Scientific Opinion

Scientific Opinion on ChromoPrecise® cellular bound chromium yeast added for nutritional purposes as a source of chromium in food supplements and the bioavailability of chromium from this source[1]

EFSA Panel on Food additives and Nutrient Sources added to Food (ANS)[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

The Panel on Food Additives and Nutrient Sources added to Food (ANS) provides a scientific opinion re-evaluating the safety of ChromoPrecise® cellular bound chromium yeast added for nutritional purposes as a source of chromium in food supplements and the bioavailability of chromium from this source. ChromoPrecise® is a yeast preparation with an enriched trivalent chromium content, obtained by culture of *Saccharomyces cerevisiae* in the presence of chromium chloride. A single tablet provides a daily intake of 100 µg chromium(III). There are limited data on the nature and identity of the organic chromium(III) compounds contained in chromium-enriched yeast and on their toxicokinetic and toxicodynamic behaviour in the body. Overall, the Panel concluded that the bioavailability in man of chromium from chromium-enriched yeast is potentially up to approximately ten times higher than that of chromium from chromium chloride. A NOAEL of 2500 mg/kg bw/day ChromoPrecise® was identified in a 90-day feeding study in rats; no evidence of adverse effects of chromium yeasts were reported in other animal studies investigating the effects of dietary supplementation with chromium yeast. ChromoPrecise® chromium yeast was non-genotoxic in a range of *in vitro* genotoxicity studies. Although no information was available on the chronic toxicity, carcinogenicity or reproductive toxicity of ChromoPrecise® chromium yeast, the ANS Panel has previously concluded that trivalent chromium is not carcinogenic, and limited data on other chromium yeasts provide no evidence of an effect on reproductive endpoints. No adverse effects have been reported in clinical efficacy trials with chromium yeasts. The Panel concluded that the use of ChromoPrecise® chromium yeast in food supplements is not of concern, despite the lack of data on the nature and identity of the organic chromium(III) compounds contained in the product, provided that the intake does not exceed 250 µg/day, as recommended by the WHO.

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KEY WORDS

ChromoPrecise® cellular bound chromium yeast, chromium(III), food supplements

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SUMMARY

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

ChromoPrecise® chromium yeast is a yeast preparation with an enriched trivalent chromium (chromium(III)) content, obtained by culture of *Saccharomyces cerevisiae* in the presence of chromium(III) chloride. A daily intake of 100 µg chromium (III) is provided in a single tablet Much of the chromium accumulated by the yeast cells from a trivalent source such as chromium chloride, as in the case of ChromoPrecise® chromium yeast, is bound to amino acids and peptides in the cell and hence is present in an organic form. The Panel noted the lack of data on the actual identity of the chromium species contained in chromium-enriched yeast and their toxicokinetic and toxicodynamic behaviour in the body.

Limited information was provided by the applicant on the bioavailability of chromium from ChromoPrecise® chromium yeast. Although there are reports in the literature that chromium from organic sources such as chromium-enriched yeast is more bioavailable than chromium from inorganic chromium compounds such as chromium chloride, in general the available data do not permit a definite conclusion on this issue. Data from a number of clinical studies that have examined effects of supplementation with chromium-enriched yeasts demonstrate that chromium from chromium-enriched yeast is absorbed to some extent and is bioavailable. The Panel concluded that there are limited data on bioavailability in man of chromium from chromium-enriched yeast but bioavailability is potentially up to ten times higher than that of chromium from chromium chloride. The Panel noted that absorption of chromium (III) from inorganic sources and the diet (an uncharacterised combination of inorganic and organic forms) was low (0.5-2% and 0.5-3% respectively).

ChromoPrecise® chromium yeast is of low acute toxicity, with an LD_{50} in the rat of > 5 000 mg/kg. In a 90-day study in rats, ChromoPrecise® chromium yeast produced mortalities at the highest dose tested of 2 500 mg/kg bw/day, which were attributed by the study authors to misdosing. The Panel agreed with this conclusion. A slight but statistically significant increase in plasma glucose, triglycerides and plasma aspartate aminotransferase (ASAT) was seen in female rats receiving 2 500 mg/kg bw/day of the chromium yeast, in the absence of histopathological findings. The study authors concluded that these changes were not of toxicological importance and that a NOAEL of 2 500 mg/kg bw/day ChromoPrecise® chromium yeast, the highest dose tested, could be identified in this study. The Panel agreed with this conclusion. No evidence of adverse effects of chromium yeasts were seen in other animal studies in which the effects of dietary supplementation with chromium yeast on animal growth, general health and immune function, and in animal models of diabetes have been investigated.

ChromoPrecise® chromium yeast was non-genotoxic in a range of genotoxicity studies, comprising bacterial and mammalian cell mutagenicity assays, a chromosome aberration test and an *in vitro* micronucleus assay. Data on genotoxicity of other chromium (III) sources indicate that there is no concern for this endpoint.

No information was available on the chronic toxicity or carcinogenicity of ChromoPrecise® chromium yeast in experimental animals or in humans. Data on chronic toxicity and carcinogenicity of other chromium(III) sources indicate that there is no concern for these endpoints. There was also no information on the effects of ChromoPrecise® chromium yeast on reproductive performance or development. Limited data on other chromium yeasts provide no evidence of adverse effects on these endpoints.

No adverse effects have been reported in clinical efficacy trials with chromium yeasts, in which a range of parameters, in addition to assessment of insulin action, glucose tolerance and lipid profile, have been investigated. These trials have involved supplementation at levels from below 20 µg chromium(III)/day up to 1000 µg chromium(III)/day for periods from 2 months to up to 7.8 years in non-diabetic and diabetic subjects.

The toxicological data available on ChromoPrecise® chromium yeast indicating an overall low degree of toxicity is underpinned by the data available on other chromium(III) compounds such as chromium(III) chloride and chromium(III) sulphate. The Panel concluded that given the absence of adverse effects in toxicological studies in animals and in clinical efficacy trials in humans, the use of ChromoPrecise® chromium yeast in food supplements is not of concern, despite the lack of data on the actual nature and identity of the organic chromium compounds contained in the product and the absence of data on chronic toxicity, carcinogenicity, reproductive and developmental toxicity,.

The Panel stresses that this conclusion only applies to ChromoPrecise® chromium yeast and not to other chromium-enriched yeasts for which equivalent safety data has not been provided.

Reported average European chromium intakes from the diet range from 61 µg/day in German males to 160 µg/day in an older Swedish study. The 97.5th percentile intake values of chromium were reported as ranging from 126 µg/day in French adults to 170 µg/day in UK adults. The first French total diet study reports average chromium intakes varying from 63 µg/day to 69 µg/day for children aged 3-10 years and 11-17 years, respectively, and high percentile intakes in the range of 107 µg/day to 119 µg/day in the same age classes (Leblanc et al., 2005)

Based on the information provided by the applicant, supplementation with ChromoPrecise® chromium yeast is intended to be in the range of 30-100 µg chromium(III)/day.

In the adult population, assuming a mean and a 97.5th percentile European dietary chromium(III) intake in the range of 60–160 µg/day and 126-170 µg/day, respectively, a consumption of an additional food supplement containing 100 µg chromium(III)/day (the upper level of daily intake of ChromoPrecise® chromium yeast for adults indicated by the applicant) would result in a total daily chromium intake varying between 160 and 260 µg chromium(III)/day in an adult at the average level of dietary exposure and between 226 and 270 µg/day for high consumers.

