TOX/2008/35

COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

GLUCOSAMINE AND HEPATOTOXICITY

Introduction

1. Glucosamine is an amino monosaccharide found in mucopolysaccharides and chitin. It is a popular food supplement derived from shell fish and from fungal sources and may be taken alone or in combination with chondroitin sulphate and is used to aid sufferers of osteoarthritis. Data from the Health Food Manufacturers Association (HFMA) suggest that the value of the UK business is nearly £50 million per annum, with 1 billion tablets are sold annually. Glucosamine is not present in the normal diet to any significant extent.

Review of glucosamine and discussions at the July COT meeting

2. Following a few reports associating hepatitis with glucosamine, including a case that became the subject of a fatal accident enquiry, the Secretariat began reviewing published data, to establish whether a link with hepatotoxicity was plausible. A summary of the relevant issues/data were presented at the July COT meeting so that Members could provide their initial views on whether and how to proceed (TOX/2008/26). Members agreed that it was worthwhile to explore the issue and requested further data on hepatitis in general, the case reports of hepatitis associated with glucosamine, the chiral form of glucosamine, chondroitin and human trial data for glucosamine and/or chondroitin. Members considered that further data on galactosamine hepatotoxicity and the effects of glucosamine on xenografted tumours were not required. The requested data are considered below and in the attached annexes.

Fatal accident enquiry and other case reports - see Annex A.

3. A case of hepatitis thought to be linked to the consumption of glucosamine and chondroitin supplements was the subject of a recent fatal accident enquiry in Scotland, though it was subsequently ruled that there was no evidence for such a link. This case is the subject of a planned publication and the authors have cited a further two cases that they consider might also be associated with these supplements. Independently of the Scottish case, the Food Standards Agency was also advised of further case in London.

4. Unlike medicines, there is no formal mechanism to notify the adverse effects of food supplements. However, adverse drug reaction “yellow cards” for food supplement products received by the Medicines and Healthcare products Regulatory Agency (MHRA) are forwarded to the FSA, where they are logged. From 1999 to date, 41 adverse reaction reports have been received for glucosamine, which include 2 reports of hepatitis and 1

report of elevated liver function tests (LFTs), 14 reports have been received for the glucosamine and chondroitin sulphate combination, of which 2 reported elevated or abnormal LFTs.

5. A further 2 case reports of hepatitis have been published in the scientific literature. It should be noted that in some of the above cases, other supplements or medication may have been consumed in addition to glucosamine.

Hepatitis – general

6. Hepatitis is inflammation of the liver. The condition may be self-contained, healing on its own or may progress to scarring (cirrhosis). Hepatitis is acute when it lasts less than 6 months and chronic where it persists longer. The most common cause of hepatitis is viral infection, but toxicants (including ethanol), other infections and autoimmune processes may also cause hepatitis. In some instances, idiosyncratic hepatitis, ie with no known aetiology may occur. Patients with hepatitis may have the condition for some time without exhibiting overt symptoms; however, symptoms can become apparent as the disease starts to affect liver functions, such as bile production, excretion of harmful substances and regulation of blood composition (Merck, 2008; British Liver Trust, 2008, Wikipedia, 2008; Patient UK, 2008).

7. The course of acute hepatitis varies from mild symptoms requiring no treatment to fulminant hepatic failure which requires transplantation. The initial symptoms are common to all types of viral infection, and may include malaise, muscle and joint aches, fever, nausea and vomiting, diarrhoea and headache. More specific symptoms include loss of appetite, dark urine, yellowing of eyes and skin due to jaundice, and abdominal discomfort. In chronic hepatitis, the majority of patients have no or few mild symptoms, with abnormal blood tests being the only manifestations. The features may be related to the extent of the liver damage and the cause of the hepatitis. Jaundice can be a late feature and indicates extensive damage; other features may include enlarged liver or spleen, ascites (fluid retention) or low grade fever. Extensive damage and scarring (cirrhosis) may lead to weight loss and a tendency to easy bruising or bleeding.

Liver function tests

8. Liver function tests commonly include measurement of the following:

Alanine aminotransferase (ALT) an enzyme present in liver cells which leaks into the blood when the liver is damaged.

Aspartate aminotransferase (AST) an enzyme also present in liver cells but which is less specific, being also found in red blood cells, cardiac and skeletal muscle. The ratio between ALT and AST may be useful in distinguishing different types of damage.

Alkaline phosphatase (ALP), an enzyme which occurs in the cells lining the biliary tract, this rises in patients with large bile track obstruction, cholestastis or obstructive liver disease.

Albumin, total protein and bilirubin.

The pattern of test results may help to diagnose which condition may be occurring. Other tests may also include blood clotting tests, gamma-glutamyl transferase (GGT) levels, immunology and the levels of proteins such as ceruloplasmin, α-1-antitrypsin or ferritin, 5' nucleotidase, serum glucose and lactate dehydrogenase.

Pathology of hepatitis

9. The morphologic appearance of the various types of hepatitis is similar thus clinical history and laboratory tests are necessary to establish causation (Ferrell, 2001). Pathological features of acute hepatitis include swelling and ballooning of hepatocytes due to hydropic degeneration, this can progress to focal necrosis, throughout the lobule, identified by aggregates of neutrophils (Wheater *et al*, 1991) or round eosinophilic bodies (councilman bodies) , Kupffer cells are very active and within the portal tracts there are increased numbers of chronic inflammatory cells. Regeneration of hepatocytes occurs with time. Acute hepatitis generally shows more spotty parenchymal inflammation than the typical chronic hepatitis but may show similar degrees of portal inflammatory changes and necrosis (Ferrell, 2001).

10. The two major types of chronic hepatitis are chronic active hepatitis (also known as chronic hepatitis with piecemeal (periportal) necrosis or interface hepatitis (Ferrell, 2001) and chronic persistent hepatitis (also known as chronic hepatitis without piecemeal necrosis or interface hepatitis). In chronic persistent hepatitis there is expansion of the portal tract by mononuclear chronic inflammatory cells (mainly lymphocytes but some plasma cells) (Wheater *et al*, 1991). The inflammation is limited to the connective tissue of the portal tract; necrosis is not observed. In chronic active hepatitis, inflammation spreads into the liver parenchyma. The continued active inflammation and hepatocyte damage commonly results in progressive fibrosis; the disease may progress to cirrhosis and liver failure if untreated. Histologically, the condition is marked by inflammation, hepatocyte necrosis and fibrosis. The mononuclear cells may form a ring around the swollen hepatocyes. Collagen deposition and bile duct proliferation may also occur.

11. Autoimmune hepatitis (AIH) can be classified into three types, depending on clinical presentation or the antibodies found (Ferrell, 2001). These often display the prominent lymphoid aggregates and duct damage seen in other types of chronic hepatitis, but patients with AIH seem to have more diffuse and severe interface hepatitis, an increased incidence of bridging and confluent necrosis and more rapid progression to cirrhosis. Infiltration of mononuclear cells also tends to be more diffuse in AIH.

12. Other types of chronic hepatitis may have characteristic but not necessarily diagnostic features such as focal copper deposits in Wilson's disease.

<u>Glucosamine enantiomers</u>

13. In paper TOX/2008/26 it was noted that commercially available glucosamine was thought to be a mixture of L and D forms whereas biological glucosamine was in the D form. This was based on a secondary internet source and it was noted that it had not been

possible to confirm this. The statement was queried by Members since as glucosamine is obtained from biological sources, it seemed more likely that it would be in the D form. Data have now been provided by the supplements industry to indicate that commercial glucosamine is all in the D form. This is based on the isolation of glucosamine from biological sources and since glucosamine in supplements conforms to pharmacopeia specifications which include a + optical rotation. The full report provided by the Health Food Manufacturers Association is attached at Annex B. It is also noted in Deal and Moskowitz (1999) that glucosamine is in the D form.

Glucosamine- Absorption, distribution, metabolism and excretion and animal toxicity data – see Annex C

14. Glucosamine sulphate is rapidly absorbed from the gut, undergoing significant first pass metabolism in the liver, glucosamine hydrochloride is less well absorbed. It is rapidly detected in the plasma and distributes to tissues including the liver, kidneys and articular cartilage. Glucosamine is phosphorylated and ultimately forms UDP *N*-acetyl glucosamine which is then incorporated into glycolipids, glycoprotein and proteoglycans. The majority of ingested glucosamine is rapidly degraded into smaller molecules such as water, urea and carbon dioxide.

15. Few data are available but glucosamine appears to be of low oral and sub chronic toxicity. No indication of liver toxicity has been observed with the exception of non-significantly elevated AST levels in Sprague Dawley rats fed a combination of glucosamine hydrochloride and chondroitin sulphate for 9 weeks (Echard *et al*, 2001). No data on chronic toxicity or reproductive and developmental toxicity have been identified.

Human trials- see Annex D

16. Glucosamine has been studied in numerous trials in human volunteers. Some of the studies measure biochemical parameters such AST and ALT but the results are rarely reported in any detail. Where side effects are noted these tend to be evenly distributed between groups. In some trials there appeared to be an excess of adverse effects, but these are generally gastrointestinal in nature.

Chondroitin sulphate – Absorption, distribution, metabolism and excretion and animal toxicity studies. see Annex E

17. Chondroitin is partially absorbed from the gut, both as intact chondroitin sulphate and as lower molecular weight fractions of depolymerised material. It is found in the plasma and in tissues such as the liver, kidneys and cartilage. There are few data on toxicity, but when tested chondroitin sulphate was of low toxicity.

Human trials- see Annex F

18. Chondroitin sulphate has been studied in a number of trials in human volunteers. Some of the studies measure biochemical parameters such AST and ALT but the results are rarely reported in any detail. In some trials there appeared to be an excess of adverse effects, but these are generally gastrointestinal in nature.

Summary and discussion

19. Glucosamine and glucosamine plus chondroitin sulphate are popular food supplements. However, a handful of case reports have suggested that they could be associated with hepatitis.

20. There are numerous clinical trials, investigating glucosamine and/or chondroitin sulphate but while liver enzymes are sometimes measured the results are rarely reported fully. However, it seems likely that any significant problem would have been identified by now.

21. There are a few toxicity studies in animals, which show no evidence of adverse effects on the liver, with the exception of non-significantly elevated AST in one study only.

Questions for the Committee

22. Members are asked to consider the following questions

a). Is there any evidence suggesting an association between hepatitis and glucosamine?

b). Is it possible to rule out such an association?

c) Would any further work be worthwhile?

Secretariat
September 2008

REFERENCES

British Liver Trust (2008), http://britishlivertrust.org.ukhome/the-liver

Deal, C.L., Moskowitz, R.W. (1999). Nutraceuticals as Therapeutic Agents in Osteoarthritis. Osteoarthritis, 25, 379-395.

Echard, B.W., Talpur, N.A., Funk, K.A., *et al* (2001). Effects of Oral Glucosamine and Chondroitin Sulfate Alone and in Combination on the Metabolism of SHR and SD Rats. Molecular and Cellular Biochemistry. 225, 85-91.

Ferrell, L. (2001). Liver Pathology:Cirrhosis, Hepatitis, and Primary Liver Tumours. Update and Diagnostic Problems. Modern Pathology, 13, 679-704

Merck (2008). Merck Manual Home Edition. http://www.merck.com/mmhe

Patient UK (2008) http://www.patient.co.uk

Wheater, P.R., Burkitt, H.G., Stevens, A. *et al* (1991). Basic Histopathology: A Colour Atlas and Text., 2nd Ed, Churchill Livingstone, Edinburgh.

Wikipedia (20080. http://en.wikipedia.org/wiki/hepatitis

COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

GLUCOSAMINE AND HEPATOTOXICITY

ANNEX A. CASE REPORTS

Some data in this annex are taken from an unpublished paper, so this annex will not be made publicly available.

