European Food Safety Authority

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SCIENTIFIC OPINION

Inability to assess the safety of zinc-enriched yeast as a source of zinc, added for nutritional purposes to foods for particular nutritional uses and foods (including food supplements) intended for the general population, based on the supporting dossiers [1]

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

(Question No EFSA-Q-2005-089, EFSA-Q-2005-191, EFSA-Q-2006-218)

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[1] For citation purposes: Scientific Statement of the Panel on Food Additives and Nutrient Sources added to Food on the inability to assess the safety of zinc-enriched yeast as a source of zinc, added for nutritional purposes to foods for particular nutritional uses and foods (including food supplements) intended for the general population, based on the supporting dossiers following a request from the European Commission. *The EFSA Journal* (2009) 1129, 1-10.

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 zinc-enriched yeast added for nutritional purposes to foodstuffs. The relevant Community legislative measure is:

- Commission Directive 2001/15/EC on substances that may be added for specific nutritional purposes in food for particular nutritional uses.[2]
- 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.[3]
- Regulation (EC) 1925/2006 of the European Parliament and of the Council on the addition of vitamins and minerals and of certain other substances to foods.[4]

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 zinc-enriched yeast added for nutritional purposes in foods for particular nutritional uses and foods (including food supplements) intended for the general population.

[2] OJ N° L 52, 22.2.2001, p. 19.
[3] OJ L 183, 12.7.2002, p.51.
[4] OJ N° L 404, 30.12.2006, p 26.

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 zinc-enriched yeast as a source of zinc, added for nutritional purposes to foods for particular nutritional uses and foods (including food supplements) intended for the general population, and on the bioavailability of zinc from this source.

This statement is based on the information on zinc-enriched yeasts provided by three petitioners.

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

Zinc-enriched yeasts are derived from cultures of specified strains of *Saccharomyces cerevisiae* grown in the presence of zinc chloride or zinc sulphate. Fermentation takes place at a specified temperature and pressure for defined periods of time. This is followed by increasing the temperature to kill the yeast. The cell wall is ruptured to release the contents which are then spray dried.

According to one of the petitioners, zinc in zinc-enriched yeast is naturally integrated by the growing yeast into its own structure and occurs therefore, in the way zinc would be present in any food material. Another petitioner described zinc-enriched yeast as "a complex of proteins, peptides and amino acids, resulting from the hydrolysis of *Saccharomyces cerevisiae*, which are bound to zinc".

One of the petitioners states that during fermentation in the presence of zinc sulphate, a specific strain of *Saccharomyces cerevisiae* produces specific zinc compounds, the metabolic fate and the biological distribution of which are similar to those of other sources of zinc in the diet.

No specific chemical identity (name, CAS Registry Number, molecular weight) was provided by any of the petitioners and the petitioners named their products differently. One of the petitioner stated that "the integration will be chemically multi-formatted by the organism and therefore, its chemical name, formula, chemical family and CAS Registry Number is undefined".

The analytical techniques which have been used to characterise the zinc-enriched yeast are Fourier Transform Infrared (FTIR) Spectroscopy, a comparative elemental analysis for carbon, hydrogen, and nitrogen (C:H:N analysis) and X-ray Photoelectron Spectroscopy (XPS).

Chemical and microbiological specifications have been provided by the petitioners The content of zinc in the products from different petitioners ranges from 0.4 to 6%. The remainder of the material is made up of ruptured yeast cells.

The manufacturing process was described in detail by one petitioner. The other petitioners provided brief details of the manufacturing process.

Zinc-enriched yeast was stated by the petitioners to be used as an ingredient in tablets, caplets, capsules, chewable tablets, effervescent powders and liquids that are food supplements. Zinc-enriched yeast was identified by one petitioner as currently used in five different products. Each tablet of these products contains between 1.2-15 mg of zinc. The proposed daily intake of these products is 1-3 tablets providing daily intakes of zinc not higher than 15 mg. The second petitioner indicated that these supplements containing zinc-enriched yeast are intended to provide in the range of 1-15 mg zinc/day. The third petitioner did not indicate specific proposals for use levels of the zinc-enriched yeast.

Some animal and human studies comparing the absorption of zinc from zinc-enriched yeasts to that from other sources were provided by the petitioners (Vinson and Bose, 1981; Vinson *et al.,* 1989; unpublished Vinson, 1991; Vinson *et al.,* 2007; Tompkins *et al.,* 2007; Yamaguchi *et al.*, 2004).