In children aged 3-17 years, assuming a mean and a 97.5th percentile dietary chromium(III) intake in the range of 63–69 µg/day and 107-119 µg/day (French data), respectively, a consumption of an additional food supplement containing 100 µg chromium(III)/day (the upper level of daily intake of ChromoPrecise® chromium yeast for adults indicated by the applicant) would result in a total daily chromium intake varying between 163 and 169 µg chromium(III)/day in an adult at the average level of dietary exposure and between 207 to 219 µg/day for high consumers.

The Panel noted that these total intakes cannot be compared with a tolerable upper intake level established by e.g. the SCF or the US Food and Nutrition Board (FNB), since such an upper level has not been established, but are below the daily intake of approximately 10 mg/person considered by EVM to be expected to be without adverse health effects. The Panel also noted that the total intakes of chromium for children aged 3-17 years have been based on consumption of the adult supplemental dose of 100 µg chromium(III)/day, which is a worst-case scenario.

The Panel has previously concluded that the intake of chromium(III) from PARNUTS and foods intended for the general population (including food supplements) should not exceed 250 µg/day, the value established by the WHO for supplemental intake of chromium that should not be exceeded. (WHO, 1996). The maximum intended use level of 100 µg chromium(III)/day proposed by the applicant for ChromoPrecise® chromium yeast is below this level. The Panel concluded that ChromoPrecise® chromium yeast as a source of trivalent chromium added for nutritional purposes to PARNUTS and foods intended for the general population (fortified foods and food supplements)

would not be of concern provided that the amount of total supplemental intake of chromium does not exceed 250 µg/day

The Panel concluded that the ability of ChromoPrecise® chromium yeast to elicit allergic responses in individuals with yeast sensitivity cannot be ascertained, therefore those individuals with yeast sensitivity should be informed of the presence of yeast protein.

The Panel considered that the specification for chromium(VI) ≤ 0.2 % of total chromium in the product should be maintained.

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BACKGROUND AS PROVIDED BY 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 ChromoPrecise® cellular bound chromium yeast as source of chromium added for nutritional purpose 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[4].

TERMS OF REFERENCE AS PROVIDED BY EUROPEAN COMMISSION

In accordance with Article 29(1) 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 ChromoPrecise® cellular bound chromium yeast as source of chromium added for nutritional purpose to food supplements

[4] OJ L 183, 17.7.2002, p 51.

ASSESSMENT

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 and bioavailability of ChromoPrecise® cellular bound chromium yeast as a source of chromium added for nutritional purposes to food supplements. The safety of chromium itself, in terms of amounts that may be consumed, is outside the remit of this Panel.

2. Technical data

2.1. Identity of the substance

ChromoPrecise® cellular bound chromium yeast is a yeast preparation obtained by culture of *Saccharomyces cerevisiae* in the presence of a trivalent chromium source. The applicant indicated that chromium(III) chloride is used as chromium source; chromium(III) chloride is a nutrient source that is authorised for use in food supplements (Directive 2002/46/EC)[5]. ChromoPrecise® cellular bound chromium yeast (hereafter referred to as ChromoPrecise® chromium yeast) is chemically defined in terms of its chromium(III) content (see Section 2.3). Chromium exists in many chemical valence states with the trivalent form being the most stable and abundant in nature, and also the form that is found in foods (SCF, 2003). No information was provided by the applicant on the nature of the organic forms in ChromoPrecise® chromium yeast.

2.2. Identification of the yeast strain

According to the information provided by the applicant, the yeast strain used to produce ChromoPrecise® chromium yeast is *Saccharomyces cerevisiae*, which is not derived from genetically modified organisms. *Saccharomyces cerevisiae* is on the EFSA list of organisms given Qualified Presumption of Safety (QPS) status (EFSA, 2008).

2.3. Specifications

Table 1 provides the specifications for ChromoPrecise® according to the information provided by the applicant.

[5]Directive 2002/46/EC of the European Parliament and of the Council of 10 June 2002 on the approximation of the laws of the Member States relating to food supplements. OJ L 183, 12.07.2002, p.51-57.

Table 1: Specifications as proposed by the applicant of Chromoprecise®.

Parameter	Limits
Appearance	Tan powder with characteristic smell and taste of yeast
Identification of cellular bound chromium yeast	Positive by Infrared spectroscopy
Assay	Chromium content: 230 – 300 mg/kg, on the dried basis
Heavy metals:	
Cadmium	≤ 1 mg/kg on the dried basis
Lead	≤ 3 mg/kg on the dried basis
Mercury	≤ 0.1 mg/kg on the dried basis
Microbiological criteria*:	
Total aerobic mesophilic count	< 10 000 CFU/g
Total yeast and mould count	< 100 CFU/g
Enterobacteria	< 100 CFU/g
E. coli	Negative in 1 g
Staphylococcus aureus	Negative in 1 g
Salmonella sp.	Negative in 10 g

* Determined according to the European Pharmacopoeia 7.0, 5.1.4. Microbiological quality of non-sterile products for pharmaceutical use.

The Panel considered that the following additional specifications should be established:

Table 2: Additional specifications as recommended by the ANS Panel

Parameter	Specification
Loss on drying	< 9 % w/w (103°C, 6 hours)
Chromium(VI)	≤ 0.2 % of total chromium[a,b]

(a): The applicant has provided data on the speciation of chromium species in ChromoPrecise® generated by two separate analytical laboratories.

(b): 0.2 % of total chromium corresponds to 0.6 mg/kg, for a yeast containing 300 mg/kg chromium.

The Panel considered that the identification test for cellular bound chromium yeast provided by the applicant requires a methodology only available to the manufacturer and cannot be performed by other laboratories.

The Panel noted the importance of compliance with the specifications for heavy metals, given the capacity of the yeast to accumulate metals present in the growth medium.

2.4. Manufacturing process

The manufacturing process for ChromoPrecise® chromium yeast involves growth of the *Saccharomyces cerevisiae* yeast in a nutrient medium in the presence of chromium chloride as chromium source, followed by pasteurisation, harvesting of the yeast, washing to remove chromium chloride that has not been taken up by the yeast cells, and spray-drying to produce the final powdered product.

2.5. Methods of analysis in food

The applicant provided details of the analytical methods used for the determination of total and extracellular chromium in the enriched yeast product. Analysis of total chromium is carried out by

using atomic absorption spectroscopy (AAS), or alternatively inductively coupled plasma atomic emission spectrometry (ICP-AES) or inductively coupled plasma mass spectroscopy (ICP-MS). Extracellular chromium is determined by ICP-AES or ICP-MS following extraction with 0.1 M HCl.

The applicant provided a validated method for the determination of total chromium and inorganic chromium species [chromium(III) and chromium(VI)] in nutraceutical supplements using the isotope dilution (ID) ICP-MS technique. The data obtained by this method are in line with the data determined by HPLC-ID-ICP-MS. (Additional technical data provided on June 2012, Precise Ingredients ApS).

2.6. Reaction and fate in foods to which the source is added

The applicant provided a report demonstrating the stability of ChromoPrecise® chromium yeast in tablet form over a 5-year period, as assessed by quantitative determination of chromium in the product, together with assessment of disintegration, appearance, average weight and microbiological contamination. The product (as a 700 mg average weight tablet containing 100 µg chromium) was tested at 25°C and 60 % relative humidity with an additional 6-month study being carried out at 40°C and 75 % relative humidity. The results showed that the product was stable over the period of testing, as judged by no change in the parameters measured. The Panel noted that the measures of stability were limited, and no assessment was made of changes in composition or degradation of the organic forms of chromium(III) bound in the yeast.

