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SCIENTIFIC OPINION

Opinion of the safety of glucosamine hydrochloride from *Aspergillus niger* as food ingredient[1]

Scientific Opinion of the Panel on Dietetic Products, Nutrition and Allergies

(Question No EFSA-Q-2008-306)

Adopted on 15 May 2009

PANEL MEMBERS

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SUMMARY

Following a request from the European Commission, the Panel on Dietetic Products, Nutrition and Allergies was asked to carry out the additional assessment for 'Glucosamine Hydrochloride from *Aspergillus niger'* as a food ingredient in the context of Regulation (EC) No. 258/97 taking account of the comments/objections of a scientific nature raised by the Member States.

The applicant intends to add glucosamine hydrochloride from the fungus *Aspergillus niger* (RGHAN) to fruit juices, fruit "smoothies", dehydrated instant drink mixes, fermented milk-based products, sports drinks and iced tea drinks, at a concentration that would provide approximately 750 mg per daily serving. The target consumers would be older people and sportsmen and sportswomen.

The RGHAN production process is similar to that used to isolate glucosamine hydrochloride from shellfish except that the source of the raw material is the biomass from an *A. niger* fermentation. *A. niger* has been used for citric acid production since the 1920s. The strain of *A. niger* used in this process is not genetically-modified, pathogenic or toxic and does not produce the mycotoxin ochratoxin A. The chitin-containing biomass is hydrolysed using food-grade concentrated hydrochloric acid at 100° C to form glucosamine hydrochloride, which is then concentrated by filtration of the biomass and evaporative crystallisation of the filtrate to yield glucosamine hydrochloride crystals. These are centrifuged, washed with water and flash dried. The product RGHAN meets the United States Pharmacopoeia-National Formulary (USP-NF) specification for glucosamine hydrochloride of > 98 %, loss on drying of < 1 %, arsenic < 3 ppm and heavy metals < 0.001 %. Analyses of five non-consecutive

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commercial batches were all within specification. Tests for pesticide residues and mycotoxins showed them to be below the level of detection. Absence of protein was demonstrated. Microbiological analyses showed RGHAN to conform with USP-NF and microbiological food standards.

The applicant calculated the mean and 95th percentile daily intakes of RGHAN for different population groups using 1986-87 data from the UK National Diet and Nutrition Survey (NDNS). The highest estimated intake values for RGHAN on an all-user basis were for children and young people aged 4-10 years; mean daily intake 543 mg/person equivalent to 22 mg/kg bw and 95th percentile of 1383 mg/person equivalent to 57 mg/kg bw. However for the target group, adults, the values for intake/person/day were for the mean 517-534 mg equivalent to 6.5 - 7.8 mg/kg bw and for the 95th percentile 1270 - 1404 mg/person equivalent to 18-19 mg/kg bw. The UK Advisory Committee on Novel Foods and Processes (ACNFP) noted that the market for foods in the categories listed in the original application has changed considerably since the 1986-87 NDNS data and recalculated the intake for adults based on more recent NDNS data from 2000 which gave values of 1056 and 2792 mg/person/day for the mean and 95th percentile users, respectively.

The toxicity of glucosamine has been studied in a number of animal species. Glucosamine has a very low acute oral toxicity. A number of repeated dose oral administration studies have been conducted in rats, dogs, rabbits and horses. The Panel considers that glucosamine has also a low chronic toxicity.

There is no evidence that glucosamine hydrochloride is genotoxic or allergenic.

A review of reports on the potential effect of glucosamine administered intravenously or intraperitoneally on glucose metabolism in rats with doses ranging from 240 to 9937 mg/kg bw showed that most of the reports observed that glucosamine altered glucose metabolism, i.e. higher blood glucose levels, reduced uptake of glucose and decreased disposal of glucose. However the oral administration of glucosamine at high doses, 1000 to 2149 mg/kg bw, does not appear to affect blood glucose levels in rats, rabbits or dogs.

A large number of human studies have been carried out with oral administration of glucosamine as a consequence of its increasing use as a dietary supplement for the treatment of osteoarthritis. Recent reviews of these trials conclude that glucosamine at the recommended intake of 1500 mg/day, equivalent to 25 mg/kg bw/day for a 60 kg person, does not elicit adverse effects in glucose regulation in healthy people in long term efficacy studies or in trials of short duration conducted in diabetic subjects.

The Panel concludes that RGHAN (glucosamine hydrochloride from *Aspergillus niger*) is safe as a food ingredient for adult consumers at the proposed intake level of 750 mg of glucosamine per day.

Consumers with diabetes or glucose intolerance should be advised to seek medical advice before consumption.

Key words: Glucosamine, *Aspergillus niger*, novel food, ingredient.

