The EFSA Journal (2009) 1127, 1-9

SCIENTIFIC OPINION

Inability to assess the safety of manganese-enriched yeast added for nutritional purposes as a source of manganese to food supplements, based on the supporting dossier [1]

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

(Question Nos EFSA-Q-2005-121, EFSA-Q-2005-189)

Adopted on 4 June 2009

PANEL MEMBERS

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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 manganese-enriched yeast added for nutritional purposes as a source of calcium to food supplements, based on the supporting dossiers, following a request from the European Commission. *The EFSA Journal* (2009) 1127, 1-9.

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 manganese-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 manganese-enriched yeast added for nutritional purposes to 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 manganese-enriched yeast added for nutritional purposes as a source of manganese in food supplements and on the bioavailability of manganese from this source.

This statement is based on the information on manganese-enriched yeasts provided by two petitioners.

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

Manganese-enriched yeasts are derived from cultures of specified strains of *Saccharomyces cerevisiae* grown in the presence of natural substrates and a source of manganese (manganese chloride).

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

Manganese-enriched yeast has no specific chemical identity (i.e. name, CAS No., molecular weight) but is chemically defined in terms of its manganese content, following culture of the yeast in the presence of manganese chloride. Both petitioners state that the manganese content is not attributable to residual amounts of the manganese source but is incorporated into the biological matrix of the yeast cell.

Both petitioners provided various data on the chemical and microbiological specifications for manganese-enriched yeast. Both applications describe manganese-enriched yeast as a tan coloured powder with no odour or a slight yeast odour, soluble in water at 20 °C, producing a hazy solution when dissolved at 5%. The petitioners indicate a total content of manganese ranging from 5.0 to 5.7%. The remaining 95% of the material is made up of enzymatically ruptured yeast cells.

A comparative elemental analysis for carbon, hydrogen, and nitrogen (C:H:N analysis) of the starter yeast and the manganese-enriched yeast is provided by one petitioner. According to this petitioner, the difference in the C:H:N ratio between the starter yeast (9.8:1.5:1) and the manganese-enriched yeast (5.5:0.9:1) supports the hypothesis that changes within the yeast due to the incorporation of the mineral into the internal structure of the yeast may have modified the overall composition of the yeast. The same petitioner provides X-ray Photoelectron Spectroscopy (XPS) data, claiming that the manganese has become incorporated into the structure of the yeast and that the manganese is not in its original form.

According to the petitioner, the binding energies are indicative of manganese becoming dissociated and forming bonds with various oxygen moieties present in the yeast structure (i.e. MnO^-, MnO_2, Mn_2O_3, $MnFe_2O_4$, and Mn_3O_4).

The other petitioner provides Fourier Transform Infrared Spectroscopy (FTIR) analysis spectra of samples of the starter yeast (as reference) and the manganese-enriched yeast. This petitioner commented that the differences between the FTIR spectra of manganese-enriched yeasts, manganese chloride and the starter yeast reference spectrum show the completion of the reaction of the manganese with the starter yeast to form manganese-enriched yeast.

The manufacturing process is adequately described by one of the petitioners; the other petitioner provided only brief details.

Analytical methods for the analysis of manganese in the manganese-enriched yeast are based on determination of total manganese by Atomic Absorption Spectroscopy (AAS).

Both petitioners report that manganese-enriched yeast is stable in foods and food supplements for a minimum of three years.

One petitioner describes several products containing manganese-enriched yeast, which would provide a daily intake of between 0.33 and 1 mg manganese/day. According to the other petitioner, manganese-enriched yeast is incorporated into supplements at levels providing a daily intake of manganese between 0.12 and 2 mg.

No evaluations were provided by either petitioner on the bioavailability of manganese from manganese-enriched yeast other than a statement provided by one petitioner that there have been no human studies undertaken to study the bioavailability of manganese from manganese-enriched yeast.

In an animal study, Vinson *et al.* (1989) investigated the relative bioavailability of trace elements and vitamins in commercial supplements. It was found that that manganese from manganese-enriched yeast has a higher bioavailability in rats than manganese from the other sources (manganese sulphate and manganese amino acid chelate) investigated.

