SCIENTIFIC OPINION

Mixture of chromium di- and tri-nicotinate as a source of chromium added for nutritional purposes in food supplements and in foods for particular nutritional uses

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

(Question No EFSA-Q-2005-079)

Adopted on 26 November 2008

PANEL MEMBERS

SUMMARY
Following a request from the Commission to the European Food Safety Authority (EFSA), the Panel on Food Additives and Nutrient Sources added to Foods (ANS Panel) was asked to provide a scientific opinion on the safety of chromium nicotinate added for nutritional purposes as a source for chromium in food supplements and in foods intended for particular nutritional uses and on the bioavailability of chromium from this source.

The present opinion deals only with the safety of a particular source of chromium and the bioavailability of chromium from this source, intended to be used in foods for particular nutritional uses (PARNUTS) and in foods supplements.

Studies in rodents reveal a low (<1%) bioavailability of trivalent chromium from various sources, including chromium nicotinate. In general, the low bioavailability of chromium found in rodents is comparable to that noted in humans, which was also reported to be low (0.5 to 2.0%). The Panel concludes that chromium bioavailability from chromium nicotinate is low and comparable to the bioavailability of trivalent chromium from other sources.

The toxicity of chromium compounds has been evaluated by various authorities including the Scientific Committee on Food (SCF), the UK Expert group on Vitamins and Minerals (EVM), the US Food and Nutrition Board and the World Health Organisation (WHO).

The SCF issued an opinion on the tolerable upper intake levels (UL) of trivalent chromium and concluded that the limited data from subchronic, chronic and reproductive toxicity studies on soluble trivalent chromium salts and the available human data do not give clear information on the dose-response relationships and therefore, a UL could not be derived.

The US Food and Nutrition Board concluded that the available data from animal and human studies are insufficient to establish an UL for soluble trivalent chromium salts.

The EVM also concluded that, overall, there were insufficient data from human and animal studies to derive a safe upper level for chromium. However, in the opinion of the EVM a total daily intake of about 0.15 mg trivalent chromium/kg bw/day (or 10 mg/person/day) was expected to be without adverse health effects.

The WHO considered that supplementation of chromium should not exceed 250 μg/day.

The safety of nicotinate (nicotinic acid) has been evaluated by various authorities, including the SCF and the EVM. The SCF established a UL for nicotinate of 10 mg/day, based on occasional flushing as the limiting adverse effect which amounts to 0.167 mg nicotinate/kg bw/day for a 60 kg person. The EVM has not established an UL for nicotinate due to insufficient data. For guidance purposes, a dose of 17 mg/day, for supplementation only, was indicated not to be expected to have any significant adverse effects. The dose is equivalent to 0.28 mg nicotinate /kg bw/day in a 60 kg adult.

The petitioner indicates that use levels foreseen in foods for particular nutritional uses and in food supplements will provide 200 μg chromium/day. Given the fact that the specifications of chromium nicotinate indicate chromium levels amounting to 10%, 200 μg chromium correspond to 2 mg of chromium nicotinate/day.

A daily dose of 2 mg chromium nicotinate would provide 200 μg trivalent chromium/day and 1.1 mg nicotinate/day. This amount of chromium would be below the level of 250 μg chromium/day considered by the WHO as a value for supplementation that should not be exceeded. The dose of 1.1 mg nicotinate/day would amount to 11% of the UL for nicotinate established by the SCF.

The Panel notes that the simultaneous use of nicotinate as a source of chromium (III) in both foods intended for particular nutritional uses and in food supplements, both at use levels up to 200 μg chromium/day, could amount to use levels of 400 μg chromium/day. This would be equivalent to 4 mg chromium nicotinate daily, providing 2.2 mg nicotinate/day. This amount of chromium would be above the level of 250 μg chromium/day considered by the WHO as a value for supplementation that should not be exceeded. The dose of 2.2 mg nicotinate/day would amount to 22% of the UL for nicotinate established by the SCF. Although the amount of nicotinate that would be consumed as a result of these proposed uses would be safe, the Panel could not conclude that these uses of chromium (III) nicotinate are of no safety concern.

