European Food Safety Authority

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SCIENTIFIC OPINION

Inability to assess the safety of chromium-enriched yeast added for nutritional purposes as a source of chromium in food supplements and the bioavailability of chromium from this source, based on the supporting dossiers [1]

Scientific Statement of the Panel on Food Additives and Nutrient Sources added to Food (ANS)

(Questions No EFSA-Q-2005-097, EFSA-Q-2005-120, EFSA-Q-2005-205, EFSA-Q-2006-211, EFSA-Q-2006-212, EFSA-Q-2006-213)

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

The European Community legislation lists nutritional substances that may be used for nutritional purposes in certain categories of foods as sources of certain nutrients.

The Commission has received a request for the evaluation of chromium enriched yeast added for nutritional purposes to food supplements. The relevant Community legislative measure is:

- Directive 2002/46/EC of the European Parliament and of the Council on the approximation of the laws of the Member States relating to food supplements [2].

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

In accordance with Article 29 (1) (a) of Regulation (EC) No 178/2002, the European Commission asks the European Food Safety Authority to provide a scientific opinion, based on its consideration of the safety and bioavailability of chromium enriched yeast as a source of chromium added for nutritional purposes in food supplements.

[2] OJ L 183, 12.7.2002, p.51.

STATEMENT

1. Introduction

Following a request from the European Commission to the European Food Safety Authority (EFSA), the Scientific Panel on Food Additives and Nutrient Sources added to Food (ANS) was asked to provide a scientific opinion on the safety of chromium-enriched yeast added for nutritional purposes as a source of chromium in food supplements and on the bioavailability of chromium from this source.

This statement is based on the information on chromium-enriched yeasts provided by six petitioners.

2. Summary of the information provided in the supporting dossiers on chromium-enriched yeasts

According to the petitioners, chromium-enriched yeasts are derived from cultures of specified strains of *Saccharomyces cerevisiae* grown in the presence of a source of trivalent chromium (chromium(III)). The source of chromium in the dossiers from five petitioners is chromium(III) chloride while one petitioner uses chromium(III) acetate. Chromium(III) acetate is not on the list of approved mineral substances which may be used in the manufacture of food supplements according to Directive 2002/46/EC. Chromium-enriched yeast has no specific chemical identity (name, CAS No., molecular weight) but is chemically defined in terms of its chromium content, following culture of the yeast in the presence of a chromium(III) salt.

Fermentation takes place at a specified temperature and pressure for defined periods of time. The temperature is then increased, to kill the yeast. Four petitioners wash the yeast after fermentation to eliminate the chromium that is not organically bound, while two petitioners do not carry out this washing procedure. After fermentation, five of the petitioners use an enzymatic digestion to rupture the yeast cell wall, releasing the contents, while one petitioner does not carry out this step.

The description of the manufacturing process for chromium-enriched yeasts submitted by the six petitioners differed significantly. Four petitioners provided adequate details of their manufacturing processes, a fifth petitioner was unable to provide details of his process, while the sixth petitioner provided only brief details.

The petitioners in general indicate that chromium in chromium-enriched yeasts is naturally integrated by the growing yeast into its own structure, and that when inorganic chromium(III) is metabolised by yeast it is no longer available by extraction with water, but it is bound to amino acids and peptides in the cell and hence present in an organic form or forms (Ding *et al.*, 2002; Hegóczki *et al.*, 1997). While the precise nature of the organic forms is not definitively known, most of the petitioners claim, supported by literature data, that the main compound in chromium-enriched yeasts containing organically bound chromium(III) is Glucose Tolerance Factor (GTF) (Davis and Vincent, 1997; Zetic *et al.*, 2001). The structure of GTF consists of one chromium(III) ion that is hexa-coordinated with two molecules of nicotinic acid and one molecule of tripeptide glutathione (glutamyl-cysteinyl-glycine). Further details on the characterisation of the fermentation products to demonstrate that the

expected forms of chromium are present in the enriched yeasts were not provided by any petitioner.

Two petitioners have submitted Fourier Transform Infrared Spectroscopy (FTIR) spectra of starter yeasts and the final product; one also providing the results of a comparative elemental analysis for carbon, hydrogen, and nitrogen (C:H:N analysis) on the starter yeasts and the final product. A third used infrared (IR) spectroscopy to characterise the product, while the other petitioners did not submit any information concerning the analytical identification of the product.