Secretariat
September 2008

COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

GLUCOSAMINE AND HEPATOTOXICITY

ANNEX B

GLUCOSE ENANTIOMERS –Report from HFMA

The attached report “Stereo-isomerism of Glucosamine” was kindly provided by the Health Food Manufacturers Association. Information on specifications is also attached.

Secretariat
September 2008

Stereo-isomerism of Glucosamine

At the meeting of the Committee on Toxicity, held on 8th July, the Committee considered the potential hepatoxicity of Glucosamine in food supplements (TOX/2008/26). The Committee identified that supplementary intakes of Glucosamine are about a third of endogenous D-glucosamine production in the body. If supplemental Glucosamine is always in the 'D' form (+ve specific optical rotation), then there should be little concern for its toxicity. However some internet sites claim that some supplements contain a racemic mixture of D-glucosamine and L-glucosamine. This information is considered below.

International standards for Glucosamine:

The United States Pharmacopoeia (USP) contains monographs for three Glucosamine ingredients used in dietary/food supplements. These are:

Glucosamine Hydrochloride USP
C6H13NO5·HCl – Specific (optical) rotation +70.0° - +73.0°

Glucosamine Sulfate Potassium Chloride
(C6H14NO5)2SO4·2KCl – Specific (optical) rotation +50.0° - +52.0°

Glucosamine Sulfate Sodium chloride
(C6H14NO5)2SO4·2NaCl – Specific (optical) rotation +52.0° - +54.0°

They each have a +ve specific optical rotation indicating the D-Glucosamine form.
The USP also contains a monograph for Glucosamine Tablets prepared from any of the three materials above or a mixture of any of them to contain between 90% and 110% of the labelled amount of Glucosamine.

The majority of Glucosamine food supplement products in the UK contain D-Glucosamine sulphate 2 KCL followed by D-Glucosamine hydrochloride. The materials are bought either complying with the USP standard or against a specification that invariably contains a range for (+ve) specific optical rotation, which is similar to the USP range for each material

Confirmation of absence of L-Glucosamine:

The HFMA has canvassed a number of suppliers of Glucosamine to its members asking whether they are aware of the availability of L-Glucosamine alone or as a racemic mixture with D-Glucosamine. None of the suppliers in Europe or China were aware of any source apart from different salts of D-Glucosamine. Most stated that the inclusion in the specification of a +ve specific optical rotation of the solution of the Glucosamine was evidence enough of it being D-Glucosamine.

Gee Lawson (A major UK industry supplier & HFMA member)
"I am not aware if any L-glucosamine in the UK or anywhere else. In the meantime, I think the fact that the products have a positive optical rotation proves that they are in the D form and cannot be a 50:50 mixture of D and L which would be no optical rotation. Please see attached specifications that include the optical rotation information for D-Glucosamine sulphate 2KCl & D-Glucosamine hydrochloride."
Dr Julian Domszy – Gee Lawson

GEE LAWSON PRODUCT SPECIFICATION

ITEM	SPECIFICATION
Product name	D-Glucosamine Sulfate 2KCL 100% through 60 mesh
Product code	GL 19329 SAP 40019317
Appearance	White crystalline powder
Assay	98.0 – 102.0%
Loss on drying	≤0.2%
Residue on ignition	27.0 – 29.0%
Specific rotation	+50° -+52°
PH	3.0 – 5.0
Heavy metals	≤10ppm
Iron	≤10ppm
Arsenic	≤1ppm
Particle size	
Through 60 mesh	100.0%
Through 80 mesh	95%
Through 115 mesh	70%
Through 170 mesh	45%
Total plate count	≤3000cfu/g
Mould & yeast	≤100cfu/g
E.coli	Negative
Salmonella	Negative
Shelf life	24 months

GEE LAWSON PRODUCT SPECIFICATION

ITEM	SPECIFICATION
Product name	D-Glucosamine HCL 40 mesh
Code	GL
Appearance	White crystalline powder
Assay	98.0 ~ 102.0%
$[\alpha]^{20}$ D	+70.0° ~ +73.0°
Loss on drying	<0.2%
Residue on ignition	<0.1%
Iron (Fe)	<10ppm
Heavy metals	<10ppm
Lead	<0.5ppm
Arsenic	<1ppm
pH	3.0 ~ 5.0
Mesh size	100% through 40 mesh
Total plate count	<3,000cfu/g
Moulds & yeast	<300 cfu/g
E.coli	Negative
Salmonella	Negative
Shelf life	2 years

Shanghai Freeman (China – supplier to the UK market)

“I'm Allen Ma, QA manager of Shanghai Freemen.
As you know, natural glucosamine is in D- form, we can ensure that all glucosamine products of our company are isolated from chitin(is available from crab, lobster or shrimp shells) and not be produced by synthetic means.
We will also ask our suppliers to provide a statement to guarantee all their glucosamine products are derived from chitin.
Up to the present, we haven’t find any L-glucosamine products in China.
The optical rotation of glucosamine is “+” means D- form.
If you have more questions, don’t hesitate to contact me.
Best regards,
Allen, QA/QC

Shanghai Freemen LifeScience Co., Ltd.”
Phone: 86 21 6438 6638 Ext.167
Fax: 86 21 6427 9623
E-mail: ma.ning@freemen.sh.cn
Website: http:/www.sflifescience.com

Internet Information:
The following two internet sites purported that glucosamine obtained from chitin was produced in a racemic mixture of L-Glucosamine & D-Glucosamine but that their products contained only the active D-Glucosamine form:

http://www.aviva.ca/article.asp?articleid=29
Published: 04/10/2006: Good Nutrition

Good Nutrition: The Key to Living with Arthritis

by Dr. Gordon Chang
There is no simple dietary source of glucosamine. The most common sources of glucosamine are in the shells and cartilage of crustaceans such as crab, lobster and shrimp. The major problem with these sources to provide glucosamine is that they are not easily digested. The other alternative is to obtain glucosamine as a dietary supplement. However not all forms of glucosamine are the same. In the human body all glucosamine exists in the D-isomer form. During the manufacturing process to make glucosamine from crustacean shells you get a mixture of D-glucosamine and L-glucosamine. Even though the body absorbs both D glucosamine and L glucosamine, it is only able to incorporate the D-glucosamine into the body tissue. The L-glucosamine is merely broken down and excreted. Therefore when choosing a glucosamine product ensure that it contains primarily D-glucosamine for maximum benefit.

http://www.integranutrition.com/glucosamine-sheet.html

OUR GLUCOSAMINE FORMULATIONS - Integra Nutrition
However, all glucosamine sulphate products are not the same. Most commercially available glucosamine products are of very low purity and generally contain both D and L stereoisomer forms of the glucosamine molecule. Commercially available products will normally contain D-glucosamine and L-glucosamine in an approximately 50/50 ratio. However, glucosamine exists and is incorporated in the body only as D-glucosamine. Hence the L-glucosamine sulphate is wasted. Since glucosamine is sold by weight, a capsule containing 500 mg of D-glucosamine provides twice the dose of bioavailable glucosamine when compared to an identical capsule consisting of regular D/L glucosamine sulphate.

These are from USA based internet sites which are often renowned for their pseudo-scientific marketing information. However, I have explored the statement that: "during the manufacturing process to make glucosamine from crustacean shells you get a mixture of D-glucosamine and L-glucosamine" and obtained information from TSI – another major supplier of Glucosamine to the European market. I stated that I had been told (by Dr Domszy at Gee Lawson) that the hydrolysis of chitin would not change the chirality of the Glucosamine as there was no cleavage at the relevant site:

TSI - "Yes, I agree with you that the hydrolysis will not change the chirality of the sugar molecule. Acid hydrolysis of chitin should result in D-glucosamine only.
Reasons:

1. Theory:
Acid hydrolysis of chitin is a two step reaction:

a. Depolymerization which is the cleavage of the 1,4 glycosidic linkage. It is an SN1 reaction which will not change the chirality of the sugar molecule.

b. Deacetylation, the reaction takes place on the N group, not the carbon, therefore will not change the chiratlity of the sugar molecule.

Biomacromolecules. 2007 Jan;8(1):309-14.

Depolymerization and de-N-acetylation of chitin oligomers in hydrochloric acid.

Einbu A, Vårum KM.
Norwegian Biopolymer Laboratory, Department of Biotechnology, Norwegian University of Science and Technology, 7491 Trondheim, Norway.

The monosaccharide 2-amino-2-deoxy-D-glucose (glucosamine, GlcN) has recently drawn much attention in relation to its use to treat or prevent osteoarthritis in humans. Glucosamine is prepared from chitin, a process that is performed in concentrated acid, such as hydrochloric acid. This process involves two acid-catalyzed processes, that is, the hydrolysis of the glycosidic linkages (depolymerization) and of the N-acetyl linkages (de-N-acetylation). The depolymerization reaction has previously been found to be much faster compared to the deacetylation, with the consequence that the chitin chain will first be hydrolyzed to the monomer 2-acetamido-2-deoxy-D-glucose (N-acetylglucosamine, GlcNAc) which is subsequently deacetylated. We have found that the chitin disaccharide GlcNAc(1-->4)GlcNAc could be completely hydrolyzed to the monosaccharide GlcNAc with negligible concomitant de-N-acetylation, and the chitin disaccharide and monosaccharide were further used to study the depolymerization reaction and the de-N-acetylation reaction, respectively. The reactions were performed in hydrochloric acid as a function of acid concentration (3-12 M) and temperature (20-35 degrees C), and 1H-NMR spectroscopy was used to monitor the reaction rates. The 1H NMR spectrum of GlcNAc in concentrated (12 M) and deuterated hydrochloric acid at 25 degrees C was assigned. The glucofuranosyl oxazolinium (3) ion was found to exist in equilibrium with the alpha- and beta-anomers of the pyranose form of GlcNAc, where 3 was present in half the total molar concentrations of the two anomeric forms of GlcNAc. At lower acid concentration (3-6 M), only trace concentrations of 3 could be detected. The rate of de-N-acetylation of GlcNAc was determined as a function of hydrochloric acid concentration, showing a maximum at 6 M and decreasing by a factor of 2 upon decreasing or increasing the acid concentration to 3 or 12 M. The activation energy for hydrolysis of the N-acetyl linkage of GlcNAc was determined to be 102 +/- 7, 116 +/- 8, and 110 +/- 8 kJ mol(-1) at 3, 6, and 12 M hydrochloric acid concentration, respectively. The results are in accordance with the proposed SN2 reaction mechanism of the acid-catalyzed hydrolysis of the N-acetyl linkage where the rate-limiting step is the addition of water to the carbonium ion. The 1H NMR spectrum of the dimer GlcNAc-GlcNAc in concentrated (12 M) and deuterated hydrochloric acid at 25 degrees C was assigned. The rate of the acid-catalyzed cleavage of the glycosidic linkage of the dimer was determined as a function of hydrochloric acid concentration, showing a 6-fold increase from 3 to 6 M HCl concentration and a further 6-

fold increase from 6 to 12 M HCl concentration, in contrast to the much smaller effect of acid concentration on the deacetylation reaction. Activation energy for hydrolysis of the glycosidic linkage of GlcNAc-GlcNAc was determined to be 110 +/- 6, 111 +/- 6, and 112 +/- 4 kJ mol(-1) at 3, 6 and 12 M hydrochloric acid concentration, respectively, that is, very similar to the activation energies determined for the deacetylation reaction. The results are in accordance with the proposed SN1 reaction mechanism of the acid-catalyzed hydrolysis of the glycosidic linkage, where the rate-limiting step is the formation of the carbonium ion.