2.7. Case of need and proposed uses

Trivalent chromium (chromium(III)) is considered an essential element in animal (mammal) and human nutrition, influencing carbohydrate, lipid, and protein metabolism via an effect on insulin action (SCF 2003). The proposed use of ChromoPrecise® chromium yeast is as a dietary supplement in the form of tablets providing around 100 µg chromium per tablet (average tablet weight 694 mg), with ChromoPrecise® chromium yeast (approximately 300 mg chromium/kg) constituting around 60 % of the tablet (approximately 400 mg).

2.8. Information on existing authorisations and evaluations

In 2009, the EFSA Scientific Panel on Food Additives and Nutrient Sources added to Food (ANS) was asked to provide a scientific opinion on the safety of a number of chromium-enriched yeasts added for nutritional purposes as a source of chromium in food supplements and on the bioavailability of chromium from these sources (EFSA, 2009). The Panel concluded that the applicants had insufficiently chemically characterised their products and therefore had 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 was not able to reach a conclusion regarding the safety of the chromium-enriched yeasts under consideration.

The German Federal Institute for Risk Assessment (BfR) has also given a specific opinion on chromium-enriched yeast (BfR, 2006), in which it concluded that the information available in the scientific literature was not sufficient to carry out a reliable risk evaluation of this source of chromium. BfR also noted that the levels of chromium reported in the literature for chromium-enriched yeasts vary considerably, and that there was little information available about the forms of chromium present in the yeast after enrichment and how this was influenced by the cultivation and enrichment process (BfR, 2006).

ChromoPrecise® chromium yeast was marketed in the EU from 1985 onwards following evaluation by the Danish Veterinary and Food Administration. Food supplements containing ChromoPrecise® were marketed in all EU Member States excluding Malta and in a number of countries outside the EU. Sale in the EU ceased in May 2009 following the EFSA opinion (EFSA, 2009).

The EFSA ANS Panel re-evaluated the safety of chromium(III) as a nutrient added to foods for particular nutritional uses (PARNUTS) and foods intended for the general population (including food supplements) (EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS), 2010). The

Panel also evaluated the safety and bioavailability of chromium(III) lactate tri-hydrate as a source of chromium(III) added for nutritional purposes to foodstuffs (EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS), 2012). The Panel concluded that the safety of chromium(III) as a nutrient added to PARNUTS and foods intended for the general population (including food supplements) is not of concern, provided that the intake of chromium(III) from these sources does not exceed 250 µg/day, the value established by the World Health Organization (WHO) for supplemental intake of chromium that should not be exceeded.

Chromium(III) chloride and chromium(III) sulphate are included in Annex II of the Food Supplements Directive 2002/46/EC as approved forms of chromium, and also within Annex Category 2 of Directive 2001/15/EC[6] as substances that may be added for specific nutritional purposes to food for particular nutritional uses.

The Societies for Nutrition of Germany (DGE), Austria (ÖGE) and Switzerland (SGE), jointly established an adequate daily intake of 30-100 µg chromium/day for adults (D-A-CH, 2000). The SCF has previously given an opinion on the tolerable upper intake level of chromium(III) (SCF, 2003). The SCF considered that the data from studies on subchronic, chronic and reproductive toxicity of soluble trivalent chromium salts in experimental animals, as well as the available human data, did not provide clear information on the dose-response relationships, and the SCF was not therefore able to derive a tolerable upper intake level for chromium. The UK Expert Group on Vitamins and Minerals (EVM) similarly concluded that overall there were insufficient data from human and animal studies to derive a safe upper level for chromium(III) (EVM, 2002, 2003). However, in the opinion of the EVM, a total daily intake of about 150 µg chromium(III)/kg bw/day (approximately 10 mg/person) would be expected to be without adverse health effects (EVM, 2003). The US Food and Nutrition Board (FNB) also concluded that the data from animal and human studies are insufficient to establish a tolerable upper intake level for soluble chromium(III) salts (FNB, 2001), while the WHO recommended that the supplementation of chromium(III) should not exceed 250 µg/day (WHO, 1996).

2.9. Exposure

Currently, trivalent chromium (Cr(III)) is used in food supplements in a number of countries in the European Union. According to the EVM, a number of multivitamin and mineral food supplements contain levels of up to 600 µg chromium(III) (EVM, 2003), and food supplements containing chromium (e.g. in the form of chromium-enriched yeast, chromium(III) lactate) were marketed in a number of EU countries at the time of the EVM report. Exposure to chromium(III) also commonly occurs via food, with the highest levels being found in meat and meat products, oils and fats, breads and cereals, fish, pulses and spices (SCF 2003; EVM 2003).

The average daily chromium intake in the diet is usually between 20 and 85 µg/day but varies between different geographic areas, and intakes up to 130 µg/day have been reported (Kumpulainen et al., 1992; Anderson et al., 1993; Schuhmacher et al., 1993). The SCF report provides information on average chromium intakes in some European countries and comparison with levels in the USA (SCF, 2003). As shown in Table 3, reported average European chromium intakes range from 61 µg/day in German males to 160 µg/day in an older Swedish study. The 97.5th percentile intake values of chromium were reported as ranging from 126 µg/day in French adults to 170 µg/day in UK adults. The first French total diet study reported average chromium intakes varying from 63 µg/day to 69 µg/day for children aged 3-10 years and 11-17 years, respectively, and high percentile intakes in the range of 107 µg/day to 119 µg/day in the same age classes (Leblanc et al., 2005)

[6] Commission Directive 2001/15/EC of 15 February 2001 on substances that may be added for specific nutritional purposes in foods for particular nutritional uses. OJ L 52, 22.2.2001, p.19-25.

Table 3: Chromium intake in European countries

Country	Mean (µg/day)	97.5th percentile (µg/day)	Reference
France	76.9 (adults) 63 (children 3-10 years) 69 (children 11-17 years)	126 107 (children 3-10 years) 119 (children 11-17 years)	Leblanc et al., 2005
Germany	61 (adult males) 84 (adult females)		D-A-CH, 2000*
Ireland	89 (adults)	153	FSAI, 2011
Spain	77 (adults)		Velasco-Reynold et al., 2008
	100 (adults)		Garcia et al., 2001*
	120 (adults)		Barbera et al., 1989*
Sweden	160 (adults)		Abdulla et al, 1989*
UK	100 (adults)	170	EVM, 2002*

*from SCF, 2003

Assuming a mean and a 97.5th percentile European dietary chromium(III) intake in the range of 60–160 µg/day and 126-170 µg/day, respectively, a consumption of an additional food supplement containing 100 µg chromium(III)/day (the upper level of daily intake indicated for ChromoPrecise® chromium yeast) would result in a total daily chromium intake varying between 160 and 260 µg chromium(III)/day in an adult at the average level of dietary exposure and between 226 and 270 µg chromium(III)/day for adult high consumers.

In children aged 3-17 years, assuming a mean and a 97.5th percentile dietary chromium(III) intake in the range of 63–69 µg/day and 107-119 µg/day (Leblanc et al., 2005), respectively, a consumption of an additional food supplement containing 100 µg chromium(III)/day (the upper level of daily intake indicated for ChromoPrecise® chromium yeast) would result in a total daily chromium intake varying between 163 and 169 µg chromium(III)/day at the average level of dietary exposure and between 207 and 219 µg chromium(III)/day for child high consumers.

Table 4: Summary information on chromium intake and anticipated potential exposure to Chromium from chromium- enriched yeasts.