Chromium-enriched yeasts are described by five petitioners as either tan, tan-brown, off-white or gray, while the product described by one petitioner is a green powder, possibly indicating that in this product green chromium chloride hydrate is adsorbed on the surface of yeast cells rather than being organically incorporated into the yeasts. The description of the solubility of the products ranges from insoluble in water, to partially soluble in water, to water soluble at 20 °C.

The content of chromium(III) in products from the different petitioners ranges from 230 to 6 000 mg/kg, most having a chromium content approximately 2 000 mg/kg. One petitioner provided comparative data on an analysis of 12 chromium-enriched yeasts from different producers on the European market, showing a content of hexavalent chromium (Cr(VI)) in investigated products in the range of 0.2-0.8% of total chromium. The majority of petitioners provided specifications for carbohydrates, proteins and lipids, which are the major constituents of the product, and also for loss on drying, ash content and lead, mercury and cadmium content. Microbiological specifications were also provided.

All petitioners provided details of the analytical methods used for the determination of chromium in the enriched yeasts and in the finished product (food supplements). The majority of petitioners indicated the use of Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES), although Flame Atomic Absorption Spectrometry (FAAS) is also used.

The proposed use of chromium-enriched yeast for all petitioners is in multivitamin- and multi-mineral food supplements, in the form of tablets and capsules. Petitioners indicated that these supplements are intended to provide in the range of 10-120 µg chromium(III)/day, although a survey undertaken by one petitioner indicated that a number of products on the market are intended to provide a daily intake of 200 µg chromium(III).

Limited information was provided by the petitioners on the bioavailability of chromium(III) from chromium-enriched yeasts, as assessed by traditional pharmacokinetic studies including measurement of blood chromium or *in vitro* uptake studies. The petitioners generally claimed that organic forms of chromium(III), such as those found in chromium-enriched yeasts, are better absorbed than inorganic chromium(III) (Mertz, 1969; Vinson and Bose, 1984; Lamson and Plaza, 2002). Evidence provided by some petitioners for the bioavailability of chromium from chromium-enriched yeast was largely based on the results of human trials and animal studies that have examined the effects of chromium supplementation on insulin action.

Limited information was also provided on the safety of chromium-enriched yeasts. The information was largely restricted to studies in humans, designed to assess the efficacy of chromium-enriched yeast at therapeutic dose levels in diabetic subjects,and the effects of dietary supplementation with chromium-enriched yeasts on animal performance and on animal models of diabetes.

3. Assessment

The Panel notes that *Saccharomyces cerevisiae* has a qualified presumption of safety (EFSA, 2008a) but considers that this presumption of safety might not be applicable to the specific conditions of culture of the yeast in the presence of a high quantity of chromium(III).

The Panel also notes that an analysis of 12 chromium-enriched yeasts from different producers on the European market has shown a content of Cr(VI) in investigated products in the range of 0.2-0.8% of total chromium.

According to the petitioners, fermentation in the presence of chromium(III) within eukaryotic cells will produce organic compounds in which chromium(III) is bound to amino acids and peptides in the cell. Most of the petitioners claim that the main compound in chromium-enriched yeast containing organically bound chromium is the glucose tolerance factor (GTF) (Mertz, 1969; Davis and Vincent, 1997; Zetic *et al.*, 2001). However, further characterisation of the fermentation products was not provided.

According to one petitioner, different C:H:N ratios and carbohydrate and protein contents of starter yeast and enriched products suggest that yeast alters its metabolism as a result of incorporating the chromium into cell structures. However, the Panel considers that the C:H:N analysis is not relevant to compare the starter yeast and the chromium-enriched yeast and that such a difference in the C:H:N ratio would not in any case provide a clear evidence of chromium incorporation or change in the structure of the yeast. In addition, the Panel noted that it is also possible that added nutrient media containing soya, and plant enzymes used to rupture the yeast cells can influence such differences. According to this petitioner, the differences between the FTIR spectra of chromium-enriched yeast and the starter yeast reference spectrum suggest changes in composition and structure within the yeast. The Panel considers that the FTIR spectra provided do not demonstrate the existence of coordinate bonds between chromium and the yeast biomass.

In considering the bioavailability of chromium(III) from chromium-enriched yeasts, the Panel concluded that the results of the human studies provided by several petitioners demonstrate that chromium(III) from chromium-enriched yeasts is absorbed and is bioavailable, with its bioavailability being comparable to that for chromium from other foods (0.5-2.0%). The Panel considered that there are insufficient data to judge whether the bioavailability from chromium-enriched yeast is greater than that from inorganic chromium(III) salts. The Panel noted however, that organic forms of chromium(III) may have higher bioavailability than inorganic chromium(III) salts. The Scientific Panel on Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC) recently noted that absorption of chromium(III) doubled when supplied as chromium amino acid chelate, in comparison with inorganic chromium (EFSA, 2008b).

