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FOOD STANDARDS AGENCY

Mr Andreas Klepsch
European Commission
By email

17 September 2007 Reference: NFU 626

Dear Mr Klepsch,

INITIAL OPINION: GLUCOSAMINE HCL FROM ASPERGILLUS NIGER

On 14 August 2006, the UK Competent Authority accepted an application from Cargill for glucosamine hydrochloride (HCl) from *Aspergillus niger* as a novel food ingredient, in accordance with Article 4 of regulation (EC) 258/97. The Advisory Committee on Novel Foods and Processes (ACNFP) reviewed this application and their opinion is attached. I apologise for the delay in submitting this opinion as the ACNFP's evaluation was extended while we obtained additional information from the applicant.

In view of the ACNFP's opinion, the UK Competent Authority requests that an additional assessment is carried out in order to determine whether glucosamine hydrochloride (HCl) from *Aspergillus niger* meets the criteria for acceptance of a novel food defined in Article 3(1) of regulation 258/97.

I am copying this letter and the ACNFP's opinion to the applicant.

Yours sincerely,

(By e-mail only)

Dr Chris Jones
For the UK Competent Authority

Room 515b, Aviation House, 125 Kingsway, London WC2B 6NH
Tel: 020 7276 8572 Fax: 020 7276 8564
E-mail: chris.jones@foodstandards.gsi.gov.uk

ADVISORY COMMITTEE ON NOVEL FOODS AND PROCESSES

OPINION ON AN APPLICATION UNDER THE NOVEL FOOD REGULATION FOR GLUCOSAMINE HYDROCHLORIDE FROM *ASPERGILLUS NIGER* AS A NOVEL FOOD INGREDIENT

Applicant: **Cargill Incorporated**

Responsible Person: **Brent Rogers**

EC Classification: **2.1**

Introduction

1. An application was submitted by Cargill Incorporated on 14 August 2006 for the authorisation of glucosamine hydrochloride (HCl) from *Aspergillus niger* as a novel food ingredient. A copy of the application dossier was placed on the FSA website for public consultation.
2. Glucosamine is a naturally occurring amino-sugar that is a major component of complex proteins called glycosaminoglycans, which form a component of cartilage.
3. In August 2004, the Committee issued an opinion that Cargill's glucosamine HCl derived from *A. niger* was substantially equivalent to the shellfish derived glucosamine that was already on the market in food supplements and foods with particular nutritional uses (PARNUTs). The Commission was notified, and supplements and PARNUTs foods containing glucosamine from this source may now be legally placed on the EU market.
4. Cargill now seeks approval to market its fungal glucosamine HCl in a range of products, mainly beverages and fermented milk-based products at levels that would provide 750mg per 100g serving.
5. The application for authorisation of this fungal glucosamine HCl was prepared pursuant to Commission Recommendation 97/618/EC of 29 July 1997 concerning the scientific aspects and presentation of information necessary to support applications for the placing on the market of novel foods and novel food ingredients. The novel ingredient (NI) has been classified as a complex novel food from a non-GM source with a history of food use of the source in the community (class 2.1). The information presented in the dossier is structured accordingly and is considered below.

I. Specification of the Novel Ingredient (NI)

Information on this aspect is provided on p.9-14, and Appendix 1 and 1A of the application dossier

6. The NI contains a minimum of 98% glucosamine hydrochloride and complies with the monograph for glucosamine hydrochloride in the US Pharmacopoeia-National Formulary (USP-NF). (This information was omitted from the original dossier, but was later added at Appendix 1A). There are 12 tests outlined in this monograph which are listed in Table I-1. Analytical results for 5 non-consecutive batches of the NI are summarised in Table I-2 and indicate that the NI meets the required specification.
7. An additional analysis has been carried out for pesticide residues and aflatoxins. All levels are within prescribed limits.

