Am J Clin Nutr* 54:836-840, 1991.
97. Anderson JW, Hamilton CC, Crown-Weber E, Riddlemoser M, Gustafson NJ. Safety and effectiveness of a multidisciplinary very-low-calorie diet program for selected obese individuals. *J Am Diet Assoc* 91:1582-1584, 1991.
98. Anderson JW, Hamilton CC, Brinkman-Kaplan V. Benefits and risks of an intensive very-low-calorie diet program for severe obesity. *Am J Gastroenterol* 87:6-15, 1992.
99. Anderson JW, Brinkman VL, Hamilton CC. Weight loss and 2-y follow-up for 80 morbidly obese patients treated with intensive very-low-calorie diet and an education program. *Am J Clin Nutr* 56:1S-3S, 1992.
100. Anderson JW, Riddell-Mason S, Gustafson NJ, Smith SF. Cholesterol-lowering effects of psyllium-enriched cereal as an adjunct to a prudent diet in the treatment of mild to moderate hypercholesterolemia. *Am J Clin Nutr* 56:93-98, 1992.
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000174

108. Anderson JW, Brinkman-Kaplan VL, Lee H, Wood CL. Relationship of weight loss to cardiovascular risk factors in morbidly obese individuals. *J Am Coll Nutr* 14:256-261, 1994.
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000188

Professional Certification

Fellow, Academy of Toxicological Sciences

Professional Affiliations

Societies

Academy of Toxicological Sciences*, **
American Association for the Advancement of Science
American Chemical Society
American College of Toxicology*
American Society of Pharmacology and Experimental Therapeutics**
(Environmental Pharmacology Committee; Liaison Committee, SOT; Toxicology Committee)
Institute of Food Technologists (Professional Member)
International Society of Regulatory Toxicology and Pharmacology*
(Councilor)
Sigma Xi
Society of Experimental Biology and Medicine*
(Councilor; Program Chairman of Southeastern Section)
Society for Risk Analysis
Society of Toxicology* **
(Member and/or Chairman: Awards, Education, Legislative Affairs, Membership,
Nominating Committees; Secretary of the Society, Councilor, and President; President,
Food Safety Specialty Section)
Virginia Academy of Science*
(Chairman, Medical Sciences Division)

* Held elected office

** Held appointed office or position

Board of Directors

ILSI (until 2002)

Board of Scientific and Policy Advisors

American Council on Science and Health (until 2000)

Journals

Editor, Food Chemical Toxicology, 1992-

Editorial Board

Environmental Carcinogenesis Reviews, 1981- 2002
Journal of Environmental Pathology, Toxicology and Oncology 1977-
Journal of Environmental Science and Health, 1979-
Journal of the American College of Toxicology, 1982-
Journal of Toxicology: Cutaneous and Ocular Toxicology, 1982- 1992
Journal of Applied Toxicology, 1989-
Pharmacology, 1978-
Pharmacology and Drug Development, 1980-
Toxicology and Applied Pharmacology, 1975-1978

Consultantships (Past, Present)

Governmental

Food and Drug Administration

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000190

National Institute of Mental Health
National Cancer Institute
Environmental Protection Agency
Department of Labor - OSHA (Chairman, Carcinogens Standards Committee)
U.S. Army - Research and Development Command

Non-Governmental

National Academy of Sciences - NRC
Committee on Toxicology (Member, Chairman)/Board on Toxicology and Environmental Health Hazards
Safe Drinking Water Committee
Evaluation of Household Substances Committee (1138 Committee)
Food Protection Committee
Food Additives Survey Committee
Committee on Risk-Based Criteria for Non-RCRA Hazardous Wastes
Committee on Risk Assessment of Flame-Retardant Chemicals

Federation of American Societies of Experimental Biology
Select Committee on GRAS Substances
Flavors and Extracts
Biotechnology Product Safety
Caprenin GRAS Committee

World Health Organization
Joint Meeting on Pesticide Residues (JMPR) (Member, Chairman)

NATO/CCMS Drinking Water Committee

Industrial

Chemical Companies; Trade Associations

University Activities

Related to Instruction

Prepared a laboratory manual in pharmacology (animal and human studies) (1960)
Introduced the use of closed circuit TV and TV tapes in pharmacology (1960)
Introduced clinical pharmacological experiments into the medical and dental programs (1960)
Planning and participation in continuing education program
(Schools of Dentistry, Medicine and Pharmacy)
Planning and administering each of the three major efforts in pharmacology
(dental, medical, pharmacy) since 1960.
Graduate Program - assisted in developing graduate training program in toxicology

Current Teaching Activities

Presents lectures on Toxicological Issues, Food Intake and Control

Not Directly Related to Instruction

Elected senator from the graduate school, then vice-president of the University Senate
Served on various committees (e.g. Curriculum, Search, Animal Care,) in each of the four major schools (Dentistry, Graduate, Medical, Pharmacy)

Research

Research was continuously funded from 1956. Sources of support included governmental (U.S.P.H.S.; N.I.H; E.P.A.; N.I.D.A.) and non-governmental (industrial). (A list of publications is attached).

000191

Awards

DOD - US Army - Chemical Research Development and Engineering Center
Distinguished Service Award, 1986

National Italian - American Foundation Award
Excellence in Medicine and Community Service, 1987

Thomas Jefferson University
Distinguished Alumnus Award, 1987

Virginia Commonwealth University - School of Basic Health Sciences
Outstanding Faculty Award, 1987

Virginia Commonwealth University - School of Basic Health Sciences, Dept. of
Pharmacology and Toxicology
Professor of the Year- 1992

American College of Toxicology
Distinguished Service Award- 1997

Virginia's Life Achievement in Science Award- April 2001

Bernard L. Oser Food Ingredient Safety Award by the Institute of Food Technologists- June 2001

International Society for Regulatory Toxicology and Pharmacology's International Achievement Award for
2001- December 2001

Society of Toxicology- Education Award- March 2002

Publications

Borzelleca, J.F. and Manthei, R.W.: Factors influencing pentobarbital sleeping time in mice. Arch. Int. Pharmacodyn. 111: 296, 1957.

Borzelleca, J.F.: Studies of the contribution of bladder absorption to the physiological changes induced by pentobarbital. J. Pharm. Exp. Ther. 129: 305, 1960.

Borzelleca, J.F.: The absorption of nicotine from the urinary bladder of the dog. Arch. Int. Pharmacodyn. 133: 444, 1961.

Borzelleca, J.F., Bowman, E.R. and McKennis, H., Jr.: The cardiovascular and respiratory effects of (-)-cotinine. J. Pharmacol. Exp. Ther. 137: 313, 1962.

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Larson, P.S., Borzelleca, J.F., Bowman, E.R., Crawford, E.M., Smith, R.B., Jr. and Henningar, G.R.: Toxicologic studies on a preparation of p-tertiary octylphenoxy-polyethoxy ethanols (Triton X-405). Toxicol. Appl. Pharmacol. 5: 782, 1963.

Borzelleca, J.F., Larson, P.S., Henningar, G.R., Hug, E.G., Crawford, E.M. and Smith, R.B., Jr.: Studies on the chronic oral toxicity of monomeric ethyl acrylate and methyl methacrylate. Toxicol. Appl. Pharmacol. 6: 29, 1964.

Borzelleca, J.F. and Cherrick, H.: The excretion of drugs in saliva. Antibiotics. J. Oral Therap. Pharmacol. 2: 180, 1965.

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Borzelleca, J.F.: Drug movement from the isolated urinary bladder of the rabbit. *Arch. Int. Pharmacodyn.* 154: 40, 1965.

Borzelleca, J.F.: Rabbit urinary bladder potentials. *Invest. Urol.* 3: 77, 1965.

Borzelleca, J.F.: Studies on the mechanisms of drug movement from the isolated urinary bladder. *J. Pharmacol. Exp. Ther.* 148: 111, 1965.

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Borzelleca, J.F. and Doyle, C.H.: Excretion of drugs in saliva. Salicylate, barbiturate, sulfanilamide. *J. Oral Therap. Pharmacol.* 3: 104, 1966.

Borzelleca, J.F. and Lowenthal, W.: Drug absorption from the rectum. II. *J. Pharm. Sci.* 55: 151, 1966.

Wooles, W.R. and Borzelleca, J.F.: Prolongation of barbiturate sleeping time in mice by stimulation of the reticuloendothelial system. *J. Reticuloendo. Soc.* 3: 41, 1966.

Wooles, W.R., Borzelleca, J.F. and Branham, G.W.: The effects of acute and prolonged salicylate administration on liver and plasma triglyceride levels and dietary-induced hypercholesterolemia. *Toxicol. Appl. Pharmacol.* 10: 1, 1967.

Borzelleca, J.F., Harris, T. and Bernstein, S.: The effect of DMSO on drug movement through the wall of the urinary bladder of the rabbit. *J. Invest. Urol.* 6: 43, 1968.

Borzelleca, J.F.: The excretion of glucose in saliva. *Dog. J. Oral Therap. Pharmacol.* 4: 338, 1968.

Kim, K.S., Borzelleca, J.F., McKennis, H. and Bowman, E.R.: Pharmacological effects of some nicotine metabolites and related compounds. *J. Pharmacol. Exp. Ther.* 161: 59, 1968.

Marcus, S. and Borzelleca, J.F.: Observations of reserpine-induced bradycardia. *Arch. Int. Pharmacodyn.* 174: 12, 1968.

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Ambrose, A.M., Borzelleca, J.F., Larson, P.S. and Hennigar, G.R.: The toxicology of a foliar fungicide, GC-4072. *Toxicol. Appl. Pharmacol.* 17: 323, 1970.

Borzelleca, J.F. and Putney, J.W., Jr.: A model for the movement of salicylate across the parotid epithelium. *J. Pharmacol. Exp. Ther.* 174: 527, 1970.

Borzelleca, J.F. and Putney, J.W., Jr.: Studies on the biotransformation of salicylic acid by the salivary gland. *Arch. Int. Pharmacodyn.* 188: 127, 1970.

Lowenthal, W., Borzelleca, J.F. and Corder, C.D., Jr.: Drug absorption from the rectum. III. Aspirin and some aspirin derivatives. *J. Pharm. Sci.* 59: 1353, 1970.

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000195

Carmine, E.L., Carchman, R.A. and Borzelleca, J.F.: Investigations into the mechanism of paraquat toxicity utilizing a cell culture system. *Toxicol. Appl. Pharmacol.* 58: 353, 1981.

Simon, G.S., Borzelleca, J.F. and Dewey, W.L.: Narcotics and diabetes II. Streptozotocin-induced diabetes selectively alters the potency of certain narcotic analgesics. Mechanism of diabetes: morphine interaction. *J. Pharmacol. Exp. Ther.* 218: 324, 1981.

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Evaluation of the health aspects of butylated hydroxytoluene as a food ingredient. 1973.

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Evaluation of the health aspects of agar-agar as a food ingredient. 1973.

Evaluation of the health aspects of certain red and brown algae as food ingredients. 1973.

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Evaluation of the health aspects of licorice, glycyrrhiza and ammoniated glycyrrhizin as food ingredients. 1974.

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Evaluation of the health aspects of dextran as food ingredients. 1975.

Evaluation of the health aspects of calcium oxide and calcium hydroxide as food ingredients. 1975.

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Evaluation of the health aspects of certain calcium salts as food ingredients. 1975.

Evaluation of the health aspects of glycerin and glycerides as food ingredients 1975

Evaluation of the health aspects of dextrin and corn dextrin as food ingredients. 1975.

Evaluation of the health aspects of sodium thiosulfate as a food ingredient. 1975.

Evaluation of the health aspects of gelatin as a food ingredient. 1975.

Evaluation of the health aspects of bile salts and ox bile extract as food ingredients. 1975.

Evaluation of the health aspects of choline chloride and choline bitartrate as food ingredients. 1975.

Evaluation of the health aspects of aluminum compounds as food ingredients. 1975.

Evaluation of the health aspects of tallow, hydrogenated tallow, stearic acid, and calcium stearate as food ingredients. 1975.

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Evaluation of the health aspects of hydrogenated fish oil as a food ingredient. 1975.

Evaluation of the health aspects of beeswax (yellow or white) as a food ingredient. 1975.

Evaluation of the health aspects of inositol as a food ingredient. 1975.

Evaluation of the health aspects of malic acid as a food ingredient. 1975.

Evaluation of the health aspects of Japan Wax as a substance migrating to food from cotton or cotton fabrics used in dry food packaging. 1976.

Evaluation of the health aspects of carnauba wax as a food ingredient. 1976.

Evaluation of the health aspects of sulfamic acid as it may migrate to foods from packaging materials. 1976.

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Evaluation of the health aspects of gum guaiac as a food ingredient. 1976.

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Evaluation of the health aspects of tall oil as it may migrate to foods from packaging materials. 1976

Evaluation of the health aspects of corn sugar (dextrose), corn syrup and invert sugar as food ingredients. 1976.

Evaluation of the health aspects of sucrose as a food ingredient. 1976.

Evaluation of the health aspects of sulfiting agents as food ingredients. 1976.

Evaluation of the health aspects of glycerophosphates as food ingredients. 1976.

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Evaluation of the health aspects of pyridoxine and pyridoxine hydrochloride as food ingredients. 1977.

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Evaluation of the health aspects of coconut oil, peanut oil, and oleic acid as they migrate to food from packaging materials, and linoleic acid as a food ingredient. 1977.

Evaluation of the health aspects of pectin and pectinates as food ingredients. 1977.

Evaluation of the health aspects of tannic acid as a food ingredient. 1977.

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Evaluation of the health aspects of sodium oleate and sodium palmitate as substances migrating to food from paper and paperboard used in food packaging. 1977.

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Evaluation of the health aspects of citric acid, sodium citrate, potassium citrate, calcium citrate, ammonium citrate, triethyl citrate, isopropyl citrate, and stearyl citrate as food ingredients. 1977.

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Evaluation of the health aspects of caffeine as a food ingredient. 1978.

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Evaluation of the health aspects of nickel as a food ingredient. 1979.

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Evaluation of the health aspects of sodium chloride and potassium chloride as food ingredients. 1979.