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BACKGROUND AS PROVIDED BY THE COMMISSION

On 14 August 2006, Cargill Inc. submitted a request under Article 4 of the Novel Food Regulation (EC) N° 258/97 to the competent authorities of the United Kingdom for placing on the market 'Glucosamine Hydrochloride from *Aspergillus niger*' as food or food ingredient.

On 21 September 2007, the competent authorities of the United Kingdom forwarded to the Commission its initial assessment report, which came to the conclusion that an additional assessment was required.

On 1 October 2007, the Commission forwarded the initial assessment report to the other Member States. Several of the Member States submitted additional comments.

The concerns of a scientific nature raised by the Member States can be summarized as follows:

- Since the laboratories which carried out the analyses in establishing compliance of commercial batches of the product with specification have not been specified in the application, it is not evident whether the analyses were conducted by accredited laboratories. The method used for the measurement of its stability in food has not been specified.
- Analytical data on the possible presence of chloropropanols in the product should be provided.
- Further information is required on the fermentation stage, which gives rise to the production of biomass, the time between the end of the fermentation and processing of the biomass and the stages of biomass preparation before hydrolysis.
- Potential risk groups can be identified e.g. persons with impaired glucose tolerance, taking coumarin anticoagulants, with cardiovascular disease or pregnant and lactating women, children and adolescents.
- The available information is insufficient to reach a firm conclusion regarding the potential effects of the novel ingredient on glucose metabolism that would be of particular concern for diabetic individuals.
- Glucosamine hydrochloride should not be permitted for food use as glucosamine is categorised as a medicinal drug and it would be very difficult to monitor and assess the intake of glucosamine if it is also used as a food ingredient.
- There is a potential risk that children may have access, and consume, foods containing the novel ingredient.

In consequence, a Community Decision is now required under Article 7, paragraph 1 of Regulation (EC) No 258/97.

TERMS OF REFERENCE AS PROVIDED BY THE COMMISSION

In accordance with Article 29 (1) (a) of Regulation (EC) No 178/2002, the European Food Safety Authority is asked to carry out the additional assessment for 'Glucosamine Hydrochloride from *Aspergillus niger*' as food ingredient in the context of Regulation (EC) N° 258/97.

EFSA is asked to carry out the additional assessment, in particular, to consider the elements of a scientific nature in the comments/objections raised by the other Member States (see Annex 3 of the request letter).

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ASSESSMENT

In accordance with the Commission Recommendation 97/618/EC glucosamine hydrochloride from *Aspergillus niger* is allocated to Class 2.1 'a complex (non-GM derived) novel food ingredient the source of the novel food having a history of food use in the community'. The assessment of the safety of this novel food ingredient is based on data supplied in the original application, the initial assessment by the UK competent authority, the concerns and objections of the other Member States and the responses of the applicant to these questions and those of the UK. The data are required to comply with the information required for novel foods of Class 2.1 i.e. structured schemes I, II, III, IX, X, XI, XII and XIII. It is noted that the novel ingredient, being a natural constituent of cartilage, is consumed by the elderly and sportsmen and sportswomen to "help maintain healthy joints". This assessment concerns only risk that might be associated with consumption and is not an assessment of the efficacy of glucosamine with regard to any claimed benefit.

I. Specification of the novel food (NF)

The molecular formula for glucosamine hydrochloride is $C_6H_{13}NO_5.HCl$ and molecular mass 215.63 Da. The trade name for the product considered in this application is REGENASURE® Glucosamine Hydrochloride from *Aspergillus niger* (abbreviated to RGHAN). RGHAN contains 83.1 % free-base glucosamine and this conversion factor is used to calculate the amount of glucosamine being consumed.

RGHAN is analysed to ensure that it conforms with the specification and purity standards based on the U.S. Pharmacopoeia – National Formulary (USP-NF) monograph for glucosamine hydrochloride. The specification is outlined in Table 1.

Table 1. **Specification for RGHAN Glucosamine Hydrochloride**

	Result	Test Method
Glucosamine hydrochloride	> 98 %	AOAC Official Method 2005.01
Loss on drying	≤ 1 %	Drying oven
Specific rotation	70.2 - 72.8	Polarimeter
pH	3 - 5	pH meter
Residue on ignition	≤ 0.1 %	Muffle furnace
Arsenic	≤ 3 ppm	Inductively Coupled Plasma (ICP) mass spectrometer with a 0.02ppm min detection limit
Heavy Metals	≤ 0.001 %	USP Method II, 231 and is a wet chemistry analysis. wet chemistry analysis

