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

STATEMENT ON GLUCOSAMINE AND HEPATOTOXICITY

Introduction

1. Glucosamine is a popular food supplement taken alone or in combination with chondroitin sulphate usually by 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 being sold annually.

2. In view of a small number of case reports linking glucosamine and hepatitis, including one that became the subject of a Scottish Fatal Accident Inquiry, the COT was asked to consider whether a causal association was plausible.

Hepatitis

3. Hepatitis is the general term for inflammation of the liver. This has a range of clinical presentations varying in duration, severity and eventual outcome. The initial symptoms of hepatitis are often non-specific but in the later stages of the disease the symptoms reflect impairment of various liver functions. Laboratory evidence of liver cell damage can often be detected in asymptomatic patients but significant impact on the synthetic, metabolic and excretory functions of the liver eventually leads to symptoms such as bruising secondary to lack of clotting factors, encephalopathy caused by failure to convert ammonia to urea, and itching when bile salts are deposited in the skin instead of being eliminated in the bile. The liver has a remarkable ability to regenerate after damage but often fails to replicate the original complex cellular architecture necessary for normal function and instead produces cirrhosis, a combination of fibrous tissue and regenerative nodules.

4. In the UK the most common causes of liver injury are fatty infiltration of the liver or viral infection, but toxicants (including alcohol), genetic storage disease and autoimmune processes can also lead to liver damage. In a proportion of patients, no ready explanation can be found for liver damage however severe. Toxicant-induced hepatitis, usually caused by drugs, is common and often resolves when the relevant chemical exposure ceases. In some cases, however, cellular damage is severe and the outcome can be fatal.

5. Identifying a cause for an episode of hepatitis depends upon a history of exposure to chemicals, drugs or contact with sources of hepatitis infection, together with laboratory investigations. Infection with many of the hepatitic viruses can be identified either by demonstrating an antigenic part of the virus or a specific antibody response to the virus in the blood. Autoimmune disease can be diagnosed from the pattern of antibodies to specific cellular components such as mitochondria and from the clinical picture of other organ involvement. Damaged liver cells tend to leak enzymes into the blood and some clue as to the site of greatest damage within the liver can be gleaned from the pattern of enzymes in the blood, with transaminases, particularly alanine aminotransferase (ALT), being released from damaged parenchymal cells and alkaline phosphatase being released from cells lining the bile ducts.

Morphology of hepatitis

6. The morphological appearances of different types of hepatitis are often similar (Ferrell, 2001). Pathological features of acute hepatitis include swelling and ballooning of hepatocytes and cell death affecting single cells, groups of cells adjacent to portal tracts, or extensive confluent areas. Kupffer cells are actively phagocytic and within the portal tracts there are increased numbers of chronic inflammatory cells. There may also be increased numbers of inflammatory cells in the hepatic parenchyma.

7. The defining feature of active chronic hepatitis is infiltration of lymphocytes from portal tracts with associated death of liver cells, so called interface hepatitis. This in time is associated with fibrosis. Sometimes the amount of inflammation is less and a biopsy fails to show interface hepatitis. The presence of plasma cells or discrete lymphoid aggregates may suggest the possibility of a viral cause. Some storage disorders, for example Wilson's disease and copper accumulation, and alpha 1 antitrypsin deficiency, show morphological evidence of a chronic active hepatitis.

Idiosyncratic drug hepatotoxicity

8. Idiosyncratic drug hepatotoxicity (IDH) occurs in1/500 to 1/50,000 individuals exposed to a particular drug (the prevalence of idiopathic hepatitis in the community is estimated to be 1/100,000) (Kaplowitz, 2005). IDH has been associated with a variety of pharmaceutical drugs as well as food supplements, notably kava kava. IDH is generally too rare to be detected in clinical trials, though elevated ALT levels may be an indicator. As a general rule, an ALT level greater than three times the upper level of normal is considered to be a sensitive indicator of liver toxicity (the marker is not completely specific since muscle injury may elevate ALT levels). While this is nearly universally described for idiosyncratic liver toxicants, it is not always predictive of overt idiosyncratic toxicity.

9. Two types of IDH occur. Allergic IDH occurs with a short latent period and involves the adaptive immune system. Symptoms may include, fever, rash or eosinophilia. Non-allergic IDH has none of the above features. There is a long latency period, where there may have been months of normal liver function test results prior to the occurrence of IDH.