2. Experiment

Both of the kinetics studies of the acid hydrolysis of chitin and the other carbohydrates yield only D isomers.

a) Kinetics of Formation of *D*(+)-Glucosamine by Acid Hydrolysis of Chitin

V. Yu. Novikov *Arctic Research Institute of Sea Fisheries and Oceanography, Murmansk, Russia*

Received June 17, 1996

Abstract - Kinetics of formation of *D*-glucosamine hydrochloride by hydrolysis of chitin were studied.

A model involving dissolution of chitin in hydrochloric acid, hydrolysis of chitin with formation of *D*-glucosamine, and degradation of *D*-glucosamine was proposed. The rate constants of the reactions were evaluated.

b) Hydrolysis of Maltose, Lactose and Sucrose all result in D isomers.

a.) Maltose
Malt sugar
α-1,4-glycosydic linkage
α-D-Glucose + D-Glucose
Hydrolysis of maltose yields two molecules of D-glucose

b.) Lactose
Milk sugar
β-1,4-glycosidic linkage
β-D-galactose + D-glucose
Reducing sugar?
Hydrolysis of lactose will yield D-galactose + D-glucose

c.) Sucrose
Table sugar
α,β-1,2-glycosidic linkage
α-D-glucose + β-D-fructose
Reducing sugar?
Hydrolysis of sucrose gives D-glucose and D-fructose

Conclusion:

It is believed that Glucosamine used in food supplements in the UK is always present as D-Glucosamine because:

1. the International standards for Glucosamine are for salts of D-Glucosamine with +ve specific optical rotation when in solution.
2. the specifications supplied with glucosamine supplied to the UK (&European) markets for use in food supplements include a range for +ve specific optical rotation when in solution, similar to that given in the USP.
3. suppliers of Glucosamine to the UK (& European) market are unaware of the availability of L-Glucosamine as a material for use in food supplements.
4. statements by US internet states that Glucosamine is produced as (the less effective) racemate from the cleavage of chitin does not concur with serious scientific evidence.

Michael Evans
HFMA Technical Adviser
4th August 2008

Glucosamine Hydrochloride
$C_6H_{13}NO_5 \cdot HCl$ 215.63
D-Glucose, 2-amino-2-deoxy-, hydrochloride.
2-Amino-2-deoxy- -D-glucopyranose 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 and +73.0 .
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 0.020 N sulphuric acid: not more than 0.24% is found.
Arsenic, *Method II* 211 : 3 µg per g.
Heavy metals, *Method II* 231 : 0.001%.
Organic volatile impurities, *Method I* 467 : meets the requirements.
Assay—
Phosphate buffer— Mix 1.0 mL of phosphoric acid with 2 L of water, and adjust with potassium hydroxide to a pH of 3.0.
file://D:\NutriTech\HFMA\Glucosamine\USP-NF Glucosamine HCl_files\usp28nf23s2_m35200.htm 04/08/2008
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-µm 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 × 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 factor for the glucosamine peak is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.
Procedure — Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assaypreparation* 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 taken 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.
Auxiliary Information— *Staff Liaison* : Lawrence Evans, III, Ph.D., Scientist
Expert Committee : (DSN) Dietary Supplements: Non-Botanicals
USP28–NF23 Page 2100
Pharmacopeial Forum : Volume No. 28(1) Page 92
Phone Number : 1-301-816-8389
file://D:\NutriTech\HFMA\Glucosamine\USP-NF Glucosamine HCl_files\usp28nf23s2_m35200.htm 04/08/2008
Glucosamine Sulfate Potassium Chloride
$(C_6H_{14}NO_5)_2SO_4 \cdot 2KCl$ 605.52
Bis(D-Glucose, 2-amino-2-deoxy-), sulfate potassium chloride complex.
Bis(2-Amino-2-deoxy- -D-glucopyranose) sulfate potassium chloride complex (-,-) [*38899-05-7*].
» Glucosamine Sulfate Potassium Chloride contains not less than 98.0 percent and not more than 102.0 percent of $(C_6H_{14}NO_5)_2SO_4 \cdot 2KCl$, 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 .
Test solution— Transfer about 50 mg of Glucosamine Sulfate Potassium Chloride to a centrifuge tube, and dissolve in 2 mL of water. Add about 0.5 mL of barium chloride TS, and centrifuge. Evaporate the supernatant, and dry the residue at 105 for 2 hours. The IR spectrum corresponds to a similar preparation of *USP Glucosamine Hydrochloride RS*, with the addition of barium chloride TS being omitted.
B: It meets the requirements of the tests for *Chloride* 191 , *Potassium* 191 , and *Sulfate* 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 +50.0 and +52.0 .
Test solution: 35 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 : between 27.0% and 29.0%.
Sodium— A solution (1 in 10), tested on a platinum wire, does not impart a pronounced yellow color to a nonluminous flame.
Arsenic, *Method II* 211 : 3 µg per g.
Heavy metals, *Method II* 231 : 0.001%.
Organic volatile impurities, *Method I* 467 : meets the requirements.

Content of sulfate— Transfer about 1 g of Glucosamine Sulfate Potassium Chloride, accurately weighed, to a 250-mL beaker, and dissolve in about 100 mL of water. Add 4 mL of 6 N hydrochloric acid. Heat the solution to boiling, and add, with constant stirring, sufficient boiling barium chloride TS to completely precipitate the sulfate. Add an additional 2 mL of barium chloride TS, and digest on a steam bath for 1 hour.
Pass the mixture through ashless filter paper, transferring the residue quantitatively to the filter, and wash the residue with hot water until no precipitate is obtained when 1 mL of silver nitrate TS is added to 5 mL of washing. Transfer the paper containing the residue to a tared crucible. Char the paper, without burning, and ignite the crucible and its contents to constant weight. Calculate the content of sulfate by multiplying the weight obtained by 0.4116. The content of sulfate is between 15.5% and 16.5%.
file://D:\NutriTech\HFMA\Glucosamine\USP-NF Glucosamine 2KCl_files\usp28nf23s2_m35208.htm 04/08/2008
Assay—
Phosphate buffer, *Mobile phase*, *Standard preparation*, and *Chromatographic system*— Proceed as directed in the *Assay* under *Glucosamine Hydrochloride*.
Assay preparation— Transfer about 125 mg of Glucosamine Sulfate Potassium Chloride, 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.
Procedure — Proceed as directed in the *Assay* under *Glucosamine Hydrochloride*.
Calculate the percentage of $(C_6H_{14}NO_5)_2SO_4 \cdot 2KCl$ in the portion of Glucosamine Sulfate Potassium Chloride taken by the formula:
$(605.52/431.26)(10{,}000C / W)(r_U / r_S)$, in which 605.52 is the molecular weight of glucosamine sulfate potassium chloride and 431.26 is twice the molecular weight of glucosamine HCl; *W* is the weight, in mg, of Glucosamine Sulfate Potassium Chloride used to prepare the *Assay preparation;* and the other terms are as defined therein.
Auxiliary Information— *Staff Liaison* : Lawrence Evans, III, Ph.D., Scientist
Expert Committee : (DSN) Dietary Supplements: Non-Botanicals
USP28–NF23 Page 2101
Phone Number : 1-301-816-8389
file://D:\NutriTech\HFMA\Glucosamine\USP-NF Glucosamine 2KCl_files\usp28nf23s2_m35208.htm 04/08/2008
Glucosamine Sulfate Sodium Chloride
$(C_6H_{14}NO_5)_2SO_4 \cdot 2NaCl$ 573.31
Bis(D-Glucose, 2-amino-2-deoxy-), sulfate sodium chloride complex.
Bis(2-Amino-2-deoxy- -D-glucopyranose) sulfate sodium chloride complex (-,-) [*38899-05-7*].
» Glucosamine Sulfate Sodium Chloride contains not less than 98.0 percent and not more than
102.0 percent of $(C_6H_{14}NO_5)_2SO_4 \cdot 2NaCl$ 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 .
Test solution— Transfer about 50 mg of Glucosamine Sulfate Sodium Chloride to a centrifuge tube, and dissolve in 2 mL of water. Add about 0.5 mL of barium chloride TS, and centrifuge. Evaporate the supernatant, and dry the residue at 105 for 2 hours. The IR

spectrum corresponds to a similar preparation of *USP Glucosamine Hydrochloride RS*, with the addition of barium chloride TS being omitted.
B: It meets the requirements of the tests for *Chloride* 191 , *Sodium* 191 , and *Sulfate* 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 +52.0 and +54.0 .
Test solution: 35 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 : between 23.5% and 25.0%.
Potassium— Acidify 5 mL of a solution (1 in 20) with 6 N acetic acid, and add 5 drops of sodium cobalt nitrite TS: no precipitate is formed.
Arsenic, *Method II* 211 : 3 µg per g.
Heavy metals, *Method II* 231 : 0.001%.
Organic volatile impurities, *Method I* 467 : meets the requirements.
Content of sulfate— Transfer about 1 g of Glucosamine Sulfate Sodium Chloride, accurately weighed, to a 250-mL beaker, and dissolve in about 100 mL of water. Add 4 mL of 6 N hydrochloric acid. Heat the solution to boiling, and add, with constant stirring, sufficient boiling barium chloride TS to completely precipitate the sulfate. Add an additional 2 mL of barium chloride TS, and digest on a steam bath for 1 hour.
Pass the mixture through ashless filter paper, transferring the residue quantitatively to the filter, and wash the residue with hot water until no precipitate is obtained when 1 mL of silver nitrate TS is added to 5 mL of washing. Transfer the paper containing the residue to a tared crucible. Char the paper, without burning, and ignite the crucible and its contents to constant weight. Calculate the content of sulfate by multiplying the weight obtained by 0.4116. The content of sulfate is between 16.3% and 17.3%.
file://D:\NutriTech\HFMA\Glucosamine\USP-NF Glucosamine 2NaCl_files\usp28nf23s2_m35210.htm 04/08/2008
Assay—
Phosphate buffer, *Mobile phase*, *Standard preparation*, and *Chromatographic system*— Proceed as directed in the *Assay* under *Glucosamine Hydrochloride*.
Assay preparation — Transfer about 100 mg of Glucosamine Sulfate Sodium Chloride, 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.
Procedure — Proceed as directed in the *Assay* under *Glucosamine Hydrochloride*.
Calculate the percentage of $(C_6H_{14}NO_5)_2SO_4 \cdot 2NaCl$ in the portion of Glucosamine Sulfate Sodium Chloride taken by the formula:
$10{,}000(573.31/431.26)(C / W)(r_U / r_S)$,
in which 573.31 is the molecular weight of the glucosamine sulfate sodium chloride and 431.26 is twice the molecular weight of glucosamine HCl; *W* is the weight, in mg, of Glucosamine Sulfate Sodium Chloride used to prepare the *Assay preparation;* and the others terms are as defined therein.
Auxiliary Information— *Staff Liaison* : Lawrence Evans, III, Ph.D., Scientist
Expert Committee : (DSN) Dietary Supplements: Non-Botanicals *USP28–NF23* Page 2101
Phone Number : 1-301-816-8389

file://D:\NutriTech\HFMA\Glucosamine\USP-NF Glucosamine 2NaCl_files\usp28nf23s2_m35210.htm 04/08/2008

COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

GLUCOSAMINE AND HEPATOTOXICITY

ANNEX C

GLUCOSAMINE – Absorption, distribution, metabolism and excretion and animal toxicology data.

1. Glucosamine (2-amino-2-deoxy-D-glucose) is an amino monosaccharide found in mucopolysaccharides (glycosaminoglycans) and chitin. Glycosoaminoglycans are large complexes of negatively charged carbohydrate chains which are incorporated into mucous secretions, connective tissue, skin, tendons, ligaments and cartilage (Anderson *et al*, 2005). In humans the endogenous production of glucosamine is in the range 4-20 g/day. The molecular weight of glucosamine is 178.17.