Evaluation of the health aspects of certain silicates as food ingredients. 1979.

Evaluation of the health aspects of manganous salts as food ingredients. 1979.

Evaluation of the health aspects of copper gluconate, copper sulfate, and cuprous iodide as food ingredients. 1979.

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Evaluation of the health aspects of hydrochloric acid as a food ingredient. 1979.

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Evaluation of the health aspects of potassium acid tartrate, sodium potassium tartrate, sodium tartrate and tartaric acid as food ingredients. 1979.

Evaluation of the health aspects of starter distillate and diacetyl as food ingredients. 1980.

Vitamin A, Vitamin A Acetate, and Vitamin A Palmitate as food ingredients. 1980.

Evaluation of the health aspects of iron and iron salts as food ingredients. 1980.

Evaluation of the health aspects of protein hydrolyzates as food ingredients. 1980.

Evaluation of the health aspects of collagen as a food ingredient. 1981.

Evaluation of the health aspects of methyl polysilicones as food ingredients. 1981.

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Evaluation of the health aspects of soya fatty acid amines as food ingredients. 1981.

Evaluation of the health aspects of activated carbon (charcoal) as a food processing aid. 1981.

Evaluation of the health aspects of smoke flavoring solutions and smoked yeast flavoring as food ingredients. 1981.

Evaluation of the health aspects of commint oil as a food ingredient. 1981.

Evaluation of the health aspects of a mixture. Evaluation of the health aspects of diferrous, dipotassium ferrous, and potassium ferrocyanides as finding agents in wine production. 1981.

Evaluation of the health aspects of wheat gluten, corn gluten, and zein as food ingredients. 1981.

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National Research Council, National Academy of Sciences
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Drinking Water and Health.
Safe Drinking Water Committee, Board on Toxicology and Environmental Health Hazards, Assembly of Life
Sciences, National Research Council, National Academy of Sciences
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National Academy Press, Washington, D.C.

Estimating Consumer Exposure to Food Additives and Monitoring Trends in Use.

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Food Additives Survey Committee, Food and Nutrition Board, Institute of Medicine, National Academy of Sciences
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Pariza, M.W., Borzelleca, J.F., Cassens, R.G., Filer, L.J., and Kritchevsky, D.,
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 1999- Graduate Faculty, University of Massachusetts Amherst, Amherst, MA

Publications: (Selected from 100+ Publications)

- Cladaras C, Hadzopoulou-Cladaras M, Avila R, Nussbaum AL, Nicolosi RJ, Zannis VI. *Complementary DNA derived structure of the amino-terminal domain of human apolipoprotein B and size of its messenger RNA transcript.* Biochemistry (1986) 25:5351-5357
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000218

SUBMISSION END

000219



Acidulants

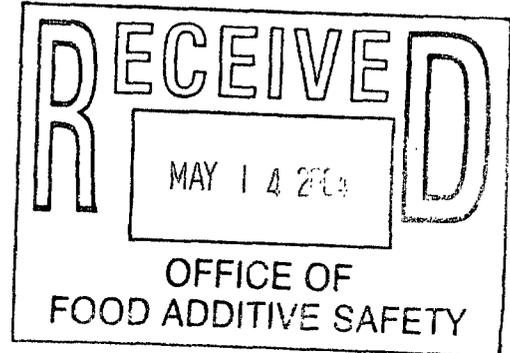
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04-04906P978129PA1N

May 10, 2004

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



Re: Clarification on Food Categories to Notification of GRAS Determination for REGENASURE™ Glucosamine Hydrochloride.

Dear Sir or Madam:

Cargill, Incorporated submitted a Notification to the Food and Drug Administration (FDA) that REGENASURE™ Glucosamine Hydrochloride is Generally Recognized As Safe (GRAS) on April 6, 2004. A food category that was included in the Expected Daily Intake (EDI) calculation was inadvertently left out of the list food categories in Table 7. An additional food category should be added to this list, specifically 21 *Code of Federal Regulations* (C.F.R.) 21 § 170.3 (n) (35), "Processed Fruits and Fruit Juices". This category includes all "commercially processed fruits, citrus, berries, and mixtures; salads, juices and juice punches, concentrates, dilutions, "ades", and drink substitutes made therefrom". To reiterate, there have been no changes to the EDI calculation, Expert Panel opinion, or the GRAS Notification other than the aforementioned food category.

A revised copy of page 21 of the GRAS Notification is provided. Also, a letter of clarification from Exponent is included. We apologize for any confusion or inconvenience this may have caused.

Please contact me at the number above if you have any questions regarding the information and conclusions in this Notification.

Sincerely,

Brent D. Rogers, Product Applications Chemist

000222

REGENASURE™ Glucosamine Hydrochloride. Additional exposure to glucosamine hydrochloride as a dietary supplement might occur, with a typical recommended dose of 1,500 mg/day. If you add this additional exposure of 1,500 mg to 3,022 mg the total would be 4,522 mg/day of product for an average 70 kg adult. This calculates out (4,522 mg/day divided by 70 kg bw) to be 65 mg/kg bw/day, which is well below the recommended ADI of 184 mg/kg bw/day (Section 6.6.4). These calculated intake values are conservative because they assume that 100% of the products ingested by the consumer contain REGENASURE™ Glucosamine Hydrochloride at the proposed level, which is not likely to occur. Also, from the EDI analysis, it appears that the highest exposure occurs in the age group of 1-6 year olds, which is an age group that is not a target for product marketing, nor would normally be considered as having an interest in the benefits of glucosamine hydrochloride consumption. The exposure for this age group for the “per user,” 90th percentile category is 98.431 mg/kg bw/day, which would be well under the recommended ADI of 184 mg/kg bw/day (Section 6.6.4).

The food categories for the beverages selected for this study can be found in the USDA’s Continuing Survey of Food Intakes by Individuals and the US FDA 21 Code of Federal Regulations section 170.3. These food categories are listed in Table 7. The list of proposed foods for REGENASURE™ Glucosamine Hydrochloride can be found in Table 8.

Table 7. List of Food Categories for Proposed Foods

<i>21 CFR § 170.3 broad food categories</i>	<i>USDA’s CSFII Food categories</i>
(16) Fresh fruits and fruit juices	Citrus Fruit Juices
(3) Beverages and bases, non alcoholic	Fruit juices and nectars excluding citrus
(35) Processed fruits and fruit juices	All juices & juice products
(36) Vegetable and vegetable juices	Tomato and tomato mixtures
(7) Coffee and tea	Non-alcoholic beverages
(31) Milk products*	Flavored milk & milk drinks, fluid

*only one product from this category

000223

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Reply to the U.S. Office

May 10, 2004

Janet Paulson
Research Chemist
Cargill
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Subject: Clarification on Food Categories: Glucosamine Hydrochloride EDI
Project No. WD00741.000

Dear Janet:

It is our pleasure to confirm the fruit juices and drinks included in the estimated daily intake (EDI) calculations for Glucosamine Hydrochloride submitted in the April 6, 2004 Notification to the FDA. Exponent's estimation of the EDI included juice of various strengths (<10% to 100%). Table 7 in your GRAS submission implied that juices in the 21CFR170.3(n) (35) ("Processed Fruits and Fruit juices, including all commercially processed fruits, citrus, berries, and mixtures; salads, juices and juice punches, concentrates, dilutions, "ades", and drink substitutes made therefrom") were not represented in the EDI estimate; however, this is not the case. An example of a food included in the intake assessment that would fall into this FDA category is "Fruit drink (include fruit punch & fruit-ade)". As explained above, the juices and juice mixtures selected by Cargill for the analysis included ades and drinks.

Sincerely,

Dr. Nancy Rachman, Ph.D.
Senior Managing Scientist

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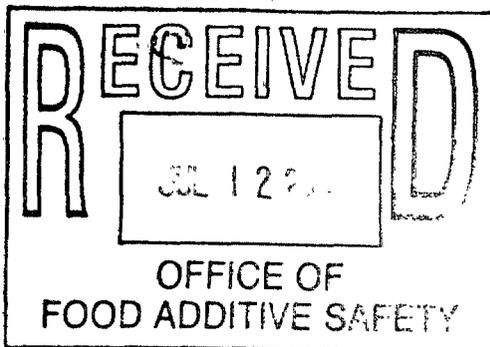
Computer Technology Services, Inc.

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Acidulants

1 Cargill Drive
Eddyville, IA 52553-5000
Tel: 641-969-3896
Fax: 641-969-3850



July 7, 2004

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

Re: Food and Drug Administration Questions on GRN 150 for Glucosamine

Dear Sir or Madam:

Cargill, Incorporated (hereafter "Cargill") submitted a Notification to the Food and Drug Administration (FDA) that REGENASURE™ Glucosamine Hydrochloride is Generally Recognized As Safe (GRAS) on April 6, 2004. On June 9, 2004 Cargill received a request by electronic mail for some additional information from Karin Ricker at the FDA. After careful consideration and further study, Cargill is now happy to provide the information requested.

Please contact me at the number above if you have any questions regarding the information.

Sincerely,

Brent D. Rogers, Product Applications Chemist

GRAS Notice 150:
Response to FDA Questions on Glucosamine GRAS Notification

Toxicology

1. *Cargill states that the endogenous production of glucosamine (GlucN) is in the range of 4-20 g/day (median, 14 g/day). They cite a personal communication (Hart, 2003) and three other publications on page 28 of the notice as the source of this information. These three reports they cite are about glycosylation of proteins by O-linked β -N-acetylglucosamine and its relation to mechanisms of insulin resistance, i.e. do not provide this information. In light of this, Cargill shall provide details on how they arrived at the 14 g/day figure.*

The endogenous production of glucosamine should be considered when making comparisons to glucosamine administration. No reports of actual measurement of endogenous glucosamine production in humans could be identified. Two experts-- Drs. Hart¹ (1-3) and Fukagawa² (4)-- were queried about this issue. They confirmed these general principles to James W. Anderson, MD by e-mail. Many experts in carbohydrate metabolism have indicated that 2-5% of carbohydrate entering the glycolytic pathway enters the hexosamine biosynthetic pathway (5,6,7). In Echard et al it is taught that "The hexosamine pathway normally accounts for approximately 2% of total cellular glucose flux,"(7) and Heart et al provides that "The hexosamine biosynthetic pathway is a minor glucose metabolic pathway that metabolizes ~3% of glucose entering the cell"(6). Virtually all of the ingested available carbohydrates—sugars and polysaccharides—are metabolized by the glycolytic pathway. In the GRAS Submission (p. 28) we estimated the endogenous production of glucosamine as follows:

"If women consume 2000 kcal/day and men consume 2500 kcal/day and 50% of this is carbohydrate, then average carbohydrate intake is 50% of 2250 kcal or 282 grams. We used a range of carbohydrate intake of 200-350 grams per day. At 200 grams of carbohydrate intake daily, endogenous production—2-5%-- would be 4-10 grams; at 350 grams the figure would be 7-17.5 grams/day. We selected 14 grams as a reasonable estimate."

Probably 10 grams is a better estimate of endogenous glucosamine production. This is calculated as 3.5% of 282 grams carbohydrate intake. Since only 20% of orally administered glucose enters the circulation, the usual dose of oral glucosamine would deliver <300 mg of glucosamine to tissues, a figure that is 3% (300 mg/10 g) of estimated endogenous production. Intake of the ADI of 184 mg/kg would deliver <25% of endogenous glucosamine production to tissues.

2. *Cargill has provided ADME information from rats, dogs and humans stating that about 90% of the ingested GlucN is absorbed, but only 20% is measured in the serum/blood. What percentage of the oral dose reaches circulation (including GlucN bound to plasma proteins and blood cells)? What happens to the 90% GlucN that reaches the liver? This has not been discussed adequately in the notice.*

¹ Hart, Gerald W., DeLamar Professor and Director, Department of Biological Chemistry, Johns Hopkins University School of Medicine, Baltimore, MD

² Fukagawa, Naomi, Associate Professor of Medicine, University of Vermont School of Medicine

GRAS Notice 150:

Response to FDA Questions on Glucosamine GRAS Notification

The Setnikar, I, Giachetti, C, and G. Zanolo. *Pharmatherapeutica* 3:#8, 538-550 (1984) paper referenced in the Notification can be used to support this expanded explanation. The figure on page 544 describes the radioactivity in plasma and red blood cells after oral administration of [14-C glucosamine sulfate] in which 44 million dpm/kg was administered. Assuming a rat weight of 200 grams, that would be about 8,000,000 dpm/animal. Figure III suggests that up to 8 hours, radioactivity in plasma is @ 24,000dpm/ml and 10,000 dpm/ml in red blood cells (RBC). In plasma, the 24,000 drops to @ 2,400 dpm/ml after 150 hours, and in RBC the 10,000 drops to 2,400 dpm/ml. Blood volume in the rat is about 4% of its body weight, hence the rat at 200 grams has 8 mls of blood.

Therefore up to 8 hours, in plasma $24,000/\text{ml} \times 8 \text{ ml} = 192,000$ / $8,000,000 \times 100 = 2.4\%$ of the original dose entering into circulation. After 150 hours, its $(2400/\text{ml} \times 8 \text{ ml}) / 8,000,000 \times 100 = 0.24\%$ in the plasma. In the RBC $(10,000/\text{ml} \times 8 \text{ ml}) / 8,000,000 \times 100 = 1.0\%$ end up in RBC up to 8 hours, and 0.24% after 150 hours. These numbers are all derived from reading off the curve so they are only estimates. The glucosamine which reaches the liver can be incorporated into plasma proteins or other proteins or a large part of it (up to 81%) is recovered as carbon dioxide indicating significant metabolism of glucosamine representing probably the fraction of GI utilized as $-\text{NH}_2$ donor substrate and possibly as an energy source. In a review article by Setnikar, I. and Rovati, L.C. Absorption, Distribution, Metabolism and Excretion of Glucosamine Sulfate. *Arzneim.-Forsch/Drug Res* 51 (II) 699-725 (2001), studies in rats after oral administration of GS showed that the greatest concentration of radioactivity was in the liver reaching a peak at 4 h post dosing. The concentration of radioactivity in the liver was anywhere from 2 to 10 times higher than in the plasma depending upon the concentration of the oral dose. This great concentration in the liver probably reflected the involvement of this organ in the elimination of GI, mainly by biotransformation. The liver is also the principal organ for incorporation of GI into plasma proteins independent of all investigated routes of administration (intravenous, intramuscular and oral). Therefore, some of the glucosamine reaching the liver can be (1) transformed into plasma proteins, (2) used as a source of energy as indicated by the amount CO_2 generated and (3) used as a NH_2 substrate.