Nutrient: Chromium	Amount	Average intake (µg/day)	High intake (µg/day)	References
Provisional estimated range of safe and adequate intake	25 to 100 (µg/day) for adults			COMA, 1991, IOM, 2001
	0,1 µg/kg bw/day and 1,0 µg/kg bw/day for children and adolescents			D-A-CH, 2000
	theoretical requirement for adults at 23 µg			COMA, 1991
Adult human basal requirement	no requirement specified			SCF, 1993
Maximum level of supplementation	250 µg/day			WHO, 1996
intake range from food in Europe for adults		61-160	126-170	Table 3
intake range from food in Europe for children (3-10/11-17 years)		63-69	107-119	Table 3
Amount of Chromium III added to supplements from ChromoPrecise® chromium yeast as indicated by the applicant	100 µg/day			Technical dossier, 2011
Source: Chromium-enriched yeasts				
Total anticipated exposure to Chromium from supplement for adults [(a)]		161-260	226-270	calculation by Panel
Total anticipated exposure to Chromium from supplement for children (3-10/11-17 years)[(b)]		163-169	207-219	calculation by Panel

(a): calculation based on proposed use level of 100 µg/day plus average dietary intake of 61-160 µg/day and high dietary intake of 126-170 µg/day for adults

(b): calculation based on proposed use level of 100 µg/day plus average dietary intake of 63-69 µg/day and high dietary intake of 107-119 µg/day for children

In order to have a daily intake of 100 µg of chromium(III) from ChromoPrecise® chromium yeast , and given a chromium(III) content in the enriched yeast ranging from 230 to 300 mg/kg (or µg/g), the anticipated intake of yeast would be between 300 and 400 mg.

3. Biological and toxicological data

Several authorities have evaluated the bioavailability and safety of trivalent chromium (IPCS, 1988; IARC, 1990; WHO, 1996; EPA, 1998; IOM, 2001; EVM 2002, 2003; SCF, 2003; ATSDR, 2008; EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS), 2010). The present opinion presents additional information available on chromium yeast, including ChromoPrecise® chromium yeast.

3.1. Bioavailability

Limited information was provided by the applicant on the bioavailability of chromium from chromium-enriched yeast, as assessed by traditional pharmacokinetic studies including measurement of blood chromium or *in vitro* uptake studies. In the case of ingested chromium-enriched yeast, including ChromoPrecise® chromium yeast, the chromium(III) contained in the yeast will be released following digestion in the gastrointestinal tract and will be available for uptake into the body. Inorganic chromium compounds are reported to be poorly absorbed in man. The absorption of ingested trivalent chromium lies in the range of 0.1–3 %, depending, among other factors, on the chemical properties of the ingested compound, the level of dietary intake, and the presence of other dietary components in the diet (SCF, 2003). The various chromium compounds found in foods consumed by humans have yet to be identified (SCF, 2003).

Mertz and Cornatzer (1971) reported that 5-10% of the chromium from brewers' yeast was absorbed, compared with 0.1-3 % reported for inorganic chromium compounds. The Panel noted that these data were reported at a time when chromium analyses in biological samples were considered to be inaccurate due to the state of analytical instrumentation (Hunt and Stoeker, 1996), and that other researchers were unable to duplicate these results (Anderson et al., 1996).

Although there are no specific studies of the relative bioavailability to animals of chromium from different supplement sources (Miles and Henry, 2000), a number of studies investigating the effects of dietary supplementation with chromium from sources such as chromium-enriched yeast on animal performance and on animal models of diabetes are available (e.g. Moonsie-Shageer and Mowat, 1993; Wenk, 1994; Crow et al., 1997; Guan et al., 2000; Arkuszewska, 2003; Saad et al., 2007). These supplementation studies in animals provide supportive evidence for the bioavailability of chromium from chromium-enriched yeast.

Evidence for the bioavailability of chromium from chromium-enriched yeast is largely based on the results of clinical trials and animal studies that have examined the effects of chromium supplementation on insulin action. The Panel considered that these studies could also be used to demonstrate the bioavailability of chromium from the source.

3.1.1. Human data

In a randomised, controlled cross-over trial in which 43 diabetic men were given daily supplements of brewers' yeast (8.4 g, providing 18 µg chromium), an autolysed brewers' yeast extract (8.4 g, providing 6 µg chromium), chromium chloride (providing 150 µg chromium) or placebo for four months, no change was seen in fasting plasma chromium after any of the treatments compared to the baseline value of 0.017 µg chromium/L (Rabinowitz et al., 1983). Erythrocyte chromium showed an increase of approximately 25 % from baseline after treatment with brewer's yeast or chromium chloride, while hair chromium showed an approximately 30 % increase with all 3 chromium supplements. Urine chromium concentration was significantly increased over baseline only after treatment with the autolysed brewers' yeast extract.

In another trial in which 24 elderly diabetic and non-diabetic subjects (aged between 63-93, mean age 78 years) were fed either chromium-enriched brewer's yeast (9 g daily, containing 10.8 µg chromium) or chromium-poor torula yeast (control) for eight weeks, both diabetic and non-diabetic subjects showed enhanced glucose tolerance following supplementation with chromium-enriched yeast compared to the control group, manifest as a reduction in blood glucose unaccompanied by changes in insulin levels (Offenbacher and Pi-Sunyer, 1980). A subsequent 10-week trial (by the same researchers) in elderly patients compared the effects of inorganic chromium (200 µg chromium daily as chromium(III) chloride) and brewer's yeast (5 g, providing 5 µg chromium(III) daily), on their glucose tolerance, plasma lipids and plasma chromium, and found no change in any of the parameters measured other than a rise in plasma chromium in the chromium chloride group (from a baseline of 0.28 to 0.50 µg chromium/l at the end of the study) (Offenbacher et al., 1985). No change in plasma chromium was seen in the subjects receiving brewer's yeast (baseline 0.27 to 0.26 µg chromium/l at

the end of the trial). However the Panel noted that the levels of chromium in the yeast used in this study were extremely low compared to the levels proposed for chromium-enriched yeast to be used as a food supplement.

Serum chromium concentrations were one of the parameters measured following daily supplementation with brewer's yeast (providing 23.3 µg chromium), chromium chloride (providing 200 µg chromium) or chromium-poor torula yeast (providing 0.54 µg chromium) in a randomised, cross-over trial involving four 8-week treatment periods with no washout between treatments in 67 subjects with type 2 diabetes mellitus (Bahijiri et al., 2000). Serum chromium increased from approximately 0.1 µg chromium/l at baseline to 0.27-0.28 µg chromium/l following supplementation with brewer's yeast or chromium chloride. At the end of the placebo periods, following chromium supplementation with brewers' yeast or chromium chloride, serum chromium concentration decreased, but remained above the baseline level with no significant difference between the two forms of chromium. Urinary chromium increased significantly, from approximately 0.38 µg chromium/l at baseline to approximately 0.71 and 1.09 µg chromium/l, following intake of brewers' yeast and chromium chloride, respectively. At the end of the placebo periods, urinary chromium remained significantly above the baseline level (0.47 µg chromium/l) in the case of the brewers yeast supplement, whereas in the case of chromium chloride urinary chromium had returned to the baseline level (0.38 µg chromium/l). The Panel noted that the different doses of chromium administered in this study (23.3 and 200 µg for brewer's yeast and chloride, respectively) resulted in equivalence in terms of bioavailability. Therefore, the Panel considered that the bioavailability in man of chromium from chromium-enriched yeast is potentially up to ten times higher than that of chromium from chromium chloride.

