

SUMMARY OF DATA FOR CHEMICAL SELECTION

Glucosamine 3416-24-8

BASIS OF NOMINATION TO THE CSWG

Glucosamine is brought to the attention of the Chemical Selection Working Group because it is a widely used dietary supplement that would be consumed over a period of many years to maximize potential beneficial effects. No chronic toxicity studies to support the safety of such long-term use were found in the available literature.

Used alone or with chondroitin sulfate, glucosamine salts alleviate pain and inflammation from osteoarthritis and reportedly have beneficial effects on degenerated joints. Thus dietary supplements containing glucosamine and chondroitin sulfate have a potential market of tens of millions of Americans who suffer from osteoarthritis, athletes and dancers who may have joint overuse, and aging baby boomers interested in maintaining their joints.

Glucosamine is an amino sugar synthesized in the cell from glucose through the hexosamine biosynthetic pathway. It is used in the body as a molecular element for special macromolecules, the proteoglycans, important constituents of the articular cartilage. Virtually no information on the potential toxicity of orally-administered glucosamine was found in the available literature. Adverse events reports from limited clinical trials have not mentioned significant toxicity in humans at the doses consumed.

SELECTION STATUS

ACTION BY CSWG: June 20, 2002

Studies requested:

- Carcinogenicity – glucosamine alone
- Carcinogenicity – glucosamine in combination with chondroitin sulfate

Priority: High

Rationale Remarks:

- CSWG will consider additional special mechanistic studies at a later date
- Widespread consumer exposure from use in dietary supplements
- Used in dietary supplements to affect chronic conditions, e.g., osteoarthritis, suggesting long-term consumer exposure
- No information whatsoever on the possible adverse or toxic effects from long term (multiyear) exposures

CHEMICAL IDENTIFICATION

Glucosamine

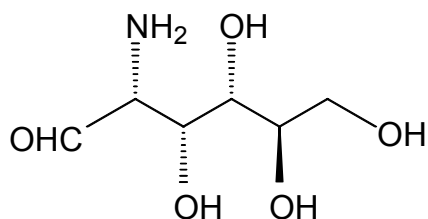
CAS Registry Numbers: 3416-24-8

Chemical Abstracts Service Name: Glucosamine (9CI)

Synonyms and Trade Name: 2-Amino-2-deoxy-D-glucose; 2-amino-2-deoxy \exists -D-glucopyranose; chitosamine; D-glucosamine; D-(+)-glucosamine (ChemFinder, 2002; ChemID, 2002; STN Easy, 2002)

Structural Class: Amino sugar; aminomonosaccharide

Structure, Molecular Formula, and Molecular Weight:



$C_6H_{13}NO_5$

Mol. wt.: 179.17

Chemical and Physical Properties:

Description: Crystals (∇ -form), colorless needles from methanol (\exists -form) (Budavari, 1996; Lewis, 1993)

Melting Point: 88 °C (∇ -form); 110 °C, decomposes (\exists -form) (Budavari, 1996; Lewis, 1993)

Solubility: Soluble in water and boiling methanol (\exists -form), slightly soluble in methanol or ethanol, insoluble in

ether and chloroform (Ξ -form) (Budavari, 1996; Lewis, 1993; Lide, 1995)

Glucosamine hydrochloride

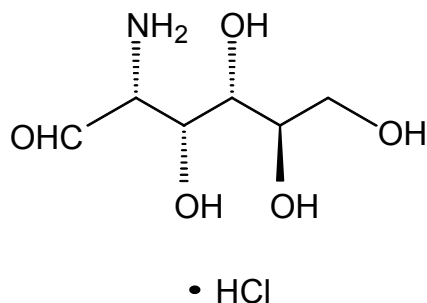
CAS Registry Numbers: 66-84-2

Chemical Abstracts Service Name: Glucosamine hydrochloride (9CI)

Synonyms and Trade Name: 2-Amino-2-deoxy-D-glucopyranose; chitosamine hydrochloride; D-glucosamine hydrochloride; D-(+)-glucosamine hydrochloride (ChemFinder, 2002; ChemID, 2002; Sigma-Aldrich, 2001a; STN Easy, 2002)

Structural Class: Amino sugar

Structure, Molecular Formula, and Molecular Weight:



Mol. wt: 215.63

Chemical and Physical Properties:

Melting Point: 190-194 °C or 300 °C (ChemFinder, 2002; Sigma-Aldrich, 2001a)

Solubility: Soluble in water, 0.1 g/ml (Sigma-Aldrich, 2001b)