3. *Cargill estimates an exposure of up to 98 mg/kg/day and an ADI of 184 mg/kg-bw/day. Is this level of exposure safe for diabetics given the existence of literature implicating GlucN in adverse effects in glucose homeostasis? What about in patients with rheumatoid arthritis and other inflammatory diseases of the joint?*

Based on *in vivo* and *in vitro* studies, especially in rodents, indicating that glucosamine may affect glucose metabolism, concerns about glucose effects in diabetic humans are reasonable. However, blood levels of glucosamine in the animal studies were 100 to 2000 times higher than levels that would be expected after oral administration of glucosamine in humans (see GRAS submission, p. 28). The studies of Monauni and colleagues(8) and Pouwels and colleagues(9) showed no effects of intravenous infusion of very large doses (10-30 grams) of glucosamine on blood glucose levels in humans. Two recent studies in humans indicate that oral glucosamine administration did not affect insulin sensitivity in non-diabetic humans (10) or blood glucose or hemoglobin A1c levels in diabetic individuals.(11) In clinical trials, blood glucose levels decreased slightly with oral glucosamine administration; in 9 trials of

GRAS Notice 150:

Response to FDA Questions on Glucosamine GRAS Notification

336 patients there was no significant effect of oral glucosamine administration on blood glucose levels.(Table 12, p. 42) In the entire group of 32 clinical trials of 3073 older patients at fairly high risk for developing diabetes, two patients on glucosamine and three patients on placebo developed diabetes. (p. 41) In our extensive review we were unable to find any evidence that oral administration of glucosamine to humans or rodents leads to abnormalities of glucose metabolism. Thus, we do not feel that oral glucosamine administration increases risk for diabetes in humans.

Glucosamine is clinically indicated for management of osteoarthritis or degenerative joint disease. It decreases pain and protects the joints from further damage. However, it does not have documented anti-inflammatory properties. Thus, it would not be indicated for use in persons with rheumatoid arthritis or other inflammatory joint diseases. We do not have evidence, nor have reason to believe, that it would pose a hazard for persons with rheumatoid arthritis.

4. *Cargill describes an unpublished short-term toxicity studies (Glaza, 2002) using its GlucN product (p. 30 of the notice). Can Cargill provide a copy of the study report?*

Cargill, Incorporated is happy to provide the Glaza study, please see the provided report.

5. *The table summarizing animal studies (Table 9 on page 31) is not clear in light of the absence of a detailed discussion of the studies in the notice. How were the studies designed? What endpoints were examined? What were the results and conclusions? Are the citations from published or unpublished studies? Was the table supposed to give LD₅₀ values?*

See the revised Table 9, which includes additional information.

6. *How did the notifiers estimate the serum level of glucosamine for the oral ingestion? It appears that they have used the IV result for estimation (page 33, 1st paragraph of the notice). If so, how did they do it?*

Only limited data are available so we imputed some estimates. The studies of Monauni et al.(8) and Pouwels et al.(9) provide the best information. Baseline levels for subjects not taking glucosamine are reported to be 0.04 mmol/l by Monauni et al.(8), <0.07 mmol/l (undetectable) by Pouwels et al.(9) and <10 ug/l (undetectable) by Setnikar et al.(12) Based on this information, we used the baseline values for persons not on glucosamine supplements as 0.04 mmol/l.(8) Setnikar et al.(12) report that administration of 6 grams of glucosamine did not produce a measurable increase in serum glucosamine values indicating that this amount did not raise levels above 10 ug/l. Later Setnikar reported that oral administration of 7.5 grams of glucosamine did not increase serum levels into the detectable range (3 ug/ml).(13)

With intravenous infusion of 9.7 grams of glucosamine over 6 hours, steady state serum levels of 0.65 mmol/l are achieved; with infusion of 30.4 grams over 6 hours, steady state serum levels of 1.42 are achieved. These doses represent the amount of free base glucosamine that was administered. Oral administration of 500 mg glucosamine three times

000231

GRAS Notice 150:

Response to FDA Questions on Glucosamine GRAS Notification

daily might achieve steady state levels equivalent to intravenous administration of 300 mg since blood levels after oral administration are only 20% of those after intravenous administration. Using these three points (0.04, 0.65 and 1.42 mmol/l), we used a regression equation to calculate the serum levels for administration of 300 mg glucosamine. This best-fit regression value for serum glucosamine concentration was 0.06 mmol/l (see attached figure). To estimate the serum glucosamine value after administration of the ADI (184 mg/kg), we calculated the value of free base glucosamine that would be administered as 138 mg/kg (see line 1, p. 44 of GRAS submission); this represents ~9.0 grams of free base glucosamine for an adult. If the serum levels are 20% of those for intravenous administration, this would represent oral administration of about 1.8 grams of free base glucosamine. The best regression fit for oral administration of 1.8 grams of free base glucosamine was 0.17 mmol/l (see attached figure).

7. *On page 36, Cargill discusses in vivo genotoxicity data from literature as well as the notifier's laboratory. How do they reconcile contradictory results from the in vivo genotoxicity reports? On the same subject, on page 37, Cargill states "the high dose of 2000 mg/kg) selected for this (in vivo micronucleus assay) study was based on relevant acute toxicity information (Glaza, 2002)". Can Cargill explain what they mean by "relevant acute toxicity information"? Can Cargill provide us the study report on this mouse in vivo micronucleus assay?*

In the studies by Banerjee (1984) and Manna (1985)*, the glucosamine was administered intraperitoneally. This is an inappropriate route to evaluate a food ingredient that is ingested. REGENASURE™ glucosamine was administered orally (by gavage), a route of exposure that simulates the route of human exposure, so it was the correct route. Since the Cargill study was with the product pertaining to GRN 150, and since the route of administration was the more appropriate one, this study is also the more meaningful one. It is not known if Banerjee followed any approved guidelines (for example, OECD), and exotic fish may not be the most appropriate model for mammalian systems. Therefore, we believe the Cargill study is the more definitive study because it tested the product that the expert panel has evaluated in this GRAS Notification, and the route of administration was the oral route and the study design followed approved guidelines. The Banerjee study may not have followed approved guidelines and it involved a less appropriate route of administration.

*When this was documented in the original bibliography of the Notification, it was incorrectly dated 1984

OECD Guideline 474 lists a limit dose of 2000 mg/kg bw for the mouse micronucleus test. We recognize that OECD is not a regulatory agency, so we should have said "and is the maximum allowable dose, the limit dose, based on internationally accepted guidelines (OECD 474)."

Cargill, Incorporated is happy to provide the mouse *in vivo* micronucleus assay. Please see report provided.

8. *Human clinical case reports. Disparate clinical data has been used by Cargill for meta-analysis without a discussion on how the clinical trials support the safety of GlucN in food. Which of the studies are important in supporting safety? Which clinical parameters and adverse effects data were used?*

000232

GRAS Notice 150:
Response to FDA Questions on Glucosamine GRAS Notification

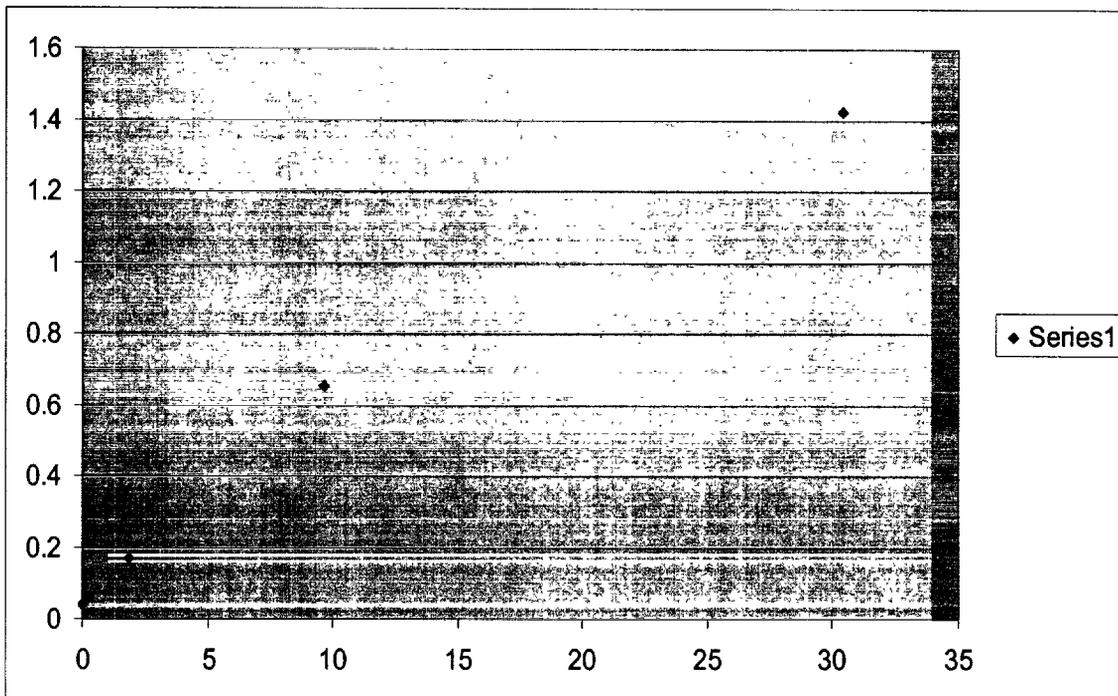
All of the human exposure data addresses the safety in humans. The clinical trial data for 3073 patients representing 979 patient years of exposure (p. 39, GRAS submission) represents an enormous safety trial. While the experimental design differences limit full interpretation of efficacy, these data represent the number of patients who were receiving glucosamine in trials for up to three years. This far exceeds the human exposure data for the vast majority of drugs approved by the FDA. Table 12 (p. 42, GRAS submission) gives the safety outcome measures. For specific safety measures the median number of patients was 663 with 587 patient years. The safety data included these assessments: clinical assessment of symptoms and adverse events; heart rate and blood pressure; serum measurements of nutrition, renal function, and liver function; complete blood counts for red cells, white cells and platelets; urinalyses; and fecal occult blood measurements.

9. *On transport of GluN and glucose by glucose transporters: Cargill provides this information on p. 28 and argues on p. 48 that "the concentration of GlucN in most cells will be lower than that in plasma". The work on page 28 (Uldry 2002) only states that the Vmax values for transport of GluN by GLUT1, 2 and 4 is lower than for glucose. However, the study reports that the Km (an indicator of the affinity that an enzyme/transporter has for a given substrate) for GlucN and glucose of GLUT1 and GLUT4 are similar, while GLUT2 (the main transporter of GluN in hepatocytes) has about 20-fold higher affinity for GlucN than glucose.*

Translation of Km and Vmax data into actual transport estimates is a challenge. With usual doses of glucosamine, serum concentrations of glucosamine will be approximately 0.06 mmol/l while glucose concentrations will be 5-7 mmol/l (fasting and postprandial) or almost 100-fold higher. If the Km for glucosamine were not much lower, glucosamine levels would never approach Km concentrations and no glucosamine would be transported. So the 20-fold higher affinity of GLUT2 for glucosamine than for glucose may be required to permit any glucosamine to enter the cells.(14) Because GLUT1 and GLUT2 have similar apparent affinities for glucosamine and glucose and the GLUT4 transported has only a 20-fold higher affinity for glucosamine and glucose, and because glucosamine concentrations in serum are only 1% of glucose concentrations, it is hard to argue that glucosamine will be concentrated in the cells—i.e. that there will be a much higher concentration in the cell than in the plasma.

Calculations of serum concentrations for glucosamine: Known values are baseline, 0.04 mmol/l, after intravenous infusion of 9.7 grams glucosamine, 0.65 mmol/l, and after intravenous infusion of 30.4 grams glucosamine, 1.42 mmol/l. We used regression analysis to estimate the values for oral administration of 1.5 grams glucosamine (equivalent to 0.3 grams intravenously) and 9.0 grams (equivalent to 1.8 grams intravenously).

GRAS Notice 150:
Response to FDA Questions on Glucosamine GRAS Notification



Grams of glucosamine equivalent to intravenous dose.

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GRAS Notice 150:
Response to FDA Questions on Glucosamine GRAS Notification

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2. Wells L, Vosseller K, Hart GW. Glycosylation of nucleocytoplasmic proteins: signal transduction and O-GlcNAc. *Science* 2001;291:2376-8.
3. Wells L, Vosseller K, Hart GW. A role for N-acetylglucosamine as a nutrient sensor and mediator of insulin resistance. *CMLS.Cell Mol Life Sci* 2003;60:1-7.
4. Fukagawa NK, Anderson JW, Young VR, Minaker KL. High-carbohydrate, high-fiber diets increase peripheral insulin sensitivity in healthy young and old adults. *Am J Clin Nutr* 1990;52:524-8.
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7. Echard BW, Talpur NA, Funk KA, Bagchi D, Preuss HG. Effects of oral glucosamine and chondroitin sulfate alone and in combination on the metabolism of SHR and SD rats. *Molecular and Cellular Biochemistry* 2001;225:85-91.
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13. Setnikar I, Rovati LC. Absorption, distribution, metabolism and excretion of glucosamine sulfate. a review. *Arzneimittel-Forschung (Drug Res)* 2001;51:699-725.
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GRAS Notice 150:
Response to FDA Questions on Glucosamine GRAS Notification

Chemistry

- 1) *The notifier provides a heavy metal specification for glucosamine HCl, in accordance with USP-NF methodology. Please provide a specification for lead. (Note: analytical results for lead are provided in the notice, but there isn't a lead specification in the notice).*

We meet the Glucosamine Hydrochloride USP-NF specifications for heavy metals measured as lead. This analysis is a qualitative test that uses a lead standard to compare our product to. Our internal specification for lead is 0.5ppm. This is a quantitative test measured by Inductively Coupled Plasma-Mass Spectrometer (ICP- MS).