The assays for glucosamine hydrochloride, loss on drying, specific rotation, pH, and residue on ignition are carried out by Cargill in-house following the methods and specifications described (USP 30 (2007) p. 946) for glucosamine hydrochloride. The Cargill laboratory was one of 12 laboratories that participated in the Association of Official Agricultural Chemists (AOAC) ring study that was conducted on the AOAC glucosamine assay method (AOAC Official Method 2005.01). All 12 of the laboratories succeeded in the study and none of the

reported test results were outliers (Zhou *et al.,* 2005). Arsenic, heavy metals, residual solvents and microbiological testing are conducted by the National Food Laboratory, Inc. The laboratory's accreditation sheet was provided by the applicant. The analytical results for five non-consecutive batches of RGHAN have been provided by the applicant and all are within specification i.e. glucosamine hydrochloride, mean value 99.5 % (range 99.0 – 102 %); loss on drying, mean 0.4 % (0.2 - 0.5 %); specific rotation, mean + 71.0° C (70.6 - 71.6° C); pH, mean 3.2 (3.1 - 3.3); residue on ignition, mean 0 %; arsenic, mean < 0 .03 ppm (< 0.02 - 0.05 ppm); heavy metals, mean < 0.001 %. Independent analytical analyses have been carried out for the USP-NF Pesticide Screen, Aflatoxin Test (detection limit < 0.5 ppb for B1, B2, G1 and G2) and for Ochratoxin A (detection limit < 1.0 ppb). All have been below the level of detection. It is likely that the acid hydrolysis of the raw material, fungal biomass, using concentrate hydrochloric acid for several hours at 100° C would degrade proteinaceous material from the source. To confirm this, the final product has been tested for the presence of protein using gel electrophoresis. (The limit of detection was not provided but the staining method used was as sensitive as the silver stain method for proteins.) There was no indication of the presence of proteins of a molecular mass of > 5 kDa.

The UK Advisory Committee on Novel Foods and Processes (ACNFP) considered the possibility of chloropropanols such as 3-monochloropropane-1,2,-diol (3-MCPD) being generated during the acid hydrolysis of the *Aspergillus niger* biomass. 3-MCPD can be formed through the action of concentrated hydrochloric acid on lipids. However the lipid content of the fungal biomass is relatively low (0.5 % dry weight - information supplied by the applicant) and the purification stages would be expected to remove water-soluble impurities e.g. 3-MCPD. The ACNFP concluded that it would not be of concern. Subsequently in response to a concern expressed by a Member State a sample of RGHAN was submitted for independent analysis and chloropropanols were below the limit of detection.

II. Effect of the production process applied to the NF

The production process is similar to that used to isolate glucosamine hydrochloride from shellfish except for the source of the raw material. The chitin-containing biomass from an *Aspergillus niger* fermentation is hydrolysed using food-grade concentrated hydrochloric acid for several hours at 100° C. This results in the depolymerisation and deacetylation of chitin to form glucosamine hydrochloride. Filtration of the digested biomass removes solid impurities yielding a filtrate containing the glucosamine hydrochloride. Evaporative crystallisation is used to remove some excess hydrochloric acid and concentrate the glucosamine, which results in its precipitation as glucosamine hydrochloride crystals. The separation and purification of the crystals is achieved by centrifugation and washing with water. The glucosamine hydrochloride crystals are then flash dried to meet the USP-NF specification of ≤1 % loss on drying. The finished product can be sifted or granulated to achieve specific particle size for distribution. The manufacturing facility employs Good Manufacturing Practices (21 CFR Part 110-FDA, 2005) for food and HACCP programmes that are updated annually.

Glucosamine hydrochloride, from all sources, is stable at acid pH and degradation begins when the temperature reaches 190° C. Consequently it is stable under conditions of pasteurisation at acid pH. Its stability in foods has been studied. Thus in lemonade and 100 % juice held at 100° C for five minutes there is 100 % recovery of glucosamine. Similarly there is 100 % recovery in isotonic drinks at pH 3.0, juice flavour at pH 3.0 and 'fitness water' at pH 2.9 all held at room temperature for 9, 24 and 17 months respectively. The applicant has also demonstrated the stability of RGHAN in yoghurt products with 100% recovery after 56 days in a number of chilled fruit yoghurts.

III. History of the organism used as the source of the NF

The *A. niger* strain used as the raw material for manufacture of RGHAN is non-genetically modified, non-pathogenic and non-toxic for humans and animals. It does not produce ochratoxin A. This species has been commonly used in food production since the 1920s. The strain used in this process is a "privately developed strain of a proprietary nature" that was specifically selected for citric acid production. The citric acid has been sold in the US, EU and further afield since 1993.

IX. Anticipated intake/extent of use of the NF

The applicant wishes to include RGHAN in fruit juices and fruit juice products, including tomato, and fruit "smoothies", dehydrated instant drink mixes (stable in dry form, pH < 7 when mixed with liquid), fermented milk-based products, yoghurts and fromage frais (~ pH 3-5), sports drinks (~ pH 2-5) and iced tea drinks (~ pH 2-6) at a concentration that would provide approximately 750 mg per daily serving. The levels of RGHAN included in each food item would vary according to the serving size e.g. 0.6 % in yoghurts with an anticipated serving size of 125 g and 0.3 % in fruit juices with a serving size of 250 g. These foods are intended for population groups such as older people and sportsmen and sportswomen. The applicant assumes that such food groups would be consumed as an alternative to supplements or PARNUTS products rather than in addition to them.