The key argument of the petitioners for the safety of chromium-enriched yeast appears to be based on chromium(III) being a natural constituent of the diet, and the long history of use of *Saccharomyces cerevisiae* in fermented food and beverages. The assumption is that, provided there is no overload of normal metabolic pathways, fermentation within eukaryotic cells will produce organic chromium(III) compounds with a metabolic fate and biological distribution similar to that of other sources of chromium in the diet. The Panel notes however, that the petitioners have insufficiently chemically characterised their products. They have not therefore demonstrated that the chromium(III) from chromium-enriched yeasts has a metabolic fate and biological distribution similar to that of other sources of chromium in the diet.

The Panel notes that the information provided by the petitioners on the toxicity of chromium-enriched yeasts was very limited. The Panel also notes that although there was no evidence of adverse effects in a number of human studies that have examined the effects of supplementation with chromium-enriched yeast (at levels of up to 1 000 µg chromium(III)/day) on insulin action, glucose tolerance and lipid profile in diabetic and non-diabetic subjects, these studies were not designed to study the safety of chromium(III)-enriched yeast. Similarly, no adverse effects have been reported in animal studies in which the effects of dietary supplementation (at levels higher than those used in the human studies) with chromium-enriched yeast on animal growth, general health and immune function, as well as in animal models of diabetes, have been investigated. There is no evidence of effects on reproductive performance or development in a small number of studies.

CONCLUSIONS

The Panel concludes that information is available demonstrating that chromium(III) is bioavailable from chromium-enriched yeasts, and that there was no evidence of adverse effects in a number of studies in humans that have examined effects of chromium-enriched yeast supplementation at levels of up to 1000 µg chromium(III)/day). However, these studies were not designed to study the safety of chromium(III)-enriched yeast.

The Panel concludes that the petitioners have insufficiently chemically characterised their products and therefore have not demonstrated that the chromium from chromium-enriched yeasts has a metabolic fate and biological distribution similar to those of other sources of chromium in the diet. Therefore, the Panel is not able to reach a conclusion regarding the safety of the chromium-enriched yeasts under consideration.

Key words:

Food supplements, chromium, chromium(III) chloride, chromium(III) acetate, yeast-transformed chromium, chromium-enriched yeast, yeast.

DOCUMENTATION PROVIDED TO EFSA

1. Technical dossier, 2005a. Dossier for safety evaluation of chromium (III) enriched yeast proposed for use in the manufacture of food supplements. April 2005. Submitted by Pharma Nord, Denmark.
2. Technical dossier, 2005b. Dossier for a request for the consideration for addition of chromium-enriched yeast (chromium-enriched Saccharomyces cerevisiae) to Annex II of Directive 2002/46/EC. March 2005. Submitted by Nature's Own Ltd, UK.
3. Technical dossier, 2005c. Dossier for safety evaluation of chromium yeast. May 2005. Submitted by Procon. Hungary.
4. Technical dossier, 2005d. Dossier on yeast enriched with chromium. August 2005 Submitted by Vireco Producing Developing Trading and Services C. Ltd. Hungary.

5. Technical dossier, 2005e. Dossier for safety evaluation of chromium yeast for use in the manufacture of food supplements. April 2005. Submitted by Beres pharmaceuticals Co. Ltd. Hungary.
6. Technical dossier, 2005f. Dossier on Bio-transformed chromium proposed for addition to Annex II of Directive 2002/46/EC of the European Parlament and of the Council relating to Food Supplements. June 2005, revision January 2008. Submitted by Higher Nature Ltd, UK.

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GLOSSARY / ABBREVIATIONS

AFC	Scientific Panel on Additives, Flavourings, Processing Aids and Materials in Contact with Food
ANS	Panel on Food Additives and Nutrient Sources added to Food
CAS	Chemical Abstracts Service
EC	European Commission
EFSA	European Food Safety Authority
FAAS	Flame Atomic Absorption Spectrometry
FTIR	Fourier Transform Infrared Spectroscopy
GTF	Glucose Tolerance Factor
ICP-AES	Inductively Coupled Plasma Atomic Emission Spectrometry
IR	Infrared