- 2) *The notifier provides analytical results for pathogenic microorganisms. Please provide specifications for these pathogens.*

The specifications for these microorganisms were established internally based on customer needs, and are listed in the table below:

Lot Number	Total Plate Count (cfu/g)	Coliform MPN method (MPN/g)	Coliform confirmation (MPN/g)	E. coli MPN method (MPN/g)	Yeast & Molds (cfu/g)	Salmonella (in 25 g)
<i>Cargill Internal specification</i>	<10 cfu/g	<3 MPN/g	<3 MPN/g	<3 MPN/g	<10 cfu/g	Negative

- 3) *The notifier states that silica is present at approximately 145 ppm in the glucosamine HCl final product (page 16 of notice). What is the source of this contaminant?*

This glucosamine process includes some glass-lined vessels, which is the source of the silica, as no other silica source exists in the process. We believe that silica is not a health concern, as silicates are commonly used in foods as anti-caking agents. A HACCP food safety plan is in place to monitor food hazards, such as a glass chip.

- 4) *Please identify the two organic acids that were mentioned under section 3.1 (page 16 of the notice).*

Citric acid and levulinic acid are the two organic acids mentioned (but not listed) under section 3.1 of the notice. Citric acid has GRAS status and is found in a variety of foods such as soda, candy and fruit drinks. Levulinic acid is a soluble by-product of our process. It is also found on the EAFUS (Everything added to Food in the US) list maintained by the FDA Center for Food Safety and Applied Nutrition. These were removed from the Notification only as a confidentiality concern, as Cargill, Incorporated believes this is information that should be kept proprietary in order to protect our product and process.

GRAS Notice 150:
Response to FDA Questions on Glucosamine GRAS Notification

Microbiology

What is the source of the organism?

Cargill, Incorporated's strain of *Aspergillus niger* is a non-GMO, internally developed and proprietary strain for food-grade citric acid production.

000237

000238

Revised Table 9. Animal Toxicity Data for Oral Administration of Glucosamine

Species	Strain	Sex	Route of Administration	Dose mg/kg/day	Duration, days	Description	Study
mice	CD-1	Males and Females	gavage	>8000	acute	LD ₅₀ for oral administration; no toxicity, no necropsy abnormalities	Leuschner q. by (Setnikar, Pacini, & Revel 1991)
Rats	Sprague Dawley	Males and Females	gavage	8000	acute	LD ₅₀ for oral administration; no toxicity observed, no necropsy abnormalities	Leuschner q. by (Setnikar, Pacini, & Revel 1991)
rabbits		Males and Females	gavage	>6000	acute	LD ₅₀ for oral administration; no toxicity, no necropsy abnormalities	Leuschner q. by (Setnikar, Pacini, & Revel 1991)
mice			gavage	>5000	acute	LD ₅₀ estimation	Sigma-Aldrich, 2001
mice			gavage	5000	acute	LD ₅₀ estimation, no mortality at this dose	Senin, Makovec, & Rovati 1987
rats		Males and Females	gavage	5000	acute	*GCI: no test material-related effects. No macroscopic findings at necropsy examination. NOEL = 5000mg/kg	Glaza, 2002 unpublished study
rats	Sprague Dawley	Males	Diet	5000	60	*GTT unchanged, no toxicity, serum triglycerides increased	Echard, Talpur, Funk, Bagchi, & Preuss 2001
rats	SHR	Males	Diet	5000	60	decrease in blood pressure	Echard, Talpur, Funk, Bagchi, & Preuss 2001
rats	Sprague Dawley	Males	gavage	2700	365	No histopathology; 7 vs 2 premature deaths (not clinically significant)	Leuschner q. by (Meininger et al. 2000)
rats	D/ART ¹ ^{av1}	Females	Diet	2500	52	GCI: no adverse effects	Beren, Hill, Diener-West, & Rose 2001
dogs			gavage	2149	183	no clinical, laboratory or histopathology abnormalities	Neumann q. by (Setnikar, Cereda, Pacini, & Revel 1991)
rabbits	White Danish Country	Males	Diet	1500	84	*GCI; no significant cholesterol effects	Stender & Astrup 1977
rabbits	White Danish Country	Males	gavage	1000	acute	single oral dose, no glucose changes	Stender & Astrup 1977
dogs	Beagles		*P.O.	200	30	2 capsules twice daily; no histopathology	McNamara, Barr, & Erb 1996
rats			Diet	60	12	normal growth rate, low doses; no toxicity; decreased growth rate at high doses in weanlings	Sugimura, Birnbaum, Winitz, & Greenstein 1959
horses			Diet	22	42	no adverse effects reported	Hanson, Paterson, & Hart 1997
horses			*P.O.	21	336	*GCI; no adverse effects	Caron, Peters, Hauptman, Eberhart, & Orth 2002
horses			Diet	18	56	no adverse effects reported	Fenton, Orth, Chlebek-Brown, Nielsen, Corn, Waite, & Canon 1999

*Glucosamine HCl indicated by GCI. GTT is glucose tolerance test. P.O. is per os (oral).

000238

Final Report

Study Title	<i>In Vivo</i> Mouse Micronucleus Assay with Cargil; Glucosamine Hydrochloride
Author	Gregory L. Erexson, PhD, DABT
Sponsor	Cargill, Inc. Cargill Acidulants, R&D 1 Cargill Drive Eddyville, Iowa 52553
Test Facility	Covance Laboratories Inc. 9200 Leesburg Pike Vienna, Virginia 22182-1699
Covance Study Number	7178-125
Genetic Toxicology Assay Number	24944-0-455OECD
Report Issued	22 December 2003
Page Number	1 of 19

000239

QUALITY ASSURANCE STATEMENT

QUALITY ASSURANCE STATEMENT

This report has been reviewed by the Quality Assurance Unit of Covance Laboratories Inc. and accurately reflects the raw data. The following inspections were conducted and findings reported to the study director (SD) and associated management.

Inspection Dates		Phase	Date Reported to SD and SD Management
From	To		
09 Jun 2003	09 Jun 2003	Protocol Review	09 Jun 2003
10 Jun 2003	10 Jun 2003	Test Article Administration	11 Jun 2003
07 Aug 2003	07 Aug 2003	Protocol Amendment Review	07 Aug 2003
07 Aug 2003	07 Aug 2003	Draft Report and Data Review	07 Aug 2003
22 Dec 2003	22 Dec 2003	Final Report Review	22 Dec 2003

representative
Quality Assurance Unit

22 Dec 2003
Date

STUDY COMPLIANCE AND CERTIFICATION

Except as noted below, the described study was conducted in compliance with the Good Laboratory Practice regulations as set forth in the Food and Drug Administration (FDA) Good Laboratory Practice Regulations, 21 CFR 58, and any applicable amendments. There were no deviations from the aforementioned regulations or the signed protocol that would affect the integrity of the study or the interpretation of the test results. The raw data have been reviewed by the Study Director, who certifies that the evaluation of the test article as presented herein represents an appropriate conclusion within the context of the study design and evaluation criteria. All test and control results in this report are supported by an experimental data record and this record has been reviewed by the Study Director.

- Exceptions:
- 1) dosing preparations were not analyzed for stability, homogeneity, or concentration.
 - 2) documentation of the stability of the test article was not provided by the Sponsor at the time of this study.

Study Director:

Gregory W. Erickson, PhD, DABT
Genetic and Molecular Toxicology
Covance-Vienna

22 December 2003
Study Completion Date

TABLE OF CONTENTS

	Page No.
ABSTRACT	5
STUDY INFORMATION	6
Sponsor	6
Test Article	6
Assay Information	6
Study Dates	6
Supervisory Personnel	6
OBJECTIVE	6
TEST SYSTEM RATIONALE	6
MATERIALS AND METHODS	7
Animals and Animal Husbandry	7
Justification of Species Selection	8
Test Article	8
Control Articles	8
Dose Rangefinding Assay	8
Micronucleus Assay	8
DATA	10
Data Presentation	10
Assay Acceptance Criteria	10
Assay Evaluation Criteria	10
RESULTS	10
Micronucleus Assay	10
CONCLUSION	11
RECORDS TO BE MAINTAINED	11
REFERENCES	12
DATA TABLES	13
HISTORICAL CONTROL DATA	17
APPENDIX 1: CERTIFICATE OF ANALYSIS	18

ABSTRACT

The objective of this study was to evaluate the test article, Cargill Glucosamine Hydrochloride, for *in vivo* clastogenic activity and/or disruption of the mitotic apparatus by detecting micronuclei in polychromatic erythrocyte (PCE) cells in Crl:CD-1[®](ICR) BR mouse bone marrow.

Based on relevant acute toxicity information provided by the Sponsor, the high dose chosen was 2000 mg/kg, the maximum allowable dose based on regulatory guidelines. In the micronucleus assay, the test article was mixed with cell culture grade water and dosed by oral gavage to six males per dose level at each scheduled harvest timepoint. The dose levels were 500, 1000, or 2000 mg/kg. Five animals dosed with the test article at 500 or 1000 mg/kg and five animals dosed with the positive control article were euthanized approximately 24 hours after dosing for extraction of the bone marrow. Five animals per harvest timepoint dosed with the test article at 2000 mg/kg and five animals per harvest timepoint dosed with the vehicle control article were euthanized approximately 24 or 48 hours after dosing for extraction of the bone marrow. At least 2000 PCEs per animal were analyzed for the frequency of micronuclei. Cytotoxicity was assessed by scoring the number of PCEs and normochromatic erythrocytes (NCEs) in at least the first 500 erythrocytes for each animal.

The test article, Cargill Glucosamine Hydrochloride, did not induce any signs of clinical toxicity in any of the treated animals at up to 2000 mg/kg (the maximum allowable dose based on regulatory guidelines). Cargill Glucosamine Hydrochloride did not induce any statistically significant increases in micronucleated PCEs at any dose level examined (500, 1000, and 2000 mg/kg). In addition, Cargill Glucosamine Hydrochloride was not cytotoxic to the bone marrow (i.e., no statistically significant decrease in the PCE:NCE ratios were observed) at any dose level.

The test article, Cargill Glucosamine Hydrochloride, was evaluated as negative in the mouse bone marrow micronucleus assay under the conditions of this assay.

STUDY INFORMATION

Sponsor
Cargill, Inc.

Test Article

Sponsor's Identification: Cargill Glucosamine Hydrochloride, Lot No. GP-17C;
CAS No. 9012-27-4
Date Received: 29 May 2003
Physical Description: Beige powder
Storage Conditions: Room temperature

Assay Information

Type of Assay: *In Vivo* Mouse Micronucleus Assay
Protocol No.: 455OECD, Edition 3
Covance Study No.: 7178-125
Genetic Toxicology Assay No.: 24944-0-455OECD

Study Dates

Initiation Date: 15 May 2003
Experimental Start Dates:
Animal Receipt: 03 June 2003
Initiation of Dosing: 10 June 2003
Experimental Termination Date: 22 June 2003

Supervisory Personnel

Study Director: Gregory L. Erexson, PhD, DABT
In-life Laboratory Supervisor: Rebecca M. Anthony, BS
Post-life Laboratory Supervisor: George P. Stojhovic, BS

OBJECTIVE

The objective of this study was to evaluate the test article, Cargill Glucosamine Hydrochloride, for *in vivo* clastogenic activity and/or disruption of the mitotic apparatus by detecting micronuclei in polychromatic erythrocyte cells in CrI:CD-1[®] (ICR) BR mouse bone marrow. The assay design was based on OECD Guideline 474, updated and adopted 21 July 1997.

TEST SYSTEM RATIONALE

The micronucleus test can serve as a rapid screen for clastogenic agents and test articles which interfere with normal mitotic cell division (Schmid, 1975; Heddle *et al.*, 1983; Heddle *et al.*,

1991). Micronuclei are small chromatin bodies, consisting of entire chromosomes and/or acentric chromosome fragments, which lag behind at mitotic anaphase. At telophase, these chromosomes and/or fragments are not segregated to either daughter nucleus and form single or multiple micronuclei in the cytoplasm. During maturation of hematopoietic cells from erythroblasts to erythrocytes, the nucleus is extruded. Micronuclei, if present, persist in the cytoplasm of these non-nucleated cells. Detection of micronuclei in non-nucleated cells eliminates the need to search for metaphase spreads in treated cell populations. Test articles affecting spindle-fiber function or formation can be detected through micronucleus induction (Schmid, 1975). In this study, enucleated immature red blood cells or polychromatic erythrocytes (PCEs) were analyzed for the presence of micronuclei.

MATERIALS AND METHODS

Animals and Animal Husbandry

Young adult male and female mice of the Crl:CD-1[®](ICR) BR strain were purchased from Charles River Laboratories, Portage, MI. This is an outbred strain that maximizes genetic heterogeneity and therefore tends to eliminate strain-specific response to test articles. The protocol for this study was approved by the Covance-Vienna IACUC.

The animals were acclimated for at least 5 days before being placed on study. The animals were housed in sanitary polycarbonate cages containing Sani-Chips[®] Hardwood Chip Laboratory bedding. The animals were housed, separated by gender, up to five animals per cage during acclimation, and by full dose group/harvest timepoint after randomization. Each batch of wood chips was analyzed by the manufacturer for specific microorganisms and contaminants. The animals were housed under the following set climatic conditions: temperature, 64 to 79°F; humidity, 30 to 70%; light cycle, 12 hours light/dark which may have been interrupted for study related activities; air changes, at least 10 per hour. A commercial diet, PMI[®] Feeds, Inc. Certified Rodent Diet[®] #5002, and tap water were available *ad libitum*. The feed was analyzed by the manufacturer for concentrations of specified heavy metals, aflatoxin, chlorinated hydrocarbons, organophosphates, and specified nutrients. The water was analyzed biannually, on a retrospective basis, for specified microorganisms, pesticides, heavy metals, alkalinity, and halogens.

The animals were randomly assigned, by a computer program, to study dose groups. Each animal was uniquely identified by ear tag. Treatment groups were identified by cage label. The animals were weighed prior to dosing and dosed based upon the individual animal weights. The body weights of the individual animals were within the range of 20 to 40 grams. The weight variation of the animals did not exceed $\pm 20\%$ of the mean weight of each sex.