The bioavailability of manganese from manganese-enriched yeast was compared to other manganese sources in a study done with 10 groups of five male weanling Sprague-Dawley rats (Vinson and Bose, 1981). All groups were fed a manganese-deficient diet for 21 days (160 µg/kg diet); nine groups were then supplemented *ad libitum* with diets containing either 2, 5 or 10 mg manganese/kg [equivalent to 0.1, 0.25 and 0.5 mg/kg bw/day] in the form of manganese sulphate ($MnSO_4$), an amino acid manganese chelate product (Nu-life) and a yeast product containing manganese (Grow - 0.05 g manganese/g yeast). The control group continued on the manganese-deficient diet for the duration of the study. Blood and liver manganese levels, measured by AAS, were estimated from the slope of a linear dose-response plot. Results showed that the average blood and liver manganese concentrations were consistently higher when manganese was supplemented as the yeast form rather than as the other sources tested (Table 1). The authors noted the high variability in blood and liver concentrations in the study and suggested that it could be due to differences in genetic background.

Table 1. Dose-responses in rats of different forms of manganese supplementation on blood and liver manganese levels (adapted from Vinson and Bose, 1981).

Manganese form	Manganese added in food [mg/kg] (~ mg/kg bw/day)	Average manganese in blood [mg/L]	Average manganese in liver [mg/kg]
$MnSO_4$	2 (0.1)	35.0 ± 5.8	72.8 ± 2.6
	5 (0.25)	45.7 ± 10.8	94.4 ± 47.6
	10 (0.5)	65.7 ± 13.5	150.8 ± 49.6
Manganese chelate	2	32.6 ± 7.4	82.8 ± 25.5
	5	42.5 ± 6.5	130.6 ± 40.0
	10	54.1 ± 4.7	191.6 ± 50.8
Manganese yeast	2	44.5 ± 16.1	121.2 ± 37.4
	5	64.6 ± 14.8	159.6 ± 25.8
	10	89.0 ± 10.9	223.6 ± 40.2
Controls	0	26.0 ± 4.8	44.4 ± 18.0

A 8-week study was conducted to assess the bioaccumulation of manganese in eggs, blood from wing vein and feathers of laying hens (Dobrzański *et al.*, 2008). Sixty Lohmann Brown layers were divided into five groups, three of them were supplemented with 80 mg manganese/kg diet (approximately 10 mg/kg bw/day) either as part of an inorganic mixture containing copper, iron and manganese (considered as control group), as a manganese-enriched yeast source, or as a mineral-enriched yeast mixture containing manganese-, copper- and iron-enriched yeasts. Feed was standardised to contain a constant amount of manganese during the study (60.5 mg manganese/kg diet). Eggs for analysis (12 per group) were collected after 25, 40 and 55 days of supplementation and manganese was measured by Inductively Coupled Plasma-Mass Spectroscopy **(**ICP-MS**)**. Results showed that manganese concentration in eggs (content or shell) or blood samples was not statistically significantly different amongst the various supplementation sources tested, although a slightly higher concentration of manganese was observed in eggs when supplemented in the form of the manganese-enriched yeast source as compared to controls. Results from hens' feathers were difficult to interpret due to environmental interferences.

In a study with 25 piglets, groups of five animals were treated to compare relative absorption of manganese from manganese-enriched yeast and from manganese oxide (Korniewicz *et al.*, 2007). Relative absorption was calculated by comparing faeces and urine manganese excretion every four days during a 3-week feed supplementation period with 50 mg manganese/kg diet (equivalent to 2 mg/kg bw/day), either as manganese-enriched yeast or as manganese oxide. Relative absorption of manganese in animal groups fed diet containing manganese-enriched yeast (~22%) was statistically significantly higher ($p<0.05$) when compared to those from the control group fed diet containing manganese oxide (~14%). By subtracting manganese intake from excreted manganese, the authors concluded that manganese retention in animals fed manganese-enriched yeast increased statistically significantly ($p<0.05$) up to approximately 20% as compared to those from the control group fed manganese oxide (increased up to approximately 13%).

No toxicological data were provided on the source. According to both petitioners, manganese-enriched yeasts are safe.

Although not explicitly stated in the dossiers, the argument for the safety of manganese-enriched yeast appears to be based on manganese 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 manganese compounds, not further defined but with a metabolic fate and biological distribution similar to that of other sources of manganese in the diet.

3. Assessment

Chemical information

The Panel notes that *Saccharomyces cerevisiae* has a qualified presumption of safety (EFSA, 2008) but considers however 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 manganese.