Discussion *Members accepted that the product met with the USP-NF specification.*

II. Effect of the production process applied to the NI

Information on this aspect is provided on p.15-20 of the application dossier

8. The production process is comparable to the one used to isolate shellfish derived glucosamine HCl and is similar except for the source of the raw material. Briefly, the chitin containing biomass from *A. niger* is hydrolysed by heating in the presence of hydrochloric acid then filtered to remove solid impurities. The remaining glucosamine is then crystallised, centrifuged and dried before packaging.
9. During the public consultation a question was raised regarding the likelihood of the production process employed giving rise to the formation of process contaminants such as acrylamide and chloropropanols. Acrylamide was ruled out because the conditions employed were not conducive to its formation, but Members were asked to consider the likelihood of chloropropanols such as 3-monochloropropane-1,2,-diol (3-MCPD), being generated during the acid hydrolysis stage of the process.

Discussion *Members accepted that the production process was the same as the one that was currently being used for the novel ingredient which is sold in dietary supplement form, and was very similar to the process used for to obtain glucosamine from shellfish. The Committee considered the possibility of 3-MCPD, being present. 3-MCPD is known to be formed through the action of concentrated hydrochloric acid on lipids and it has previously been found in foods such as acid-hydrolysed vegetable protein. The applicant explained the fungal biomass has relatively low lipid content (0.5% dry wt) and that the subsequent steps in the purification process would be expected to remove any impurities. As 3-MCPD is water-soluble, any residues would be removed with the mother liquor during the crystallisation and the final stage, in which the crystals are washed with water, would also remove any additional impurities. The Committee concluded that the production process did not give any cause for concern.*

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

Information on this aspect is provided on p.20-21 of the application dossier

10. The source organism is a strain of the fungus *Aspergillus niger* that is referred to as non-toxic and non-pathogenic for humans and other animals. The dossier refers to *A. niger* as having a history of safe use generally in food production since the 1920's. The strain used to produce the NI has been used in the US and other countries for citric acid production since 1993.

Discussion *Members accepted that A. niger was widely used in the food industry, and that there were no concerns regarding the general safety of the fungus. However, the Committee expressed concern that there was a low level risk of allergenicity if proteins were present in the final product. (see Paragraph 40 below).*

IX. Anticipated intake/extent of use of the NI

Information on this aspect is provided on p.22-31 of the application dossier

11. The applicant intends to use the NI in fruit juice and fruit juice products, dehydrated instant drink mixes, fermented milk based products such as yoghurts and fromage frais, sports drinks and iced tea drinks, at levels that would provide 750mg per daily serving. These categories of foods which will be fortified with the NI are intended for population groups that seek nutritional support to maintain healthy joints. These groups include older people and sportsmen or women. The applicant is of the view that these food categories are intended to be consumed as an alternative to, rather than as well as, food supplements or PARNUTs foods. The proposed uses are summarised in Table IX.2-1 of the application dossier.

12. Although these would not form part of the target population, the applicant also provided intake estimates calculated using the UK NDNS data for young children (1997), schoolchildren (1992-1993) and adults (1986 –1987). Intakes have been calculated for 'all persons' (i.e. all people in the surveyed population) and 'all users' (i.e. all people in the surveyed population who have consumed the foods that might contain the NI).

13. Figures in Table IX.3-1 for 'all users' show that on a mg per person per day basis the theoretical highest mean and 95th percentile intakes of approximately 543 mg per day and 1542mg per day of the NI may occur in young people/children between the ages of 4 and 10. This "worst case" estimate is based on such children being regular consumers of all of these products, which the applicant states would not be the case. In the other population groups intakes are similar with mean daily intakes consumption ranging from 473 to 534 mg/day and 95th percentile intakes ranging from 1270 to 1542 mg/day.

14. Calculations on a body weight basis also show that children/young people have the highest potential level of consumption at 19.05 mg/kg per day for all person consumption and 21.72 mg/kg per day for all user consumption. As above, intakes are similar for different age groups with the greatest potential consumption being in male teenagers. Among the proposed beverage uses, fruit juices and yoghurt are the major contributors to intake of the NI in all groups.

15. Based on these intake estimates, the applicant has concluded that the safe endpoints indicated from all safety studies (see Section XIII below) would not be exceeded by consumption of the NI at the recommended maximum levels.