Personnel handling the animals or working within the animal facilities were required to wear suitable protective garments and equipment.

Justification of Species Selection

The mouse has been routinely utilized as an animal model of choice for the mammalian bone marrow erythrocyte micronucleus assay.

Test Article

Test Article Analysis. The Sponsor was responsible for the determination and documentation of the identity, strength, purity, stability and uniformity of the test article and the determination of stability, homogeneity and concentration of the dosing preparations. The certificate of analysis is presented in Appendix 1.

Two sets of duplicate samples (2.0 mL each) were taken from the low-, mid-, and high-dose formulations. The samples will be stored at approximately -60 to -80°C until disposition instructions are received from the Sponsor.

Test Article Handling. Prior to dosing, the top stock of the test article, Cargill Glucosamine Hydrochloride, was prepared by adding the appropriate volume of the vehicle, cell culture grade water, to a pre-weighed quantity of the test article and mixed, forming a solution. Lower concentrations were obtained by dilution with the vehicle. The formulations were held at room temperature prior to dosing.

Control Articles

Vehicle Control Article. The vehicle control article for the micronucleus assay was cell culture grade water (BioWhittaker, Lot No. 01100526; expiration date: 10 February 2005, CAS No. 9004-32-4). The same supplier and lot were used throughout the entire study. The vehicle control animals were dosed with the vehicle control by the same route as, and in parallel with, the test article, and in amounts equal to the maximum volumes administered to the experimental animals.

Positive Control Article. Cyclophosphamide (Sigma, Lot No. 91K1176, CAS No. 6055-19-2), the positive control article for the micronucleus assay, was dissolved in deionized water (BioWhittaker, Lot No. 01100526; expiration date: 10 February 2005, CAS No. 9004-32-4) at approximately 8 mg/mL and administered once by oral gavage at approximately 10 mL/kg to achieve a dose level of approximately 80 mg/kg.

Dose Ranging Assay

Since the Sponsor had relevant acute toxicity information for the test article, a dose ranging assay was not performed.

Micronucleus Assay

Based on Sponsor information, only males were used in the micronucleus assay. The high dose, unless non-toxic, should have produced some indication of toxicity, e.g., toxic signs, death, or depression of the ratio of PCEs to normochromatic erythrocytes (NCEs). The use of a high dose,

as defined above, increased the likelihood that a weak clastogen could be detected. No doses higher than 2000 mg/kg were tested.

Extraction of Bone Marrow. At the appropriate harvest timepoints, the animals were euthanized by CO₂ inhalation followed by incision of the diaphragm. The hind limb bones (tibias) were removed for marrow extraction from five surviving animals in each treatment and control group. For each animal, the marrow flushed from the bones was combined in an individual centrifuge tube containing 3 to 5 mL fetal bovine serum (one tube per animal). Animals not needed for bone marrow collection were euthanized at the completion of the assay.

Preparation of Slides. Following centrifugation to pellet the tissue, the supernatant was removed by aspiration and portions of the pellet were spread on slides and air-dried. The slides were fixed in methanol, stained in May-Grünwald solution followed by Giemsa, and protected by permanently mounted coverslips. For control of bias, all slides were coded prior to analysis.

Slide Analysis. Slides prepared from the bone marrow collected from five animals per group at the designated harvest timepoints were scored for micronuclei and the PCE to NCE cell ratio. The micronucleus frequency (expressed as percent micronucleated cells) was determined by analyzing the number of micronucleated PCEs from at least 2000 PCEs per animal. The PCE:NCE ratio was determined by scoring the number of PCEs and NCEs observed while scoring at least the first 500 erythrocytes per animal.

The criteria for the identification of micronuclei were those of Schmid (1976). Micronuclei were darkly stained and generally round, although almond- and ring-shaped micronuclei occasionally occurred. Micronuclei were sharp bordered and generally between one-twentieth and one-fifth the size of the PCEs. The unit of scoring was the micronucleated cell, not the micronucleus; thus, the occasional cell with more than one micronucleus was counted as one micronucleated PCE, not two (or more) micronuclei.

The staining procedure permitted the differentiation by color of PCEs and NCEs (bluish-gray and red, respectively).

The historical background frequency of micronucleated cells was expressed as percentage micronucleated cells based on the number of PCEs analyzed. The historical background frequency of micronuclei in the CD-1[®] strain at this laboratory is about 0.0 to 0.4%, which is within the range reported in the published data (Salamone and Mavourmin, 1994).

DATA

Data Presentation

Data are summarized by dose group for the different timepoints and are presented in Table 1. Individual animal data are presented in Tables 2 and 3. Historical control data are presented after the data tables.

Assay Acceptance Criteria

Acceptable Controls. The vehicle control group had less than approximately 0.4% micronucleated PCEs and the group mean was within the historical control range. The positive control group had a statistically significantly higher ($p \leq 0.01$) number of micronucleated PCEs than the vehicle control group and was consistent with historical positive control data.

Acceptable High Dose. Generally the high dose should reach the limit dose or produce some indication of toxicity, e.g., toxic signs and/or mortality in the test article dosed animals and/or a reduction in the PCE:NCE ratio. If there are solubility constraints, the highest dose tested will be the solubility limit or higher doses if a well-dispersed suspension is obtained that does not settle out rapidly.

Assay Evaluation Criteria

Assay data analysis was performed using an analysis of variance (Winer, 1971) on untransformed proportions of cells with micronuclei per animal and on untransformed PCE:NCE ratios when the variances were homogeneous. Ranked proportions were used for heterogeneous variances. If the analysis of variance was statistically significant ($p \leq 0.05$), a Dunnett's t-test (Dunnett, 1955; 1964) was used to determine which dose groups, if any, were statistically significantly different from the vehicle control. Analyses were performed separately for each sampling time.

The criteria for a positive response was the detection of a statistically significant increase in micronucleated PCEs for at least one dose level, and a statistically significant dose-related response. A test article that did not induce both of these responses was considered negative. Statistical significance was not the only determinant of a positive response; the Study Director also considered the biological relevance of the results in the final evaluation.

RESULTS

Micronucleus Assay

Dose Selection. The dose levels used were specified by the Sponsor at 500, 1000, and 2000 mg/kg.

Dosing Information. The animals used in the micronucleus assay were dosed on 10 June 2003. Forty-two animals, approximately 8 weeks old at the time of dosing, with a weight range of

28.0 to 37.3 g, were used in this assay. An outline of the dosing scheme and harvest timepoints is shown in the following table:

Dosing Scheme for the Micronucleus Assay with Cargill Glucosamine Hydrochloride

Target Treatment (mg/kg)	Stock Concentration (mg/mL)	Route of Administration	Dosing Volume (mL/kg)	Animals/Harvest Timepoint ^a	
				24 Hour Male	48 Hour Male
500	50	oral gavage	10	6	-
1000	100	oral gavage	10	6	-
2000	200	oral gavage	10	6	6
Vehicle Control, Cell culture grade water	0	oral gavage	10	6	6
Positive Control, Cyclophosphamide, 80	8	oral gavage	10	6	-

^a Six animals were dosed to ensure the availability of five animals for analysis.

Animal Observations. All animals in all the dose groups appeared normal immediately after dosing and remained healthy until the appropriate harvest timepoints.

Results and Interpretation. The test article, Cargill Glucosamine Hydrochloride, did not induce any signs of clinical toxicity in any of the treated animals at up to 2000 mg/kg (the estimated maximum tolerated dose). Cargill Glucosamine Hydrochloride did not induce any statistically significant increases in micronucleated PCEs at any dose level examined (500, 1000, and 2000 mg/kg). In addition, Cargill Glucosamine Hydrochloride was not cytotoxic to the bone marrow (i.e., no statistically significant decrease in the PCE:NCE ratios were observed) at any dose level.

The positive control, cyclophosphamide, induced statistically significant increases in micronucleated PCEs as compared to that of the vehicle controls, with a mean and standard error of $3.26 \pm 0.44\%$.

CONCLUSION

The test article, Cargill Glucosamine Hydrochloride, was evaluated as negative in the mouse bone marrow micronucleus assay under the conditions of this assay.

RECORDS TO BE MAINTAINED

All raw data, documentation, records, the protocol, and the final report generated as a result of this study will be archived in the storage facilities of Covance-Vienna for at least 1 year following submission of the final report to the Sponsor. After the 1-year period, the Sponsor

may elect to have the aforementioned materials retained in the storage facilities of Covance-Vienna for an additional period of time or sent to a storage facility designated by the Sponsor.

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Winer, B.J., *Statistical Principles in Experimental Design*, Second Edition, McGraw-Hill, New York (1971).

DATA TABLES

Table 1: Micronucleus Data Summary Table

Assay No.: 24944-0-455OECD

Test Article: Cargill Glucosamine Hydrochloride

Date Dosed: 10 Jun 03

Treatment	Dose	Harvest Time	% Micronucleated PCEs	Ratio PCE:NCE
			Mean of 2000 per Animal \pm S.E. Males	Mean \pm S.E. Males
Controls				
Vehicle	Water	24 hr	0.02 \pm 0.01	0.43 \pm 0.07
		48 hr	0.01 \pm 0.01	0.54 \pm 0.09
Positive	CP 80mg/kg	24 hr	3.26 \pm 0.44*	0.47 \pm 0.09
Test Article	500mg/kg	24 hr	0.00 \pm 0.00	0.57 \pm 0.06
		24 hr	0.02 \pm 0.02	0.35 \pm 0.01
	2000mg/kg	24 hr	0.00 \pm 0.00	0.51 \pm 0.04
		48 hr	0.00 \pm 0.00	0.40 \pm 0.06

* Significantly greater than the corresponding vehicle control, $p \leq 0.01$.

CP = Cyclophosphamide

PCE = Polychromatic erythrocyte

NCE = Normochromatic erythrocyte

Table 2: Micronucleus Test - Individual Animal Data

Assay No.: 24944-0-455OECD
 Test Article: Cargill Glucosamine Hydrochloride
 Date Dosed: 10 Jun 03

Treatment		Animal Number	# Mn PCEs/ 2000 PCEs	Ratio PCE:NCE
24 Hour Harvest		Male		
Vehicle Control	Water	9545	0	0.43
		9550	1	0.39
		9553	1	0.29
		9559	0	0.33
		9567	0	0.71
Positive Control	CP 80mg/kg	9551	89	0.61
		9554	43	0.24
		9557	66	0.73
		9564	80	0.37
		9566	48	0.38
Test Article	500mg/kg	9530	0	0.77
		9542	0	0.61
		9543	0	0.55
		9546	0	0.48
		9548	0	0.42
	1000mg/kg	9537	0	0.35
		9552	0	0.34
		9555	2	0.34
		9560	0	0.35
		9561	0	0.37
	2000mg/kg	9531	0	0.49
		9532	0	0.51
		9539	0	0.40
		9540	0	0.63
		9549	0	0.53

CP = Cyclophosphamide
 PCE = Polychromatic erythrocyte
 # MN PCEs = Micronucleated PCEs
 NCE = Normochromatic erythrocyte

Table 3: Micronucleus Test - Individual Animal Data

Assay No.: 24944-0-455OECD
 Test Article: Cargill Glucosamine Hydrochloride
 Date Dosed: 10 Jun 03

Treatment		Animal Number	# Mn PCEs/ 2000 PCEs	Ratio PCE:NCE
48 Hour Harvest	Male			
Vehicle Control	Water	9535	0	0.53
		9536	0	0.61
		9541	1	0.81
		9544	0	0.29
		9547	0	0.45
Test Article	2000mg/kg	9533	0	0.39
		9534	0	0.37
		9538	0	0.26
		9556	0	0.35
		9565	0	0.63

PCE = Polychromatic erythrocyte
 # MN PCEs = Micronucleated PCEs
 NCE = Normochromatic erythrocyte

HISTORICAL CONTROL DATA

Mouse Micronucleus - 1/2002 through 6/2002

		% MICRONUCLEATED PCES FROM 2000 PCES PER ANIMAL MEAN ± S.E. MALES		PCE:NCE RATIO MEAN ± S.E. MALES	
POOLED VEHICLE CONTROLS					
24 hour harvest	Minimum	0.00		0.13	
	Maximum	0.25		1.17	
	Average	0.049±0.004		0.564±0.020	
	N	125		125	
48 hour harvest	Minimum	0.00		0.11	
	Maximum	0.20		1.16	
	Average	0.048±0.005		0.570±0.021	
	N	113		113	
POSITIVE CONTROLS					
Cyclophosphamide 24 hour harvest	Minimum	0.90		0.08	
	Maximum	4.90		1.00	
	Average	2.669±0.085		0.533±0.015	
	N	121		121	

PCE = Polychromatic erythrocyte
 NCE = Normochromatic erythrocyte
 N = Number of animals

**APPENDIX 1:
CERTIFICATE OF ANALYSIS**

Cargill, Incorporated
Worldwide Acidulants
1 Cargill Drive
Eddyville, IA 52553-5000

Certificate of Analyses



D-Glucosamine Hydrochloride

Lot Number GP-017C

Date 9/24/02

PRODUCT DESCRIPTION

Cargill D-Glucosamine Hydrochloride is produced from a natural source and is non-shellfish, non-animal derived.

ANALYTICAL

	<u>Specification</u>	<u>Result</u>
Specific Rotation	+70.0° to +73.0° [α] _D ²⁰ (2.5%, H ₂ O, ≥3.5 hrs)	71.5°
pH	3.0 to 5.0 (20 mg/mL)	3.3
Loss on Drying	<1.0 % by weight	0.6%
Residue on Ignition	<0.1 % by weight	0.02%
Heavy Metals ¹	<0.001 % by weight	<0.001 % by weight
Assay ²	98-102% dry basis	99.4%
Arsenic ¹	≤ 3 ppm	< 0.5 ppm
Sulfate	< 0.24%	< 0.24%
ID – IR spectrum	matches	matches
ID – chloride	meets	meets
ID – hplc retention time	matches	matches

MICROBIOLOGICAL ANALYSIS

Total Plate Count	<1000 cfu/g	<1000 cfu/g
-------------------	-------------	-------------

APPROVED BY _____

1. Heavy metals and arsenic are done by ICP.
2. Assay completed by outside lab using interim method pending completion of USP monograph method validation. Assay is calculated on a dry basis per USP method.