Following 30 days feeding on a low zinc diet, male weanling Sprague-Dawley rats (n=5/group) then received a diet supplemented with 50, 100, or 250 mg zinc/kg diet for 30 days in the form of zinc sulphate, zinc amino acid chelate or zinc-enriched yeast (63 g zinc/kg product). The dietary concentrations of zinc were equivalent to doses of 5, 10 or 25 mg zinc/kg bw/day. A control group continued on a low zinc diet until termination. Statistical analysis of the data was not reported in the study (Vinson and Bose, 1981). At termination, the average zinc levels in the blood and the liver were higher in the groups receiving the three sources of zinc compared to the control group. The levels of zinc in the blood in the groups receiving daily doses of 5 mg zinc/kg bw/day from the different sources did not differ. In the 10 or 25 mg zinc/kg bw/day groups, the highest zinc levels in the blood were reported for the zinc-enriched yeast groups as compared to the groups receiving the other sources. At all three dose levels, the average content of zinc in the liver was the highest in the rats receiving zinc-enriched yeast compared to groups receiving the same doses of zinc from the other two sources. The relative bioavailability of zinc in the blood was 101% from zinc amino acid chelate and 172% from zinc-enriched yeast compared to the bioavailability of zinc from zinc sulphate, which was defined as 100%. The relative bioavailability of zinc in the liver was 129% from zinc amino acid chelate and 187% from zinc-enriched yeast compared to the bioavailability of zinc from zinc sulphate, which was defined as 100%. According to the authors these results supported the concept that zinc from the zinc-enriched yeast was the most bioavailable of the examined sources of zinc (Vinson and Bose, 1981; unpublished Vinson, 1991).

Following four weeks feeding on a zinc-deficient diet, male weanling rats (strain not stated) were divided into nine groups (n=6) receiving diets supplemented with zinc at dietary concentrations of 0 (control; kept on zinc–deficient diet), 50, 100 or 250 mg/kg diet (equivalent to zinc doses of 0, 5, 10 or 25 mg/kg bw/day) in the form of either zinc-enriched yeast, zinc orotate or zinc gluconate for five consecutive weeks. Statistical analysis of the data was not reported in this study. At the end of the repletion period the average body weight gains were similar in the zinc-enriched yeast group (175 ± 74 g) and the zinc orate group (173 ± 56 g) which were higher in comparison to the zinc gluconate group (137 ± 71 g). After termination, the average plasma level of zinc in the low-dose groups was the lowest in animals receiving the zinc-enriched yeast. No difference in the plasma zinc levels was seen for the middle-dose groups treated with the different sources. In the high-dose groups, the highest plasma concentration of zinc was recorded in animals receiving the zinc-enriched yeast (10.4 ± 2.7 mg/L (SD), *versus* 7.15 ± 1.50 mg/L zinc orate or 8.48 ± 2.37 mg/L zinc gluconate). The average liver content of zinc in the low-dose groups was the lowest in animals receiving the zinc-enriched yeast. No difference in the liver zinc levels was seen for

the middle-dose groups treated with the different sources. In the high-dose groups, the highest liver level of zinc was in animals receiving zinc orotate (39.3 ± 2.9 mg/kg tissue) and the liver zinc levels in animals receiving the highest doses of zinc-enriched yeast (35.8 ± 6.4 mg/kg tissue) or zinc gluconate (35.0 ± 3.0 mg/kg tissue) were comparable. The relative bioavailability of zinc was 368% for zinc-enriched yeast and 244% for zinc orotate compared to the bioavailability from zinc gluconate, which was defined as 100% (unpublished Vinson, 1991).

Following four weeks feeding on a zinc-deficient diet, 57 male weanling rats (strain not stated) were divided into groups receiving diets supplemented with zinc at dietary concentrations of 0 (control kept on zinc–deficient diet), 50, 100 or 200 mg/kg diet (equivalent to zinc doses of 0, 5, 10 or 20 mg/kg bw/day) from zinc gluconate or zinc-enriched yeast for five consecutive weeks. At termination the body weight and body weight gain in zinc treated groups were higher than in the zinc-deficient group ($p<0.0001$) and the overall weight gain was greater for the zinc-enriched yeast groups compared to the zinc gluconate groups. Absolute liver weights were significantly higher in all zinc-treated groups compared to those in the zinc–deficient group. Zinc levels in the liver were significantly higher in the high-dose zinc group receiving the zinc-enriched yeast compared to the zinc-deficient group (33.9 ± 1.3 mg/kg tissue (SD) *versus* 25.2 ±1.1 mg/kg tissue). Zinc from the zinc-enriched yeast was 3.7 times more bioavailable than zinc from zinc gluconate based on changes in the zinc content in the liver (Vinson *et al.,* 2007).