Sixty eight adult diabetic and non-diabetic subjects were given 1 000 µg chromium/day as chromium-enriched yeast or placebo for 6 months in a randomised, controlled trial (Cheng et al., 2004). The authors reported that urinary chromium excretion increased significantly, from 0.17– 0.19 ng/mg creatinine, at baseline, to 1.15–1.50 ng/mg creatinine, after 6 months chromium supplementation. Plasma chromium concentration also increased significantly, from 1.8-2.2 µg chromium/l, at baseline, to 5.7-5.9 µg chromium/l. Following 6 months chromium supplementation, fasting glucose and glycosylated haemoglobin were stated to differ in the chromium and placebo groups of the euglycaemic, mildly hyperglycaemic and severely hyperglycaemic groups. The Panel noted that the baseline plasma chromium concentrations reported by Cheng et al. (2004) were approximately 10-fold higher than those reported in other studies.

In a randomised, double-blind trial in which 40 adult men and women with type 2 diabetes mellitus received either 400 µg chromium/day in the form of chromium-enriched yeast or placebo for 3 months, plasma chromium concentration in the yeast-supplemented group increased from 0.18 to 0.33 µg/l, while in the control group plasma chromium increased from 0.19 to 0.24 µg/l (Racek et al., 2006). The increase in the placebo group was attributed to a likely higher chromium content of the diet during the summer period, when the study finished. According to the authors chromium supplementation resulted in a significant decrease in fasting serum glucose compared with placebo, while fasting serum insulin decreased after both treatments, although to a greater extent following chromium yeast supplementation (Racek et al., 2006).

Król et al. (2011) examined the efficacy of supplementation with chromium-enriched brewer's yeast on body mass, carbohydrate, lipids and mineral indices in type 2 diabetic patients. The trial involved 20 subjects (11 males and 9 females aged 37–63) given 500 µg chromium/person/day in divided doses in the form of chromium-enriched brewer's yeast (BioChromDIA) or placebo (yeast alone) for eight weeks in a double-blind, placebo-controlled crossover design. Serum chromium increased by approximately 0.40 µg/l, or 116% (from a mean of 0.35 to 0.76 µg/l) in subjects receiving chromium yeast supplementation compared to an increase of approximately 0.05 µg/l in the placebo group (Król et al., 2011).

3.1.2. Conclusion on bioavailability

Overall, the Panel considered that the results of the clinical studies described above, together with supportive data in animals, demonstrate that chromium from chromium-enriched yeast is absorbed and is bioavailable. The Panel concluded that there are limited data on bioavailability in man of chromium from chromium-enriched yeast but bioavailability is potentially up to ten times higher than that of chromium from chromium chloride (Bahijiri et al., 2000). The Panel noted that absorption of chromium (III) from inorganic sources and the diet (an uncharacterised combination of inorganic and organic forms) were low (0.5-2 % and 0.5-3 %, respectively) (EFSA, 2009; SCF, 2003).

3.2. Absorption, distribution, metabolism and excretion

No specific toxicokinetic studies on chromium-enriched yeast were provided by the applicant. However the Panel considers that the toxicokinetic profile of chromium from chromium-enriched yeast is likely to be similar to that of trivalent chromium from food. The majority of dietary chromium (> 97 %) is not absorbed and is excreted via the faeces. Dietary factors such as starch, ascorbic acid, minerals, oxalate, and amino acid intake can have a significant influence on chromium absorption, and carbohydrate intake has been shown to influence chromium urinary excretion and tissue concentration (Davis et al., 2000).

Following limited absorption of trivalent chromium, as discussed in Section 3.1, chromium binds to plasma proteins, such as transferrin, and is transported to the liver. In humans, chromium concentrates in the liver, spleen, soft tissue, and bone; a similar pattern is seen in rats with incorporation in the kidneys and testes in addition to the liver, spleen and bone (FNB, 2001). Mertz and co-workers proposed a three-compartment model with half-lives of 0.5, 5.9 and 83 days based on studies of radiolabelled chromium ($^{51}CrCl_3$) in rats (Mertz et al., 1965). Urine is the main excretory route for absorbed chromium in both animals and humans, with small amounts being lost in perspiration and bile. Additionally chromium is excreted in human milk (FNB, 2001). Urinary chromium excretion reflects the dietary chromium intake in a dose dependent manner (Uusitupa et al., 1983; Kumpulainen et al., 1992).

As indicated above, chromium binds to transferrin (Harris 1977, cited in FNB, 2001) and interactions between iron and chromium are therefore possible, resulting in impairment of iron storage and metabolism. These interactions have been studied in animals fed a range of trivalent chromium compounds (inorganic and organic) in their diet at relatively high levels (Anderson et al., 1996, 1997); the Panel considered that they are unlikely to be important in the case of chromium-enriched yeast supplements containing low levels of chromium (< 100 µg).

3.3. Toxicological data

The toxicity of chromium compounds has been reviewed by several authorities (IPCS, 1988; IARC, 1990; WHO, 1996; EPA 1998; IOM, 2001; EVM, 2002, 2003; SCF, 2003; ATSDR, 2008; EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS), 2010) The data summarised below provides an overview on specific toxicological and safety studies carried out on chromium-enriched yeast, including ChromoPrecise® chromium yeast.

3.3.1. Acute toxicity

The oral LD_{50} of ChromoPrecise® chromium yeast, administered by gavage as a preparation in deionised water to three female Wistar rats, was > 5 000 mg/kg (Technical dossier, 2011). No treatment-related effects were reported. No adverse symptoms or mortality were reported following administration of chromium-enriched brewer's yeast to rats and mice at a dose level of 21.5 g/kg bw (Li et al., 1990). Chromium content of the yeast was not specified and no further experimental details were provided in the publication.

3.3.2. Short-term and subchronic toxicity

3.3.2.1. Animal data

A 90-day oral toxicity study according to OECD TG 408 has been carried out with ChromoPrecise® chromium yeast in rats (Technical dossier, 2011). Four groups of 10 male and 10 female adult Wistar Crl:WI rats were administered 0, 2.5, 250 or 2 500 mg/kg bw/day of the chromium yeast by gavage as a preparation in deionised water for 90 days. Animals were examined for daily clinical signs, mortality and detailed clinical examinations were recorded weekly and functional tests were carried out pre-treatment and at termination. Body weight and food consumption was recorded weekly. Haematological and clinical chemistry measurements were carried out on blood samples taken at termination, macroscopic examination was carried out at necropsy and organ weights were recorded. Histopathological examination of selected tissues was also performed. Two males and three females receiving 2 500 mg/kg bw/day of the chromium yeast were found dead during the study; deaths were attributed to misdosing, according to the study authors. Technical difficulties with administration of the yeast preparation were also indicated by the finding of mild to moderate granulomatous bronchopneumonia in a number of test animals, without a marked dose-relationship. Other than these findings, animals receiving ChromoPrecise® chromium yeast showed little evidence of treatment-related effects. There was no effect on body weight or body weight gain, or on food consumption, and no treatment-related effects on haematological parameters were recorded, other than a significant increase in blood neutrophils and a significant decrease in lymphocytes in female rats receiving 250 or 2 500 mg/kg bw/day chromium yeast, and a non-significant increase in neutrophils in males in these two groups. These findings were attributed by the study authors to be a reaction to the inflammatory changes in the lungs induced by the dosing errors; the Panel considered that this was a plausible explanation. Clinical chemistry results showed a slight but statistically significant increase in plasma glucose, triglycerides and plasma ASAT in females receiving 2 500 mg/kg bw/day of the chromium yeast. Organ weights were unaffected by treatment, and there were no macroscopic or microscopic findings at termination that could be attributed to ChromoPrecise® chromium yeast, other than the misdosing findings referred to above. The study authors concluded that a NOAEL of 2 500 mg/kg bw/day ChromoPrecise® chromium yeast, the highest dose tested, could be identified in this study, and the Panel agreed with this conclusion, considering the minor clinical chemistry findings in females receiving 2 500 mg/kg bw/day of the chromium yeast in the absence of histopathological findings to be non-adverse.