Absorption

2. Glucosamine is extensively absorbed orally, with a significant proportion undergoing first-pass metabolism in the liver (Anderson *et al*, 2005). Over 90% of a radiolabelled dose is absorbed with 10% appearing in the faeces, subsequently 20-30% of the dose appears in the urine and up to 70% appears in exhaled CO_2, 8-12% of the dose is retained in the tissues (Deal and Moskowitz, 1999). The pattern of distribution and excretion of glucosamine is comparable after oral and iv administration but the plasma and tissue concentrations are about 5 times lower because of the first pass effect.

Animal

3. The bioavailability of glucosamine in rats and dogs is estimated to be about 25 **%** (Setnikar *et al*., 1993). The bioavailability of glucosamine in dogs given a single dose of a glucosamine hydrochloride and low molecular weight chondroitin sulphate combination was approximately 12% (Adebowale *et al*, 2002).

4. Following oral administration to rats, radiolabelled glucosamine was detected in the plasma, peaking 4 hours after administration (Setnikar *et al*, 1984). The level of radioactivity in the plasma then declined slowly with biphasic kinetics. Faecal excretion was limited; demonstrating high bioavailability of oral glucosamine. A high level of radiolabelled CO_2 was excreted suggesting that the glucosamine was rapidly broken down into smaller components.

5. The bioavailability of orally administered glucosamine hydrochloride in the rat was estimated to be only 21% (Aghazadeh-Habashi *et al*, 2002). However, these authors

suggested that incomplete absorption was occurring, since reduced bioavailability was not observed after i.p. administration. However, the dose used was high (350 mg/kg) and the salt used was glucosamine hydrochloride this may not be inconsistent as it has been suggested that the sulphate salt of glucosamine is readily absorbed from the small intestine since the majority is in an non-ionised form, but the hydrochloride salt is thought to be less well absorbed, (Maher, 2000, Deal and Moskowitz, 1999).

Human

6. Eighteen subjects with osteoarthritis were given 1500 mg doses of commercially available glucosamine sulphate (Biggee, *et al*, 2005). Glucosamine could not be detected at baseline, but was detected in 17/18 subjects after dosing, beginning to rise at 30-45 minutes after dosing and peaking at 90-180 minutes, with a range of 1.9 -11.5 µM (0.34-2 µg/mL). Glucose and glucosamine appear to compete competitively for transport from the gastrointestinal tract, (Biggee, *et al*, 2007). The authors also suggest that ingestion of glucose with glucosamine might increase serum levels since competitive inhibition of glucosamine uptake into the liver might allow more glucosamine into the peripheral circulation.

Distribution

7. Steady state pharmacokinetic parameters indicate that glucosamine distributes to the vascular and extra-vascular compartments.

8. Radiolabelled glucosamine initially appears in the plasma compartment and subsequently distributes into tissues. In dogs given ^{14}C labelled glucosamine (Setnikar *et al,* 1986) the radioactivity was almost completely absorbed from the gastrointestinal tract, rapidly appearing in the plasma before being incorporated into α and ß-globulins. Radioactivity was then detected in the liver, kidneys and articular cartilage.

9. In rats given an oral dose of ^{14}C labelled glucosamine, radioactivity was observed in the liver, kidneys, cartilage, epiphysis of the femoral head and in other extra vascular tissues (Setnikar *et al*, 1984)

10. Following iv infusion, in humans the highest levels of glucosamine were found in the liver, kidneys and articular cartilage (Barclay *et al*, 1998**;** Setnikar *et al*, 1986, 1993)

11. Glucosamine is actively transported from the extracellular tissues into cells by glucose transporter (GLUT), a process facilitated by insulin. Three members of the GLUT family can transport glucosamine (GLUT 1, 2 and 4) (Uldry *et al*, 2002). While GLUT1 and GLUT4 have similar affinities for glucose and glucosamine, GLUT 2 has an approximately 20 fold higher affinity for glucosamine.

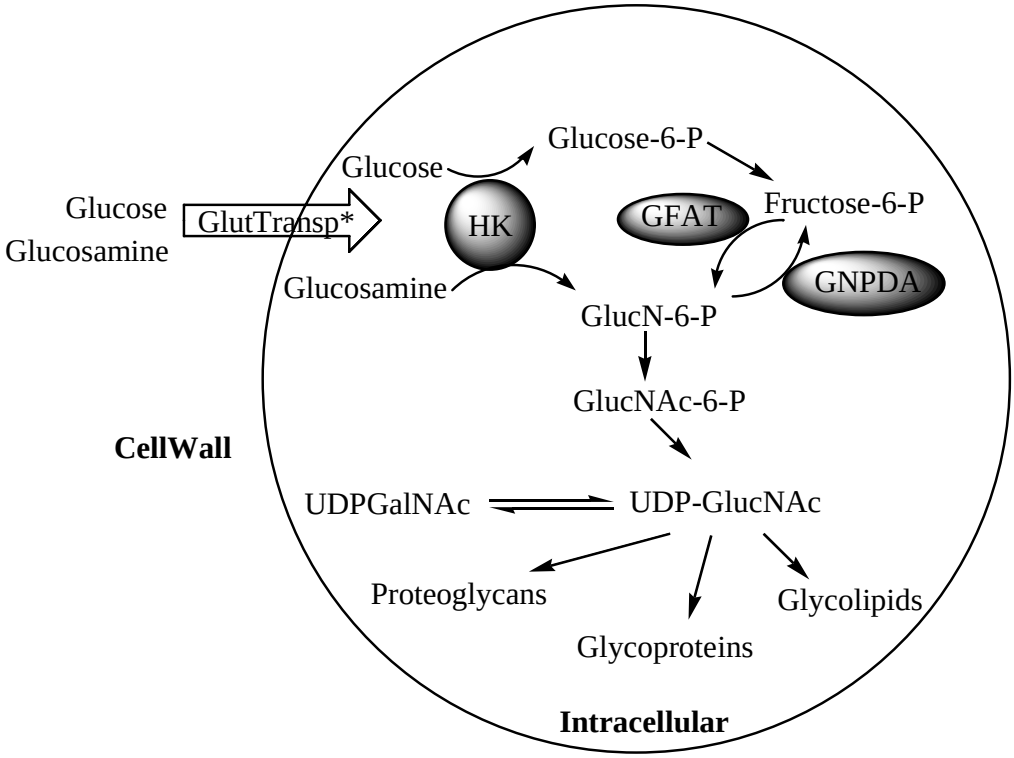
Metabolism

See Fig. 1 below.

12. Glucosamine is phosphorylated by one of a family of hexokinases (HK) to glucosamine-6-phosphate (Anderson *et al*, 2005). Endogenous glucosamine is formed from glucosamine -6-phosphate which is formed from fructose-6-phosphate and glutamine by glucosamine -6-phosphate synthase (also known as glucosamine: fructose-6-P amidotransferase or GFAT).

13. GFAT irreversibly catalyses the first and rate-controlling step in the formation of uridine diphosphate –*N*- acetylglucosamine (UDP-GlucNAc) a precursor of all macromolecules containing amino sugars. Glucosamine -6-phosphate is readily converted back to fructose-6-phosphate by glucosamine -6-phosphate deaminase (GNPDA). Glucosamine -6-phosphate is acetylated to *N*- acetylglucosamine 6-phosphate by glucosamine phosphate *N*- acetyltransferase and subsequently converted to UDP glucosamine by UDP *N*- acetylglucosamine pyrophosphorylase. In some tissues, glucosamine -6-phosphate may be converted to glucosamine-1-phosphate by phosphacetylglucosamine mutase during the formation of UDP-GlucNAc**.** Exogenous glucosamine enters the pathway at a point distal to GFAT activity.

Fig 1. Glucosamine metabolism (taken from Anderson *et al*, 2005).



KEY: GlucN (glucosamine); GlucNAc-6-p (*N*-acetyl glucosamine-6-phosphate); UDP (uridine diphosphate) UDP GalNAc (UDP- *N* –acetylgalactosamine)

14. Bovine primary chondrocytes were incubated with different concentrations of glucose, glucosamine or glucosamine sulphate in medium containing high or low levels of glucose (Qu *et al*, 2007). The intra-cellular levels of UDP hexoses, UDP hexosamine, UDP glucoronic acid, glycosaminoglycan synthesis rate and aggrecan mRNA expression were also measured. The idea of the experiment was to assess whether glucosamine or related compounds could increase proteoglycan synthesis at physiologically relevant levels. Incubation of chondrocytes in low glucose medium with 1mM glucose, glucosamine or glucosamine sulphate increased the concentration of UDP hexosamine, the highest increase occurring after the addition of glucosamine sulphate for 30 minutes. This was not seen at the lower concentrations of 100 µM. The authors concluded that at physiologically relevant levels, glucosamine sulphate could not increase UDP hexosamine and UDP glucoronic acid levels. The intracellular contents of UDP hexosamine and UDP glucoronic acid were higher after 8 days incubation compared to 2 days, suggesting that the UDP glucoronic acid level could be the rate limiting step for glycosaminoglycan synthesis.

15. D-glucosamine, particularly at high concentrations causes an increase of UDP- *N*-acetyl-D-glucosamine and UDP- *N*- acetyl-D-galactosamine in the liver (reviewed Decker and Keppler, 1974). A reduction in uridine-phosphate and UDP-hexose pools occurs as a consequence of D-glucosamine metabolism. The extent of the uridine phosphate trapping depends on the enzyme patterns of the tissue concerned. The effect of glucosamine on liver is less pronounced than that of galactose analogues. The metabolism of glucosamine is highly regulated by rates of transport into various tissues and key enzymatic steps (Anderson *et al*, 2005). GFAT is strongly inhibited by UDP-GlucNAc, its end product.

Excretion

16. A substantial quantity of glucosamine is rapidly broken down into smaller compounds such as water, carbon dioxide and urea and excreted in expired air, in urine and to a lesser extent in faeces. The majority of glucosamine recovered in faeces following oral dosing is presumed to be unabsorbed.

Animals

17. In a pharmacokinetic study in dogs (Setnikar *et al,* 1986; Setnikar and Rovati, 2001) 21% of a single oral dose of C^{14} labelled glucosamine was excreted, with 1.2% of the parent compound being excreted intact in the urine. Only a small amount of glucosamine was excreted in the faeces. Notable amounts were converted into CO_2 and excreted into expired air. Comparable effects were observed in rats.

Humans

18. Following an iv. dose of 800 mg glucosamine in humans, the majority of the glucosamine not incorporated into tissues was excreted in the urine, with small percentages of faecal elimination (Barclay *et al*, 1989). Elimination half life was estimated to be 15 hours in human volunteers (Persiani *et al*, 2005).

Effect of supplement administration on tissue glucosamine levels.

19. Following repeated oral administrations of standard crystalline glucosamine sulphate powder to healthy volunteers, glucosamine was rapidly bioavailable (Persiani *et al*, 2005). In this study, 12 volunteers were given three consecutive daily doses of 750, 1500 and 3000 mg glucosamine sulphate. Glucosamine levels were determined in plasma at intervals up to 48 hours after the last dose. Absorption was linear in the dose range 750-1500 mg but was less than expected at the 3000 mg level possibly due to the saturation of absorption processes. The maximum average concentrations measured were up to 30 fold higher than endogenous levels following the 1500 mg dose, reaching the 10 µM level after approximately 3 hours. Glucosamine persisted in the plasma for at least 48 fours after dosing, so that an elimination half life could not be calculated. However, taking the steady state into account, this was tentatively estimated to be 15 hours.