The Panel notes that recent reviews and evaluations of chromium (III) point at conflicting outcomes of genotoxicity assays and report diverging views and conclusions on the consequences of this genotoxicity issue for the ultimate safety assessment of chromium (III). The Panel is aware that given this situation, the safety of chromium (III) might need to be re-evaluated in light of the recent reviews and evaluations.
Key words:
# TABLE OF CONTENTS

Panel Members .......................................................................................................................... 1  
Summary ..................................................................................................................................... 1  
Table of Contents ....................................................................................................................... 4  
Background as provided by the Commission ............................................................................... 5  
Terms of reference as provided by the Commission .................................................................... 5  
Acknowledgements ..................................................................................................................... 5  
Assessment .................................................................................................................................. 6  
1. Introduction ............................................................................................................................ 6  
2. Technical data ......................................................................................................................... 6  
   2.1. Chemistry ...................................................................................................................... 6  
   2.2. Specifications ............................................................................................................... 7  
   2.3. Manufacturing Process ............................................................................................... 7  
   2.4. Methods of analysis in food .......................................................................................... 7  
   2.5. Reaction and fate in foods to which the source is added ................................................. 7  
   2.6. Case of need and intended levels of use ....................................................................... 7  
   2.7. Exposure ..................................................................................................................... 8  
   2.8. Information on existing authorisations and evaluations ............................................... 9  
3. Biological and toxicological data ............................................................................................ 10  
   3.1. Bioavailability ............................................................................................................ 10  
   3.2. Toxicological data ....................................................................................................... 11  
      3.2.1. Acute toxicity ....................................................................................................... 11  
      3.2.2. Subacute and subchronic toxicity ........................................................................ 12  
      3.2.3. Reproductive and developmental toxicity ............................................................. 13  
      3.2.4. Genotoxicity ........................................................................................................ 13  
      3.2.5. Chronic toxicity ................................................................................................... 14  
      3.2.6. Human studies ..................................................................................................... 15  
      3.2.7. Other studies ........................................................................................................ 15  
4. Discussion .............................................................................................................................. 15  
Conclusions ............................................................................................................................... 17  
Documentation provided to EFSA ............................................................................................. 18  
References .................................................................................................................................... 18  
Glossary / Abbreviations ............................................................................................................ 24
BACKGROUND AS PROVIDED BY THE COMMISSION

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

The Commission has received a request for the evaluation of chromium polynicotinate added for nutritional purposes to foodstuffs. The relevant Community legislative measures are:

- 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\(^2\)

TERMS OF REFERENCE AS PROVIDED BY THE COMMISSION

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

ACKNOWLEDGEMENTS


---

ASSESSMENT
The present opinion deals only with the safety of a particular source of chromium and the bioavailability of the nutrient cation from this source, intended to be used in foods for particular nutritional uses and in food supplements.

The source referred to by the petitioner as chromium polynicotinate is actually a mixture of chromium di- and tri-nicotinate and this mixture which has been assessed in this opinion will be referred to as ‘chromium nicotinate’.

Nicotinic acid is one of the vitamers of niacin (vitamin B₃) which also includes nicotinamide. Because niacin data may also refer to this other vitamer (nicotinamide) data on niacin exposure, and its safety cannot be used to evaluate exposure to and safety of nicotinic acid.

1. Introduction
Chromium nicotinate is a trivalent chromium compound consisting of a complex of chromium and nicotinic acid.

Chromium exists in many chemical valence states, but the trivalent form is most abundant in nature, most stable, and also the form that is found in foods (Norseth, 1981; SCF, 2003). It is found in μg quantities throughout the food supply, primarily in fruits, vegetables and grain products (SCF, 2003).

2. Technical data

2.1. Chemistry
The petitioner indicates that chromium nicotinate is a mixture of di- and tri-nicotinate, with the tri-nicotinate dominating. The tri-nicotinate form consists of three molecules of deprotonated niacin and a molecule of Cr³⁺. The molecular weight of chromium tri-nicotinate is 418.36 g/mol.