Glucosamine sulfate

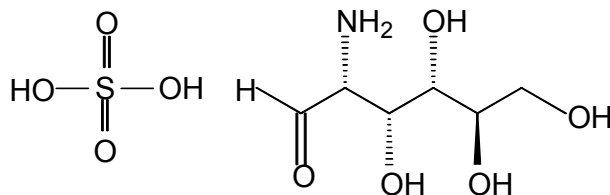
CAS Registry Number: 29031-19-4

Chemical Abstracts Service Name: Glucosamine sulfate (9CI)

Synonyms and Trade Name: 2-Amino-2-deoxy-D-glucose sulfate (salt)(ChemID, 2002)

Structural Class: Amino sugar

Structure, Molecular Formula, and Molecular Weight:



$C_6H_{13}NO_5 \cdot xH_2SO_4$

Mol. wt.: 277.24

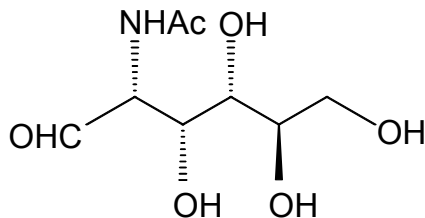
Acetylglucosamine

CAS Registry Numbers: 7512-17-6
Chemical Abstracts Service Name: Acetylglucosamine (9CI)

Synonyms and Trade Name: 2-Acetoamido-2-deoxy- β -D-glucopyranose; N-acetyl-D-(+)-glucosamine; N-acetyl- β -D-glucosaminide; N-acetylchitosamine (ChemFinder, 2002; ChemID, 2002; STN Easy, 2002)

Structural Class: Amino sugar

Structure, Molecular Formula, and Molecular Weight:





Mol. Wt.: 221.21

Chemical and Physical Properties:

Description: Needles from methanol (Budavari, 1996; Sigma-Aldrich, 2001b)

Melting Point: 201-210 °C (Budavari, 1996; ChemFinder, 2002; Sigma-Aldrich, 2001b)

Solubility: Soluble in water, 0.1 g/ml (Sigma-Aldrich, 2001b)

Technical Products and Impurities: Glucosamine hydrochloride (≥99.0%) and acetylglucosamine (≥99.0%) are available from Sigma-Aldrich (Sigma-Aldrich, 2001b).

Most glucosamine supplements contain glucosamine hydrochloride or glucosamine sulfate although some contain acetylglucosamine (Integrative Medicine, 2001; The Natural Pharmacist, 2001).

An evaluation of 4 glucosamine and 12 glucosamine/chondroitin sulfate supplements found that the amount of glucosamine salt (hydrochloride or sulfate) was 95-135% of the labeled amount in all but one product which contained only 75% of the labeled amount (Consumers Union of US, Inc., 2002).

Another study of glucosamine hydrochloride or sulfate supplements reported that the actual amount of glucosamine salt was 90-115% of the labeled amount for 12 products. In contrast, 2 products contained only 25 and 75% of the labeled amount (Adebowale *et al.*, 2000).

EXPOSURE INFORMATION

Production and Producers:

Manufacturing Process: Glucosamine supplements are usually obtained from chitin, a polysaccharide formed of acetylglucosamine units. Chitin is found in the shells of shrimp, lobster, and crabs (Genis, 2002; Integrative Medicine, 2001; Manbir Online, 2002).

Deacetylation of chitin produces chitosan, a polysaccharide that contains less acetylglucosamine residues and, therefore, is soluble in low pH solutions. Both glucosamine and acetylglucosamine are derivatives of chitin and chitosan (Genis, 2002).

Producers and Importers: Chemical Sources International (2001) lists the following figures for US suppliers: 3 for glucosamine, 20 for glucosamine hydrochloride, 4 for glucosamine sulfate, and 16 for acetylglucosamine.

According to recent issues of chemical directories, glucosamine is manufactured and/or distributed by American Ingredients, Inc.; Biopolymer Engineering; Buckton Nutrition, Inc.; Carbomer, Inc.; F.H.Taussig, Inc.; FTL International, Inc.; Kaltron/Pettibone; M.M.P. Inc.; Pharmed Medicare (P) Ltd.; Premium Ingredients; Paul Schueller Internatioanl Inc.; Stauber Performance Ingredients, Inc.; Wilke International, Inc. (Block, 2001; Hunter, 2001; Tilton, 2001).