Based on the use levels and serving sizes the applicant has calculated mean and upper percentiles of intake for different population groups using 1986-87 data contained in the UK National Diet and Nutrition Survey (NDNS). Calculations have been provided by the applicant for the mean and 90th, 95th and 97.5th percentile all-person i.e. across the population and all-user intakes i.e. across consumers of the specific food groups. Calculations were made to determine the estimated intake of RHGAN per person and per kg body weight from all food uses combined. A summary of relevant data is in Table 2.

Table 2. **Summary of the estimated intake of RGHAN by UK users from all the proposed food categories.**

Population Group	All user estimated intakes[1]			
	Per person (mg/day)		Per kg bw (mg/day)	
	Mean	95th percentile	Mean	95th percentile
Children/young people aged 4-10 years	543	1383	22	57
Female adolescents aged 11-18 years	474	1361	9.3	26
Male adolescents aged 11-18 years	520	1542	10	29
Female adults aged 16-64 years	517	1270	7.8	19
Male adults aged 16-64 years	534	1404	6.5	18

[1]. The values shown are for RGHAN. For free base glucosamine values these figures would have to be multiplied by 0.83.

The highest estimated intake values for RGHAN on an all-user basis are for children and young people aged 4-10 years with a mean of 543 mg/person/day and a 95th percentile of 1383 mg/person/day equivalent to 22 and 57 mg/kg bw/day for the mean and 95th percentile respectively. However these calculations are based on such children being specifically targeted for consumption of these products and consuming products containing RGHAN from all the food categories. For the other population groups, intakes are broadly similar.

The UK ACNFP noted that the market for the foods in the categories listed in the original application has changed considerably since the 1986-1987 NDNS data were collected. The Agency recalculated estimated intakes using data from a more recent NDNS survey of UK adults (2000) which gave significantly higher values for the mean and 95th percentile of 1056 and 2792 mg/person/day respectively equivalent to 860 and 2270 mg/person/day of free-base glucosamine i.e. about twice the values from the 1986-87 data. In response the applicant provided a simplified list of food applications and use levels as shown in Table 3.

Table 3. **List of food applications and food levels**

Product	**Maximum levels of added RGHAN incorporation in 100 g food**
Fruit juices and "smoothies"	375 mg
Soft drinks (including ready-to-drink iced teas)	300 mg
Fermented milk and fermented soy based products	750 mg
Dried beverage mixtures	300 mg
Sports drinks	300 mg

The applicant emphasised that products containing RGHAN would be marketed to adults and not to children.

X. Information from previous human exposure to the NF or its source

The applicant reported that there is widespread consumption of glucosamine in the form of supplements throughout the world (Biggee and McAlindon, 2004) including the EU. The applicant lists examples of glucosamine food supplements currently on the UK market with recommended daily intakes of up to 1600 mg but points out that food products containing RGHAN would provide an alternative to food supplements and not an additional source.

XI. Nutritional information on the NF

The novel food ingredient has no nutritional value other than as a source of carbohydrate.

XII. Microbiological information on the NF

The applicant has confirmed that the RGHAN meets the USP-NF specifications and microbiological food standards. The microbiological analysis of five batches of product showed numbers for total plate counts < 10 cfu/g, yeasts and moulds < 10 cfu/g, coliforms < 3 mean probable number (MPN)/g. *Escherichia coli* < 3 MPN/g, *Staphylococcus aureus* < 10 cfu/g and absence of *Salmonella* spp. in 25 g were all below the level of detection.

XIII. Toxicological information on the NF

Toxicological evaluation of *Aspergillus niger*

In a review of the safety of *A. niger,* Schuster *et al*. (2002) report that 3-10 % of strains examined produce the mycotoxin ochratoxin A and that all producer strains should be checked for ochratoxin A production. They concluded that strains not producing ochratoxin A would be safe production strains. The strain used to produce RGHAN is not an ochratoxin A producer.

Toxicological evaluation of Glucosamine

Much of the toxicological data on glucosamine hydrochloride presented by the applicant is from a review of the toxicology of glucosamine and its safety in humans (Anderson *et al*., 2005) and a recent human study, the glucosamine/chondroitin arthritis intervention trial (GAIT) study (Clegg *et al*., 2006).