Synonyms of chromium nicotinate are O-coordinated chromium (poly)nicotinate, O-coordinated chromium di-nicotinate, O-coordinated chromium nicotinate, O-coordinated chromium di-pyridine-m-carboxylate, tetra aquo-dinicotinato chromium complex, chromium (poly)nicotinate, chromium di-nicotinate, chromium nicotinate, oxygen coordinated niacin bound chromium complex, niacin bound chromium, or any derivations thereof.

Chromium nicotinate is listed in Chemical Abstracts as triaquahydroxybis(3-pyridinecarboxylato-O) chromium (III). The CAS Registry Number of chromium di-nicotinate is 148485-16-9.

Nicotinate, also known as nicotinic acid, is an organic compound with the molecular formula HO₂CC₅H₄N.
2.2. Specifications

The petitioner indicates that chromium nicotinate is a non-fibrous, odourless and tasteless lavender (intense grey-purple) coloured powder. The compound has limited solubility in water and ethanol.

The petitioner indicates that chromium nicotinate is a mixture of di- and tri-nicotinate and provides specifications indicating a moisture content of less than 8%, a niacin content of 550 ± 50 mg/g (55 %), a chromium content of not less than 100 mg/g (10 %), the presence of sodium at 8-10 %, of chloride at 12-16 % and of lead at less than 4 mg/kg, of arsenic at less than 5 mg/kg, of mercury at less than 0.3 mg/kg and of cadmium at less than 0.3 mg/kg. Microbial assays reveal a total plate count of less than 3000 CFU/g, yeast and moulds at less than 100 CFU/g and negative results for *Escherichia coli*, *Salmonella* and *Staphylococcus aureus*.

The Panel notes that the specifications for lead and mercury are higher than those established in the Commission Regulation (EC) No 629/2008. Maximum levels of lead and mercury in food supplements as sold should be 3.0 mg/kg and 0.1 mg/kg respectively.

2.3. Manufacturing Process

Chromium nicotinate is synthesised from niacin and chromium(III)chloride hexahydrate by a method adequately described by the petitioner.

2.4. Methods of analysis in food

The petitioner indicates that the method of analysis for chromium is by ICP (Inductively Coupled Plasma) spectroscopy and that niacin (nicotinate) can be detected and quantified by HPLC. Graphite Furnace Atomic Absorption Spectrometry can be used to determine the chromium content in foods (Miller-Ihli, 1996).

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

Experimental data provided by the petitioner relate to the stability of the raw material and demonstrate the stability of this material for at least three years. The petitioner indicates that under normal conditions, at room temperature and out of direct sunlight, no degradation of chromium nicotinate is anticipated. Except for conditions with pH values exceeding normal physiological levels, and excessive heat (>80 °C), no reactions or reaction products are anticipated. Stability experiments at 40 °C and 75 % relative humidity revealed that under these conditions the chromium content remained unaffected for at least 6 months; experiments at 30 °C and 65 % relative humidity revealed that under these conditions the amount of chromium remained unaffected for at least, 36 months.

2.6. Case of need and intended levels of use

Chromium nicotinate is intended for use in foods and dietary supplements as a stand-alone ingredient or in multi-ingredient formulas, as a powder, in tablets, two-piece hard gelatine...
capsules or soft gelatine capsules. Chromium nicotinate can also be mixed or blended into a variety of prepared foods.

The petitioner indicates that use levels will be such that intake would be consistent with Estimated Safe and Adequate Daily Dietary Intake (ESADDI) and Recommended Daily Intake (RDI) levels.

The petitioner also indicates that use levels in foods for particular nutritional uses and in food supplements will provide 200 μg of chromium/day. Given the fact that the specifications of chromium nicotinate indicate chromium levels to amount to 10%, this corresponds to levels of 2 mg of chromium nicotinate per day.

2.7. Exposure

Chromium

Trivalent chromium occurs naturally in food. The chromium content of foods is not included in existing food composition databases. Therefore, the intake can only be assessed using total diets or duplicate portion techniques. In the UK a total diet study (TDS) showed that the highest concentration of chromium was found in meat products (230 μg/kg), followed by oils and fats (170 μg/kg), bread (150 μg/kg), nuts and miscellaneous cereals (140 μg/kg), fish, sugar and preserves (130 μg/kg) (EVM, 2002).