16. The Food Standards Agency notes that the market for the foods in the categories listed in Table IX.2-1 has changed markedly since the 1986–1987 NDNS data was collected. An Agency review using data from the more recent NDNS survey of British adults (2000) gave significantly higher estimates for mean and 95th percentile intakes of 1056 and 2792 mg per day respectively. The Committee noted these values and expressed concern that the estimates may be conservative since it was assumed that consumers would not also consume dietary supplements containing glucosamine. Members also expressed concern that appetising foods with added glucosamine may be consumed by children and that the applicant did not provide an adequate explanation of what they considered to be a safe upper level of consumption.

17. In response the Applicant provided a simplified list of food applications and use levels, as shown in the following table:

Product	**Maximum levels of incorporation**
Fruit juice and fruit smoothie type products	375 mg per 100 g
Soft drinks (including ready to drink iced teas)	300 mg per 100 g
Fermented milk-based products	750 mg per 100 g
Dried beverage mixtures	300 mg per 100 g
Sport Drinks	300 mg per 100 g

18. The applicant also emphasised that products containing the NI would be marketed as a support to joint health in adults and not marketed at children. The applicant noted that if the products were to be marketed at children then this would require the submission of a dossier under EU Health & Nutrition Claims legislation. The applicant was also of the view that even if there was occasional consumption by children (such as a child consuming a product intended for an adult in the home) then there was no reason to presuppose that this would be a risk to health. The applicant highlighted that the dietary supplements containing up to 1500mg glucosamine were widely available on the UK market, and that in addition to the metabolism of glucosamine being both well understood and tightly regulated in the body, there were also numerous scientific studies carried out demonstrating safe consumption at these levels and at levels of up to 3200mg/day. The applicant also noted that whilst the dietary survey data may indicate higher levels of intake, these were a 'worst case scenario' and realistically high levels would not exceed 1500mg/day.

Discussion *Members accepted the additional information as providing the necessary reassurance that high level consumption (in excess of 1500mg/day) was unlikely to occur on a regular basis, but remained concerned that high level consumption could have implications for adults with type 2 diabetes (both diagnosed and undiagnosed). (This issue is discussed in detail at Section XIII.)*

X. Information from previous human exposure to the NI

Information on this aspect is provided on p.32-34 of the application dossier.

19. The applicant is of the view that there is widespread consumption of the NI in the form of supplements throughout the world, including within the EU; however, there is no formally established maximum recommended daily intake for glucosamine. Examples of products currently on the market and the recommended daily intakes are given in Table X.1-1. The proposed foods containing the NI are intended to provide an alternative, and not an additional, source of glucosamine to supplements and PARNUTs foods.

Discussion *Members accepted that the NI, and its counterpart which is obtained from shellfish is widely available throughout the world. Members accepted that the purpose of the NI was to provide an alternative and not an additional source of glucosamine.*

XI Nutritional information on the NI

Information on this aspect is provided on p.35-37 of the application dossier.

20. The nutritional value of the NI is given in Table XI-1 of the application dossier. The NI has little nutritional value other than a source of carbohydrate and its inclusion in various food categories is intended to provide an alternative source of glucosamine

Discussion *Members accepted that the nutritional properties of the NI did not give cause for concern.*

XII. Microbiological information on the NI

Information on this aspect is provided on p.38-39 of the application dossier.

21. The NI meets the USP-NF microbiological specification, and microbiolgical food standards. The applicant demonstrates this by tabulating counts of yeast and moulds, total coliforms, *Escherichia coli*, *Staphylococcus aureus* and *Salmonella* in 5 separate batches of the product (Table XII.1-1). The results demonstrate that the necessary specifications have been met.

Discussion *Members accepted that the NI met the requisite microbiological specification.*

XIII. Toxicological information on the NI

Information on this aspect is provided on p.40-66 of the application dossier

(a) Toxicological evaluation of *A.niger*

Application Dossier p.41

22. The strain of *A. niger* used to produce the NI was selected based on its safety. The strain does not produce ochratoxin A and the absence of this mycotoxin from the final product is confirmed by the results of tests carried out and presented in Appendix 2 of the application dossier (Incorrect reference to Appendix 3 in the dossier).