20. Twelve osteoarthritic patients received 14 consecutive once daily doses of 1500 mg crystalline glucosamine sulphate powder (Persiani *et al*, 2007). Plasma and synovial fluid were collected at baseline and steady state (3 hours after the last dose). Mean endogenous concentrations in plasma and synovial fluid were 57 ng/ml and 36.5 ng/ml respectively and varied between patients (41-121 ng/ml and <10-67 ng/ml respectively). Three hours after dosing, glucosamine levels increased substantially in all patients, with mean increases of 20.5 and 21.5 fold in plasma and synovial fluid respectively. In plasma, the median post-treatment concentration was 1282 ng/ml (range 600-4061 ng/ml) and in synovial fluid 777 ng/ml (range 577-3248 ng/ml). Plasma and synovial glucosamine concentrations were highly correlated.

21. Assuming a commercial dose of 1500 mg glucosamine sulphate (6.5.mmol) was totally and rapidly distributed in the intra and extra cellular fluid, the maximum concentration reached would be 136 µM (Qu *et al*, 2007). In practice, extensive metabolism would make this much lower.

Animal toxicology studies

Acute

22. The oral LD50 for glucosamine has been estimated to be >8000 mg/kg bw in rats and mice and >6000 mg/kg in rabbits (Setnikar, 1991a) dosed by stomach tube. In an unpublished study (Glaza, 2002 cited in Anderson, 2005), no adverse clinical, macro or microscopic effects were observed in 5 rats of each sex given a single dose of 5000 mg/kg glucosamine by stomach tube.

Sub-acute and sub-chronic

23. Sprague Dawley rats were given chemically defined diets, containing 25, 50, 80, 120, 160 or 200 g/kg glucosamine as solutions of 50% solids for 24 days (Sugimura *et al*, 1959). The glucosamine was a replacement for glucose and was administered by inverted feeding tube *ad libitum* or force fed (details not provided). Glucosamine depressed the

growth rates of the animals. A second group of rats had Walker carcinomas transplanted into them; glucosamine treatment did not inhibit tumour growth (as had been previously reported). No toxic signs were observed in the animals, all animals remaining "clean and active".

24. In a study, intended to look at effects on insulin resistance, groups of eight male Sprague Dawley (SD) or Spontaneously Hypertensive (SH) rats were given baseline diet or diet containing 0.5% glucosamine,.0.4% chondroitin sulphate or a combination of both at the same percentage for 9 weeks (Echard *et al*, 2001). Based on average weights and food consumption, a glucosamine concentration of 0.5% was estimated to be 10-20 times the human dose of 1500 mg for a 70 kg male (equivalent to 21.4 mg/kg- suggesting the rats were exposed to 200-400 mg/kg bw).The authors considered that adjusting to the metabolic characteristics of the rat, the glucosamine intake could be estimated to be 3-7 times the human dose. Among the biochemical parameters measured were AST and ALT levels which appeared elevated in the SD rats receiving the combination of glucosamine and Chondroitin sulphate. However, the increases were not statistically significant when analysed by ANOVA.

	ALT (units/ml)	AST (units/ml)
SD rats		
Control	36 ± 6.6	150 ± 42
Glucosamine	44 ± 7.3	136± 36
CS	52 ± 10	150± 26
Combination	84 ± 25.4	322±122
SH rats		
Control	95.3 ± 21.6	324 ± 70.6
Glucosamine	132 ± 43.9	305± 86
CS	107±25.1	265± 37
Combination	132 ± 55.2	394±101

All livers (control and treated) of SD and SH rats showed hepatocyte vacuolisation with a predominantly portal distribution. This observation is not discussed further. No comments were made regarding adverse clinical signs

25 Groups of 9 male or female Sprague Dawley rats were treated daily with 0, 500, 1000 or 2000 mg/kg chitosan oligosaccharide by gavage for 4 weeks (Kim *et al*., 2001). (Chitin is a polymer of *N*-acetylglucosamine, chitosan is derived by deacetylation by alkali, resulting in a co-polymer of ß-(1→4)-2-acetamido-D-glucose and ß-(1→4)-2-amino-D-glucose (the latter usually exceeding 80%). There were no difference between treated and control animals in behaviour, external appearance, body weights, food consumption, urinalysis, haematology, blood biochemistry (measurements included AST and ALP, the latter being slightly, but non-significantly decreased in the 1000 mg/kg females only),

relative organ weights and histopathology. The NOAEL was considered to be greater than 2000 mg/kg.

26. Groups of 10 male or female F344 rats were given diet containing 0, 0.625, 1.25, 2.5 or 5% *N*-acetylglucosamine for 13 weeks (Lee *et al*., 2004). No indications of toxicity were observed in a range of parameters including clinical signs, haematology, serum biochemistry, and histopathology. On this basis a NOAEL of 5% equivalent to 2476 and 2834 mg/kg bw/day for male and female rats respectively was established. *N*-acetylglucosamine is rapidly hydrolysed to glucosamine *in vivo*. Liver weights were significantly reduced in treated males but this was not dose-related.

27. Groups of 10 male or female F344 rats were given diet containing 0, 0.04, 0.2, or 1% *N*-oligoglucosamine for 90 days (Naito *et al*, 2007). Oligoglucosamine is a preparation of hydrolysed chitosan consisting of D-glucosamine oligomers (molecular weight is stated to be in the 100 Das implying no more than 4 linked molecules). This is used as a food additive used in Japan. No adverse clinical signs were observed in the low and mid dose groups. In the animals given 1% oligoglucosamine erythema and swelling of the snout and forelimbs was observed as was loss of fur on the forelimbs. This was attributed to dermal responses to the glucosamine adhering to the fore limbs during feeding. These findings were also associated with reduced food intake and body weights as a result of the feeding difficulties. This was more marked in the males and was apparent from day 22 onwards; the effect was consistent with the reduced food intake also observed. Final body weights were reduced by about one third in the males, but were unaffected in the females. In the 1% group platelet count, lymphocyte count and differential neutrophil count were elevated, possibly due to inflammation. Abnormalities in urinalysis and clinical chemistry (decreased total protein and albumin/globulin ratios but no changes in the levels of AST or ALT) were observed as was a small thymus and spleen, dark spots on the glandular stomach mucosae, a pale Harderian gland and reduced testes (relative liver weights were unaffected). The authors considered that it was uncertain whether all the changes were due to malnutrition in the top dose rats. The NOAEL was stated to be 0.2% (equivalent to 124 mg/kg bw/day in males and 142 mg/kg bw/day in females).

Chronic studies

28. Two unpublished studies were cited by Setnikar (1991b). In the first of these, rats were given an oral dose of 300, 900 or 2700 glucosamine sulphate for 52 weeks, In the top dose groups, 7/60 premature deaths were reported compared to 2/60 in the controls; this was not found to be statistically significant. No drug related macroscopic or histopathologic findings were observed in these animals or in the survivors examined at the end of the experiment. No further details are provided. In the second study, dogs were given a daily dose of 159, 478 or 2149 mg/kg for 26 weeks. No “clinical, laboratory or histopathologic toxic sign could be detected” even at the top dose. No further details are provided.

Other

29. In a study investigating anti-oxidant effects, pre-treatment of male ICR mice with 1500 mg/kg glucosamine, chitosan oligosaccharide or *N*-acetyl glucosamine reduced the hepatotoxicity associated with carbon tetrachloride treatment (Yan *et al*, 2006). The animals were given the oligosaccharide pre-treatment daily for 12 days before being given an i.p. dose of 20 mg/kg CCl_4 a variety of renal and hepatic parameters were then measured. The pre-treatments reduced the elevated ALT, AST, creatinine and uric acid levels associated with CCl_4 treatment (though not to the control level) (pre treatment only groups were not included). However, the decrease in AST levels associated with glucosamine pre-treatment was not significant *N*-acetyl glucosamine had the most protective effect. The pre-treatments also reduced the level of lipid peroxidation produced by the CCl_4. The treatments also reduced the depletion of total sulfhydryl (the effect of *N*-acetyl glucosamine was not significant) but had a more varied effect on non-protein sulfhydryl and consistent with this CCl_4 reduced the total anti-oxidant capability (T-AOC), an effect which was reduced by the pre-treatments. *N*-acetyl glucosamine pre-treatment increased T-AOC to above the level of the control. The pre-treatments did not reduce CCl_4 induced DNA fragmentation. Metallothioneine (MT) levels in the liver were increased both after the pre-treatment and after CCl_4 administration. Microscopic examination revealed that the hepatic damage induced by CCl_4 was reduced by pre-treatment. *N*-acetyl glucosamine had the most protective effect. The authors speculated that MT induction could be induced by metal accumulation in the liver as a result of the chitosan oligosaccharides binding metal ions.

Secretariat
September 2008

REFERENCES

Adebowale, A., Du, J., Liang, Z., *et al*, (2002). The Bioavailability and Pharmacokinetics of Glucosamine Hydrochloride and Low Molecular Weight Chondroitin Sulfate After Single and Multiple Doses to Beagle Dogs. Biopharmaceutics and Drug Disposition, 23, 217-225.

Anderson, J.W., Nicolosi, R.J., Borzecella, J.F. (2005) Glucosamine Effects in Humans: a Review of Effects on Glucose Metabolism, Side Effects, Safety Considerations and Efficacy. Food and Chemical Toxicology, 43, 187-201.

Aghazadeh-Habashi, A., Sattari, S., Pasutto, F.M. *et al*, (2002). Single Dose Pharmackinetics and Bioavaialability in the Rat. J Pharm Pharmaceut Sci, 5, 181-184.

Barclay, T.S., Tsourounis, C., McCart, G.M. (1998). Glucosamine. Annals of Pharmacotherapy, 32, 574-579.

Biggee,B.A., Blinn, C.M., McAlindon,T ., *et al*, (2005). Low Levels of Human Serum Glucosamine After Ingestion of Glucosamine Sulphate Relative to Capability for Peripheral Effectiveness. Ann Rheum Dis, 65, 222-226.

Biggee,B.A., Blinn, C.M., Nuitte, M., *et al*, (2007). Changes in Serum Levels of Glucosamine and Sulphate after Ingestion of Glucosamine Sulphate with and without simultaneous Ingestion of Glucose. Ann Rheum Dis, 66, 1403-1404.

Deal, C.L., Moskowitz, R.W. (1999). Nutraceuticals as Therapeutic Agents in Osteoarthritis. Osteoarthritis, 25, 379-395.

Decker, K., and Keppler, D, (1974). Galactosamine Hepatitis: Key Role of the Nucleotide Deficiency Period in the Pathogenesis of Cell Injury and Cell Death. Rev, Physiol. Biochem, Pharmacol 71, 77-106.

Echard, B.W., Talpur, N.A., Funk, K.A., *et al* (2001). Effects of Oral Glucosamine and Chondroitin Sulfate Alone and in Combination on the Metabolism of SHR and SD Rats. Molecular and Cellular Biochemistry. 225, 85-91.

Kim, S-K., Park. P-J., Yang, H-P. *et al* (2001). Subacute Toxicity Study of Chitosan Oligsaccharide in Sprague-Dawley Rats. Arzneim-Forsh/Drug Res, 51, 769-774.

Lee , K-Y., Shibutani. M., Takagi, H. *et al* (2004). Subchronic Toxicity Study of Dietary *N*-acetylglucosamine in F344 rats. Food and Chemical Toxicology, 42, 687-695.

Maher, T.J. (2000). Glucosamine Continuing Education Module. Natural Healing Track. May 2000, 2-7.

Naito , Y., Tago, K., Nagata, T. *et al* (2007). A 90-day *Ad libitum* Administration Toxicity Study of Oligoglucosamine in F344 rats Subchronic. Food and Chemical Toxicology, 45, 1575-1587.

Persiani, S., Roda, E., Rovati, L.C. *et al* (2005). Glucosamine Oral Bioavailability and Plasma Pharmacokinetics after Increasing Doses of Crystalline Glucosamine Sulfate in man. Osteoarthritis and Cartilage, 13, 1041-1049.