CARGILL CONFIDENTIAL
If you have any questions, please call 1-641-969-3896.

Final Report

Study Title	Acute Oral Toxicity Study Glucosamine Hydrochloride in Rats (EPA/OECD Guidelines)
Author	Steven M. Glaza, BS
Sponsor	Cargill World Wide Acidulants, R&D 1 Cargill Drive Eddyville, Iowa 52553-5000
Test Facility	Covance Laboratories Inc. 3301 Kinsman Boulevard Madison, Wisconsin 53704-2595
Laboratory Study Identification	Covance 7350-100
Report Issued	26 July 2002
Page Number	1 of 46

000258

COMPLIANCE STATEMENT

**Acute Oral Toxicity Study Glucosamine Hydrochloride in Rats
(EPA/OECD Guidelines)**

All aspects of this study were in accordance with the Food and Drug Administration Good Laboratory Practice Regulations for Nonclinical Laboratory Studies as set forth in Title 21 of the United States Code of Federal Regulations, Part 58; with the exception that stability information for the test material was not provided to Covance at the time of study conduct and that the analysis of the test material mixture for concentration, homogeneity/solubility, and stability was not conducted.

Steven M. Glaza, BS
Study Director
Covance Laboratories Inc.

U

Date 26 JUL 02

000259

QUALITY ASSURANCE STATEMENT

This report has been reviewed by the Quality Assurance Unit of Covance Laboratories Inc. The following inspections were conducted and findings reported to the study director (SD) and associated management.

Inspection Dates			Phase	Date Reported to SD and SD Management
Start Date	End Date			
06 May 2002	06 May 2002		Protocol Review	06 May 2002
07 May 2002	07 May 2002		Animal Observation/Scores	08 May 2002
04 Jun 2002	04 Jun 2002		Protocol Amendment Review	04 Jun 2002
11 Jun 2002	12 Jun 2002		Draft Report Review	12 Jun 2002
11 Jun 2002	12 Jun 2002		Data Review	12 Jun 2002
23 Jul 2002	23 Jul 2002		Revised Draft Report Review	23 Jul 2002

Representative
Quality Assurance Unit

26 July 02
Date

000260

KEY PERSONNEL

**Acute Oral Toxicity Study Glucosamine Hydrochloride in Rats
(EPA/OECD Guidelines)**

Sponsor's Representative:	Janet Paulson Cargill World Wide Acidulants, R&D
Study Director:	Steven M. Glaza, BS
Study Toxicologist:	JoAnna Bultman, BA, LAT
Supervisor, Small-Animal Metabolism:	Jeffrey B. Hicks, LAT
Supervisor, Dose Formulations:	Dixie Bushee, BS, LATG
Associate Director, Laboratory Animal Medicine:	Donna J. Clemons, DVM, MS, DACLAM
Supervisor, Postlife Laboratory:	Michele Marggi

000261

CONTENTS

	Page
COMPLIANCE STATEMENT	2
QUALITY ASSURANCE STATEMENT	3
KEY PERSONNEL	4
ABSTRACT	7
STUDY CONDUCT	7
Purpose	7
Protocol Adherence	7
Regulatory Guidelines	7
Study Timetable	7
Major Computer Systems	7
Record Retention	8
TEST MATERIAL	8
Test Material	8
Reserve (Archive) Samples and Disposition	8
TEST SYSTEM	8
Species and Justification	8
Identification and Acclimation	9
Husbandry	9
EXPERIMENTAL DESIGN	9
Animal Selection	9
Rationale for Route of Administration	9
PROCEDURES	10
Dose Preparation	10
Method of Administration	10
Observation of Animals	10
Clinical Observations	10
Body Weights	10
Termination	10
RESULTS AND DISCUSSION	10
Observation of Animals	10
Clinical Observations	10
Body Weights	11
Anatomic Pathology	11
CONCLUSION	11
SIGNATURE	11
COMMENTS ON THE DATA	12

000262

CODES, ABBREVIATIONS, AND UNITS..... 13
 General Codes and Abbreviations..... 14

TABLES..... 15
 Table 1: Mortality Summary 16
 Table 2: Individual Body Weights/Body Weight Gains (g) 17
 Table 3: Individual Clinical Signs..... 18
 Table 4: Incidence of Macroscopic Observations 19

APPENDIX 1 20
 Protocol Deviations 21
 Protocol 22
 Protocol Amendment No. 1 31
 Report of Analysis..... 33

APPENDIX 2 37
 Individual Anatomic Pathology Data 38

000263

ABSTRACT

The acute oral toxicity of glucosamine hydrochloride, Lot No. GP-11, was evaluated in male and female rats when administered as a single gavage dose at a dose level of 5,000 mg/kg of body weight (mg/kg). There were no test material-related effects on mean body weights or body weight changes during the study. All animals appeared clinically normal throughout the study with the exception of one female that was sacrificed on Day 9 due to an unexpected pregnancy/litter delivery. There were no macroscopic findings at the necropsy examinations conducted at termination.

Based on the results of this study, the no-observable-effect level (NOEL) for glucosamine hydrochloride, Lot No. GP-11, administered as a single gavage dose to rats was 5,000 mg/kg.

STUDY CONDUCT

Purpose

The purpose of this study was to assess the acute oral toxicity produced when the test material is administered by the oral route (gavage) to rats.

Protocol Adherence

This study was conducted in accordance with the Protocol dated 29 April 2002. The protocol and protocol amendment are in Appendix 1. The dose, method, frequency, and duration of administration utilized in this study were chosen based on the requirements of the regulatory test guidelines. All procedural times presented in this report fall within the acceptable ranges as specified in Covance standard operating procedure (SOP).

Regulatory Guidelines

The study design was based on the United States Environmental Protection Agency Office of Prevention, Pesticides, and Toxic Substances (OPPTS), Series 870, Health Effects Testing Guidelines, No. 870.1100, August 1998, and the Organisation of Economic Co-operation and Development (OECD) Guidelines for the Testing of Chemicals, Guideline 401.

Study Timetable

Study Initiation Date:	29 April 2002
Experimental Start Date:	07 May 2002
Experimental End Date:	21 May 2002
Study Completion Date:	26 July 2002

Major Computer Systems

Metasys, a facility management system, was used to monitor and control environmental conditions and water-flow within the facility (e.g., animal rooms), and the Metasys or the REES environmental monitoring system was used to monitor and document facility

000264

storage conditions (e.g., refrigerators, freezers, constant temperature rooms). The Path/Tox System (PTS), supplied by Xybion Medical Systems Corporation, was used for the direct on-line capture of anatomic pathology data. The Talisman application was used for dose preparation information. The Report Generation System application was used to transfer the information from PTS in Microsoft Word for reporting purposes (if applicable). All version numbers of the applications can be found in the log book for the application.

Record Retention

The raw data, documentation, records, protocol, and the final report generated as a result of this study will be archived in the storage facility of Covance-Madison. At least 1 year after submission of the final report, the Covance Archives staff will contact the sponsor. At that time, the sponsor may choose to have the aforementioned materials returned or archived for an additional period of time; a fee will be charged based on the archive disposition option selected by the sponsor. Raw data stored on durable media and the protocol, study correspondence, and the original final report will be retained by Covance.

The following supporting records will be retained at Covance but will not be archived with the study data.

Animal room environmental/maintenance records (see Protocol Deviations for exceptions)

Instrument calibration and maintenance records

TEST MATERIAL

Test Material

The test material, glucosamine hydrochloride, Lot No. GP-11, is a tan powder. It was received at Covance on 30 April 2002 and stored at room temperature.

A report of analysis documenting the purity of the test material is presented in Appendix 1. Information on synthesis methods, composition, or other characteristics that define the test material is on file with the sponsor.

Reserve (Archive) Samples and Disposition

Reserve samples of the test material were not required to be taken. Any unused test material was utilized for additional analytical chemistry testing performed by Covance per authorization received from the sponsor.

TEST SYSTEM

Species and Justification

Male and female Crl:CD®(SD)IGS BR rats were obtained from the Portage, Michigan, facility of Charles River Laboratories on 29 April 2002. At initiation of treatment, the

000265

animals were approximately 7 to 9 weeks old; the males weighed from 220 to 231 g, and the females weighed from 194 to 208 g.

Historically, the rat has been one of the animals of choice because of the large amount of background information on this species.

Identification and Acclimation

The animals were identified by animal number and corresponding ear tag throughout the study.

After receipt, the animals were acclimated for a period of 8 days.

Husbandry

Environmental controls for the animal room were set to maintain 18 to 26°C, a relative humidity of 30 to 70%, and a 12-hour light/12-hour dark cycle. In cases where variations from these conditions existed, they were documented and considered to have had no adverse effect on the study outcome.

During acclimation and throughout the study, the animals were separated by sex and group-housed in suspended, stainless-steel cages.

Certified rodent diet #8728C, Harlan Teklad was provided *ad libitum*, except for 17 to 20 hours before test material administration. The lot numbers are recorded in the data. The diet is routinely analyzed by the manufacturer for nutritional components and environmental contaminants. Results of specified nutrient and contaminant analyses are on file with Covance-Madison.

Water was provided *ad libitum*. Samples of the water are analyzed for specified microorganisms and environmental contaminants. The results are on file with Covance-Madison.

There were no known contaminants in the diet or water at levels that would have interfered with this study.

EXPERIMENTAL DESIGN

Animal Selection

Animals were selected for the study based on health and body weight requirements according to Covance SOPs. An adequate number of extra animals were purchased so that no animal in obviously poor health was placed on test.

Rationale for Route of Administration

Historically, the oral route has been one of the routes used to assess the acute toxicity of various materials.

000266

PROCEDURES

Dose Preparation

The required amount of test material was suspended in reverse osmosis water to a concentration of 250 mg/mL. The test material mixture was warmed to approximately 52°C to aid in solubilizing the test material. The mixture was then cooled to room temperature prior to dose administration.

Method of Administration

Individual doses were calculated based upon each animal's fasted body weight taken before test material administration and administered by gavage at a dose volume of 20 mL/kg. All animals received the same test material concentration (250 mg/mL). The animals had food withheld for 17 to 20 hours before test material administration. The prepared test material dosing mixture was stored at room temperature until administration. During dose administration the test mixture was mixed with a stir bar and stir plate. The day of treatment was designated as Day 1.

Observation of Animals

Clinical Observations

Each surviving animal was observed twice daily (a.m. and p.m.) for mortality and moribundity.

At approximately 1, 2.5, and 4 hours postdose and once daily thereafter, clinical observations were made for each surviving animal.

Body Weights

Body weights were recorded before experimental initiation (Day 1), and on Days 8 and 15 for surviving animals.

Termination

One female was terminated on Day 9 due to an unexpected pregnancy/litter delivery. This animal and the litter were sacrificed and discarded without a necropsy examination. At termination of the inlife phase (Day 15), all surviving animals were euthanized by an overexposure to carbon dioxide. All animals surviving to Day 15 were subjected to an abbreviated macroscopic necropsy examination. The necropsy included a macroscopic examination of the external surface of the carcass; and all organs and tissues in the thoracic, abdominal, pelvic, and oral cavities. After necropsy, the animals were discarded.

RESULTS AND DISCUSSION

Observation of Animals

Clinical Observations

There were no test material-related clinical observations. Animal No. C05932 was sacrificed on Day 9 due to pregnancy. Given the normal gestation period for rats, it is

000267

assumed that this animal was bred prior to its receipt at Covance. The early sacrifice of this animal had no effect on the outcome of the study.

Body Weights

There were no test material-related changes in body weights or body weight gains.

Anatomic Pathology

There were no macroscopic findings in the animals sacrificed and necropsied at termination.

CONCLUSION

Based on the results of this study, the no-observable-effect level (NOEL) for glucosamine hydrochloride, Lot No. GP-11, administered as a single gavage dose to rats was 5,000 mg/kg.

SIGNATURE

Steven M. Glaza, BS
Study Director
Covance Laboratories Inc.

U - _____
Date 26 JUL 02

COMMENTS ON THE DATA

Various models of calculators, computers, and computer programs were used to analyze data in this study. Because different models round off or truncate numbers differently, values in some tables (e.g., means, standard deviations, or individual values) may differ slightly from those in other tables, from individually calculated data, or from statistical analysis data. Neither the integrity nor the interpretation of the data was affected by these differences.

Some tabular data were compiled using Excel® Version 7.0 software.

The units for the dose levels in the inlife summary tables are mg/kg.

000269

CODES, ABBREVIATIONS, AND UNITS

General Codes and Abbreviations

Note: The following lists of codes, abbreviations, and units are used by Covance. Some, but not necessarily all, of this information may be needed for this report.

000270

General Codes and Abbreviations

WK	Week
No.	Number of measurements in a group
Mean; MEAN	Arithmetic mean
-, NA	No value; not applicable; not present
#	Number

000271

TABLES

000272

Table 1: Mortality Summary

Dose Level (mg/kg)	Sex	Mortality Result No. Died/No. Dosed
5000	M	0/5
5000	F	1 ^a /5

a Animal sacrificed on Day 9 due to accidental pregnancy

BEST ORIGINAL COPY

000273

Table 2: Individual Body Weights/Body Weight Gains (g)

Animal Number	Day 1**	Day 8		Day 15	
	Weight	Weight	Gain*	Weight	Gain*
Males (5000 mg/kg)					
C05925	220	315	95	359	139
C05926	230	303	73	354	124
C05927	224	325	101	388	164
C05928	229	321	92	372	143
C05929	231	327	96	385	154
Mean	227	318	91	372	145
Females (5000 mg/kg)					
C05930	208	230	22	253	45
C05931	200	242	42	251	51
C05932	201	276	75a	NT	NT
C05933	195	232	37	245	50
C05934	194	234	40	243	49
Mean	200	243	35	248	49

* Gain from Day 1 body weight.