10. No animal model exists for either type of idiosyncratic drug hepatotoxicity.

Glucosamine and hepatitis

11. A case of hepatitis thought to be linked to the consumption of glucosamine and chondroitin supplements became the subject of a Fatal Accident Inquiry in Scotland, though it was subsequently ruled that there was no evidence for such a link. In this case a 64 year old male with a 1 week history of nausea and vomiting, developed jaundice (Smith and Dillon, 2008). The patient was not taking any prescribed medication, but had been taking glucosamine and chondroitin supplements for 1 month. Derangement in liver function was predominantly hepatocellular, and ALT, bilirubin and alkaline phosphatase levels were elevated. On cessation of glucosamine, ALT levels decreased but bilirubin continued to rise. The patient then developed acute renal failure and coagulopathy; hepatic and renal failure progressed; and the patient developed encephalopathy and a metabolic acidosis. Although scheduled for transplantation, the patient continued to decline and developed peritoneal and blood-borne sepsis, dying the following day. Post-mortem examination revealed extensive liver necrosis

12. This case is the subject of a planned publication and the authors cite a further two cases that they consider might also be associated with glucosamine supplements. Independently of the Scottish cases, the Food Standards Agency was also advised of a further case in London.

Published case reports

13. In addition to the four cases noted above, two case reports have been published in the scientific literature.

14. A 52 year old male presented with cholestatic hepatitis (Ossendza *et al*, 2007), having taken 3 capsules a day of glucosamine forte[1] (glucosamine sulphate) for 19 days. The dose of glucosamine taken is unclear. Liver enzyme levels were elevated and liver biopsy showed centri- and medio-lobular lesions, combining a moderate canalicular cholestasis, necrotic hepatocytes and a discrete inflammation with a lymphohistiocyte microgranuloma and a few eosinophilic leucocytes that “may be compatible with a drug-induced origin”. Liver tests were normalised 8 weeks after glucosamine was discontinued. The authors considered that the time course, the hypereosinophilia, the presence of eosinophilic leucocytes in the hepatic inflammatory infiltrate and the favourable outcome suggested an allergic mechanism.

15. A 55 year old woman was hospitalised as a result of abnormal liver function tests (Fujii *et al*, 2008). Transaminases and biliary enzymes were markedly elevated and the patient had hyper-ferritinaemia. The patient had been taking soybean extract, glucosamine sulphate and lutein (extracted from marigold) for a period of 6 months;

[1] HFMA advise that glucosamine forte is an additional descriptor for a number of glucosamine products indicating higher dosages, eg 1500 mg.

none of these supplements contained iron. On cessation of the supplements, liver function returned to normal without medication in approximately 4 weeks. Laparoscopic examination of the liver revealed a whitish uneven surface which was suggestive of chronic liver damage. A liver biopsy showed marked inflammation in the portal areas and hepatic lobules; grade 2 siderosis in the hepatocytes and iron storage in the Kupffer cells was also observed. Further investigation revealed that the patient was heterozygous for the H63D mutation in the HFE (haemochromotosis) gene, which is involved in iron metabolism. The authors proposed that an interaction between the supplements and iron overload may have underlain the liver damage.

Adverse drug reaction reports

16. Unlike medicines, there is no formal procedure for the notification of adverse effects of foods, including food supplements. However, adverse drug reaction reports for food supplement products which are received by the Medicines and Healthcare products Regulatory Agency (MHRA) as part of their ADROIT ("yellow card") scheme are forwarded to the FSA, where they are logged along with other reports received directly from consumers. Further investigation or enforcement action can then be undertaken by the FSA as appropriate. The adverse drug reaction reports are not independently verified.

17. From 1999 to 2008, 41 adverse drug reaction reports had been received for glucosamine; these included 2 reports of hepatitis and 1 report of abnormal liver function tests (LFTs). Fourteen reports had been received for the glucosamine and chondroitin sulphate combination, of which 2 noted elevated or abnormal LFTs.

18. In summary, there are 5 case reports of hepatitis either in the literature or awaiting publication, one case directly reported to the FSA and 6 adverse drug reaction reports of hepatitis or abnormal liver function tests for either glucosamine or the glucosamine and chondroitin sulphate combination. These cases presented as acute hepatitis and no other causes were identified.