Persiani, S., Rotini, R., Trisolino, G. *et al* (2007). Synovial an Plasma Glucosamine Concentrations in Osteoarthritic Patients Following Oral Crystalline Glucosamine Sulfate at Therapeutic Dose. Osteoarthritis and Cartilage, 15, 764-772.

Qu, C.J., Jauhiainen, M., Auriola, S., *et al* (2007). Effects of Glucosamine Sulfate on Intra-cellular UDP Hexosamine and UDP-glucuronic acid Levels in Bovine Primary Chondrocytes. Osteoarthritis and Cartilage, 15, 773-779.

Setnikar, I., Giacheti, C., Zanolo, G. (1984). Absorption, Distribution and Excretion of Radioactivity after Single Intravenous or Oral Absorption of [^{14}C] Glucosamine to the Rat. Pharmatherapeutica, 3, 538-550.

Setnikar, I., Giacheti, C., Zanolo, G. (1986). Pharmacokinetics of Glucosamine in the Dog and in Man. Arzneim Forsch/Drug Res, 36, 729-735.

Setnikar, I., Cereda, M.A., Pacini, A., *et al* (1991a). Antireactive Properties of Glucosamine Sulfate. Arzneim-Forsh/Drug Res, 41, 157-161.

Setnikar, I., Pacini, A., Revel. L. (1991b). Antiarthritic Effects of Glucosamine Sulfate. Arzneim-Forsh/Drug Res, 41, 542-545.

Setnikar, I., Palumbo, R., Canali. S., *et al* (1993). Pharmacokinetics of Glucosamine in Man. Arzneim-Forsh/Drug Res, 43, 1109-1113.

Sugimura, T., Birnbaum, S.M., Winitz, M., *et al* (1959). Quantitative Nutritional Studies with Water-Soluble Chemically Defined Diets.IX. Further Studies on D-Glucosamine- Containing Diets. Archives of Biochemistry and Biophysics, 83, 521-527.

Uldry, M., Ibberson, M., Hosokawa, M., *et al*, (2002). GLUT2 is a High Affinity Glucosamine Transporter. FEBS Letters, 524, 199-203.

Yan, Y., Wanshun, L., Baoqin, H. *et al* (2006). Protective Effects of Chitosan Oligsaccharide and its Derivatives Against Carbon Tetrachloride-Induced Liver Damage in Mice. Hepatology Research, 35, 178-184.

COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

GLUCOSAMINE AND HEPATOTOXICITY

ANNEX D

GLUCOSAMINE – Human volunteer trials

Glucosamine Clinical trials

1. Glucosamine has been investigated in a number of clinical trials. These have been tabulated below. The great majority of the trials have been conducted in patients with osteoarthritis, with a few being conducted in athletes or servicemen. Where clinical chemistry has been conducted (in about half the studies), there have been no adverse effects observed and no significant differences measured. In general, these data are not provided and in it is unclear whether there are any outlying elevated idiosyncratic responses that may not have been reported. Where the paper reports that data were analysed individually (Hughes and Carr, 2002; Noack *et al*, 1994) no adverse effects were apparent. This was also noted in Reichelt *et al*, 1994 where glucosamine was given i.m.

2. In one of the few patients where results were reported, groups of 15 in-patients were given injections of 400 mg glucosamine sulphate (i.m. or intra-articular) or a piperazine/chlorbutanol combination for one week followed by two weeks of oral doses of glucosamine sulphate (3x 500 mg) or placebo for a further 2 weeks (Crolle and D'Este, 1980). There were no differences in ALT or AST levels between groups or following treatment (see below).

	Glucosamine group		Placebo group	
	Before	After	Before	After
ALT(U/L)	25.5±22.1	21.9±12.6	24.0±19.3	20.9±13.7
AST (U/L)	6.1±6.0	3.5±2.2	5.3±5.1	5.6±8.8

In a very similar trial by D'Ambrosio and colleagues (1981) AST and ALT levels were also unaffected by treatment. in groups of 15 patients given placebo or glucosamine respectively.

	Glucosamine group		Placebo group	
	Before	After	Before	After
ALT(U/L)	14.8±1.7	12.8±1.4	21.1±12.4	12.4±1.2
AST (U/L)	24.9±2.6	16.0±1.9	30.9±4.4	15.4±2.3

3. In a 6 month study by Herrero-Beaumont *et al* (2007) patients were given 1500 mg glucosamine sulphate, 3000 mg acetoaminophen or placebo (n =106, 108,104 respectively). Routine laboratory tests for liver function (assessed by transaminase and gamma glutamyl transferase levels (GGT) at baseline, 3 months, 6 months and end of study) indicated that more people in the acetoaminophen group developed abnormalities in liver function than the other 2 groups; abnormalities were detected in 21 patients compared with 2 in the glucosamine and 6 in the placebo group. Two patients (1 placebo, 1 acetoaminophen with) were withdrawn from the study at 3 months with clinically significant levels (ALT in 1 placebo patient at 2 x normal, GGT at 3 x normal in 1 acetoaminophen patient). Clinically significant levels (2-3 x above upper reference levels) of GGT were measured in one acetaminophen and 1 glucosamine patient but these did not require study withdrawal.

4. Many trials measure haematology since chondroitin and glycosoaminopolysulfate have a heparanoid structure (Leffler *et al*, 1999).

5. The largest randomised trial was conducted by Clegg *et al* (2006)[1]. In this, 1583 patients were randomised to receive placebo, glucosamine, chondroitin, glucosamine plus chondroitin or celexicob for 24 weeks. A range of biochemical parameters were measured including liver enzymes. The results were not fully reported but it was stated that adverse events were mild and evenly distributed between groups

[1] The authors have been contacted regarding individual data, but a response has not yet been received.

Table 1 Human trials with glucosamine

Numbers/design	Dose/day	Duration	Clinical Chemistry?	Comments	Reference
15/ group	400 mg glucosamine sulphate or piperazine/chlorbutanol by injection followed by 3 x 500mg glucosamine sulphate Or placebo	1 week 2 weeks	No differences in haematology, blood urea nitrogen, blood glucose, ALT or AST between groups or following treatment.		Crolle and D'Este (1980)
15/ group	400 mg glucosamine sulphate or piperazine/chlorbutanol by injection followed by 6 x 250mg glucosamine sulphate or placebo	1 week 2 weeks	No differences in haematology, blood urea nitrogen, blood glucose, ALT or AST between groups or following treatment.		D'Ambrosio *et al* (1981)
200 Double-blind placebo controlled,	3x 500 mg glucosamine sulphate or 3 x 400 mg ibuprofen	4 weeks	No "significant changes" in haematology or laboratory tests compared to baseline.	Increased adverse events in the ibuprofen group.	Müller-Faβbender *et al* (1994)

252 Double-blind placebo controlled,	3x 500 mg glucosamine sulphate or placebo	4 weeks	No significant changes in mean values or individual data for routine laboratory tests (haematology, clinical chemistry urinalysis).	No difference in adverse events between groups	Noack *et al* (1994)
106 Double-blind placebo controlled, athletes with acute injury	1500 mg glucosamine sulphate	4 weeks		Subjects "reported no acute side effects"	Ostojic *et al* (2007)
178 Double-blind placebo controlled	1500 mg glucosamine sulphate or 1200 mg ibuprofen.	4 weeks	No differences in urine analysis (glucose, proteins, urobilinogen), haematology, glutamic-pyruvic transminase, blood urea nitrogen.	Fewer adverse effects in the glucosamine group. Reported effects were nausea, stomach discomfort and sleepiness.	Qui *et al* (1998).
80 Double-blind placebo controlled	3x 500 mg glucosamine sulphate,	30 days	No differences in urine analysis, haematology,	No significant differences in adverse effects between the groups.	Drovanti *et al* (1980).

1208 patients in multi-centre open trial	3 x 500 mg glucosamine sulphate	50 days ± 14		Well tolerated by more patients than other treatments. A range of side effects reported, none hepatic.	Tapadinhas *et al* (1982)
20 Double-blind placebo controlled	3 x 500 mg glucosamine sulphate,	6-8 weeks	No differences in urine analysis, haematology, erythrocyte sedimentation rate		Pujalte *et al* (1980)
40 Double-blind placebo controlled	1500 mg glucosamine sulphate,	8 weeks	Haematology unaffected by treatment	Heartburn, epigastric pain and nausea	Vaz (1982).
120 Double-blind placebo controlled followed by Open label study		8 weeks		No significant differences in adverse effects between the groups. Effects largely GI	Houpt *et al* (1999)
95 Double-blind placebo controlled	1500 mg glucosamine sulphate or 1800 mg repragen (polyherbal supplement)	8 weeks	"laboratory based assays" conducted. Results unchanged from baseline.	No significant adverse events attributable to treatment.	Mehta *et al* (2007)

98 Double-blind placebo controlled	500 mg x 3 glucosamine sulphate,	2 months	Haematology unaffected by treatment	No significant differences in adverse effects between the groups. Effects largely GI	Rindone *et al* (2000).
45 DBPC, 114 (59 placebo, 55 treatment	1500 mg Glucosamine hydrochloride + 1200 mg Chondroitiin Sulfate	12 weeks		Comparable number of adverse effects (largely GI) in both groups	Nguyen *et al* (2001)
108 Open trial	2x 250 mg glucosamine sulphate,	Twice weekly injections for 5 weeks 12 weeks		Exhaustion In 1 subject	Vajranetra (1983).
46 Double-blind placebo controlled	2000 mg glucosamine sulphate	12 weeks		No differences in distribution of side effects between groups. Mild effects and of short duration, no withdrawals	Braham *et al* (2003)
70 Double-blind placebo controlled	1500 mg glucosamine sulphate, Aquamin, combination or placebo	12 weeks		No differences in adverse effects between groups	Frestedt *et al* (2008)

205 Double-blind placebo controlled, internet based trial	1500 mg glucosamine sulphate	12 weeks		No differences in number and type of adverse effects between groups.	McAlindon *et al* (2004)
51 Double-blind placebo controlled, patients with Rheumatoid arthritis	3 x 500 mg glucosamine hydrochloride	12 weeks		Stated to be well tolerated.	Nakamura *et al* (2007)
45 Double-blind placebo controlled	3 x 500 mg glucosamine sulphate or ibuprofen	3 months		2 dropouts due to dizziness, stomach upset.	Thie *et al* (2001)
1583 Double-blind placebo controlled	1500 mg glucosamine sulphate, 1200 mg chondroitin sulphate, glucosamine + chondroitin, or celecoxib	24 weeks	Liver enzyme levels measured, results not provided	Adverse events mild and evenly distributed between groups	Clegg *et al*, 2006
137 Double-blind placebo controlled Discontinuation trial in patients previously taking glucosamine	Up to 1500 mg glucosamine sulphate (dose usually taken) or placebo.	24 weeks or first disease flare		No serious adverse effects reported and no differences between groups.	Cibere *et al* (2004)
80 Double-blind placebo controlled	3 x 500 mg Glucosamine sulphate	6 months	No significant differences between groups in blood or urine tests. No adverse	No significant differences in adverse events between the groups.	Hughes and Carr (2002)

			effects in individual patients.		
318 Double-blind placebo controlled	1 x1500 mg Glucosamine sulphate (plus 2x placebo), 3 x 1000 g acetoaminophen or placebo.	6 months	Increased number of abnormal LFTs in acetoaminophen patients. No significant differences between groups in other tests	No significant differences in adverse events between the groups.	Herrero-Beaumont *et al* (2007)
222 Double-blind placebo controlled	1500 mg Glucosamine sulphate/day or 2 x 750 mg/day	2 years		Adverse effects "generally mild" and similar in both groups.	Rozendaal *et al* (2008)
212 Double-blind placebo controlled	1500 mg Glucosamine sulphate	3 years	"No great abnormalities" in routine laboratory tests	No significant differences in adverse effects between the groups.	Reginster et al (2001)
202 Double-blind placebo controlled (55 control and 66 treatment completed trial	1500 mg Glucosamine sulphate/day	3 years	"Routine laboratory safety tests" performed.)	No significant differences in adverse effects between the groups. No liver effects in placebo group. High drop out rate	Pavelká et al (2002)

Table 2. Clinical trials with glucosamine combinations

Numbers/design	Dose/day	Duration	Clinical Chemistry?	Comments	Reference
45 DBPC, 114 (59 placebo, 55 treatment	1500 mg Glucosamine hydrochloride + 1200 mg Chondroitiin Sulfate	12 weeks		Comparable number of adverse effects (largely GI) in both groups	Nguyen *et al* (2001)
34 Double-blind placebo controlled	1500 mg Glucosamine sulphate/day + 1200 mg chondroitin sulphate + 228mg manganese ascorabte	16 weeks (8 week crossover)	Haematology, occult blood	Survey of symptoms consistent with toxicity completed. No differences found between the groups in symptoms or haematology.	Leffler *et al* (1999)

REFERENCES

Braham, R., Dawson B., Goodman, C. (2003) The Effect of Glucosamine, Supplementation on People Experiencing Regular Knee Pain. British Journal of Sports Medicine, 37, 45-49.