** Fasted body weights

a Value not included in calculated the mean

NT Not Taken

000274

Table 3: Individual Clinical Signs

Animal Number	Observation	Hour			Day														
		1.0	2.5	4.0	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
Males (5000 mg/kg)																			
C05925	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
C05926	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
C05927	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
C05928	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
C05929	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
Females (5000 mg/kg)																			
C05930	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
C05931	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
C05932*	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	-	-	-	-	-	-	-	
C05933	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
C05934	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	

✓ Condition existed.

* Animal sacrificed on Day 9 due to accidental pregnancy

000275

Table 4: Incidence of Macroscopic Observations

ACUTE ORAL TOXICITY STUDY GLUCOSAMINE HYDROCHLORIDE IN RATS
(EPA/OECD GUIDELINES)

ORGAN AND KEYWORD(S) OR PHRASE	--- NUMBER - OF - ANIMALS - AFFECTED ---	
	SEX: MALE	FEMALE
TABLE INCLUDES: SEX=ALL; GROUP=ALL; WEEKS=ALL DEATH=ALL; SUBSET=ALL		
GROUP: -1- -1-		
NUMBER: 5 4		
** TOP OF LIST **		
GENERAL COMMENT (GC)	NUMBER EXAMINED: 5 4	
	NOT REMARKABLE: 0 0	
NO MACROSCOPIC LESIONS	5	4
** END OF LIST **		

000276

APPENDIX 1

Protocol Deviations
Protocol
Protocol Amendment No. 1
Certificate of Compliance

000277

Protocol Deviations

Protocol

Record Retention. "The following supporting records will be retained at Covance but will not be archived with the study data. Animal room environmental/maintenance records."

Actual Procedure

The maintenance records were archived with the study data.

Protocol

Test Material. Purity and Stability. "The sponsor will provide information regarding the purity and stability of the test material."

Actual Procedure

Stability information for the test material was not provided to Covance at the time of study conduct. However, a stability study was being planned by the sponsor at the time this final report was issued.

These deviations are not expected to have affected the results of the study.

000278



Protocol

Study Title	Acute Oral Toxicity Study Glucosamine Hydrochloride in Rats (EPA/OECD Guidelines)
Study Director	Steven M. Glaza, BS
Sponsor	Cargill World Wide Acidulants, R&D 1 Cargill Drive Eddyville, Iowa 52553-5000
Sponsor's Representative	Janet Paulson
Testing Facility	Covance Laboratories Inc. 3301 Kinsman Boulevard Madison, Wisconsin 53704-2595
Covance Study Identification	Proposal 40590A1 Covance 7350-100
Version	Final
Protocol Issued	29 April 2002

000279

STUDY IDENTIFICATION

Study

Acute Oral Toxicity Study of Glucosamine Hydrochloride in Rats (EPA/OECD Guidelines)

Purpose

To assess the acute oral toxicity produced when the test material is administered by the oral route (gavage) to rats.

Sponsor

Cargill World Wide Acidulants, R&D
1 Cargill Drive
Eddyville, Iowa 52553-5000

Sponsor's Representative

Janet Paulson
Cargill World Wide Acidulants, R&D
Telephone No.: 641.969.3917
Facsimile No.: 641-969.3850

Study Location

Covance Laboratories Inc.
3301 Kinsman Boulevard
Madison, Wisconsin 53704-2595

Study Director

Steven M. Glaza, BS
Covance Laboratories Inc.
3301 Kinsman Blvd.
Madison, Wisconsin 53704-2595
Telephone No.: 608.241.7292
Facsimile No: 608.242.7936

Study Toxicologist

JoAnna Bultman, BA, LAT
Covance Laboratories Inc.
3301 Kinsman Blvd.
Madison, Wisconsin 53704-2595
Telephone No.: 608.242.2712 ext. 2699
Facsimile No: 608.242.7936

Proposed Study Timetable

Experimental Start Date	07 May 2002
Experimental Termination Date	21 May 2002
Unaudited Draft Report Date	25 June 2002

REGULATORY COMPLIANCE

The study will be conducted in compliance with the Food and Drug Administration Good Laboratory Practice Regulations as set forth in Title 21 of the United States Code of Federal Regulations, Part 58, issued 22 December 1978, and with any applicable amendments with the exception that analysis of the test material mixtures for concentration, homogeneity/solubility, and stability will not be conducted.

ANIMAL CARE AND USE STATEMENT

All procedures in this protocol are in compliance with the Animal Welfare Act, the Guide for the Care and Use of Laboratory Animals, and the Office of Laboratory Animal Welfare. In the opinion of the sponsor and study director, the study does not unnecessarily duplicate any previous work.

MAJOR COMPUTER SYSTEMS

The major computer systems to be used on this study may include, but not limited to, the following systems. Metasys, a facility management system, will be used to monitor and control environmental conditions and water-flow within the facility (e.g., animal rooms), and the Metasys or the REES environmental monitoring system will be used to monitor and document facility storage conditions (e.g., refrigerators, freezers, constant temperature rooms). The Path/Tox System (PTS), supplied by Xybion Medical Systems Corporation, will be used for the direct on-line capture of anatomic pathology data. The Talisman application will be used for dose preparation information. The Report Generation System application will be used to transfer the information from PTS in Microsoft Word for reporting purposes (if applicable). All version numbers of the applications can be found in the log book for the application.

000281

QUALITY ASSURANCE

The protocol, study conduct, and the final report will be audited by the Covance Quality Assurance Unit.

TEST MATERIAL

Identification

Glucosamine hydrochloride. The specific lot number will be documented in the raw data.

Physical Description

Crystalline solid

Purity and Stability

The sponsor will provide information regarding the purity and stability of the test material.

Storage Conditions

Room temperature

Test Material Safety

The sponsor will provide relevant occupational safety information known about the test material [e.g., Material Safety Data Sheet (MSDS), safety instructions, test material identity].

RESERVE (ARCHIVE) SAMPLES

If this study is more than four weeks in experimental duration, a reserve sample of each batch/lot of the test material will be taken and stored at Covance, in a freezer set to maintain a temperature of -10 to -30°C. The reserve samples will be maintained as outlined in the Record Retention section.

000282

DISPOSITION OF TEST MATERIAL

Any unused test material will be returned to the following person after issuance of the final report, unless otherwise directed by the sponsor:

Janet Paulson
Cargill World Wide Acidulants, R&D
1 Cargill Drive
Eddyville, Iowa 52553-5000
Telephone No.: 641.969.3917
Facsimile No.: 641-969.3850

TEST ANIMALS AND HUSBANDRY

Animals

Species

Rat

Strain and Source

CrI:CD⁵(SD)IGS BR, Charles River Laboratories

Age at Initiation of Treatment

Young adult

Weight at Initiation of Treatment

200 to 300 g

Number and Sex

5 males and 5 females for the initial dose level

5 males and/or 5 females for any additional dose levels (if required)

Identification

Individual numbered ear tag

Husbandry

Housing

Separated by sex and group housed in suspended, stainless-steel cages

000283

Diet

Certified rodent diet (#8728C, Harlan Teklad) *ad libitum* except for 17 to 20 hours before test material administration. The diet components are routinely analyzed by the manufacturer for nutritional composition and environmental contaminants. Results of the specified nutrient and contaminant analyses are on file at Covance-Madison.

Water

Ad libitum. Samples of the water are periodically analyzed for specified microorganisms and environmental contaminants. The results are on file at Covance-Madison.

Contaminants

There are no known contaminants in the diet or water at levels that would be expected to interfere with this study.

Environment

Environmental controls for the animal room will be set to maintain a temperature of 18 to 26°C, a relative humidity of 30 to 70%, and a 12-hour light/12-hour dark cycle.

Acclimation

At least 5 days

Environmental Enrichment and Dietary Supplements

The animals will not be given additional supplements as a form of environmental enrichment. Animals may be given various cage enrichment devices.

Selection of Test Animals

Based on health and body weight according to Covance standard operating procedures (SOP). An adequate number of extra animals will be purchased so that no animal in obviously poor health is placed on test.

Justification for Species Selection

Historically, the rat has been one of the animals of choice because of the large amount of background information on this species.

TREATMENT PROCEDURES

Dose Level

Initially, a single dose level of 5000 mg/kg of body weight (Group 1) will be administered to five males and five females. Based on the results of the initial dose level, additional dose levels may be added at the direction of the study director after consultation with the sponsor in order to meet the objectives of the study.

000284

Dose Mixture Preparation and Administration

All animals will receive the same concentration of test material per dose level. For the 5000 mg/kg dose level, the test material will be suspended in reverse osmosis water to a concentration of 250 mg/mL and administered at a dose volume of 20 mL/kg. Slight warming of the test material mixture (up to approximately 60°C) may be used in solubilizing the test material (the mixture may appear cloudy after preparation). The mixture will be cooled to room temperature prior to administration. Individual doses will be calculated based upon each animal's fasted body weight taken before test material administration and administered by gavage. The animals will have food withheld for 17 to 20 hours before test material administration. The prepared test mixtures will be stored at room temperature until administration. During dose administration the test mixtures will be mixed with a stir bar and stir plate. The day of treatment will be designated as Day 1.

Reason for Route of Administration

Historically, the oral route has been one of the routes used to assess the acute toxicity of various materials.

OBSERVATION OF ANIMALS**Clinical Observations**

At approximately 1, 2.5, and 4 hours after test material administration and daily thereafter for at least 14 days for clinical signs and twice daily (a.m. and p.m.) for mortality. Observations may be extended when directed by the study director.

Body Weights

Before experimental initiation (Day 1), on Days 8 and 15, and at death (when survival exceeds 1 day).

TERMINATION**Pathology**

All animals, whether dying during the study, sacrificed in a moribund condition, or euthanized at its respective experimental termination (Day 15), will be subjected to an abbreviated macroscopic necropsy examination and all abnormalities will be recorded. The animals to be sacrificed will be euthanized by an overexposure to carbon dioxide. After necropsy, the animals will be discarded and no tissues will be saved.

Statistical Evaluation

LD₅₀ calculations (when applicable), will be determined by a modified Behrens-Reed-Muench cumulant method (Covance Statistics Library). No other statistical evaluations are required.

000285

REPORT

A final report including those items listed below will be submitted.

Description of the test material
Description of the test system
Procedures
Dates of experimental initiation and termination
Tabulation of mortality data by sex and dose level
Description of any toxic effects
Tabulation of mean body weights by sex and dose level
LD₅₀ calculations for each sex with 95% confidence intervals (when applicable)
Macroscopic pathology findings

RECORD RETENTION

The raw data, documentation, records, protocol, specimens, and the final report generated as a result of this study will be archived in the storage facility of Covance-Madison. At least 1 year after submission of the final report, the Covance Archives staff will contact the sponsor. At that time, the sponsor may choose to have the aforementioned materials returned or archived for an additional period of time; a fee will be charged based on the archive disposition option selected by the sponsor. Raw data stored on durable media and the protocol, study correspondence, and the original final report will be retained by Covance.

The following supporting records will be retained at Covance but will not be archived with the study data.

Animal room environmental/maintenance records
Instrument calibration and maintenance records

000286

PROTOCOL APPROVAL

Jarret Paulson /
Sponsor's Representative
Cargill World Wide Acidulants, R&D

_____ Date

5/02/02

Steven M. Glaza
Study Director
Covance Laboratories Inc.

U - _____ Date

29 APR 02

000287



Protocol Amendment No. 1

Study Title	Acute Oral Toxicity Study Glucosamine Hydrochloride in Rats (EPA/OECD Guidelines)
Study Director	Steven M. Glaza, BS
Sponsor	Cargill World Wide Acidulants, R&D 1 Cargill Drive Eddyville, Iowa 52553-5000
Study Monitor	Janet Paulson
Testing Facility	Covance Laboratories Inc. 3301 Kinsman Boulevard Madison, Wisconsin 53704-2595
Covance Study Identification	Covance 7350-100
Page Number	1 of 2

000288

This amendment modifies the following portion of the protocol.

Effective 07 May 2002

1. **TEST ANIMALS AND HUSBANDRY, Weight at Initiation of Treatment.** To modify the weight range at initiation of treatment to reflect the actual weight range, delete the text in this section and replace with the following.

194 to 300 g

AMENDMENT APPROVAL

Janet Paulson
Sponsor's Representative
Cargill World Wide Acidulants, R&D

6/05/02

Date

Steven M. Glaza, BS
Study Director
Covance Laboratories Inc.