Glucosamine hydrochloride is manufactured and/or distributed by Aceto Corp.; Aerchem, Inc.; AF Nutraceutical Group, Inc.; AIDP, Inc.; Alfa Aesar/Johnson Matthey; Alfa Chem; Arrow Chem; Ashland Distribution Company; Barrington Chemical Corp.; Biopolymer Engineering; Botanicals International, Inc.; Charles Bowman & Co.; Fabrichem, Inc.; Flavine International, Inc.; Irma Corp.; Jarrow Formulas, Inc.; Kaltron Pettibone; Kowa American; Marcor Development Corp.; Markan Global Enterprises, Inc.; Maypro Industries, Inc.; Pharmachem Laboratories, Inc.; Pharmed Medicare (P) Ltd.; RIA

International LLC; Rochem International, Intl.; Seltzer Chemicals, Inc.; Spectrum Chemical Mfg. Corp.; F.H. Taussig, Inc.; TR-Metro Chemicals Inc.; Wilke International, Inc., Xenos BioResources, Inc. (Block, 2001; Hunter, 2001; Tilton, 2001).

Glucosamine sulfate is manufactured and/or distributed by AF Nutraceuticals Group, Inc.; AIDP, Inc.; AMT Labs, Inc.; Aceto Corp.; Aerchem, Inc.; Alfa Chem; Amax Nutritional, Inc.; Anmar International Ltd.; Ashland Distribution Company; Biopolymer Engineering; Biosynergy Nutraceuticals; Botanicals International, Inc.; Charles Bowman & Co.; Buckton Scott USA, Inc.; CBC (America) Corp.; Compound Solutions, Inc.; CPB International, Inc.; CTC Organics; Eby Sales International, Inc.; Fabrichem, Inc.; Ferro Pfansthiel Laboratories, Inc.; Flavine International Inc.; JFC Technologies; Jarrow Formulas, Inc.; Markan Global Enterprises, Inc.; Marcor Development Corp.; Pharmed Medicare (P) Ltd.; Pharmline, Inc.; RIA International LLC; Rochem International, Inc.; Seltzer Chemicals, Inc.; Spectrum Chemical Mfg. Corp.; Stauber Performance Ingredients, Inc.; F.H. Taussig, Inc.; Universal Preserv-A-Chem, Inc.; Westco Fine Ingredients, Inc. (Block, 2001; Hunter, 2001; Tilton, 2001).

Acetylglucosamine is manufactured and/or distributed by Aceto Corp.; Advanced Synthesis Technologies, SA; AIDP, Inc.; Alfa Aesar/Johnson Matthey; Botanicals International, Inc.; CBC (America) Corp.; CPB International, Inc.; CTC Organics, Fabrichem, Inc.; Ferro Pfansthiel Laboratories, Inc.; Flavine International Inc.; H&A Chemicals; Jalie Corporation; Kyowa Hakko USA, Inc.; Marcor Development; Maypro Industries, Inc.; M.M.P., Inc.; OMG, Americas; Optima Specialty Chemical, LLC; Pharmed Medicare (P) Ltd.; Pharmline, Inc.; Seltzer Chemicals, Inc.; Spectrum Chemical Mfg. Corp.; TCI America; Westco Fine Ingredients, Inc. (Block, 2001; Hunter, 2001; Tilton, 2001).

The US International Trade Commission (USITC) reported glucosamine hydrochloride as an end-use chemical for the years 1991 and 1992 with no US production data in the ten

most recent volumes of *Synthetic Organic Chemicals, US Production and Sales* for the years 1984-1993. This source is no longer published. No quantitative information on annual production was found in any other available literature (US International Trade Commission, 1993 & 1994).

Production/Import/Export Level: Glucosamine, glucosamine hydrochloride, and acetylglucosamine are listed in the EPA Toxic Substances Control Act (TSCA) Inventory (ChemID, 2002). Between September 21, 2000 and April 4, 2002, the Piers Imports database listed 367 entries for glucosamine compounds. In contrast, between November 22, 2000 and December 4, 2001 only 19 entries in the Piers Exports database reported exports of glucosamine compounds (Dialog Information Services, 2002).

Use Pattern: The market for dietary supplements containing glucosamine and chondroitin sulfate is large. Between July 1998 and May 1999, retail sales in the US were estimated to be more than \$500,000,000 (Adebowale *et al.*, 2000). These dietary supplements are marketed primarily for pain and inflammation relief in osteoarthritis and related autoimmune diseases that affect the joints. As many as 40 million Americans have been reported to suffer from osteoarthritis. In Europe, glucosamine has been approved as prescription drug for over a decade (Adebowale *et al.*, 2000; Hippocrates, 2000).

Glucosamine supplements are used alone or in combination with chondroitin sulfate and/or dimethyl sulfone. Performance-enhancing products may contain other ingredients such as creatine (Good 4 All, 2000; Herbal Information Center General Store, 2001; Johnson & Johnson, 2001; Life enhancement, Inc., 2001; Nutripeak, 2002a; The NutraSense Company, 2001).