Cibere, J., A., Kopec, J., Thorne, *et al* (2004) Randomized, Double-Blind, Placebo- Controlled Glucosamine Discontinuation Trial in Knee Osteoarthritis. Arthritis and Rheumatism, 51, 738-745.

Clegg, D.O., Reda, D.J., Harris, C.L. *et al* (2006) Glucosamine, Chondroitin Sulfate, and the Two in Combination for Painful Knee Osteoarthritis. New England Journal of Medicine, 354, 795-808.

Crolle, G. and D'Este, E. (1980) Glucosamine Sulphate for the Management of Arthrosis: A Controlled Clinical Investigation. Current Medical Research and Opinion, 7, 104-109.

D'Ambrosio, E., Casa, B., Rompani, R. *et al* (1981) Glucosamine Sulphate: A Controlled Clinical Investigation in Arthrosis. Pharmatherapeutica, 2, 504-508.

Drovanti, A., Bignamini, A.A., Rovati, A.l. (1980). Therapeutic Activity of Oral Glucosamine Sulfate in Osteoarthritis: A Placebo-Controlled Double-Blind Trial Investigation. Clinical Therapeutics, 3, 260-272.

Frestedt, J.L., Walsh, M., Kuskowski, M.A., *et al* (2008). A Natural Mineral Suuplement Provides Releif from Knee Osteoarthritis: a Randomized Controlled Pilot Trial. Nutrition Journal, 17, 7-9.

Herrero-Beaumont, G., Ivorra, J.A.R., del Carmen Trabado, M. *et al* (2007). Glucosamine Sulfate in the Treatment of Knee Osteoarthritis Symptoms. Arthritis and Rheumatism, 56, 555-567.

Houbt, J.B., McMillan, R., Wei, C., *et al* (1999). Effect of Glucosamine Hydrochloride in the Treatment of Pain of Osteoarthritis of the Knee. J Rheumatology, 26, 2423-2430.

Hughes, R. and Carr A. (2002). A Randomised, Double-Blind, Placebo-Controlled trial of Glucosamine Sulphate as an Analgesic in Osteoarthritis of the Knee. Rheumatology, 41, 279-284.

Leffler, C.T., Philippi, A.F, Leffler, S.G. *et al* (1999). Glucosamine, Chondroitin, and Manganese Ascorbate for Degenerative Disease of the Knee or Lower Back: A Randomised, Double-Blind, Placebo-Controlled Pilot Study. Military Medicine, 164, 85-91.

McAlindon, T., Formica, M., LaValley, M. *et al* (2004). Effectiveness of Glucosamine for Symptoms of Knee Osteoarthritis: Results from an Internet-Based Randomised, Double-Blind Controlled Trial. Am J Med, 117, 643-649.

Nguyen, P., Mohamed, S.E., Gardiner, D., *et al* (2001). A Randomized Double-Blind Clinical Trial of the Effect of Chondroitin Sulfate and Glucosamien Hydrochloride on Tempromandibular Joint Disorders: A Pilot Study. Orofacial Pain, 19, 130-139.

Mehta, K., Gala, J.,Bhasale, S. (2007). Comparison of Glucosamine Sulfate and a Polyherbal Supplement for the Relief of Osteoarthritis of the Knee: A Randomised, Controlled Trial [ISRCTN25438351]. BMC Complementary and Alternative Medicine, 7, 34-47.

Müller-Faβbender, H., Bach, G.L., Haase, W. *et al* (1994). Glucosamine Sulfate Compared to Ibuprofen in Osteoarthritis of the Knee. Osteoarthritis and Cartilage, 2, 61-69.

Nakamura, H., Masuko, K., Yudoh, K. *et al* (2007). Effects of Glucosamine Administration on Patients with Rheumatoid Arthritis. Rheumatol Int, 27, 213-218

Noack. W., Fischer, M., Förster, K.K., *et al* (1994). Glucosamine Sulfate in Osteoarthritis of the Knee. Osteoarthritis and Cartilage, 2, 51-59.

Ostojic, S.M., Arsic, M., Prodanovic, S. *et al* (2007). Glucosamine Administration in Athletes: Effects on Recovery of Acute Knee Injury. Research in Sports Medicine, 15, 113-124.

Pavelkã, K., Gatterovã, J., Olejarovã, M. *et al* (2002). Glucosamine Sulfate Use and Delay of Progression of Knee Osteoarthritis. Arch Intern Med, 162, 2113-2123.

Pujalte, J.M., Llavore, E., Ylescupidez.F.R. (1980) Double-blind Clinical Evaluation of Oral Glucosamine Sulphate in the basic Treatment of Osteoarthritis. Current Medical Research and Opinion, 7, 110-114. Q

Qui, G.X., Gao, S.N., Giacovelli, G.,Efficacy and Safety of Glucosamine Sulfate versus Ibuprofen in Patients with Knee Osteoarthritis. Arzneim Forsch 48, 469-474.

Reginster, J.Y., Deroisy, R., Rovati, L.C., *et al*.(2001) Long-term Effects of Glucosamine Sulphate on Osteoarthritis Progression: a Randomised, Placebo-controlled Clinical Trial. The Lancet, 357, 251-256.

Reichelt, A., Förster, K.K., Fischer, M. *et al*, (1994). Efficacy and Safety of Glucosamine Sulfate in Osteoarthritis of the Knee. Arzneim Forsch 44, 75-80.

Rindone, J.P., Hiller, D., Collacott, E., *et al* (2000). Randomised, controlled trial of Glucosamine for Treating Osteoarthritis of the Knee. West J Med, 172, 91-94.

Rozendaal, R.M., Koes, B.W., van Osch, E.H., *et al* (2008). Effect of Glucosamine Sulphate on Hip Osteoarthritis. Annals of Internal medicine, 148, 268-277.

Tapadinhas, M. J., Rivera, I. C., Bignamini, A. A. (1982) Oral Glucosamine Sulphate in the Management of Arthrosis : Report on a Multi-centre Open Investigation in Portugal. Pharmatherapeutica, 3, 157-168.

Thie, N.M.R., Prasad, N.G., Major, P.W. (2001). Evaluation of Glucosamine Sulphate Compared to Ibuprofen for the Treatment of Temporomandibular Joint Osteoarthritis: A Randomized Double-blind Controlled 3 Month Clinical Trial. J Rheumatology, Current Medical Research and Opinion, 8, 145-149.

Vajranetra (1983). Clinical Trial of a Glucosamine Compounds for Osteoarthritis of Knee Joints. J Med Ass Thailand, 57, 409-418.

Vaz (1982). Double-blind Clinical Evaluation of the Relative Efficacy of Ibuprofen and Glucosamine Sulphate in the Management of Osteoarthritis of the knee in Out-patients. Current Medical Research and Opinion, 8, 145-149.

COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

GLUCOSAMINE AND HEPATOTOXICITY

ANNEX E

CHONDROITIN – Absorption, istribution, metabolism and excretion and animal toxicology studies.

1. Chondroitin sulphate is a long chain polymer of a repeating disaccharide unit: galactosamine sulphate and glucuronic acid (Abdel Fattah and Hammad, 2001). It is the most abundant glycosoaminoglycan in the connective tissue, including in articular cartilage. Condrosulf®, a commercial preparation of chondroitin sulphate, has a relative molecular mass of 14, 000 and a sulphate to carboxyl ration 0.95 due to the high percentage of monosulphated disaccharides (38% 6-monosulfate and 55% 4-monosulfate) and a low percentage of disulfated disaccharises in the polysaccharide chains (Conte *et al*, 1995).

Absorption, distribution, metabolism and excretion

Animal

2. Over 70% of an oral dose of chondroitin sulphate (radio-labelled with ^{3}H) given to dogs or rats was absorbed being found in the urine and tissues (Palmieri *et al*, 1990; Conte *et al*, 1995). The radioactivity in the plasma was associated with high, intermediate and low molecular weight fractions (Conte *et al*, 1995). The peak level in the plasma for the high molecular mass compounds was reached after 1-2 hours, but after 36 hours high molecular weight compounds were still present in the plasma. 24 hours after dosing, the levels of radioactivity were higher in the intestine, liver, kidney, synovial fluid and cartilage compared to other tissues. In the urine, compounds with a mass equivalent to the administered chondroitin sulphate and partially hydrolysed chondroitin sulphate were measured, a large peak with a molecular mass, equal to or lower than N-acetylgalactosamine was also observed, the concentration increasing gradually with time after administration. A figure is not given, but after 72 hours it appears that approximately 12 and 24% of the radioactivity was measured in the urine of the dog and rat respectively. Radiolabelling of chondroitin sulphate (Palmieri, *et al*, 1990) indicated the presence of chondroitin sulphate poly, oligo and monosaccharides as well as tritiated water were present in the plasma, urine, synovial fluid and cartilage. The level of low molecular weight material increased

with time from administration. The high molecular weight material represented at least 10% of the orally administered chondroitin sulphate. A tropism of the radioactivity towards glycosaminoglycan rich tissues such as joint cartilage was observed.

3. Partially depolymerised chondroitin sulphate was largely excreted in the urine of rats given oral doses (Conte *et al*, 1991b). This largely occurred within the first 24 hours after dosing, with 40% being excreted by that time. Urinary excretion then declined and about 40% of the radiolabel remained in the tissues. The levels of radioactivity were higher in the liver and kidneys, and levels in the synovial fluid and cartilage were high compared to other tissues such as adipose and brain.

4. The bioavailability of chondroitin sulphate in dogs given a single dose of a glucosamine hydrochloride and low molecular weight chondroitin sulphate combination was approximately 4.8-5% and 200-278%, after multiple dosing over 14 days (Adebowale *et al*, 2002). It was concluded that the low molecular weight chondroitin sulphate showed significant accumulation after multiple dosing.

Human

5. A dose of 3g chondroitin sulphate was given to 12 human volunteers (Conte *et al*, 1991b). Two peaks were measured in the plasma. The elimination half life was approximately 6 hours. Absolute bioavailability was calculated to be 13.2%. A peak of oligo and polysaccharides with a molecular weight lower than 5000 Da derived from partial digestion of the chondroitin was also present. Taking these results with those from other routes of administration and from animal models, the authors concluded that a fraction of the exogenous chondroitin sulphate was absorbed together with high molecular weight polysaccharides resulting from a partial depolymerization and desulfation of the compound; an even more consistent fraction was absorbed as low molecular weight compounds resulting from a more marked depolymerization and desulfation. The compound was excreted in the urine.