<u>Glucosamine</u>

19. 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 of 4-20 g/day. The molecular weight of glucosamine is 178.17. Glucosamine is not present in the normal diet to any significant extent.

20. Commercially available glucosamine supplements are generally sulphate or hydrochloride salts and are derived from shellfish or fungal sources.

Absorption, distribution, metabolism and excretion.

21. Glucosamine sulphate is rapidly absorbed from the gut, undergoing significant first pass metabolism in the liver (Anderson *et al*, 2005). However, glucosamine hydrochloride is less well absorbed (Deal and Moskowitz, 1999).

22. Studies with radio-labelled glucosamine show that following ingestion it is rapidly detected in the plasma (Setnikar *et al,* 1984) and is then distributed to tissues including the liver, kidneys and articular cartilage (Setnikar *et al,* 1984; Setnikar *et al,* 1986). Glucosamine is phosphorylated and ultimately forms UDP *N*-acetyl glucosamine which is then incorporated into glycolipids, glycoprotein and proteoglycans (Anderson *et al*, 2005).

23. The majority of ingested glucosamine is rapidly degraded into smaller molecules such as water, urea and carbon dioxide. Non-absorbed glucosamine is excreted in the faeces (Setnikar *et al,* 1986; Setnikar and Rovati, 2001).

Animal toxicity

24. Glucosamine has been investigated in only a few acute and sub-chronic studies in laboratory animals, which indicated that it is of low oral toxicity. Where liver function has been specifically assessed (Echard *et al*, 2001; Kim *et al,* 2001: Naito *et al,* 2007), glucosamine, chitosan oligosaccharides and *N*-oligoglucosamine respectively were not associated with significantly elevated ALT or AST levels or other liver related adverse effects. No data on chronic toxicity or reproductive and developmental toxicity have been identified.

Human volunteer studies

25. Glucosamine has been studied in numerous trials in human volunteers (29 were reviewed for the original COT discussion paper (TOX/2008/35). The great majority of these trials have been conducted in patients with osteoarthritis, with a few being conducted in athletes or servicemen. The studies have lasted from two weeks to three years in duration.

26. Where it is reported that clinical chemistry was conducted (in approximately half the studies), no significant differences were found between the treatment and placebo groups and no adverse effects were observed. In general, these data are not reported in full and it is unclear whether any individuals had elevated liver enzyme levels following treatment that might not have significantly affected the group means. However, where papers report that data were analysed individually (Hughes and Carr, 2002; Noack *et al*, 1994) it is stated that no adverse effects were apparent. This was also noted in Reichelt *et al*, 1994 where glucosamine was given by intra-muscular (i.m.) injection.

27. In one of the few trials where clinical chemistry 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 (3 x 500 mg) or placebo for a further 2 weeks (Crolle and D'Este, 1980). There were no differences in ALT or AST levels between the groups at baseline or changes following treatment. 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.

28. In a 6 month study by Herrero-Beaumont *et al* (2007), patients were given 1500 mg glucosamine sulphate, 3000 mg acetaminophen (paracetamol) per day or placebo (n =106, 108 and 104 respectively). Routine laboratory tests for liver function (assessed by transaminase and gamma glutamyl transferase (GGT) levels at baseline, 3 months, 6 months and end of study) indicated that more people in the paracetamol 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 paracetamol) 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 paracetamol patient). Clinically significant enzyme levels (2-3 x upper reference levels) of GGT were measured in one paracetamol and 1 glucosamine patient but these did not require withdrawal from the study.

29. The largest randomised trial was conducted by Clegg *et al* (2006). In this, 1583 patients were randomised to receive placebo, glucosamine, chondroitin, glucosamine plus chondroitin or celecoxib 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.

Allergy

30. Since glucosamine is derived from shellfish, there has been concern that glucosamine supplements could trigger allergy in shellfish-allergic individuals. However, it is uncertain whether, due to the extraction process, there is sufficient protein allergen present to pose a threat to sensitised individuals. In a small study by Gray *et al* (2004) 6 subjects allergic to shellfish did not experience a positive reaction to a skin prick challenge with glucosamine. In a small double-blind placebo-controlled food challenge study by Villacis and colleagues (2006), 15 subjects with shrimp allergy were given 1500 mg glucosamine-chondroitin where the glucosamine had been derived from shrimp, or placebo, where the glucosamine had been derived from synthetic sources. No signs of immediate or delayed (at 24 hours) hypersensitivity reactions were observed.