03 JUN 02

Date

Covance Laboratories Inc.
 3301 Kinsman Blvd.
 Madison, WI 53704
 Tel: 608/241-4471 Fax: 608/241-7227

REPORT OF ANALYSIS

COVANCE
 THE DEVELOPMENT SERVICES COMPANY

SAMPLE NUMBER: 20500644

BATCH NUMBER: 20500644

DATE ENTERED: 05/03/02

REPORT PRINTED: 06/25/02

JANET PAULSON
 CARGILL
 1 CARGILL DRIVE
 EDDYVILLE, IA 52553-5000

GLUCOSAMINE HCL: LOT #GP-11

NITROGEN-DUMAS METHOD

	ASSAY 1	6.39	% NITROGEN
	ASSAY 2	6.49	% NITROGEN
			ON A DRY WEIGHT BASIS
MOISTURE, 100 DEGREE VAC. OVEN		.7	GM/100 G
TOTAL FAT, ACID HYDROLYSIS		< .1	GM/100 G
ASH		< .1	GM/100 G
CRUDE FIBER		< 100.	MG/100 G
GLUCOSAMINE		99.0	%
			AS HYDROCHLORIDE

SUGAR PROFILE

FRUCTOSE	< .1	GM/100 G
GALACTOSE	< .1	GM/100 G
GLUCOSE	< .1	GM/100 G
SUCROSE	< .1	GM/100 G
LACTOSE	< .1	GM/100 G
MALTOSE	< .1	GM/100 G
AFLATOXIN		

Covance Laboratories Inc.
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Madison, WI 53704
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COVANCE
THE DEVELOPMENT SERVICES COMPANY

SAMPLE NUMBER: 20500644

PAGE 2

GLUCOSAMINE HCL: LOT #GP-11

<u>ASSAY</u>	<u>ANALYSIS</u>	<u>UNITS</u>
B1	< .5	PPB
B2	< .5	PPB
G1	< .5	PPB
G2	< .5	PPB

USP PESTICIDE SCREEN

USP PESTICIDE SCREEN

<u>COMPOUND NAME</u>	<u>MG/KG</u>
ALACHLOR	< .02
ALDRIN AND DIELDRIN (SUM OF)	< .05
AZINPHOS-METHYL	< 1.0
BROMOPROPYLATE	< 3.0
CHLORDANE (SUM OF CIS- AND TRANS- ISOMERS AND OXYCHLORDANE)	< .05
CHLORFENVINPHOS	< .5
CHLORPYRIFOS	< .2
CHLORPYRIFOS-METHYL	< .1
CYPERMETHRIN (AND ISOMERS)	< 1.0
DDT (SUM OF ISOMERS)	< 1.0
DELTAMETHRIN	< .5
DIAZINON	< .5
DICHLORVOS	< 1.0
DITHIOCARBAMATES (AS CS2)	< 2.0
ENDOSULFAN (SUM OF ENDOSULFAN ISOMERS AND ENDOSULFAN SULFATE)	< 3.0
ENDRIN	< .05
ETHION	< 2.0
FENITROTHION	< .5
FENVALERATE	< 1.5
FONOFOS	< .05
HEPTACHLOR (SUM OF HEPTACHLOR AND HEPTACHLOR EPOXIDE)	< .05
HEXACHLOROBENZENE	< .1
HEXACHLOROCYCLOHEXANE ISOMERS (OTHER THAN GAMMA)	< .3
LINDANE (GAMMA-HEXACHLOROCYCLOHEXANE)	< .6
MALATHION	< 1.0
METHIDATHION	< .2
PARATHION	< .5
PARATHION METHYL	< .2
PERMETHRIN	< 1.0
PHOSALONE	< .1

Covance Laboratories Inc.
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COVANCE
 THE DEVELOPMENT SERVICES COMPANY

PAGE 3

SAMPLE NUMBER: 20500644

GLUCOSAMINE HCL: LOT #GP-11

USP PESTICIDE SCREEN

(CONTINUED)

PIPERONYL BUTOXIDE	<	3.0
PRIMIPHOS-METHYL	<	4.0
PYRETHRINS (SUM OF)	<	3.0
QUINTOZENE (SUM OF QUINTOZENE, PENTACHLOROANILINE AND METHYL PENTACHLOROPHENYL SULFIDE)	<	1.0

METHOD REFERENCE

USP/NF METHOD 561, US PHARMACOPEIA SUPPLEMENT 9, NOVEMBER 15, 1998,
 PP. 4644-4646 (MODIFIED).

METHOD REFERENCES

NITROGEN-DUMAS METHOD

AOAC International, '968.06 Protein (Crude) in Animal Feed' (modified),
 Official Methods of Analysis, (ed.) Patricia Cunniff, Sixteenth Ed.,
 Vol. 1, AOAC International, Gaithersburg, MD (1995).

MOISTURE, 100 DEGREE VAC. OVEN

AOAC International, '925.09 Moisture in Flour' (modified), Official
 Methods of Analysis, (ed.) Patricia Cunniff, Sixteenth Ed., Vol. 2,
 AOAC International, Gaithersburg, MD (1995).

TOTAL FAT, ACID HYDROLYSIS

Official Methods of Analysis of AOAC INTERNATIONAL, 17th Ed., Method 954.02,
 AOAC INTERNATIONAL: Gaithersburg, Maryland, (2000), modified.

ASH

AOAC International, '923.03 Ash of Flour' (modified), Official Methods of
 Analysis, (ed.) Patricia Cunniff, Sixteenth Ed., Vol. 2, AOAC International,
 Gaithersburg, MD (1995).

CRUDE FIBER

AOAC International, '962.09 Fiber (Crude) in Animal Feed and Pet Food'
 (modified), Official Methods of Analysis, (ed.) Patricia Cunniff, Sixteenth
 Ed., Vol. 1, AOAC International, Gaithersburg, MD (1995).

GLUCOSAMINE

Dionex Chromatography Database 4.2.0, Record 755.

000292

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COVANCE
THE DEVELOPMENT SERVICES COMPANY

PAGE 4

SAMPLE NUMBER: 20500644

GLUCOSAMINE HCL: LOT #GP-11

METHOD REFERENCES (CONTINUED)

SUGAR PROFILE

1. Mason, B. S., and Slover, H. T., 'A Gas Chromatographic Method for the Determination of Sugars in Foods' (modified), Journal of Agricultural and Food Chemistry, 19(3):551-554 (1971).
2. Brobst, K. M., 'Gas-Liquid Chromatography of Trimethylsilyl Derivatives, Methods in Carbohydrate Chemistry' (modified), Volume 6, Academic Press, New York, New York, (1972).

AFLATOXIN

Official Methods of Analysis of AOAC International, 17th Ed. Methods 991.31 and 990.33, AOAC International: Gaithersburg, Maryland, (2000) modified.

USP PESTICIDE SCREEN

U.S. Pharmacopeia 24, General Chapter <561> "Vegetable Drugs", pp. 1888-1890, USP 24/NF 19, Rockville, MD (2000).

000293

APPENDIX 2

Individual Anatomic Pathology Data

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APPENDIX 2

Individual Anatomic Pathology Data

PAGE: 1

ACUTE ORAL TOXICITY STUDY GLUCOSAMINE HYDROCHLORIDE IN RATS
(EPA/OECD GUIDELINES)

ANIMAL NUMBER: C05925 SEX: MALE DOSE GROUP: 1 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 05/21/02 STUDY DAY OF DEATH: 15 STUDY WEEK OF DEATH: 3 TERMINAL BODY WEIGHT: NOT ENTERED
DATE AND TIME OF NECROPSY: 05/21/02 7:54 PROSECTOR: (b) (6) RECORDER: TONE EVERTS
POST-FIX WEIGHER: NOT AVAILABLE PATHOLOGIST: AS ASSIGNED WEIGHER: NOT AVAILABLE

*** ANIMAL HAS NO LAST INLIFE OBSERVATIONS RECORDED ***

* * * G R O S S P A T H O L O G Y O B S E R V A T I O N S * * *		
ORGAN NAME	SEVERITY, KEYWORD(S) OR PHRASE	FREE-TEXT COMMENTS AND NOTES
GENERAL COMMENT (GC)	-NO MACROSCOPIC LESIONS	

000295

APPENDIX 2

Individual Anatomic Pathology Data

PAGE: 2

ACUTE ORAL TOXICITY STUDY GLUCOSAMINE HYDROCHLORIDE IN RATS
(EPA/OECD GUIDELINES)

ANIMAL NUMBER: C05926 SEX: MALE DOSE GROUP: 1 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 05/21/02 STUDY DAY OF DEATH: 15 STUDY WEEK OF DEATH: 3 TERMINAL BODY WEIGHT: NOT ENTERED
DATE AND TIME OF NECROPSY: 05/21/02 7:52 PROSECTOR: (b) (6) RECORDER: TONE EVERTS
POST-FIX WEIGHER: NOT AVAILABLE PATHOLOGIST: AS ASSIGNED WEIGHER: NOT AVAILABLE

*** ANIMAL HAS NO LAST INLIFE OBSERVATIONS RECORDED ***

* * * G R O S S P A T H O L O G Y O B S E R V A T I O N S * * *
ORGAN NAME SEVERITY, KEYWORD(S) OR PHRASE FREE-TEXT COMMENTS AND NOTES

GENERAL COMMENT (GC) -NO MACROSCOPIC LESIONS

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APPENDIX 2

Individual Anatomic Pathology Data

PAGE: 5

T
EACUTE ORAL TOXICITY STUDY GLUCOSAMINE HYDROCHLORIDE IN RATS
(EPA/OECD GUIDELINES)

ANIMAL NUMBER: C05929 SEX: MALE DOSE GROUP: 1 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
 DATE OF DEATH: 05/21/02 STUDY DAY OF DEATH: 15 STUDY WEEK OF DEATH: 3 TERMINAL BODY WEIGHT: NOT ENTERED
 DATE AND TIME OF NECROPSY: 05/21/02 7:54 PROSECTOR: (b) (6) RECORDER: TONE EVERTS
 POST-FIX WEIGHER: NOT AVAILABLE PATHOLOGIST: AS ASSIGNED WEIGHER: NOT AVAILABLE

*** ANIMAL HAS NO LAST INLIFE OBSERVATIONS RECORDED ***

*** GROSS PATHOLOGY OBSERVATIONS ***

ORGAN NAME	SEVERITY, KEYWORD(S) OR PHRASE	FREE-TEXT COMMENTS AND NOTES
GENERAL COMMENT (GC)	-NO MACROSCOPIC LESIONS	

000299

APPENDIX 2

Individual Anatomic Pathology Data

ACUTE ORAL TOXICITY STUDY GLUCOSAMINE HYDROCHLORIDE IN RATS
(EPA/OECD GUIDELINES)

PAGE: 6

ANIMAL NUMBER: C05930	SEX: FEMALE	DOSE GROUP: 1	SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 05/21/02	STUDY DAY OF DEATH: 15	STUDY WEEK OF DEATH: 3	TERMINAL BODY WEIGHT: NOT ENTERED
DATE AND TIME OF NECROPSY: 05/21/02 7:57	PROSECTOR: (b) (6)	R	RECORDER: TONE EVERTS
POST-FIX WEIGHER: NOT AVAILABLE	PATHOLOGIST: AS ASSIGNED		WEIGHER: NOT AVAILABLE

*** ANIMAL HAS NO LAST INLIFE OBSERVATIONS RECORDED ***

* * * G R O S S P A T H O L O G Y O B S E R V A T I O N S * * *		
ORGAN NAME	SEVERITY, KEYWORD(S) OR PHRASE	FREE-TEXT COMMENTS AND NOTES
GENERAL COMMENT (GC)	-NO MACROSCOPIC LESIONS	

000300

APPENDIX 2

Individual Anatomic Pathology Data

ACUTE ORAL TOXICITY STUDY GLUCOSAMINE HYDROCHLORIDE IN RATS
(EPA/OECD GUIDELINES)

PAGE: 7

ANIMAL NUMBER: C05931 SEX: FEMALE DOSE GROUP: 1 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 05/21/02 STUDY DAY OF DEATH: 15 STUDY WEEK OF DEATH: 3 TERMINAL BODY WEIGHT: NOT ENTERED
DATE AND TIME OF NECROPSY: 05/21/02 7:50 PROSECTOR: (b) (6) I RECORDER: TONE EVERTS
POST-FIX WEIGHER: NOT AVAILABLE PATHOLOGIST: AS ASSIGNED WEIGHER: NOT AVAILABLE

*** ANIMAL HAS NO LAST INLIFE OBSERVATIONS RECORDED ***

* * * G R O S S P A T H O L O G Y O B S E R V A T I O N S * * *
ORGAN NAME SEVERITY, KEYWORD(S) OR PHRASE FREE-TEXT COMMENTS AND NOTES

GENERAL COMMENT (GC) -NO MACROSCOPIC LESIONS

000301

APPENDIX 2

Individual Anatomic Pathology Data

ACUTE ORAL TOXICITY STUDY GLUCOSAMINE HYDROCHLORIDE IN RATS
(EPA/OECD GUIDELINES)

PAGE: 8

ANIMAL NUMBER: C05933 SEX: FEMALE DOSE GROUP: 1 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 05/21/02 STUDY DAY OF DEATH: 15 STUDY WEEK OF DEATH: 3 TERMINAL BODY WEIGHT: NOT ENTERED
DATE AND TIME OF NECROPSY: 05/21/02 7:49 PROSECTOR: (b) (6) RECORDER: TONE EVERTS
POST-FIX WEIGHER: NOT AVAILABLE PATHOLOGIST: AS ASSIGNED WEIGHER: NOT AVAILABLE

*** ANIMAL HAS NO LAST INLIFE OBSERVATIONS RECORDED ***

* * * G R O S S P A T H O L O G Y O B S E R V A T I O N S * * *		
ORGAN NAME	SEVERITY, KEYWORD(S) OR PHRASE	FREE-TEXT COMMENTS AND NOTES
GENERAL COMMENT (GC)	-NO MACROSCOPIC LESIONS	

000302

APPENDIX 2

Individual Anatomic Pathology Data

ACUTE ORAL TOXICITY STUDY GLUCOSAMINE HYDROCHLORIDE IN RATS
(EPA/OECD GUIDELINES)

PAGE: 9

ANIMAL NUMBER: C05934 SEX: FEMALE DOSE GROUP: 1 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 05/21/02 STUDY DAY OF DEATH: 15 STUDY WEEK OF DEATH: 3 TERMINAL BODY WEIGHT: NOT ENTERED
DATE AND TIME OF NECROPSY: 05/21/02 7:51 PROSECTOR: (b) (6) RECORDER: TONE EVERTS
POST-FIX WEIGHER: NOT AVAILABLE PATHOLOGIST: AS ASSIGNED WEIGHER: NOT AVAILABLE

*** ANIMAL HAS NO LAST INLIFE OBSERVATIONS RECORDED ***

* * * G R O S S P A T H O L O G Y O B S E R V A T I O N S * * *
ORGAN NAME SEVERITY, KEYWORD(S) OR PHRASE FREE-TEXT COMMENTS AND NOTES

GENERAL COMMENT (GC) -NO MACROSCOPIC LESIONS

000303

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Acidulants

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September 9, 2004

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

Re: Withdrawal of GRN 150

Dear Sir or Madam:

On April 6, 2004, Cargill, Incorporated submitted a Notification to the Food and Drug Administration (FDA) that glucosamine is Generally Recognized As Safe (GRAS). Since submitting the Notification, Cargill has continued to assess additional and alternative processing steps. Pursuant to the potential for process-related changes in the manufacture of glucosamine, Cargill would like to withdraw the Notification.

It is Cargill's opinion that the process changes under consideration would not alter the expert panel's determination that glucosamine is GRAS. Nevertheless, it is appropriate for this information to be reviewed and considered by the expert panel.

Cargill, Incorporated respectfully requests notice of the receipt of this letter. Please contact me at the number above if you have any questions.

Sincerely,

Cargill, Incorporated
Brent D. Rogers, Product Applications Chemist

000305