Large food manufacturers have begun to add glucosamine compounds to fruit juices. For example, Pepsi's widely available SoBe Sport System® line, which contains glucosamine, is promoted for use among young athletes. In 2000, SoBe Sport System® became the

official beverage of the USA Track & Field National Team. Elation®, another fruit-flavored beverage containing glucosamine, is produced by Coca-Cola and Procter & Gamble (Barnes and Winter, 2001; PharmaTimes, 2002; South Beach Beverage Company, 2002).

According to the International Nomenclature of Cosmetic Ingredients (INCI), acetylglucosamine is used as a skin conditioner in cosmetics (INCI, 2000). It is also an ingredient in creams and moisturizers aimed to relieve minor pain or improve skin appearance (Herbalhut.com, 2002, 2001; IntegraDerm, 2002). Glucosamine supplements intended to improve hair and nails are also commercially available (Nutripeak, 2002b).

Several clinical trials have shown that glucosamine salts, administered orally, improve stiffness of the joints, narrow joint space and relieve pain in persons with osteoarthritis (Adebowale *et al.*, 2000; Deal & Moskowitz, 1999; Kelly, 1998; Maher, 2000; McAlindon *et al.*, 2000).

In 2000, McAlindon and coworkers conducted a meta-analysis of human clinical trials that tested glucosamine or chondroitin for knee or hip osteoarthritis treatment. Studies were identified from a search of MEDLINE (1966-June1999) and the Cochrane Controlled Trials Register. The 17 studies selected for inclusion had to be double-blinded, randomized, placebo-controlled trials of 4 or more weeks duration. Although the authors found some degree of efficacy, they also noted that quality issues and likely publication bias suggest that reports of these benefits are exaggerated.

In 2001, Reginster and coworkers conducted a clinical trial of glucosamine sulfate in 212 osteoarthritis patients. After 3 years of glucosamine sulfate treatment, the subjects showed a significant improvement in pain and physical function and no further joint-space narrowing. Stiffness remained unchanged.

A total of 4,380 patents using glucosamine were on file with the US Patent and Trademark Office as of November 2001 (USPTO, 2001).

Human Exposure:

Consumer Exposure: The principal sources of human exposure to glucosamine products occur from the ingestion of dietary supplements and sports beverages. These products are intended to treat or prevent degenerative joint diseases, chronic conditions that increase with age and are common in athletes. Thus these products appeal to a wide audience, from aging osteoarthritis patients to junior high, high school, and elite athletes (AbdelFattah *et al.*, 2001; Adebowale *et al.*, 2000; South Beach Beverage Company, 2002).

The standard dose used in Europe is 1,500 mg/day of glucosamine sulfate (Adebowale *et al.*, 2000; Hippocrates, 2000; Dunford, 2002). In the US, a dose of 1,500 mg/day of glucosamine hydrochloride or sulfate with a maintenance dose of 750 mg/day is often recommended (Dunford, 2002; Rotta Pharmaceuticals, 2002; The NutraSense Company, 2001;). The recommended dosage for acetylglucosamine is 500-1,500 mg/day (Life enhancement, Inc., 2001; Nutrimart.com, 2002).

Consumers using cosmetics containing acetylglucosamine would have dermal contact with small amounts of this chemical (Herbal Hut, 2002; Home Shopping Network, 2001; INCI, 2000; IntegraDerm, 2002).

Occupational Exposure: The National Occupational Exposure Survey (NOES), which was conducted by the National Institute for Occupational Safety and Health (NIOSH) between 1981 and 1983, estimated that 16,143 workers, including 13,164 females, in 1,651 facilities representing 2 industries were potentially exposed to glucosamine hydrochloride in the workplace (Sigma-Aldrich, 2001a). The NOES database does not contain information on the frequency, level, or duration of exposure to workers of any

chemical listed therein, and it does not reflect recent increased usage of glucosamine products as dietary and animal feed supplements.

Environmental Occurrence: Glucosamine is the principal component of *O*-linked and *N*-linked glycosaminoglycans (sialic acid, hyaluronan, keratan sulfate, heparan sulfate, dermatan sulfate, chondroitin sulfate). Glycosaminoglycans form the matrix of all connective tissue. Glucosamine is also the precursor for the biosynthesis of all the hexosamines that will form sialic acids and proteoglycans (Deal & Moskowitz, 1999; Kelly, 1998).

No information on any other environmental occurrence of glucosamine compounds was identified in the available literature.