Metabolism of glucosamine and toxicokinetics

Exogenous glucosamine is actively transported from extracellular tissue into cells by glucose transporters (Uldry *et al*., 2002); a process facilitated by insulin (Heart *et al*., 2000). Once in the cell glucosamine is phosphorylated to glucosamine-6-phosphate. Glucosamine-6-phosphate can be produced endogenously from fructose-6-phosphate and glutamine. In humans the endogenous production of glucosamine is in the range of 4 - 20 g/day (median values of ~ 14 g/day or 230 mg/kg bw/day for a 60 kg adult).

Some animal studies suggest that glucosamine administered intravenously or intraperitoneally may produce insulin resistance and hyperglycaemia by affecting insulin secretion and action (Echard *et al*. 2001; IOM, 2003). However, such animal studies have achieved blood and tissue levels in excess of 100 times higher than would be expected with oral glucosamine doses used in humans (Heart *et al*., 2000; Monauni *et al*., 2000; Nelson *et al*., 2000; Echard *et al*., 2001).

A number of pharmacokinetic studies have been carried out in rats (Aghazadeh-Habashi and Sattari, 2002), dogs (Setnikar *et al*., 1986) and human volunteers (Setnikar *et al*., 1986, 1993; Setnikar and Rovati, 2001). The results of the studies are comparable and suggest that glucosamine is metabolised *via* analogous pathways and that rats and dogs represent appropriate models for establishing the safety of glucosamine in humans (Setnikar and Rovati, 2001). Glucosamine is detectable in most tissues examined after oral administration including the liver, kidney and joint cartilage. About 90 % of glucosamine taken orally is absorbed of which a significant fraction undergoes first-pass metabolism in the liver. Blood levels achieved after oral glucosamine are only 20 % of those achieved with intravenous administration (Setnikar and Rovati, 2001; Aghazadeh-Habashi and Sattari, 2002; IOM, 2003).

Animal toxicity studies

Acute toxicity studies

In an acute oral toxicity study using male and female rats glucosamine hydrochloride (99 % purity) did not induce adverse effects after administration at a dose of 5000 mg/kg bw. Glucosamine hydrochloride thus has a very low acute oral toxicity.

The toxicity of glucosamine sulphate has also been studied in rats following intravenous (iv) or intraperitoneal (ip) administration (unpublished study reports cited by Setnikar *et al*., 1991a). The LD_{50} for ip injection is ~5200 mg/kg bw and for iv injection is ~1700 mg/kg bw.

Subacute, subchronic and chronic toxicity studies

A number of oral administration studies have been conducted in rats (Sugimura *et al*., 1959; Leuschner and Neumann, 1987; Beren *et al*., 2001; Echard *et al*., 2001; Lee *et al*., 2004; McNamara *et al*., 1996), rabbits (Stender and Astrup, 1977) and horses (Hanson *et al*., 1967; Fenton *et al*. 1999; Caron *et al*., 2002) to determine the effects of glucosamine over an extended period of time. According to the applicant, the no observed adverse effect level (NOAEL) for free-base glucosamine in a 52-week study using rats was 2130 mg/kg bw/day. The full study reports, however, were not provided by the applicant.

Effects of glucosamine on glucose metabolism in rats, rabbits and dogs

The rat model has often been selected for study because it is highly sensitive to the effects of parenteral administration of glucosamine on glucose metabolism (IOM, 2003). The IOM reviewed 14 reports on the potential effect of glucosamine on glucose metabolism in rats administered intravenously or intraperitoneally at doses ranging from 240 to 9937 mg/kg bw. Twelve of these reports with doses ranging from 240 to 9937 mg/kg bw showed that glucosamine altered glucose metabolism i.e. increased the blood glucose levels, reduced the uptake of glucose and decreased the disposal of glucose. In two of the studies infusion of 564 mg/kg bw did not affect blood glucose levels, and 250 mg/kg bw did not induce hyperglycaemia but glucose metabolism was altered. However, oral administration of glucosamine at high doses (1000 to 2149 mg/kg bw) did not affect blood glucose levels in rats (Echard *et al*., 2001), rabbits (Stender and Astrup, 1977) or dogs (Setnikar and Rovati, 1991a).

Genotoxicity studies

The applicant has evaluated the mutagenic activity of the RGHAN glucosamine hydrochloride in the *Salmonella-Escherichia coli* reverse mutation assay (Mecchi, 2003). The tester strains used were *Salmonella enterica* var. Typhimurium TA98, TA100, TA1535 and TA1537 and *E. coli* WP2 uvrA. The doses of RGHAN were 100, 333, 1000, 3300 and 5000 µg per plate, with and without S9 mix. According to the applicant and to Anderson *et al*. (2005) who referred to Mecchi et al. (2003) there was no evidence of mutagenicity. This is in agreement with a previous study with D-glucosamine using only the *E. coli* WP2 strain reviewed by Brusick *et al*., (1980).

It is noted by the applicant that some studies suggest that glucosamine and other sugars can have clastogenic effects *in vitro*. For example Nanjou *et al*. (1984) have demonstrated that glucosamine can induce DNA strand breaks in bacteriophage.