The 1997 UK TDS reported that the mean chromium exposure was 100 μg/day and 170 μg/day at the 97.5th percentile. The French TDS of 2001 indicated a mean exposure of 77 μg/day for adults (aged >15 years old) and 68 μg/day for children (aged 3 – 14 years old) and, at the 97.5th percentile, an exposure of 126 μg/day for adults and 124 μg/day for children (Leblanc, 2004). In duplicate diets in Germany, Sweden and Spain mean chromium intakes varied from 61 to 160 μg/day (SCF, 2003). In the UK EVM Report, maximum total chromium exposure was estimated to be 770 μg/day (dietary intake excluding water: 170 μg/day (97.5th percentile), water: up to 2 μg (assuming consumption of 2 litres of water/day at a maximum UK concentration < 1 μg/L) and supplements: up to 600 μg/day) (EVM, 2003).

The petitioner indicates that levels to be used in foods for particular nutritional uses (PARNUTS) and in foods supplements will provide 200 μg of chromium/day.

Nicotinate

Nicotinate is one of the vitamers of niacin, which also includes nicotinamide. Dietary intake data are presented as niacin equivalents (IOM, 2001; SCF, 2002). The total niacin equivalents in the diet are taken as the sum of preformed niacin (i.e. nicotinic acid plus nicotinamide) plus 1/60 of the tryptophan content (SCF, 1993). Data on dietary intake of nicotinate from a regular diet are not available.

Based on a daily dose of 2 mg chromium nicotinate, as a source of chromium to be added for nutritional purposes in foods intended for particular uses and in foods supplements, the potential exposure to nicotinate corresponds to 1.1 mg nicotinate per day.
2.8. Information on existing authorisations and evaluations

The petitioner indicates that currently a Generally Recognised as Safe (GRAS) notification for chromium nicotinate to the U.S. Food and Drug Administration (FDA) is being finalised and that a Product Master File submission (2004) has been sent to the Natural Health Products Directorate (NHPD) of Health Canada.

Chromium

For chromium, the SCF stated that since data on essentiality and metabolism of chromium were so sparse, the Committee was not able to specify any requirements (SCF, 1993). The UK Committee on Medical Aspects of Food Policy calculated from balance studies a theoretical requirement for adults of 23 μg/day by using regression equations and concluded that a safe and adequate level of intake lies above 25 μg for adults, and between 0.1 μg/kg bw/day and 1.0 μg/kg bw/day for children and adolescents, respectively (COMA, 1991). The Societies for Nutrition of Germany (DGE), Austria (ÖGE), and Switzerland (SGE), jointly established an adequate daily intake of 30-100 μg/day for adults (D-A-CH, 2000). In the US, the Food and Nutrition Board derived Adequate Intakes (AI) for chromium for different age groups, e.g. 35 μg/day and 25 μg/day for 19 to 50 year old men and women, respectively (IOM, 2001).

Under European legislation (Directive 2002/46/EC), chromium (III) chloride and chromium (III) sulphate are included in the list of substances that can be used in the manufacture of foods for particular nutritional uses and in food supplements. Chromium (III) chloride and chromium (III) sulphate are included in Regulation (EC) No 1925/2006 on the addition of vitamins and minerals and of certain other substances to food as well as in Commission Directive 2001/15/EC on substances that may be added for specific nutritional purposes in foods for particular nutritional uses.

In 2003, the SCF was not able to derive a UL for chromium because available human data and the data from subchronic, chronic and reproductive toxicity studies in experimental animals of soluble trivalent chromium salts did not provide clear information on the dose- response relationships (SCF, 2003). Also the US Food and Nutrition Board concluded that the data from animal and human studies were insufficient to establish a UL for soluble chromium (III) salts (IOM, 2001). The EVM concluded that overall there are insufficient data from human and animals studies to derive a safe upper level for chromium. However, in the opinion of the EVM a total daily intake of about 0.15 mg trivalent chromium/kg bw/day (or 10 mg/person/day) would be expected to be without adverse health effects (EVM, 2003). The WHO considered that supplementation of chromium should not exceed 250 μg/day (WHO, 1996).