Young male Wistar rats (n=5/group) received by gavage single doses of 5, 10, 50 or 100 mg zinc/kg bw in the form of zinc oxide or zinc-enriched yeast (100 000 mg zinc/kg product) or 10, 50 or 100 mg zinc/kg bw from zinc sulphate. Other groups of animals were given repeated daily doses of zinc oxide, zinc-enriched yeast or zinc sulphate over a seven day period, using the same dose levels as used in the single-dose protocol. In comparison to baseline levels, serum zinc concentrations were significantly increased by a single oral administration of one of the three compounds providing zinc doses of 100 mg/kg bw/day. A significant increase was observed one hour after administration. A significant increase was also recorded with the lowest dose of zinc (5mg/kg bw) from zinc-enriched yeast or from zinc oxide groups. A single oral administration of 100 mg zinc/kg bw from all the sources caused a significant increase in liver zinc content. A significant increase in femoral-diaphyseal and metaphyseal zinc contents was observed with the administration of the zinc–enriched yeast or zinc sulphate. When zinc yeast or zinc oxide (100 mg zinc/kg bw) was administered once daily for seven days to rats, a significant increase in zinc levels in the serum, liver, and femoral-metaphyseal tissues was seen. A significant increase in calcium content and alkaline phosphatase activity in the femoral-diaphyseal and-metaphyseal tissues occurred with the administration of zinc-enriched yeast or zinc oxide; the effect of zinc-enriched yeast on both parameters was greater than that of zinc oxide (Yamaguchi *et al*., 2004).

A short-term study in human volunteers (21-44 years old, n=3 males and n=2 females) with three zinc sources reported the relative bioavailability of zinc to be 175% from zinc-enriched yeast and 111% from zinc gluconate compared to the bioavailability of zinc from zinc sulphate, which was defined as 100%. The relative bioavailability of zinc was calculated from the area under the concentration-time curve (32.1 ± 16.0 (arbitrary units) for zinc-enriched yeast, 20.3 ± 8.6 for zinc gluconate, and 18.3 ± 10.9 for zinc sulphate). The blood concentration-time curves indicated that zinc from the zinc-enriched yeast was more slowly absorbed compared to zinc absorption from the two other sources as it took more time to reach the maximum zinc concentration after ingestion of the zinc-enriched yeast than after ingestion of the other two zinc compounds. The authors argued that the peptides and free

amino acids present in the yeast may associate or bind with zinc and slow its absorption analogous to the situation of trace elements in foods which have been shown to be more slowly absorbed than the isolated salts of the trace elements (Vinson *et al*., 1989; unpublished Vinson, 1991).

In a trial which used a randomised, two-way crossover design, healthy adult male subjects (n=3/group) received a single dose of 20 mg zinc either in the form of zinc gluconate or zinc-enriched yeast (54.7 g zinc/kg product according to a certificate of analysis of the same commercial product provided to EFSA by the authors of the study). After a seven day washout period each subject received the opposite supplement. Urine, blood and faecal samples were collected over a 48 hour period after a single dose of each of the supplements. No difference was observed in urine excretion of zinc after treatment with zinc gluconate or zinc-enriched yeast. Both supplements increased blood levels of zinc. Compared to zinc-enriched yeast, treatment with zinc gluconate resulted in higher zinc concentrations in the blood in the first six hours but also showed greater losses in the faeces. The net zinc balance (quantity of zinc ingested – (total zinc in the urine + total zinc in faeces)) after 48 hours for zinc-enriched yeast was 9.46 mg but for zinc gluconate it was -2.00 mg, indicating that zinc gluconate supplementation contributed to a net loss of zinc. The relative bioavailability of zinc (quantity of zinc ingested – total zinc in faeces/quantity of zinc ingested x 100) from the zinc-enriched yeast was 70 and 33% at 24 and 48 hours, respectively, whereas the relative bioavailability of zinc from zinc gluconate was 43 and -5.3% for the same time points, respectively. The authors concluded that the zinc from zinc-enriched yeast supplements is more bioavailable than zinc from zinc gluconate salts (Tompkins *et al.*, 2007).

Based on these studies, the petitioners claim that zinc from zinc-enriched yeast is more bioavailable than zinc from inorganic zinc salts or zinc amino acid chelates. A description of the specifications of the zinc-enriched yeast used in the Vinson studies has been provided by one of the petitioners.

Limited toxicological data were provided on zinc-enriched yeasts (Sri *et al*., 1993) from one of the petitioners.