In a mouse bone marrow chromosome aberration assay carried out by Banerjee and Manna (1984) glucosamine hydrochloride administered by intraperitoneal injection to Swiss albino mice at a dose of 10 mg/kg bw significantly increased the chromosomal aberration frequency compared with distilled water. In the opinion of the Panel these studies, which were not carried out according to the usual standard, are of limited value for the risk assessment due to relevant methodological flaws (e.g. only one dose level, lack of positive controls).

The applicant conducted a micronucleus assay in mice in accordance with OECD Guideline 474. The animals were dosed using RGHAN glucosamine hydrochloride mixed with water by gavage. The doses were 500, 1000 or 2000 mg/kg bw. RGHAN did not induce any signs of clinical toxicity in any animal up to the maximum dose nor were any statistically significant increases in micronucleated PCEs observed. The test material was not toxic to the bone marrow (i.e. there was no statistically significant decrease in the ratios of polychromatic and normochromatic erythrocytes) at any dose level.

Human studies

The applicant has listed and reviewed 37 studies that include data on 3783 patients treated with glucosamine for 1191 patient years for periods ranging from 12 days to three years. The most common dose was 1500 mg/day, and the highest 2656 mg/day.

Human studies – adverse events

A number of non-specific symptoms are commonly reported in glucosamine supplementation trials. These include constipation, diarrhoea, nausea, dyspepsia, excessive gas, abdominal distension, abdominal cramps, headache, skin rash and pruritus. Nineteen studies from the literature reviewed by the applicant report specific side effect data comparing glucosamine to placebo. In 12 of these, symptoms were less common in glucosamine treated subjects than in those treated with placebo. Two studies report that symptoms were more common with glucosamine than placebo.

Clegg *et al*. (2006) recently carried out a large clinical trial. The study was a multi-centre, double-blind, placebo and celecoxib-controlled Glucosamine Arthritis Intervention Trial (GAIT), evaluating both efficacy and safety. Patients (1583) were randomised to one of four treatment groups receiving 1500 mg glucosamine hydrochloride daily, 1200 mg chondroitin sulphate daily, glucosamine and chondroitin daily, 200 ml celecoxib daily or placebo for 24 weeks. Adverse and serious adverse effects were assessed by the investigator at each study visit. Safety monitoring included complete blood counts, measurement of serum aspartate transferase, alanine aminotransferase, glucose, creatinine, partial thromboplastin time and urine analysis at each study visit. Throughout the study there were no gastrointestinal adverse events that could be attributed to the intervention. The number of withdrawals in the glucosamine group was 9 versus 11 in the placebo group. The results of this study support the database from earlier studies supporting the safety of chronic glucosamine supplementation. Reviews of the literature by Richy *et al* (2003) and Towkeed *et al.* (2005) also supported safety.

The applicant listed 16 studies reporting specific safety endpoints including liver and kidney function assessments, haematological and cardiovascular parameters. None of the studies reported adverse health effects.

Effects of glucosamine on glucose metabolism in humans

The applicant lists a number of clinical trials that reported fasting blood glucose levels in subjects receiving glucosamine supplementation. Reginster *et al*., (2001) reported that blood glucose values were slightly lower in patients receiving glucosamine (106 subjects) although the difference was not significant. Also the other clinical trials indicated no significant changes in clinical chemistry values. In two studies (Monauni *et al*., 2000; Pouwels *et al*., 2001) with 10 and 6 subjects respectively, large amounts of glucosamine (7200 or 9700 mg of the free base) infused over five hours produced no change in blood glucose levels. In one of these studies (Monauni *et al*., 2000) the dose of glucosamine was increased to 30500 mg (436 mg/kg) for five healthy volunteers; this was well tolerated by four individuals while one developed mild symptoms (headache). Tannis *et al*. (2004) reported that daily doses of 1500 mg of glucosamine sulphate over 12 weeks were associated with no significant changes in fasting plasma glucose, insulin levels or results of an oral glucose tolerance test. A study by Yu *et al*. (2003) showed that 1500 mg glucosamine for 28 days had no effect on glucose tolerance or insulin sensitivity of 10 non-diabetic subjects. In total 18 studies either directly or indirectly reported that a daily intake of 1500 mg glucosamine salt had essentially no effect on fasting glucose levels in humans.

The UK ACNFP drew the applicant's attention to a review of the effects of glucosamine on glucose control (Stumpf and Lin, 2006). The authors concluded that the findings in rats that iv administered glucosamine could alter glucose metabolism and induce insulin resistance have not been confirmed in long-term efficacy studies using oral glucosamine for treatment of osteoarthritis or in trials of short duration conducted in diabetic patients. The long-term effects of glucosamine in patients with diabetes have yet to be established in well-controlled studies.