Nicotinate

Niacin is listed in Directive 2002/46/EC (EC, 2002) as amended, as a vitamin permitted in the manufacture of food supplements. The dietary requirement is expressed in niacin equivalents. The SCF opinion on substances added for nutritional purposes which have been proposed for use in the manufacture of ‘PARNUTS’ recommended intakes of niacin equivalents between 9 and 18 mg/day (SCF, 1993).

In 2002 the SCF concluded that it is likely there is no requirement for any preformed niacin in the diet under normal conditions and that endogenous synthesis from tryptophan will meet requirements (SCF, 2002). They also indicated that it has been reported that the dose of free nicotinic acid which produces flushing consistently in clinical studies is 50 mg/day. The available data indicate that flushing would be unlikely to occur repeatedly in subjects given
Mixture of chromium di- and tri-nicotinate as a source of chromium

less than 50 mg/day, but occasional flushing was reported at a dose of 30 mg of nicotinic acid/day. The SCF has established a UL for nicotinic acid of 10 mg/day, based on the available data indicating occasional flushing, the limiting adverse effect, at 30 mg per day. An uncertainty factor (UF) of 3 was applied to allow for the fact that a slight effect was reported, that the study was performed in a small number of subjects, and to also take into account the steep dose-response relationship (SCF, 2002).

The EVM has not established a UL for nicotinic acid due to insufficient data. For guidance purposes, based on a LOAEL of 50 mg/day, due to the absence of a NOAEL, an uncertainty factor of 3 is used. Thus, a dose of 17 mg/day, for supplementation only, would not be expected to have any significant adverse effects. The dose is equivalent to 0.28 mg/kg bw/day for a 60 kg adult. This guidance level is given for supplements only, as adverse effects appear to be related to acute, bolus intakes of nicotinic acid, rather than more sustained exposures as would occur with ingestion of nicotinic acid via food (EVM, 2003).

In the UK there is mandatory fortification of flour (except wholemeal and certain other specified types) with nicotinic acid at a level of not less than 1.6 mg/100 g flour for restoration purposes (EVM, 2003).

3. Biological and toxicological data

Several reviews have included evaluations on the bioavailability and safety of trivalent chromium (ATSDR, 2000; EPA, 1998; EVM, 2002, 2003; IARC, 1980; IOM, 2001; IPCS, 1988; SCF, 2003; WHO, 1996;) or of nicotinate (CRN, 2004; EVM, 2003; HSDB, 2007;SCF, 1999, 2002). Therefore the present opinion only presents the findings from additional studies on chromium nicotinate.

3.1. Bioavailability

The petitioner indicates that as with other trivalent forms of chromium, the exact mode of absorption and distribution of chromium nicotinate in humans is unknown. However, as the nicotinate complex will be partially broken down to its components, trivalent chromium and nicotinic acid in stomach acid, the petitioner postulates that chromium and nicotinic acid are absorbed by the usual mechanisms, in addition to a component absorbed as the complex.

In a study reported in the literature 16 healthy elderly volunteers, divided into three groups were given either 200 µg chromium, 100 mg nicotinic acid, or 200 µg chromium + 100 mg nicotinic acid daily for 28 days and evaluated on days 0 and 28 (Urberg and Zemel, 1987). Fasting glucose and glucose tolerance were unaffected by either chromium or nicotinic acid alone. In contrast, the combined chromium-nicotinic acid supplement caused a 15 % decrease in a glucose area integrated total (p<0.025) and a 7 % decrease in fasting glucose. None of the treatments exerted any effect on fasting or one-hour insulin levels. It was concluded that these data suggest that the inability to respond to chromium supplementation may result from sub- optimal levels of dietary nicotinic acid.

There have been two studies to evaluate absorption and biological activity of chromium nicotinate in rodents (Olin et al., 1994; Polansky et al., 1993).