Regulatory Status: No standards or guidelines have been set by NIOSH or the Occupational Safety and Health Administration (OSHA) for occupational exposure to or workplace allowable levels of glucosamine. Glucosamine was not on the American Conference of Governmental Industrial Hygienists (ACGIH) list of compounds for which recommendations for a threshold limit value (TLV) or biological exposure index (BEI) are made.

Since 1994, dietary supplements have been regulated under the Dietary Supplement Health and Education Act (DSHEA). The DSHEA requires no proof of safety for dietary supplements on the market prior to October 15, 1994. Labeling requirements for dietary supplements allow warnings and dosage recommendation as well as substantiated “structure or function” claims. All claims must prominently note that they have not been evaluated by the FDA, and they must bear the statement “This product is not intended to diagnose, treat, cure, or prevent any disease” (FDA, 1995). Glucosamine compounds are not approved drugs in the US.

EVIDENCE FOR POSSIBLE CARCINOGENIC ACTIVITY

Human Data: No epidemiological studies or case reports investigating the exposure of glucosamine compounds and cancer risk in humans were identified in the available literature.

A number of trials have shown that glucosamine is well tolerated and that most side effects are gastrointestinal (Kelly, 1998; Leffler *et al.*, 1999, Maher, 2000). A recent clinical trial involving 212 subjects with osteoarthritis found no differences in reporting adverse events between those who received glucosamine sulfate (1,500 mg/day) for 3 years and those who received placebo (Reginster *et al.*, 2001). Patients with chronic knee or low back pain tolerated treatment with 1,500 mg/day of glucosamine hydrochloride, 1,200 mg/day of chondroitin sulfate, and 228 mg/day of manganese ascorbate for 8 weeks, and they showed no significant differences in vital signs, occult blood testing, or hematologic parameters compared with the placebo group (Leffler *et al.*, 1999).

The National Center for Complementary and Alternative Medicine (NCCAM) and the National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS) are conducting a multicenter study to investigate the efficacy of Pfanstiehl Laboratories glucosamine hydrochloride and/or Bioibérica, S.A. chondroitin sulfate for treating knee pain associated with osteoarthritis. The study will be a 24-week clinical trial with a total of 1,588 individuals. In addition, one-half of the patients will receive blinded treatment for eighteen more months (NIH News Release, 2000).

Animal Data: No 2-year carcinogenicity studies of glucosamine compounds in animals were identified in the available literature.

The LD₅₀ values for glucosamine hydrochloride are given in Table 1.

Table 1. Acute Toxicity Values for Glucosamine Hydrochloride

mouse	oral	15,000
mouse	iv	1,100
mouse	sc	6,200

Source: Sigma-Aldrich (2001a)

The oral LD₅₀ of glucosamine sulfate in the mouse was reported to be >5,000 mg/kg, a dose that did not produce mortality (Senin *et al.*, 1987).

Between 1987 and 1998, one ‘Adverse Drug Experience’ report relating to glucosamine ingestion in dogs was filed with the Food and Drug Administration (FDA). Three of the four died, with at least one exhibiting the following signs: anorexia, anemia, hematochezia, and vomiting among other clinical signs (FDA, 2001).

Short-Term Tests: A limited number of studies of glucosamine was found in the available literature.