Other authors have investigated the effects of dietary supplementation with chromium yeast on animal growth, general health and immune function, and in animal models of diabetes (e.g. Moonsie-Shageer and Mowat, 1993; Kegley and Spears, 1995; Arthington et al., 1997; Guan et al., 2000; Lemme et al., 2000; Liu et al., 2012). Although the level of supplementation in these animal studies was generally 5-15 times higher than the anticipated maximum proposed supplementation level with ChromoPrecise® chromium yeast in the normal population (up to 100 µg chromium/day), they provide little information on the safety of chromium-enriched yeast, although no adverse effects were reported.

3.3.2.2. Human data

Supplementation with chromium-enriched yeast has been reported by many authors to increase insulin sensitivity and to decrease fasting serum glucose and endogenous insulin production. Chromium(III) supplementation has also been reported to reduce elevated cholesterol and triglycerides in a dose-dependent manner.

A number of clinical studies have evaluated the effects of supplementation with chromium-enriched yeast for periods from 2 months to up to 7.8 years at levels from below 20 µg chromium/day up to 1 000 µg chromium/day, in both diabetic and normal (control) subjects (e.g. Liu and Morris, 1978; Offenbacher and Pi-Sunyer, 1980; Elwood et al., 1982; Grant and McMullen;1982; Rabinowitz et al., 1983; Vinson and Bose, 1984; Offenbacher et al., 1985; Clausen, 1988; Roeback et al., 1991; Uusitupa et al., 1992; Bahadori et al., 1997; Bahijiri et al., 2000; Trow et al. 2000; Gaede et al., 2003a, b;

Mindrescu and Ciocan, 2004; Cheng et al., 2004; Racek et al., 2006; Król et al., 2011; Sharma et al., 2011).

The majority of these studies have been designed to explore the efficacy of chromium-enriched yeast supplementation on insulin and glucose homeostasis in diabetic subjects, although a number of studies have explored potential effects on lipid parameters such as cholesterol and triglycerides. Although in these studies generally no adverse effects were reported, these studies were not designed to study the safety of chromium-enriched yeast. The Panel was provided with post-marketing surveillance data from 2005 to 2011 by the applicant. The Panel noted that these are not indicative of any significant adverse effect associated with the consumption of ChromoPrecise® chromium yeast (Technical dossier, 2011).

3.3.3. Genotoxicity

ChromoPrecise® chromium yeast has been tested in a number of *in vitro* genotoxicity assays (Technical dossier, 2011). In a bacterial mutagenicity study conducted in accordance with OECD TG 471, using the Salmonella typhimurium strains TA98, TA100, TA1535, TA1537 and TA102 and test concentrations of 31.6, 100, 316, 1 000, 2 500 or 5 000 µg/plate in the presence or absence of metabolic activation, there was no evidence of a mutagenic response at any test concentration of ChromoPrecise® chromium yeast. ChromoPrecise® chromium yeast was similarly non-mutagenic in a mammalian cell gene mutation assay in the mouse lymphoma cell line L5178Y (TK+/) conducted in accordance with OECD TG 476 and using test concentrations up to 5000 µg/mL in the presence or absence of metabolic activation. Relative total growth of the L5178Y (TK+/) cells was inhibited approximately 60 % in only one of the two independent studies, at test concentrations of 3 500 µg/ml and above, without a marked dose response trend in cytotoxicity There was no indication of a mutagenic effect of ChromoPrecise® chromium yeast, either in the presence or absence of metabolic activation, in this study. Also, no change in colony size indicative of a clastogenic effect was detected. ChromoPrecise® chromium yeast did not induce chromosome aberrations in human lymphocytes (TG 473) at test concentrations of up to 2 500 µg/ml in the presence or absence of metabolic activation. Toxicity was evident at a test concentration of 5 000 µg/ml. Finally, in an *in vitro* micronucleus assay conducted in accordance with OECD TG 487, using human lymphocytes and test concentrations of up to 3 500 µg/ml ChromoPrecise® chromium yeast, in the presence or absence of metabolic activation, there was no evidence of induction of micronucleus formation.

In a limited and poorly-reported study, groups of male and female mice (n=5/sex/group) were administered 2.5, 5 or 10 g chromium/kg bw as chromium-enriched yeast, or with 80 mg/kg cyclophosphamide (positive control), and 1 000 bone marrow erythrocytes from each animal were examined for micronuclei (Li et al., 1990). The route of administration was not reported, but the Panel assumed that it was by gavage. The publication also did not report the sampling interval after dosing or other relevant experimental details. The authors reported that chromium did not induce micronuclei, whereas there was a statistically significant increase in the positive control group. In several *in vitro* genotoxicity assays (the Ames test with *Salmonella typhimurium* strains TA97, TA98, TA100, and TA102, the Rec assay in *B. subtilis*, the SOS chromotest, and a prophage λ induction test in *E. coli*) reported in the same publication, exposure to chromium-enriched yeast covering a 100-fold concentration range produced negative results, whereas genotoxic effects were seen in positive controls.

Data on genotoxicity of other chromium (III) sources indicate overall that there is no concern for this endpoint (EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS), 2010).

3.3.4. Chronic toxicity and carcinogenicity

No chronic toxicity or carcinogenicity data are available for ChromoPrecise® chromium yeast or other chromium-enriched yeasts.

Data on chronic toxicity and carcinogenicity of other chromium(III) sources indicate overall that there is no concern for these endpoints (EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS), 2010).

3.3.5. Reproduction and developmental studies

No data are available on the reproductive or developmental toxicity of ChromoPrecise® chromium yeast.

In a limited and poorly-reported study with male mice (strain not reported) fed either 0, 1.25, 2.5 or 5.0 g chromium/kg, as a chromium-enriched yeast, or 20 mg/kg cyclophosphamide (positive control), chromium did not affect the frequency of a number of individual spermatozoal abnormalities (9 common abnormalities were screened) or the total number of abnormalities, whereas a positive response was seen with cyclophosphamide (Li et al., 1990). There is no indication in the study report as to whether the chromium levels cited relate to levels in the enriched yeast or to exposure on the basis of body weight, although the Panel concluded that the former is more likely. Nor is there any indication of the period of exposure. Despite the deficiencies in this study, it appears that high intakes of chromium from chromium-enriched yeast had no adverse effects on sperm morphology in the mouse.

The effect of supplementation of the diet of pregnant pigs with 330 µg chromium(III)/kg in the form of chromium-enriched yeast was investigated, starting approximately one week before parturition and continuing during lactation (Savoini et al., 1998). The pigs received 1.8 kg feed (~ 600 µg chromium(III)/day) daily until parturition, and an average of 4.8 kg feed (~ 1600 µg chromium(III)/day) daily during lactation. The numbers of piglets born, born alive, alive on day 15 and alive at weaning were numerically higher in the chromium group, though the differences were not statistically significant. Litter weights at days 3 and 15, and at weaning were not significantly different. No effect was seen on the condition of the sows or on a number of immunological parameters.

Data on the reproductive and developmental toxicity of other chromium(III) sources indicate overall that there is no concern for these endpoints (EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS), 2010).