(b) Toxicological evaluation for glucosamine

23. Much of the data by the applicant regarding the toxicology of glucosamine and its safety in humans is taken from a recent review of the literature and from a recent human study, the Glucosamine/chondroitin Arthritis Intervention Trial (GAIT) study.

Glucosamine metabolism
Application Dossier p.42-43

24. Exogenous glucosamine is actively transported into cells by glucose transporters, a process that is facilitated by insulin. Glucosamine is a component of the hexosamine pathway, an important branch of glycolysis. Glucosamine metabolism is highly regulated by differing rates of transport into different tissues according to glucose transporter affinity.

25. Some animal studies suggest that glucosamine administration may produce insulin resistance and hyperglycaemia. However, most *in vitro* and animal studies have achieved blood and tissue levels 100 to 2000 times higher than would be expected with the glucosamine doses used in humans.

Absorption, Distribution, Metabolism and Excretion (ADME) studies
Application Dossier p.43-46

26. A number of ADME studies have been carried out in animal models and human volunteers and the results of these studies are comparable. Collectively, the studies indicate that a large proportion of orally administered glucosamine is absorbed but has a limited bioavailability as a significant proportion undergoes first pass metabolism in the liver. Consequently, tests in rats have shown that the blood levels of glucosamine after oral administration are only about 20% of those achieved by the intravenous route. Glucosamine is detectable in most tissues examined after oral administration to rats including the liver, kidney and joint cartilage.

Animal toxicity studies: acute (oral)
Application Dossier p.46-47

27. The LD_{50} of glucosamine for rats and mice exceeds 5000mg/kg and for rabbits exceeds 6000mg/kg. The NOAEL for the NI was determined by one rat study to be 5000mg/kg. Table XIII.2.2.1-1 summarises the single dose acute oral toxicity studies carried out on glucosamine.

Animal toxicity studies: subchronic and chronic (oral)
Application Dossier p.47-50

28. A number of studies in various animal species have looked at the effects of glucosamine over an extended time period (12 – 365 days). These studies are summarised in Table XIII.2.2.2-1. Based on these studies the NOAEL for rats and dogs (for free base glucosamine) has been established as at least 2130mg/kg and 1696mg/kg body weight/day respectively.

Animal toxicity studies: parenteral administration
Application Dossier p.51

29. The effects of intravenous (IV) or intraperitoneal (IP) administration of glucosamine has been examined in rats and mice. The LD_{50} data are summarised in the Table below.

Species	**Rat**		**Mouse**	
Route administered	IV	IP	IV	**R**IP
LD_{50} (mg/kg bodyweight)	~1700	>5200	~1600	>6600

1.

30. The rat model has often been selected for study as it is particularly sensitive to the effects of parenteral glucosamine administration on glucose metabolism. Of 14 reports reviewed, glucose metabolism was altered in 12, resulting in higher blood glucose levels, reduced uptake of glucose and decreased disposal of glucose. The dosage of glucosamine reported in these studies ranged from 240 to 9937 mg/kg. However, the reduced bioavailability of orally administered glucosamine means that the levels reached in the blood are typically only 20% of those reached through parenteral routes. Blood glucose levels were not significantly altered in studies where high doses of glucosamine (1000 to 2149 mg/kg bodyweight) were administered orally to rats, rabbits or dogs.

Animal toxicity studies: mutagenicity and genotoxicity
Application Dossier p.51-53

31. The applicant has carried out an *in vitro* study of the mutagenic activity of the NI using the *Salmonella- E. coli* / mammalian-microsome reverse mutation assay. In tests on 5 batches of the NI there was no increase in the mean number of revertants; this is in agreement with a previously published study, although there is also some evidence that glucosamine may have clastogenic[1] effects *in vitro.*

32. The applicant has also carried out an *in vivo* micronucleus assay in mice using the NI at doses up to 2000mg/kg. No clinical signs of cytotoxicity were found at the doses used. Based on these negative findings of genotoxicity in vitro and in vivo the applicant concludes that the NI is non-genotoxic. The applicant points out that a positive result was obtained in a mouse chromosomal aberration study using only a single dose. Weighed against the body of available evidence the applicant does not consider this result to be significant.