Data presented by one petitioner on analytical results on three mineral-enriched yeasts would suggest that their crude chemical composition in terms of ash, protein, fat, minerals (except those added to enrich the yeast) and amino acids composition are comparable to the non-enriched yeast (Dobrzański *et al*., 2003). However, the Panel considers that the XPS data provided by the petitioner can give some information on the content of the crystal compounds in enriched yeast, but do not provide a significant contribution to its chemical characterisation. The Panel considers that the FTIR spectra do not demonstrate the existence of coordinate bonds between manganese and the yeast biomass.

Bioavailability information

Overall the Panel concludes that the results of available experimental studies suggest that manganese from manganese-enriched yeast is absorbed and is bioavailable to an extent comparable to that from other manganese sources tested.

Toxicological information

The Panel notes that neither safety data nor suitable supporting references were provided by either of the petitioners to support the assumption of safety.

CONCLUSIONS

The Panel concludes that overall the bioavailability of manganese from manganese-enriched yeast is at least similar to that from other manganese sources (i.e. manganese sulphate and manganese amino acid chelate).

The Panel also concludes that due to the lack of appropriate dossiers supporting the use of manganese-enriched yeast in food supplements, the safety of the manganese-enriched yeasts under consideration cannot be assessed.

Key words:

Manganese, manganese-enriched yeast, manganese chloride, yeast, food supplements.

ACKNOWLEDGEMENTS

The European Food Safety Authority wishes to thank the members of the Working Group A on Food Additives and Nutrient Sources added to Food of the ANS Panel for the preparation of this opinion: F. Aguilar, N Bemrah, P. Galtier, J. Gilbert, S. Grilli, R. Guertler, G.E.N. Kass, C. Lambré, J.C. Larsen, J-C. Leblanc, A. Mortensen, I. Pratt, I. Stankovic.

DOCUMENTATION PROVIDED TO EFSA

1. Technical dossier, 2005a. Bio-transformed Manganese Proposed for Addition to Annex II of Directive 2002/46/EC of the European Parliament and of the Council Relating to Food Supplements. Submitted by Higher Nature Ltd UK. Original submission June 2005. Additional information submitted January 2008 and October 2008.

2. Technical dossier, 2005b. A request for consideration of the addition of Manganese-enriched yeast (Manganese-enriched *Saccharomyces cerevisiae)* to Annex II of the Food Supplements Directive (2002/46/EC). Submitted by Nature's Own Limited, UK. Original submission May 2005.

REFERENCES

Dobrzański Z, Opaliński S, Górecka H, Korczyński M, Kołacz R, Trziszka T, 2008. Bioavailability of Fe, Cu and Mn from yeast enriched in bioelements used in laying hens feeding. Electronic Journal of Polish Agricultural Universities (EJPAU). 11 (2). Available at: http://www.ejpau.media.pl/volume11/issue2/art-12.html

Dobrzański Z, Korniewicz A, Dolińska B, Górecki H, Jamroz D, Ryszka F, 2003. The chemical content and feeding value of enriched with chromium, selenium and zinc yeast *Saccharomyces cerevisiae*. Proceedings XI International Congress ISAH. 23-27 February 2003, Mexico.

EFSA (European Food Safety Authority), 2008. Opinion of the Scientific Panel on Biological Hazards on the maintenance of the list of QPS microorganisms intentionally added to food or feed. The EFSA Journal 923, 1-48.

Korniewicz D, Dobrzański Z, Chojnacka K, Korniewicz A, Kolacz R. 2007. Effect of dietary yeasts enriched with Cu, Fe and Mn on digestibility of main nutrients and absorption of minerals by growing pigs. American Journal of Agricultural and Biological Sciences 2, 267-275. Available at: http://www.scipub.org/fulltext/AJAB/AJAB24267-275.pdf

Vinson JA, Bose P, 1981. Comparison of the bioavailability of trace elements in inorganic salts, amino acid chelates and yeast. Proceedings on Mineral Elements, 615-621.

Vinson JA, Bose P, Lemoine L, Hsiao H, 1989. Relative bioavailability of trace elements and vitamins found in commercial supplements. In: Nutrient Availability: Chemical and Biological Aspects. Southgate D., Johnson I and Fenwick G (Eds). Special publication No. 72. Royal Society of Chemistry, Cambridge (UK), 125-127.

Glossary / Abbreviations

AAS	Atomic Absorption Spectroscopy
ANS	Scientific Panel on Food Additives and Nutrient Sources added to Food
CAS	Chemical Abstracts Service
EC	European Commission
EFSA	European Food Safety Authority
FTIR	Fourier Transform Infra Red
ICP-MS	Inductively Coupled Plasma Mass Spectroscopy
XPS	X-ray Photoelectron Spectroscopy