The absorption and retention of three trivalent chromium compounds, chromium chloride (CrCl3), chromium nicotinate (CrNic) and chromium picolinate (CrPic) were observed over a 12-hour period in a CD rat model (Olin et al., 1994). Male rats (150 – 170 g) were gavaged
with 44 μCi (2.7 nmoles) $^{51}$Cr as CrCl$_3$·6H$_2$O, CrNic or CrPic prepared in a 25% egg white slurry. Rats were killed at 1, 3, 6 and 12 hours post-gavage. Cardiac blood was collected and liver, kidneys, pancreas, testes and gastrocnemius were removed, weighed and assayed for $^{51}$Cr. The amount of $^{51}$Cr in these tissues, along with that in urine (collected from the 6 and 12 hour groups), was used to calculate $^{51}$Cr absorbed/retained. There were no differences between males (n = 100) and females (n = 24) and therefore data were combined. On average, 90% of the dose was recovered. Cr in all forms was very poorly absorbed with less than 1% absorption at all time points. The highest retentions for individual tissues were observed in muscle, followed by the liver and blood. For the majority of the time points and tissues, the average percentage of retained $^{51}$Cr was higher in CrNic- gavaged rats than in CrCl- or CrPic-gavaged rats. Tissues collected one hour post-gavage from CrNic rats had retention percentages that were 3.2 to 8.4 fold higher than in the CrPic or CrCl groups. Three hours post-gavage, CrNic rats had blood, muscle and pancreatic $^{51}$Cr retentions that were 2.4 to 8-fold higher than CrPic rats. By 6 and 12 hours post-gavage, the absorbed/retained tissues were 1.8 to 3.8 fold higher in CrNic than in CrPic rats. The percentage of the $^{51}$Cr dose retained in body tissues and fluids with respect to the administered dose was less than 1% for all chromium sources at all time points. The study concluded that there can be significant differences in the bioavailability of different chromium compounds in the rat. However, overall chromium in all forms was very poorly absorbed (less than 1%) at all time points.

In another study, absorption of radioactive chromium trinicotinate (CrNic), chromium tripicolinic acid (CrPic), chromium dinicotinic acid glycine cysteine glutamic acid (CrNAGLYCYSGLU) and chromium chloride (CrCl$_3$) was determined in 6-week old male Wistar rats (Polansky et al., 1993). Weanling rats were fed a cornstarch-based diet containing less than 30 ng chromium/g for 3 weeks. Each rat was then given 2 µg of chromium by gavage; the rats were then sacrificed after 4 and 24 hours and the GI tract removed and the body, minus head and GI, counted. In all cases rates of absorption were less than 1% and there were no significant differences between the groups. Altogether these studies reveal a low bioavailability of chromium from the various sources, including chromium nicotinate.

### 3.2. Toxicological data

The toxicity of chromium compounds has been reviewed by several institutions (ATSDR, 2000; EPA, 1998; EVM, 2002; EVM, 2003; IARC, 1980; IOM, 2001; IPCS, 1988; SCF, 2003; WHO, 1996). The safety of nicotinic acid has been evaluated by various authorities including the SCF (1999) and the EVM (2003). Therefore this opinion only presents animal and human data specific for chromium nicotinate.

#### 3.2.1. Acute toxicity

The petitioner indicates that chromium nicotinate is safe at very high doses, that the most recent studies and observations continue to show a highly beneficial efficacy/safety ratio for chromium supplementation and that the LD$_{50}$ of intravenous nicotinic acid bound chromium complexes in rats is approximately 1g/kg bw.
3.2.2. Subacute and subchronic toxicity

There have been a limited number of studies that fit into the category of subchronic toxicity, three studies in rodents (rat) and one in non-rodents (calves). None of the studies were designed to evaluate the safety of chromium nicotinate.