3.3.6. Hypersensitivity reactions to chromium

As reported by EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) (2010), several studies have indicated that, if chromium salts do not appear to be food allergens, consumption of a diet rich in chromium or oral challenges with potassium dichromate may increase the severity of symptoms of contact dermatitis, and that hand eczema improves with diets low in chromate in sensitive patients (Sharma, 2009). Most of the studies investigating chromate allergy have used chromium salts and mostly chromium(VI). However, chromium dermatitis seems rather a result of a combined chromium(VI) and chromium(III) allergy (Hansen et al., 2003, 2006).

3.3.7. Safety of the yeast source in chromium-enriched yeast

The quantity of yeast ingested as a result of the use of supplements containing ChromoPrecise® chromium yeast will be approximately 400 mg daily. This is comparable to the quantity of non-enriched brewer's yeast consumed as a dietary supplement. The product is pasteurised and microbiological risks are therefore not foreseen. *Saccharomyces cerevisiae* is also on the EFSA list of organisms given Qualified Presumption of Safety (QPS) status (EFSA, 2008).

Digestion of the yeast cells can be anticipated to release cellular constituents (amino acids and peptides, sugars/carbohydrates, vitamins and minerals), all of which are anticipated to be endogenous in the human body. Thus consideration of the safety of the source can be restricted to the assessment of the chromium-containing compounds released from the cell, as detailed above.

Some individuals may be sensitive to yeast protein, as shown by skin prick tests and radioallergosorbent testing (RAST), particularly those who have had a yeast infection such as *Candida*, or people who may be exposed to yeasts by inhalation or people with atopic dermatitis. However, serious allergic responses to yeast including anaphylaxis are reported to be rare, despite the fact that yeast has been used as a food ingredient since records began. While it is theoretically possible that chromium-modified yeast proteins may present an enhanced risk of allergenicity, there are no data to support this hypothesis, and the applicants have not provided any reports of such reactions. The Panel concluded that the use of supplements containing ChromoPrecise® chromium yeast is unlikely to present a significant risk of inducing allergy. The ability of ChromoPrecise® chromium yeast to elicit allergic responses in individuals with yeast sensitivity cannot be ascertained, therefore those individuals with yeast sensitivity should be informed of the presence of yeast protein.

4. Discussion

The intended use of ChromoPrecise® chromium yeast is as a source of trivalent chromium added for nutritional purposes in food supplements. The Panel noted that much of the chromium accumulated by the yeast cells from a trivalent source such as chromium chloride, as in the case of ChromoPrecise®, is bound to amino acids and peptides in the cell and hence is present in an organic form (Hegóczki et al,. 1997; Ding et al,. 2002). The Panel noted the lack of data on the actual nature and identity of the organic chromium compounds contained in ChromoPrecise® chromium yeast.

Limited information was provided by the applicant on the bioavailability of chromium from ChromoPrecise® chromium yeast. Although there are reports in the literature that chromium from organic sources such as chromium-enriched yeast is more bioavailable than chromium from inorganic chromium compounds such as chromium chloride, in general the available data do not permit a definite conclusion on this issue. Data from a number of clinical trials that have examined effects of supplementation with chromium-enriched yeasts on insulin action, glucose tolerance and lipid profile in diabetic subjects demonstrate that chromium from chromium-enriched yeast is absorbed to some extent and is bioavailable. The Panel concluded that there are limited data on bioavailability in man of chromium from chromium-enriched yeast but is potentially up to ten times higher than that of chromium from chromium chloride. (Bahijiri et al., 2000). The Panel noted that absorption of chromium (III) from inorganic sources and the diet (an uncharacterised combination of inorganic and organic forms) was low (0.5-2 % and 0.5-3 %, respectively) (EFSA, 2009; SCF 2003).

ChromoPrecise® chromium yeast is of low acute toxicity, with a LD_{50} in the rat of > 5 000 mg/kg. In a 90-day study in rats, ChromoPrecise® chromium yeast produced mortalities at the highest dose tested of 2 500 mg/kg bw/day, which were attributed by the study authors to misdosing. The Panel agreed with this conclusion and noted that technical difficulties with administration of the yeast preparation were also indicated by the finding of mild to moderate granulomatous bronchopneumonia in a number of test animals, without a marked dose-relationship. A significant increase in blood neutrophils and a significant decrease in lymphocytes in females receiving 250 or 2 500 mg/kg bw/day chromium yeast, and a non-significant increase in neutrophils in males in these two groups were attributed by the study authors to be a reaction to the inflammatory changes in the lungs induced by the dosing errors; the Panel considered that this was a plausible explanation. A slight but statistically significant increase in plasma glucose, triglycerides and plasma ASAT was seen in females receiving 2 500 mg/kg bw/day of the chromium yeast, in the absence of histopathological findings. The study authors concluded that these changes were not of toxicological importance and that a NOAEL of 2 500 mg/kg bw/day ChromoPrecise® chromium yeast, the highest dose tested, could be identified in this study. The Panel agreed with this conclusion.

No evidence of adverse effects of chromium yeasts has been seen in animal studies in which the effects of dietary supplementation with chromium yeast on animal growth, general health and immune function, and in animal models of diabetes have been investigated.

ChromoPrecise® chromium yeast was not genotoxic in a range of genotoxicity studies, comprising bacterial and mammalian cell mutagenicity assays, a chromosome aberration test and an *in vitro* micronucleus assay.

Data on genotoxicity of other chromium(III) sources indicate overall that there is no concern for this endpoint (EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS), 2010).

No information is available on the chronic toxicity or carcinogenicity of ChromoPrecise® chromium yeast in experimental animals or in humans. Data on chronic toxicity and carcinogenicity of other chromium(III) sources indicate overall that there is no concern for these endpoints (EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS), 2010).

There is also no information on the effects of ChromoPrecise® chromium yeast on reproductive performance or development. Limited data on other chromium yeasts provide no evidence of adverse effects on these endpoints.

No adverse effects have been reported in clinical efficacy trials with chromium yeasts, in which a range of parameters, in addition to assessment of insulin action, glucose tolerance and lipid profile, have been investigated. These trials have involved supplementation at levels from below 20 µg chromium(III)/day up to 1 000 µg chromium(III)/day for periods from 2 months to up to 7.8 years in non-diabetic and diabetic subjects.

The toxicological data available on ChromoPrecise® chromium yeast indicating an overall low degree of toxicity is underpinned by the data available on other chromium(III) compounds such as chromium(III) chloride and chromium(III) sulphate. The Panel concluded that given the absence of adverse effects in toxicological studies in animals and in clinical efficacy trials in humans, the use of ChromoPrecise® chromium yeast in food supplements is not of concern, despite the lack of data on the actual identity of the chromium compounds contained in ChromoPrecise® chromium yeast.

The Panel noted that the SCF was unable to derive a tolerable upper intake level for chromium(III), because of the deficiencies in the database. The Panel also noted that the EVM similarly concluded that overall there were insufficient data from human and animal studies to derive a safe upper level for chromium, but that in the opinion of the EVM a total daily intake of about 150 µg chromium(III)/kg bw/day (approximately 10 mg/person) would be expected to be without adverse health effects (EVM, 2002, 2003). The US FNB also concluded that the data from animal and human studies are insufficient to establish a tolerable upper intake level for soluble chromium(III) salts (FNB, 2001), while WHO considered that supplementation of chromium should not exceed 250 µg/day (WHO, 1996). The EFSA ANS Panel considered that the intake of chromium(III) from PARNUTS and foods intended for the general population (including food supplements) should not exceed 250 µg/day (EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS), 2010), as recommended by WHO (WHO, 1996).