3. Assessment

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

According to one of the petitioners, fermentation in the presence of zinc chloride or zinc sulphate within eukaryotic cells will produce zinc compounds, not further defined but with a metabolic fate and biological distribution similar to those of other sources of zinc in the diet.

According to the same petitioner, the difference in the C:H:N ratio between the starter yeast and the zinc-enriched yeast supports the hypothesis that changes within the yeast due to the incorporation of zinc into the internal structure of the yeast may have modified the overall composition of the yeast. However the Panel considers that the C:H:N analysis is not relevant to compare the starter yeast and the zinc-enriched yeast and that such a difference in the C:H:N ratio would not in any case provide a clear evidence of incorporation of zinc or change in the structure of the yeast.

The same petitioner also stated that the XPS spectra submitted indicate that zinc is not in an elemental form but rather bound within the structure to organic moieties. The Panel considers that the XPS spectra provided can give some information on the crystallinity of zinc-enriched yeasts, but do not provide a significant contribution to its chemical characterisation.

According to another petitioner, the differences between the FTIR spectra of zinc-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 zinc and the yeast biomass.

According to the petitioners zinc-enriched yeast is safe. Although not explicitly stated in the dossiers the argument for the safety of zinc-enriched yeast appears to be based on zinc being a normal 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 zinc compounds with a metabolic fate and biological distribution similar to those of other sources of zinc in the diet.

The Panel considers that the petitioners have insufficiently chemically characterised their products and therefore have not demonstrated that the zinc from zinc enriched yeast has a metabolic fate and biological distribution similar to those of other sources of zinc in the diet.

The petitioners state that zinc from zinc-enriched yeasts is more bioavailable than zinc from inorganic zinc salts or zinc amino acid chelates, and is absorbed in the intestine. The rat studies presented by the petitioners support the higher bioavailability of zinc from zinc-enriched yeast compared to that from other zinc sources. Two human studies showed that zinc from zinc-enriched yeast increases blood zinc levels but the concentrations are lower in the first hours after ingestion compared to the concentrations following ingestion of zinc gluconate. On the other hand the relative bioavailability of zinc was higher from zinc-enriched yeast than from the zinc sources tested.

The only available toxicological data on zinc-enriched yeast is an acute study in mice that suggests that zinc-enriched yeast is a less-toxic source of zinc relative to other inorganic sources (Sri *et al.*, 1993). Zinc content in the yeast used in this study was reported to be 15 000 mg zinc/kg product. The Panel noted that the chemical characterisations in the publication submitted by the petitioner were insufficient to determine whether the test substance was sufficiently similar to the zinc-enriched yeasts evaluated in this opinion. The Panel is therefore unable to base conclusions on the safety of zinc-enriched yeast on this study.

CONCLUSIONS

The Panel concludes overall that the bioavailability of zinc from zinc-enriched yeasts is at least similar to that from other zinc sources (i.e. zinc sulphate, zinc oxide, zinc orotate, zinc gluconate, zinc amino acid chelate).

The Panel also concludes that due to the lack of appropriate dossiers supporting the use of zinc-enriched yeast in foods for particular nutritional uses and foods (including food supplements) for the general population, the safety of the zinc-enriched yeasts under consideration cannot be assessed.

Key words:

Food supplements, zinc, zinc chloride, zinc sulphate, yeast-transformed zinc, zinc-enriched yeast.

DOCUMENTATION PROVIDED TO EFSA

1. Technical dossier 2005a. Dossier on zinc-enriched yeast (zinc-enriched *Saccharomyces cerevisiae*) proposed for addition to Annex II of Directive 2002/46/EC of the European Parliament and of the Council Relating to Food Supplements. March 2005. Submitted by Nature's Own Limited, UK.
2. Technical dossier 2005b. Dossier on Bio-transformed zinc proposed for addition to Annex II of Directive 2002/46/EC of the European Parliament and of the Council Relating to Food Supplements. Original submission June 2005; revised versions January 2008 and November 2008. Submitted by Higher Nature Ltd, UK.
3. Technical dossier 2005c. Dossier on yeast enriched with zinc. August 2005. Submitted by Vireco Producing Developing Trading and Services Co. Ltd, Hungary.

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GLOSSARY / ABBREVIATIONS

ANS	Scientific Panel on Food Additives and Nutrient Sources added to Food (ANS)
CAS	Chemical Abstracts Service
EC	European Commission
EFSA	European Food Safety Authority
FTIR	Fourier Transform Infra Red
XPS	X ray Photoelectron Spectroscopy