Human studies: clinical
Application Dossier p.53-57

33. The applicant has summarised the extensive literature on human clinical studies in Table XIII.2.3.1-1. In summary the applicant considers glucosamine to be well tolerated with no serious effects reported.

[1] Clastogenic = causing changes to chromosomes (e.g. breaks in chromosomes, change in chromosome number)

Human studies: adverse events
Application Dossier p.57-60

34. 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 and skin rash or pruritis. The studies in the literature reporting side effect data comparing glucosamine to placebo are summarised in Table XIII.2.3.2-1. In 12 of the 19 studies symptoms were less common in glucosamine treated subjects than those given placebo. Only two studies reported the reverse.

35. Further reviews of the side effects, effectiveness and toxicity of glucosamine are cited and data summarising these studies are shown in Tables XIII.2.3.2-1 and –2. A recently completed clinical trial, the largest to date, examining both efficacy and safety is cited as supporting the safety of chronic glucosamine supplementation. The Committee queried why the applicant had dismissed the findings of an *in vivo* study by Nguyen *et al.,* (2001) which indicated a higher proportion of subjects with adverse reactions than in other studies. In response, the applicant suggested that this could be attributed to the relatively high dropout rate and highlighted a comment by the authors that of the nine individuals who dropped out of the study, only three of them dropped out for reasons that could be attributed to the study. The applicant also speculated that additional use of chondroitin sulphate in this study could have been a contributing factor. The applicant also noted that the adverse reactions reported were mild and were consistent with reactions to shellfish (the source of the glucosamine used in this study).

Human studies: objective endpoints
Application Dossier p.60-61

36. The results of 16 studies reporting various specific safety endpoints, including toxicological assessments, haematological and cardiovascular parameters are summarised in Table XIII.2.3.3.1. No adverse effects were reported for any of the parameters measured in any of these studies.

Human studies: glucosamine hydrochloride versus sulphate
Application Dossier p.64

37. There appears to be no evidence that there is any difference in the efficacy or safety of either form of glucosamine. The only difference that needs to be considered is the quantity of free base in each preparation.

Human studies: effects of glucosamine on glucose metabolism
Application Dossier p.62-63

38. Clinical trials reporting fasting blood glucose levels in subjects receiving glucosamine supplementation are shown in Table XIII.2.3.3-1. In total 18 studies, either directly or indirectly, have reported that glucosamine supplementation has no effect on fasting blood glucose levels in humans (see para 29 above). A

review published in 2006[2] concluded that the data from these studies are limited and it remains to be determined whether long-term glucosamine intake has detrimental effects in patients with more severe diabetes. These authors recommended that patients initiating glucosamine supplementation should monitor their glucose levels closely. Further studies have appeared in the scientific literature after the dossier was drawn up, including one suggesting that a single oral dose of 1500 mg of glucosamine sulphate (equivalent to 970 mg of glucosamine base) may interfere with glucose metabolism in susceptible individuals[3], such as those with type 2 diabetes. In response to concerns raised by the Committee, the applicant provided a supplementary report which provided a critical review of the available literature on this issue.

Human studies: high intakes and long term use
Application Dossier p.63-64

39. The results of studies involving high intake or long term use of glucosamine are summarised. High intakes appear to be well tolerated and there was no difference in the frequency of adverse events in glucosamine-supplemented groups and placebo controls in long term studies.

Discussion Members accepted the toxicological studies provided by the applicant as being sufficient to demonstrate the general safety of the NI. Members also accepted the additional clarification regarding the adverse results noted in the study by Nguyen et al (2001).

The Committee noted that the target population for products containing glucosamine would include middle-aged or elderly people, including a significant proportion of diabetics, including a number whose condition has not been diagnosed. The Committee was therefore concerned by the reports that glucosamine might affect glucose metabolism in diabetics. Members took note of the additional review provided by the applicant but were of the view that the available scientific studies were inadequate to determine the likelihood of a significant effect of glucosamine on glucose metabolism amongst individuals with Type 2 diabetes. Furthermore, a December 2006 opinion from the European Medicines Evaluation Authority[4] advised that this potential interaction should be highlighted to patients who are taking medicinal products containing glucosamine.