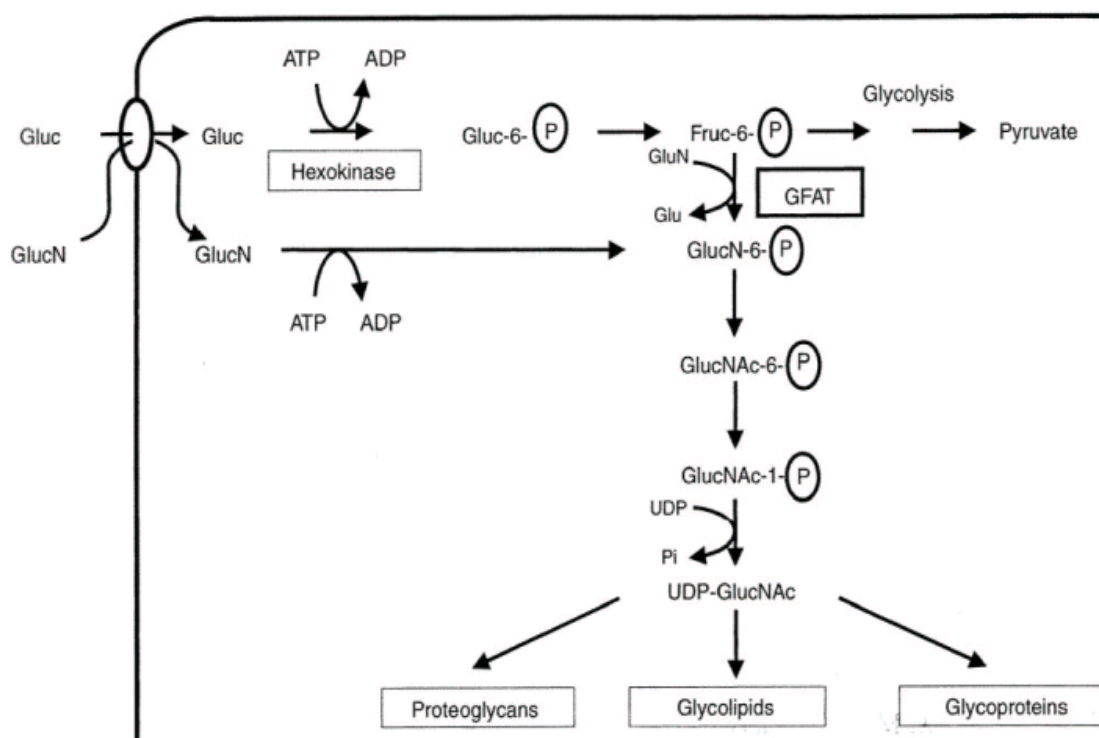
- Glucosamine did not induce DNA repair in an *E. coli* WP2 strain (Genetic Toxicology, 2002).
- 0.1% Glucosamine hydrochloride injected intraperitoneally (ip) into Swiss albino mice at 1 ml/100 g induced chromosomal aberrations (CA) in bone marrow cells (Banerjee & Manna, 1984).
- In tilapia fish (*Oreochromis mossambica*) glucosamine hydrochloride injected ip at a concentration of 0.1%, 1 ml/100 g, induced micronuclei in red blood cells. The authors felt, however, that further critical evaluation of the micronucleus assay in fish needed to be done (Manna *et al.*, 1985).

Metabolism:

Glucosamine is produced in the body through the hexosamine biosynthetic pathway. In this

pathway, glucose enters the cells through the glucose transporter and is metabolized to fructose-6-phosphate by a hexokinase. Fructose-6-phosphate forms glucosamine-6-phosphate in a reaction catalyzed by the rate-limiting enzyme glutamine:fructose-6-phosphate-amidotransferase (GFAT) where glutamine serves as a donor of the amino group (Figure 1) (Schleicher & Weigert, 2000).

Fig. 1. Hexosamine Biosynthetic Pathway



Hexosamine biosynthetic pathway. Central role of acetylglucosamine-6-phosphate (GlucN-6-P) in the metabolism of amino sugars and the synthesis of proteoglycans, glycolipids, and glycoproteins. Abbreviations: Gluc = Glucose. Gluc-6-P = Glucose-6-phosphate. Fruc-6-P = Fructose-6-phosphate. GlucN = Glucosamine. GlucNAc-6-P = Acetylglucosamine-6-phosphate. GlucNAc-1-P = Acetylglucosamine-1-phosphate. UDP = Uridine. GluN = Glutamine. GFAT = Glutamine:fructose-6-phosphate-amidotransferase

Physiological glucosamine levels in extracellular fluids are negligible; however, if glucosamine is added to cells, it is rapidly taken up by the glucose transporter and phosphorylated to produce glucosamine-6-phosphate, bypassing the rate-limiting enzyme GFAT and entering into the hexosamine biosynthetic pathway (Schleicher and Weigert, 2000).

An *in vitro* study using entire intestinal tissue showed that transport of glucosamine (no salt specified) was carrier-mediated and independent of sodium ion. In contrast, acetylglucosamine was absorbed by simple diffusion without deacetylation of the molecule (Tesoriere *et al.*, 1972).

Acetylglucosamine was reported to be rapidly digested by intestinal bacteria, binding to dietary lectins forming a complex found in the feces, and catabolized by the intestinal cell. For these reasons, it was thought that intestinal absorption of acetylglucosamine is not efficient, although no studies were found in the available literature that proved this assumption (ICNR, 2002).

In vivo studies on the absorption of glucosamine salts were conducted in animals and humans. Setnikar and coworkers (1986) analyzed the rate of absorption and pharmacokinetics of glucosamine sulfate, reporting the following findings:

- When [¹⁴C]-glucosamine sulfate was given orally to dogs, radioactivity appeared in the plasma within 15 minutes, peaking at 24 hours. Within the first hour, radioactive materials were thought to represent unchanged glucosamine, but afterwards, the radioactivity was bound to plasma glycoproteins. Four hours after administration, the greatest radioactivity was found in the liver, followed by the kidneys. Other organs and tissues, including the articular cartilage, showed an active uptake of radioactivity,

Intravenously (iv) administered [¹⁴C]-glucosamine sulfate showed the same kinetic and distribution patterns of radioactivity in dogs as observed after oral administration.