Fifteen male albino rats were placed on a synthetic rat food diet for two weeks (US Patent 5,194,615, 1993). The rats were then divided into three groups of five. Group 1 was maintained on the synthetic rat food diet (control group). Group 2 was fed the synthetic diet with 10 mg/kg chromium nicotinate added to the drinking water, calculated to amount to 1.5 mg/kg bw/day assuming 200 g bw and consumption of 30 ml drinking water/day. Group 3 was fed the synthetic diet with 10 mg/kg chromium chloride added to the drinking water, calculated to amount to 1.5 mg/kg bw/day assuming 200 g bw and consumption of 30 ml drinking water/day. This diet regime was maintained for six weeks. Each animal was weighed every two weeks. The growth rates of the rats from each group demonstrated that the diet supplemented with chromium nicotinate had a marked effect on the total body weight and total fat content of the rats. The growth rates of subjects from groups 1 and 3 were almost parallel, until the toxicity of chromium chloride caused a decrease in the weight of the animals in group 3. At the end of six weeks the animals were sacrificed, dissected and the organs were assayed for chromium content showing that chromium contents in the heart, liver, spleen, kidney and muscle of rats receiving chromium nicotinate or chromium chloride were up to at most 6.3 fold higher than those in the control group. Blood was collected from each rat and the serum assayed for glucose, triglycerides and cholesterol. There were no reports of gross effects on the body organs examined. The tissue levels of chromium for the chromium supplemented groups ranged from less than 1.1- to 6-fold increases compared to the control group and varied between the two chromium treated groups, being generally lower in the chromium nicotinate group than in the chromium chloride group (84, 69, 146, 39 and 75 % of the value for the chromium chloride group for the heart, liver, spleen, kidney and muscle respectively). Based on these results the authors concluded that chromium nicotinate is metabolised differently than chromium chloride and that it flows to different body pools.

A growth trial was carried out to test the effect of trivalent chromium on the body composition of growing rats (Fekete et al., 2001). At the same time, an evaluation of different measurement methods (weight of epididymal fat pad, adipocyte morphometry, total body electrical conductivity) was performed. Outbred Wistar rats of 30 days of age were fed diets of different (0, 10 and 20 %) protein levels. The diets were supplemented with 4 mg/kg chromium as chromium nicotinate. After a 5 day adjustment period, the experimental feeding lasted 15 days. It was found that chromium addition increased feed intake. The treatment caused changes in body composition, increasing fat and protein deposition.

The effects of grape seed extract (GSE) and a chromium niacin complex (more details not available) on systolic blood pressure (SBP), circulating lipids, and indices of glucose homeostasis were assessed in rats (Tyson et al., 2000). It was noted that GSE and chromium niacin complex alone and in combination can significantly lower SBP in normotensive and hypertensive rats and can affect the glucose-insulin and rennin-angiotensin systems without producing any obvious toxicity.

Twenty-four Holstein bull calves split into three groups were fed a milk replacer diet to assess the effects of chromium on calf performance and metabolism of glucose over a 70-day period (Kegley et al., 1997). Treatment consisted of no supplemental chromium (control) or 0.4 mg/kg dry matter of CrCl$_3$ or 0.4 mg/kg dry matter of chromium nicotinate. Two calves failed to adapt to the diet and were removed from the study. One calf from the CrCl$_3$ group died from a genetic abnormality and one calf from the control group died from a respiratory
Mixture of chromium di- and tri-nicotinate as a source of chromium

disease. Performance data were analysed at 14-day intervals for the entire study. After analysis of these data and the blood samples taken at approximately monthly intervals, it was concluded that chromium supplementation did not markedly affect the performance (body weight) of calves, but the chromium-nicotinate and CrCl₃ did intensify the response to i.v. administered insulin. There were no other gross adverse effects, other than the deaths noted above.