<u>Chondroitin sulphate</u>

31. 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. Commercial chondroitin sulphate is largely obtained from the cartilaginous tissue of animals such as cows, pigs, chicken and fish.

Absorption, distribution, metabolism and excretion

32. Chondroitin sulphate is partially absorbed from the gut, both as intact chondroitin sulphate and as lower molecular weight fractions of depolymerised material (Conte *et al*, 1995). Studies with radio-labelled chondroitin sulphate show that following ingestion, chondroitin is found in the plasma and in tissues such as the liver, kidneys and cartilage (Palmeri *et al*, 1991). Partially depolymerised chondroitin sulphate is excreted in the urine (Conte *et al*, 1991).

Animal toxicity

33. Chondroitin sulphate has not been tested for toxicity in an isolated form, but no adverse effects were apparent in rats fed hydrolysed chicken sternal cartilage for up to 3 months (Schauss *et al*, 2007). The parameters measured included biochemistry, but it is unclear whether this included AST and ALT levels.

Human volunteer studies

34. Chondroitin sulphate has been studied in a number of randomised trials in human volunteers (11 were reviewed in the original COT discussion paper TOX/2008/35). Some of the studies measured biochemical parameters, which may have included AST and ALT, but these were only specifically noted in two reports (Uebelhart *et al,* 1998; Clegg *et al*, 2006) and the results are not described in detail. None of the reports comment on individual results so it is uncertain whether or not elevated enzyme levels occurred in some individuals.

35. 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 celecoxib 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.

Galactosamine

36. Galactosamine sulphate is part of the repeating sub-unit of chondroitin sulphate. The sugar D-galactosamine is a model hepatotoxin (Keppler *et al*, 1968). To achieve liver damage experimentally, D-galactosamine is generally administered in six separate ip injections over a 24 hour period. D-galactosamine treatment results in elevated ALT, AST and bilirubin levels, while protein levels are decreased. Histologically, pan-lobular focal hepatic necrosis is observed, which resembles the features of human viral hepatitis. The liver damage increases in severity in the 48 hours after the first dose. However, four weeks after galactosamine treatment, there is no observable difference between control and experimental animals.

37. The mechanism of D-galactosamine hepatotoxicity has not been fully established (Coen *et al*, 2007). The most established view is that depletion of hepatic uridine nucleotides occurs, as a result of the formation of UDP aminosugars, resulting in inhibition of RNA and protein synthesis.

38. The toxicity of D-galactosamine is not idiosyncratic and the type of liver damage observed experimentally is not comparable to that described in the case reports above. Therefore it is unlikely that galactosamine is involved in the hepatotoxicity that has been associated with glucosamine or glucosamine plus chondroitin supplements.

Conclusions

39. Glucosamine or glucosamine plus chondroitin supplements are widely used by some population groups without any clear evidence of significant adverse effects occurring. A small number of individual case reports have linked glucosamine with hepatitis, and in most of these cases cessation of the supplement has been followed by an improvement in the patient's condition. However, hepatitis is not specific and it is possible that other unidentified exposures were involved in all of the case reports associated with glucosamine

40. Data from the numerous trials in human volunteers for both glucosamine and chondroitin, as well as the more limited animal toxicology data, do not indicate any adverse effects of glucosamine on the liver. Glucosamine occurs naturally within the human body and no plausible mechanism by which it might cause hepatotoxicity is apparent.

41. Current evidence does not suggest that glucosamine is likely to be a cause of hepatitis although a causal link cannot be completely excluded. It should be noted, however, that the likelihood of an individual user of glucosamine experiencing adverse effects is, at most, very low.

42. At present, it is unlikely that further research will resolve the uncertainty since if hepatitis is caused by glucosamine then it appears to occur by an idiosyncratic mechanism. Thus any human study would need to be extremely large to demonstrate the hazard due to the rarity of the outcome and the many potential confounding factors such as the use of other medication.

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