- In humans, 38.3% of the 800 mg of glucosamine sulfate administered by iv injection

was excreted through the urine, mostly in the first 2 hours.

- In humans, oral administration of 6 g of glucosamine sulfate produced substantial amounts glucosamine in the urine but undetectable levels in the plasma.

Regarding the work by Setnikar and coworkers, Kelly (1998) noted that oral doses of glucosamine concentrate in the liver where it is incorporated into plasma proteins, degraded, or used in biosynthetic processes. Kelly noted that a substantial quantity of the absorbed glucosamine is probably degraded to smaller compounds, such as water, carbon dioxide and urea when it first entered the liver. A review article by Maher (2000) reported that 65% of the total radioactivity from oral administration of radioactive glucosamine (no salt specified) can be recovered as exhaled carbon dioxide, with 10% retained in tissues.

Maher (2000) noted that the sulfate salt is readily absorbed from the small intestine since the majority is in an unionized form but the hydrochloride salt is thought to be less well absorbed.

Other Biological Effects:

Cell Growth Inhibition:

Bekesi and Winzler (1970) reported that glucosamine hydrochloride can inhibit tumor growth in animals under some circumstances.

- In rats implanted with Walker 256 carcinosarcoma, glucosamine hydrochloride infusion resulted in a high rate of tumor regression and an increase in survival time in the CD strain and inhibited tumor growth in the Schmidt Sprague-Dawley strain.

Acetylglucosamine infusion had no effect on the growth of tumor cells in CD rats.

- Continuous infusion of glucosamine hydrochloride to Swiss-Webster mice bearing sarcoma 180 resulted in tumor regression in 56% of the mice and an increase in survival.
- CD rats and Swiss-Webster mice successfully treated with glucosamine were resistant to a second implantation of the tumors.
- Extensive necrosis of tumor tissues was observed in tumor-bearing rats and mice even though gross examination of major organs showed no treatment-related toxic effects.

- Blood glucose levels increased significantly during glucosamine hydrochloride infusion and returned to normal after 24 hours.

A follow-up study by Molnar and Bekesi (1972) also noted disruption and necrosis of the tumor mass 5 days after infusion with glucosamine hydrochloride (350 mg/kg/hr for 40 hrs) in Walker 256 tumor-bearing Sprague-Dawley rats. Reversible abnormalities in hepatic parenchymal cells and cytoplasmic vacuolization in renal proximal tubular epithelium were observed in non-tumor tissues immediately after infusion.

Glucosamine hydrochloride inhibited DNA synthesis, RNA synthesis, and protein synthesis in L5178Y mouse leukemic cells (Bossmann, 1972). Glucosamine hydrochloride also inhibited *in vitro* protein, RNA, and DNA synthesis in sarcoma 180 ascites cells, Walker 256 tumor tissue, and C3H spontaneous mammary tumor tissue even though acetylglucosamine had only minor inhibitory effects. Glucosamine hydrochloride and acetylglucosamine inhibited *in vitro* protein synthesis in normal liver tissue by 30% (Bekesi *et al.*, 1969).

Effects on Glucose Metabolism: Glucosamine, which enters the hexosamine pathway downstream of the rate-limiting step, has been routinely used to mimic the insulin resistance caused by high glucose and insulin (Hresko *et al.*, 1998).

McClain and Crook (1996) reviewed studies describing the effects of glucosamine in *in vitro* cellular regulation and intact animals.

1. Preexposure to glucosamine induced insulin resistance in skeletal muscle, the tissue responsible for the majority of insulin-dependent glucose utilization.
2. Preexposure to glucosamine abolished the ability of insulin to stimulate glycogen synthesis even though insulin stimulation of glycogen synthase and insulin receptor number/activation were not affected.
3. Glucosamine infusion decreased the glucose disposal rate (GDR) in normal rats, but

did not affect the GDR in partially pancreatectomized diabetic rats.

4. Rats infused with glucosamine showed impaired translocation of the glucose transporter GLUT4, in a manner similar to that found in human insulin resistance.
5. Glucosamine enters the cell through the glucose transporters and is then phosphorylated by hexokinase. However, nonphysiological concentrations of glucosamine are required to deplete cellular levels of uridine triphosphate, causing marked changes in intracellular glucose utilization. At these concentrations, protein glycosylation is also inhibited.
6. Glucosamine increased transforming growth factor \forall (TGF- \forall) mRNA levels in rat aortic smooth muscle cells and transforming growth factor \exists (TGF- \exists) transcription in cultured renal glomerular and proximal tubule cells. TGF- \exists has been implicated in the pathogenesis of diabetic nephropathy.