Another recent publication examined the effects of oral glucosamine on serum glucose and insulin levels at the beginning and during a three-hour oral glucose tolerance test (Biggee *et al*., 2007). Sera from 16 patients with osteoarthritis, but no other diagnosed medical condition, who had fasted overnight, were collected through a three-hour period of continued fasting and during a three-hour period after ingestion of 75 g glucose with or without 1500 mg glucosamine sulphate. Three participants who were not previously known to have abnormalities of glucose tolerance demonstrated significant ($p = 0.04$) elevations in glucose levels after ingestion of glucosamine sulphate. The other 13 participants had mean incremental elevations that were not significant ($p = 0.20$). Glucosamine sulphate had no effect on insulin levels. The authors suggested that glucosamine might affect glucose levels and consequent glucose uptake in patients who have untreated diabetes or glucose intolerance.

In response the applicant commissioned a supplementary report that provided a critical review of the available literature and further reviews have subsequently been identified by the Panel.

In a review by Marshall *et al*. (2006) the authors concluded that despite theoretical risks based on animal models given high iv doses of glucosamine, oral doses of glucosamine/chondroitin (1500 mg/1200 mg daily) do not adversely affect short-term glycaemic control for patients whose diabetes is well controlled or for those without diabetes or glucose intolerance. They also concluded that while no compelling theoretical or incidental data suggest that long-term results should be different, further studies are required to clarify the effects of glucosamine on patients with poorly controlled diabetes or glucose intolerance.

According to Dahmer and Schiller (2008) most human studies have failed to confirm adverse effects of glucosamine at usual levels of consumption, 1500 mg/day (Monauni *et al*., 2000; Scroggie *et al*., 2003; Marshall *et al*., 2006; Muniyappa *et al*., 2006; Stumpf and Lin, 2006; Albert *et al*., 2007; Biggee *et al*., 2007; Pham *et al*., 2007) but they recommend that glucosamine should be used with caution in patients taking diabetes medication or warfarin. However, four intervention studies do not reveal adverse effects of glucosamine on glucose metabolism in diabetic patients.

Albert *et al*., 2007 studied 2 subjects with type 1 and 10 subjects with type 2 diabetes. They were randomly assigned to a double-blind, placebo-controlled, cross-over trial of 500 mg glucosamine or placebo orally three times a day for two weeks followed by a four week washout phase and a two week cross-over to the alternative treatment. Fasting serum glucose remained at the same level during the drug and placebo phases, which can be seen to support the view that commonly consumed levels of glucosamine do not have significant effects on glucose metabolism in diabetic patients during two weeks supplementation.

A randomised placebo-controlled, double-blind, cross-over trial of oral glucosamine at standard doses (500 mg three times a day) in 20 lean and 20 obese subjects has been published (Muniyappa *et al*., 2006). Glucosamine or placebo treatment for six weeks was followed by a one week washout and cross-over to the other arm. When compared with placebo, glucosamine did not cause insulin resistance or endothelial dysfunction in lean subjects or significantly worsen these findings in obese subjects.

A randomised placebo-controlled, double-blind, clinical trial to evaluate possible effects of glucosamine supplementation on glycaemic control in a selected population of 13 male and 13 female elderly patients with type 2 diabetes has been carried out by Scroggie *et al.* (2003). Most of the patients were being treated with one or two drugs for glycaemic control. For 90 days, patients received either placebo or a combination of 1500 mg of glucosamine hydrochloride with 1200 mg of chondroitin sulphate daily. The authors concluded that oral glucosamine supplementation does not result in clinically significant alterations in glucose metabolism in patients with type 2 diabetes.

In a study by Yu *et al*. (2003), seven obese and seven lean subjects participated. Three of the obese subjects had impaired glucose tolerance. Each subject had a baseline 4-h meal tolerance test and a frequently sampled intravenous glucose tolerance test, before and after four weeks of glucosamine sulphate (500 mg three times a day). No detrimental effects were observed on glucose metabolism. The authors concluded that it is unlikely that long-term use of glucosamine in diabetic subjects would lead to adverse effects on glucose metabolism; however, since they did not study diabetic patients, definitive conclusions cannot be drawn.

The Panel notes that the few studies with a limited number of subjects provide inconclusive results on the effects of oral glucosamine on glucose metabolism or glucose tolerance. In the majority of the studies glucosamine did not cause insulin resistance and there were no adverse effects on glucose metabolism.

Hepatitis

The Panel also notes a recent statement of the Food Standards Agency's Committee on Toxicity (CoT) on glucosamine and hepatoxicity in response to a small number of case reports which have linked glucosamine and hepatitis (FSA, 2009). Results from the numerous trials performed with glucosamine in volunteers, as well as the more limited animal toxicology data, do not indicate that glucosamine has adverse effects on the liver. Glucosamine occurs naturally within the human body and no plausible mechanism by which it might cause hepatotoxicity is apparent. The panel concludes that there is little concern of adverse effects of glucosamine on the liver, which is in accord with the conclusions of the CoT.