Reported average European chromium intakes from the diet range from 61 µg/day in German males to 160 µg/day in an older Swedish study. The 97.5th percentile intake values of chromium were reported as ranging from 126 µg/day in French adults to 170 µg/day in UK adults. The first French total diet study reports average chromium intakes varying from 63 µg/day to 69 µg/day for children aged 3-10 years and 11-17 years, respectively, and high percentile intakes in the range of 107 µg/day to 119 µg/day in the same age classes (Leblanc et al., 2005)

Based on the information provided by the applicant, supplementation with ChromoPrecise® chromium yeast is intended to be in the range of 30-100 µg chromium(III)/day.

In the adult population, assuming a mean and a 97.5th percentile European dietary chromium(III) intake in the range of 60–160 µg/day and 126-170 µg/day, respectively, a consumption of an additional food supplement containing 100 µg chromium(III)/day (the upper level of daily intake of

ChromoPrecise® chromium yeast for adults indicated by the applicant) would result in a total daily chromium intake varying between 160 and 260 µg chromium(III)/day in an adult at the average level of dietary exposure and between 226 and 270 µg/day for high consumers.

In children aged 3-17 years, assuming a mean and a 97.5th percentile dietary chromium(III) intake in the range of 63–69 µg/day and 107-119 µg/day (LeBlanc et al. 2005), respectively, a consumption of an additional food supplement containing 100 µg chromium(III)/day (the upper level of daily intake of ChromoPrecise® chromium yeast for adults indicated by the applicant) would result in a total daily chromium intake varying between 163 and 169 µg chromium(III)/day in an adult at the average level of dietary exposure and between 207 to 219 µg/day for high child consumers.

The Panel noted that these total intakes cannot be compared with a tolerable upper intake level established by e.g. the SCF or FNB, since such an upper level has not been established, but are well below the daily intake of approximately 10 mg/person considered by EVM to be expected to be without adverse health effects.

The Panel has previously concluded that the intake of chromium(III) from PARNUTS and foods intended for the general population (including food supplements) should not exceed 250 µg/day, the value established by the WHO for supplemental intake of chromium that should not be exceeded. (WHO, 1996). The maximum intended use level of 100 µg chromium(III)/day proposed by the applicant for ChromoPrecise® chromium yeast is below this level. The Panel concluded that ChromoPrecise® chromium yeast as a source of chromium added for nutritional purposes to PARNUTS and foods intended for the general population (fortified foods and food supplements) would not be of concern provided that the amount of total supplemental intake of chromium does not exceed 250 µg/day.

In order to have a daily intake of 100 µg of chromium(III) from ChromoPrecise® chromium yeast , and given a chromium(III) content in the enriched yeast ranging from 230 to 300 mg/kg (or µg/g), the anticipated intake of yeast would be approximately 400 mg. The Panel considered that this exposure to yeast constituents as a result use of supplements containing chromium-enriched yeast is low and that the cellular constituents of the yeast are anticipated to be endogenous in the human body. While it is theoretically possible that chromium-modified yeast proteins may present an enhanced risk of allergenicity, there are no data to support this hypothesis. The applicant has not provided any reports of such reactions.

The Panel concluded that the use of supplements containing ChromoPrecise® chromium yeast is unlikely to present a significant risk of inducing allergy. The ability of ChromoPrecise® chromium yeast to elicit allergic responses in individuals with yeast sensitivity cannot be ascertained, therefore those individuals with yeast sensitivity should be informed of the presence of yeast protein.

The Panel considered that the specification for chromium (VI) ≤ 0.2 % of total chromium in the product should be maintained.

CONCLUSIONS

The present opinion deals with the safety of a particular source of trivalent chromium, ChromoPrecise® chromium yeast, to be used in food supplements, and the bioavailability of chromium from this source.

The Panel concluded that there are limited data on bioavailability in man of chromium from chromium-enriched yeast but bioavailability is potentially up to ten times higher than that of chromium from chromium chloride. The Panel noted that absorption of chromium(III) from inorganic sources and the diet (an uncharacterised combination of inorganic and organic forms) was low (0.5-2 % and 0.5-3 % respectively).

The Panel has also concluded that, given the lack of adverse effects in new toxicological studies, in previous animal studies and in clinical efficacy trials in humans, the use of ChromoPrecise® chromium yeast in food supplements is not of safety concern, despite the lack of data on the nature and identity of the organic chromium compounds contained in the yeast and the absence of data on chronic toxicity, carcinogenicity, reproductive and developmental toxicity.

The Panel stresses that this conclusion only applies to ChromoPrecise® chromium yeast and not to other chromium-enriched yeasts for which equivalent safety data have not been provided.

The Panel has previously concluded that the intake of chromium(III) from PARNUTS and foods intended for the general population (including food supplements) should not exceed 250 µg/day, the value established by the WHO for supplemental intake of chromium that should not be exceeded. (WHO, 1996). The maximum intended use level of 100 µg chromium(III)/day proposed by the applicant for ChromoPrecise® chromium yeast is below this level. The Panel concluded that ChromoPrecise® chromium yeast as a source of trivalent chromium added for nutritional purposes to PARNUTS and foods intended for the general population (fortified foods and food supplements) would not be of concern provided that the amount of total supplemental intake of chromium does not exceed 250 µg/day.

The Panel also concluded that the ability of ChromoPrecise® chromium yeast to elicit allergic responses in individuals with yeast sensitivity cannot be ascertained, therefore those individuals with yeast sensitivity should be informed of the presence of yeast protein.

The Panel considered that specifications provided should be extended by including specifications for loss on drying and for chromium(VI): maximum content ≤ 0.2 % of total chromium.

DOCUMENTATION PROVIDED TO EFSA

1. Technical Dossier, Safety evaluation of ChromoPrecise® cellular bound chromium(III) enriched yeast. Precise Ingredients ApS, March 2011
2. Additional technical data provided on June 2012, Precise Ingredients ApS.

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ABBREVIATIONS

AAS	Atomic Absorption Spectroscopy
ANS	Panel on Food Additives and Nutrient Sources added to Food
ASAT	Aspartate aminotransferase
ATSDR	US Agency for Toxic Substances and Disease Registry
BfR	Bundesinstitut für Risikobewertung/German Federal Institute for Risk Assessment
COMA	UK Committee On Medical Aspects of Food Policy
D-A-CH	Deutsche Gesellschaft für Ernährung (DGE), Österreichische Gesellschaft für Ernährung (ÖGE), Schweizerische Gesellschaft für Ernährungsforschung (SGE), Schweizerische Vereinigung für Ernährung (SVE)/ Societies for Nutrition of Germany (DGE), Austria (ÖGE) and Switzerland (SGE and SVE)
EC	European Commission
EFSA	European Food Safety Authority
EPA	US Environment Protection Agency
EVM	UK Expert Group on Vitamins and Minerals
FNB	US Food Nutrition Board
FSAI	Food Standard Authority of Ireland
GTF	Glucose Tolerance Factor
IARC	International Agency for Research on Cancer
ICP-AES	Inductively Coupled Plasma-Atomic Emission Spectroscopy
ICP-MS	Inductively Coupled Plasma-Mass Spectroscopy
ID	Isotope Dilution
IOM	US Institute of Medicine
IPCS	International Programme on Chemical Safety
LD_{50}	Lethal Dose, 50%
NOAEL	No Observed Adverse Effect Level
NTP	US National Toxicology Programme
OECD TG	Organisation for Economic Co-operation and Development Test Guideline
PARNUTS	Particular Nutrient Uses
QPS	Qualified Presumption of Safety
RAST	Radioallergosorbent Testing
SCF	Scientific Committee on Food
WHO	World Health Organization