Members noted that glucosamine is currently on the market in the form of dietary supplements, but any concern over a possible effect in diabetics would be greater if it was added as an ingredient to a range of foods since adverse reactions were less likely to be picked up by clinicians than if the glucosamine was being consumed as a food supplement.

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[2] Stumpf JL, Lin SW (2006) Ann. Pharmacother. 40(4) 694-698,

[3] Biggee BA, Binn CM, Nuite M, SILbert JE, McAlindon TE (2007) Ann. Rheum. Dis. 66(2) 260-262.

[4] http://www.emea.europa.eu/pdfs/human/referral/glucomed/GlucomedBackgroundSummary-en.pdf

Allergenicity

Application Dossier p.65

40. An expert opinion on the potential allergenicity of the NI has been provided by an allergy specialist[5], who concludes that: there is no reason to be concerned about the possible allergenicity of the NI.

41. Conventional methods for protein analysis cannot be used for the NI due to interference by the glucosamine. In order to demonstrate the absence of protein in the NI, the applicant therefore carried out SDS-PAGE analysis of a single batch followed by sequential staining of the gel with Sypro Ruby and Coomassie blue (Application dossier, Appendix 3).

42. Members were of the view that the use of SDS-PAGE gels was not the most sensitive way to measure protein levels in the novel ingredient. The Committee accepted that nitrogen-based methods could not be used but suggested the use of an alternative method such as Mass Spectrometry. The applicant highlighted that the production process employed used high temperature and acidity which was likely to denature any potential allergenic protein and noted that there had been no reports of allergenicity from sales of the NI as a supplement. The applicant subsequently provided LC-MS data demonstrating that the NI did not contain any protein.

Discussion *Members accepted that the LC-MS data provided adequate reassurance that the NI does not contain detectable amounts of protein.*

Proposed labelling

Information on this aspect is provided on p.10 & 33 of the application dossier

43. In the earlier application for substantial equivalence (see paragraph 3 above) the applicant agreed to label the product as “Non-Shellfish Glucosamine Hydrochloride” with a footnote referring to its source “from the fungus *Aspergillus niger*”.

44. In this application the applicant requested reference to fungus be removed and proposed to simplify the labelling to, “Non-Shellfish Glucosamine Hydrochloride” with a footnote referring to its source “from *Aspergillus niger*”. This was justified on the grounds that there is no trace of the organism in the final product and there is therefore no need to label on the grounds of allergenicity. Furthermore, the applicant pointed out that products such as citric acid and soya sauce, which are also manufactured by fermentation of *Aspergillus,* have a long and safe history of use and are not labelled to indicate their fungal source.

Discussion *The Committee noted the applicant's argument but remained of the view that the applicant should be encouraged to mention the fungal source of glucosamine when labelling the product*

[5] Professor S.L. Taylor of the University of Nebraska

Overall Discussion

44. The Committee noted that this NI had previously been considered by the Committee when the applicant had requested an opinion on the equivalence of the NI compared with the existing counterpart which is obtained from shellfish. Although the applicant had previously obtained a positive opinion on equivalence, this application was for a number of new food categories and as a full novel food application (Article 1 of (EC)258/97) and required greater scrutiny in order to determine whether the criteria for authorisation of a novel ingredient were met, namely that the ingredient must not:

    - Present a risk to the consumer
    - Mislead the consumer
    - Be nutritionally disadvantageous (compared with existing ingredients that it might replace).

45. The Committee considered that the available information is insufficient to reach a firm conclusion regarding the possible effect of the novel ingredient on glucose metabolism, which would be of particular concern for diabetic individuals. The Committee was satisfied with the safety of the novel ingredient in other respects, and saw no reason to change the previously agreed wording for labelling of this ingredient.

Conclusion

46. The Advisory Committee on Novel Foods and Processes is of the view that additional assessment is required in order to judge whether glucosamine hydrochloride, for use as an ingredient in a range of foods and beverages, meets the criteria for acceptance of novel foods and food ingredients.

September 2007