Glucosamine hydrochloride potentiated the diabetogenic effect of streptozotocin in SJLXB6 mice, resulting in extensive pancreatic \exists cell death. Infusion of glucosamine hydrochloride also induced moderate \exists cell apoptosis in Sprague-Dawley rats, and the glucosamine-treated animals showed depletion of immunoreactive insulin in the \exists cells (Liu *et al.*, 2000).

These studies used large amounts of glucosamine, often administered iv over a short time. Therefore, extrapolation of the observed effects to the use of glucosamine as an oral supplement is difficult. However, an increase in fasting insulin level was reported in six patients taking glucosamine sulfate for twelve weeks (AbdelFattah & Hammad, 2001). Glucosamine (no salt specified) also produced dose-dependent oxidative stress in cultured rat pancreatic islet cells (Kaneto *et al.*, 2001).

In Vitro Effects on Chondrocyte Metabolism: The basis for the anti-inflammatory activity elicited by ingestion of glucosamine compounds has not been demonstrated using *in vivo* models. *In vitro* studies suggest that glucosamine compounds may inhibit IL-1 mediated

inflammation in chondrocytes.

Glucosamine sulfate significantly increased protein synthesis and protein kinase C activity in human chondrocyte cultures from osteoarthritic knee joints. In this model, glucosamine sulfate decreased proinflammatory phospholipase A2 and collagenase activities, but did not change the content of cyclic adenosine monophosphate (cAMP) nor the production of nitric oxide (NO) which is involved in interleukin-1 (IL-1) inflammatory and catabolic effects in the joint (Piperno *et al.*, 2000).

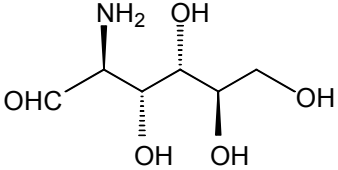
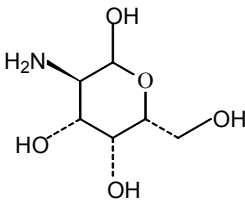
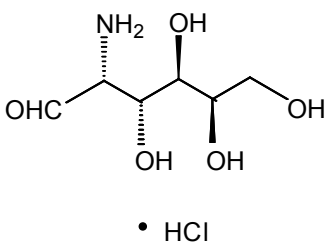
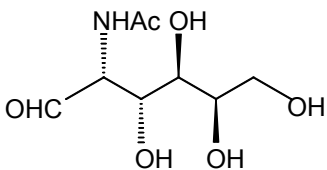
Acetylglucosamine inhibited the production of certain IL-1 mediators of inflammation, including NO, cyclooxygenase-2 (COX-2), and interleukin-6 (IL-6), in normal human articular chondrocytes. Glucosamine also suppressed IL-1-mediated production of NO in the same system (Shikhman *et al.*, 2001).

Glucosamine hydrochloride inhibited IL-1-mediated aggrecanase activity in a rat chondrosarcoma cell line (Sandy *et al.*, 1998).

Structure-Activity Relationships:

Four amino sugars structurally related to glucosamine were selected for review. These chemicals were mannosamine, galactosamine, galactosamine hydrochloride, and acetyl galactosamine. No information on carcinogenic activity or genotoxicity was found for any of these compounds in a search of the National Library of Medicine TOXNET databases, including TOXLINE. No information on any of these chemicals was located on the 1999 CD-Rom version of the *Survey of Compounds Which Have Been Tested for Carcinogenic Activity* (CancerChem).

Table 4. Pharmacological and Toxicological Information on Four Structurally Related Amino Sugars

Compound	Pharmacological/ Toxicological Effects
<p>Mannosamine [CAS No. 579-33-9]</p> 	<p>No data found in the available literature</p>
<p>Galactosamine [CAS No. 90-76-6]</p> 	<p>Used to induce experimental liver injury (Abdul-Hussain & Mehendale, 1992; Sell <i>et al.</i>, 1974)</p>
<p>Galactosamine hydrochloride [CAS No. 1772-03-8]</p>  <p>• HCl</p>	<p>Used to induce experimental hepatitis (Kalpana <i>et al.</i>, 1999, 2002)</p>
<p>Acetyl galactosamine [CAS No. 31022-50-1]</p> 	<p>No data found in the available literature</p>

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