Allergenicity

Conventional methods for protein analysis cannot be used for the glucosamine hydrochloride product due to the amino group in the glucosamine that interferes with the assay. The applicant demonstrated the absence of protein in RGHAN using SDS-PAGE followed by sequential staining of the gel with Coomassie Blue and Sypro Ruby (no limit of detection was given although the statement was made that the staining method was at least as sensitive as the silver stain for protein). The applicant provided LC-MS data to demonstrate the absence of protein in a sample of purified glucosamine-hydrochloride. Model experiments with bovine serum albumin spiked to a mycelium matrix confirmed that under the hydrolysis conditions employed to produce glucosamine no intact protein would be expected to remain. Limits of detection for the analytical methods applied have not been provided. The applicant also drew attention to the fact that the product was already on sale as a food supplement and noted that there have been no reports of allergenicity. The Panel notes that absence of protein does not fully exclude the possibility of allergenicity.

DISCUSSION

Glucosamine is one of the most popular dietary supplements sold in the USA (Dahmer and Schiller, 2008) and is commonly available in the EU. Glucosamine hydrochloride from *A. niger* (RGHAN) labelled a vegetarian product is currently available in the EU. The applicant now intends to market RGHAN as a novel ingredient in fruit juices and "smoothies", soft drinks, fermented milk-based products, dried beverage mixtures and sports drinks at 750 mg per daily serving.

The specification and the manufacturing process do not cause the Panel concern. Analyses of several batches using recognised methods have confirmed that the manufacturing process is well controlled and the product meets specification. There are no anticipated problems with mycotoxins, microbial or pesticide contaminants. Analyses for chloropropanols and protein have demonstrated their absence subject to the limits of detection.

Intake data derived from the UK National Diet and Nutrition Survey for 1986-87, showed that adult users aged 16-64 years would consume RGHAN at the mean and 95th percentile levels 530 and 1400 mg/day, equivalent to 6.5 and 18 mg/bw/day. The UK ACNFP that carried out the initial assessment revised these figures using more recent data to 1056 and 2792 mg/day equivalent to 17.6 and 46.5 mg/kg bw/day. This is a worst-case scenario where users would consume all the food items containing RGHAN, rather than only one or two. The applicant assumes that the targeted users, the elderly and sportsmen and sportswomen who consumed glucosamine as a novel food would not also consume it as a food supplement. The applicant confirms that the marketing of the novel foods would not be aimed at children.

According to the applicant, the NOAEL value for free-base glucosamine in a 52-week study using rats was 2130 mg/kg bw/day.

The main concern of some Member States is the effect of the novel food on glucose metabolism, which would be of particular concern for diabetic patients. The commonly recommended daily intake of glucosamine in food supplement form is 1500 mg/day (25 mg/kg bw/day). Human trials have shown no adverse effects in long-term efficacy studies in healthy individuals or in short-term trials with diabetic subjects. Currently the glucosamine hydrochloride from *A. niger,* on sale in Europe as a food supplement, warns against its use by diabetics until medical advice has been sought and that it should not be used by individuals under the age of 18 years. The same restrictions should apply to RGHAN used as a novel food ingredient.

CONCLUSIONS AND RECOMMENDATIONS

The Panel concludes that RGHAN (glucosamine hydrochloride from *Aspergillus niger*) is safe as a food ingredient for adult consumers at the proposed intake level of 750 mg of glucosamine per day.

Consumers with diabetes or glucose intolerance should be advised to seek medical advice before consumption.

DOCUMENTATION PROVIDED TO EFSA

1. Letter from the European Commission to the Chairman of the European Food Safety Authority with the request for an opinion on the safety of 'Glucosamine Hydrochloride from *Aspergillus niger*' as a food ingredient. SANCO E4/AK/bs (2008) D/540163.

2. Initial assessment report by the Advisory Committee on Novel Foods and Processes (UK) concerning the assessment of 'Glucosamine Hydrochloride from *Aspergillus niger'* as a food ingredient.
3. Letters from Member States with comments on the initial assessment report on 'Glucosamine Hydrochloride from *Aspergillus niger'* as a food ingredient from the Advisory Committee on Novel Foods and Processes (United Kingdom).
4. Response to Member States comments on the UK Advisory Committee on Novel Foods and Processes Opinion for 'Glucosamine Hydrochloride from *Aspergillus niger'* as a food ingredient.
5. Application under Regulation No. 258/97 for the use of 'Glucosamine Hydrochloride from *Aspergillus niger'* as a food ingredient.
6. Expert review of glucosamine and glucose tolerance in normal, pre-diabetic and diabetic individuals.

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