6. In human volunteers (Conte *et al*, 1995) given a dose of 0.8 g chondroitin sulphate/day or 0.4 g twice a day the plasma chondroitin level was increased compared to the pre-dose value. The t ½ was estimated to be approximately 10 hours.

Animal toxicity studies

Acute

7. Groups of 5 Sprague Dawley rats of each sex were fed a single 5000 mg/kg dose of hydrolysed chicken sternal cartilage preparation (which contains collagen type II, chondroitin sulphate (approximately 18%) and hyaluronic acid- the preparation is intended to be used as a food supplement) (Schauss *et al*, 2007). The animals were monitored for 14 days after dosing. Body weight gain was normal and no lesions were apparent after macroscopic examination at necropsy.

Sub-chronic

8. In a sub-chronic study by the same authors (Schauss *et al*, 2007) groups.of 10 Sprague Dawley rats of each sex were fed 0, 30, 300 or 1000 mg/kg hydrolysed chicken sternal cartilage preparation daily for 90 days. dverse effects. No mortality, clinical signs or adverse effects were apparent during the study. No treatment related changes in body weight, haematology or biochemistry were observed. Some changes in organ weights were observed but these were not dose related. No gross or microscopic changes were observed. It was noted that hepatocyte vacuolation was found in 4 males and 1 female of the top dose group and 2 males of the control group and was not considered to be dose-related or adverse.

Other

9. The protective anti-oxidant effect of chondroitin sulphate against carbon tetrachloride (CCl_4) induced liver injury was investigated (Ha and Lee, 2003). Sprague Dawley rats were pre-treated with 100 or 200 mg/kg chondroitin sulphate given ip, before receiving CCl_4 14 days afterwards. The chondroitin sulphate treatment protected against oxidative damage in a dose-related way as assessed by a reduction in the induced malondialdehyde levels, and increased superoxide dismutase, catalase, reduced glutathione, oxidised glutathione and glutathione peroxidase in the liver. Histopathological examination supported these findings with the deranged and necrotic tissue apparent in the CCl_4 group, being less marked or absent in the treated group. Again, the protective effect appeared to be dose-related. A group treated with chondroitin only was not included.

Secretariat

September 2008

REFERENCES

Abdel Fattah W., Hammad, T. (2001). Chondroitin Sulfate and Glucosamine: A review of Their Safety Profile. JANA, 3, 16-523.

Adebowale, A., Du, J., Liang, Z., *et al*, (2002). The Bioavailability and Pharmacokinetics of Glucosamine Hydrochloride and Low Molecular Weight Chondroitin Sulfate After Single and Multiple Doses to Beagle Dogs. Biopharmaceutics and Drug Disposition, 23, 217-225.

Conte, A., de Bernardi., Palmeri, L *et al* (1991a). Metabolic Fate of exogenous Chondroitin Sulfate. Arzneim-Forsch/Drug Res, 41, 768-772.

Conte, A., Palmeri, L., Segnini, D., *et al* (1991b). Metabolic Fate of Partially Depolymerized Chondroitin Sulfate. Drug Exptl Clin Res, 17, 27-33.

Conte, A., Volpi, N., Palmeri, L., *et al* (1995). Biochemical and Pharmacokinetics Aspects of Oral Treatment with Chondroitin Sulfate. Arzneim-Forsch/Drug Res, 45, 918-925.

Ha, B.J. and Lee, J.Y. (2003) The Effect of Chondroitin Sulfate Against CCl_4 Induced Hepatotoxicity. Biol Pharm Bull, 26, 622-626.

Palmeri, L., Conte, A., Giovannini., *et al* (1990). Metabolic Fate of Exogenous Chondroitin Sulfate in the Experimental Animal . Arzneim-Forsch/Drug Res, 40, 319-323.

Schauss, A. G., Merkel, D. J., Glaza, S. M. *et al* (2007). Acute and Subchronic Oral Toxicity Studies in rats of a Hydrolyzed Chicken Sternal Cartilage Preparation Food and Chemical Toxicology, 45, 315-321.

COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

GLUCOSAMINE AND HEPATOTOXICITY

ANNEX F

CHONDROITIN – Human trials

Human studies

1. A number of trials have been conducted in human volunteers. These have been tabulated in Table 3 below. In general, tests on liver function have either not been conducted or conducted but not fully reported.

2. The largest trial was conducted by Clegg *et al* (2006). In this, 1583 patients were randomised to receive placebo, glucosamine, chondroitin, glucosamine plus chondroitin or celexicob for 24 weeks. A range of biochemical parameters were measured including liver enzymes. The results were not fully reported but it was stated that adverse events were mild and evenly distributed between groups.

3. A meta analysis by Bana *et al* (2006) reported that the tolerability of chondroitin sulphate was good to excellent. However it was noted that several studies suggested that there was a higher incidence of intestinal disorders in the treatment groups. A range of adverse effects not related to treatment were also reported. None of these were related to the liver.

Secretariat
September 2008

Table 3 Human trials with chondroitin sulphate.

Numbers/design	Dose/day	Duration	Clinical Chemistry?	Comments	Reference
DBPC, 114 (59 placebo, 55 treatment	2 x 500 mg Chondroitiin Sulfate	3 months	No differences in clinical chemistry or haematology	Significantly more adverse effects (respiratory and GI) in the CS group	Mazieres *et al*, 2001
DBPC,403 (44 placebo, 40 and 43 in the two treatment groups	1x 1200 mg or 3x400 mg Chondroitiin Sulfate	3 months	Not done	Comparable number of adverse effects (largely GI) in both groups	Bourgeois *et al*, 1998
60 patients/group DBPC	800 mg	3 months twice a year	No treatment related effects on haematology, serum bilirubin, urea and creatinine in treatment group.	Comparable number of adverse effects (largely GI) in both groups	Uebelhart *et al*, 2004.
12 patients/group	800 mg Chondroitin Sulfate compared to naproxen 500 mg/day	24 weeks	Not done	No discussion of adverse effects.	Rovetta *et al*, 2002
1583 Double-blind placebo controlled	1500 mg glucosamine sulphate, 1200 mg chondroitin sulphate, glucosamine + chondroitin, or celecoxib	24 weeks	Liver enzyme levels measured, results not provided	Adverse events mild and evenly distributed between groups	Clegg *et al*, 2006 Hochberg *et al*, 2008

DBPC, 309 (154 placebo, 153 treatment	2 x 500 mg Chondroitiin Sulfate	24 weeks	Not done	Comparable number of adverse effects (largely GI) in both groups	Mazieres *et al*, 2007
40 patients/group DBPC	2x 400 mg Chondroitiin Sulfate	6 months	Not done	No difference in "clinical side effects" between groups	Bucsi and Poor, 1998.
50 patients/group open, randomised trial,	1200 mg Stuctrum (Chondroitiin Sulfate) or ibuprofen	6 months	Unclear	More side effects in ibuprofen group. Nausea and diarrhoea in Structrum group.	Alekseeva *et al* (1999) English Abstract of Russian study
DB PC, 23 placebo, 23 treatment	800 mg Chondroitiin Sulfate	1 year	No differences in blood, renal or liver biological parameters.		Uebelhart *et al*, 1998
12 patients/group	800 mg Chondroitin Sulfate compared to naproxen 500 mg/day	2 years	Not done	No discussion of adverse effects.	Rovetta *et al*, 2004
150 patients/group DBPC	800 mg Chondroitiin Sulfate	2 years	"Routine laboratory tests" Results not stated.	No difference in adverse effects between groups	Michel *et al*, 2005

REFERENCES

Alekseeva, L.I., Benevolenskaia L.I., Nasonov, E.L., *et al* (1999). Structum (Chondroitin Sulfate) a New Agent for the Treatment of Osteoarthrosis. Ter Arkh, 71, 51-53. [English abstract of Russian study]

Bana,G., Jamard, B., Verroull, E., *et al* (2006). Chondroitin Sulfate in the Management of Hip and Knee Osteoarthritis: An Overview, Advances in Pharmacology, 53, 507-522.

Bucsi, L. and Poór, G (1998). Efficacy and Tolerability of Oral Chondroitin Sulfate as a Symptomatic Slow Acting-Drug for Osteoarthritis (SYSADOA) in the.Treatment of Knee Osteoarthritis Osteoarthritis and Cartilage, 6 (Supplement A1), 31-36.

Bourgeois, P., Chales, G., Dehais, J.*et al* (1998). Efficacy and Tolerability of Chondroitin Sulfate 1200 mg/day vs Chondroitin Sulfate 3 x 400 mg/day.vs Placebo. Osteoarthritis and Cartilage, 6 (Supplement A1), 25-30.

Bucsi, L., Poor, G. (1998). Efficacy and Tolerability of Oral Chondroitin Sulfate as a Symptomatic Slow-Acting Drug for Osteoarthritis (SYSADO) in the Treatment of Knee Osteoarthritis. Osteoarthritis and Cartilage, 6 (Supplement A1), 25-30.

Clegg, D.O., Reda, D.J., Harris, C.L. *et al* (2006) Glucosamine, Chondroitin Sulfate, and the Two in Combination for Painful Knee Osteoarthritis. New England Journal of Medicine, 354, 795-808.

Hochberg, M.C., Clegg, D.O. (2008). Potential Effects of Chondroitin Sulfate on Joint Swelling: A Gait Report. Osteoarthritis and Cartilage, 16, S22-S24.

Mazieres, B., Combe, B., Phan Van, A., *et al* (2001). Chondroitin Sulfate in Osteoarthritis of the Knee: A Prospective, Double-Blind, Placebo-Controlled Multicenter Clinical Study. J Rheumatol, 28, 173-181.

Mazieres, B., Hucher, M., Zaim, M. *et al* (2007). Effect of Chondroitin Sulfate in Symptomatic Knee Osteoarthritis: A Multicentre, Randomised, Double-Blind, Placebo-Controlled Ann Rheum Dis, 66, 639-645.

Michel, B.A., Stucki, G., Frey D. *et al* (2005). Chondroitins 4 and 6 Sulfate in Osteoarthritis of the Knee. A Randomised Controlled Trial. Arthritis and Rheumatism, 52, 779-786.

Nguyen, P., Mohamed, S.E., Gardiner, D., *et al* (2001). A Randomized Double-Blind Clinical Trial of the Effect of Chondroitin Sulfate and Glucosamien Hydrochloride on Tempromandibular Joint Disorders: A Pilot Study. Orofacial Pain, 19, 130-139.

Rovetta, G., Monteforte, P., Molfetta G. *et al* (2002) Chondroitin Sulfate in Erosive Osteoarthritis of the Hands: Behaviour of Erosions, Osteophytes, Pain and Hand Dysfunction. Rugs Exptl Clin Res, 30, 11-16.

Rovetta, G., Monteforte, P., Molfetta G. *et al* (2004) A Two Year Study of Chondroitin Sulfate in Erosive Osteoarthritis of the Hands. Int J Tissue React, 24, 29-32.

Uebelhart, D., Thonar, E.J., Delmas, P.D., *et al* (1998). Effects of Oral Chondroitin Sulfate on the Progression of Knee Osteoarthritis: A Pilot Study. Osteoarthritis and Cartilage, 6 (Supplement A1), 39-46.

Uebelhart, D., Malaise, M., Marcolongo R., *al* (2004). Intermittent Treatment of Knee Osteoarthritis with Oral Chondroitin Sulfate: A One Year, Randomized, Double-blind Multicenter Study versus Placebo. Osteoarthritis and Cartilage, 12 269-276.