3.2.3. Reproductive and developmental toxicity

There has been one study to evaluate placental transfer of chromium (US Patent 5,194,615). In this study fifteen timed pregnant albino rats were divided into three groups. After an equilibration period of one week they were then divided into three groups of five animals. Group 1 was maintained on the synthetic rat food diet (control group). Group 2 was fed the synthetic diet with 10 mg/kg chromium nicotinate added to the drinking water, calculated to amount to 1.5 mg/kg bw/day assuming 200 g bw and consumption of 30 ml drinking water/day. Group 3 was fed the synthetic diet with 10 mg/kg chromium chloride added to the drinking water, calculated to amount to 1.5 mg/kg bw/day assuming 200 g bw and consumption of 30 ml drinking water/day. This diet regime was maintained for one week, after which the animals were weighed, sacrificed and the organs and fetuses analysed for chromium content. Blood was collected from each rat and the serum assayed for glucose, triglycerides and cholesterol. The data show that chromium from both chromium salts is transported across the placenta. The data also revealed that the fetal tissue chromium concentration in the chromium nicotinate group achieved a 30% increase compared to the control group and a 21% increase compared to the chromium chloride group. It was also concluded that the chromium nicotinate did not achieve high tissue concentrations and that it was not concentrated in the tissues. No adverse effects were reported (US Patent 5,194,615).

3.2.4. Genotoxicity

In very large amounts, certain trivalent chromium compounds are toxic and have been shown to cause chromosomal damage (Freidman et al., 1987; Léonard and Lauwerys, 1980; Levis et al., 1978; Norseth, 1981; Olin et al., 1994; Stearns et al., 1995).

Stearns et al. (1995) reported clastogenic effects in Chinese hamster ovary cells upon exposure to high concentrations of chromium picolinate added to the medium. Clastogenicity was not exhibited by CrCl₃, chromium nicotinate or nicotinic acid. Niacin-bound chromium achieved 18-fold greater cell concentrations than chromium picolinate and did not produce chromosomal aberrations compared to chromium picolinate (Stearns et al., 1995).

Comparative induction of oxidative stress in cultured macrophage cells (J774A.1) by chromium picolinate and chromium nicotinate, were evaluated by Bagchi et al. (1997). The macrophage cells were treated with a range (0 to 50 µg/ml) of concentrations of the two salts for 0 and 24 hours at 37 °C. Small dose-dependent increases in lipid peroxidation, superoxide anion and hydroxyl radical production and DNA fragmentation were observed with both chromium salts. Greater increases in superoxide anion production and DNA fragmentation were observed with chromium picolinate in comparison to chromium nicotinate. Neither chromium salt resulted in a significant decrease in cell viability in comparison to the control.
but it was concluded that both of these chromium salts induce low levels of oxidative stress in cultured macrophage cells (Bagchi et al., 1997).

The concentration-dependent effects of chromium picolinate and chromium polynicotinate on production of reactive oxygen species (ROS) and DNA fragmentation were compared in murine macrophage cells. Chromium picolinate exhibited higher production of the noxious superoxide anion and increased DNA fragmentation than the O-coordinated CrNic form. The toxic effects were attributed to the picolinate moiety (Olin et al., 1994).

Others have also suggested that the chromosomal toxicity of high-concentration trivalent chromium compounds may be due to pro-oxidant action that can be suppressed with antioxidants (Friedman et al., 1987; Stohs and Bagchi, 1995).

The Panel notes that recent reviews and evaluations of chromium (III) (Eastmond et al., 2008; Levina and Lay, 2008) point at conflicting outcomes of genotoxicity assays and report diverging views and conclusions on the consequences of this genotoxicity issue for the ultimate safety assessment of chromium (III). The Panel is aware that given this situation the safety of chromium (III) might need to be re-evaluated in light of these recent reviews and evaluations.

3.2.5. Chronic toxicity

Preuss et al. (2001) found that rats fed large amounts of chromium nicotinate for over a year, exhibited no evidence of toxicity. One hundred and four hybrid (Brown Norway/Fischer 344) rats were dosed after a weaning and acclimatisation period of 5 weeks. Two groups were examined. Group 1 received a baseline diet and Group 2 received a baseline diet supplement with chromium nicotinate (5 mg/kg diet, claimed to be equivalent to an adult human dose of 4 mg), zinc monomethionine (18 mg/kg diet, equivalent to an adult human dose of 15 mg) and grape seed extracts (GSE). At the end of the study, blood chemistry was measured. Also in the study randomly selected rats were used from each group for evaluation of lipid peroxidation/free radical formation (hepatic TBARS formation). Systolic blood pressure (SBP) measurements were made twice a week. Body weight gains were virtually the same between the two groups over the one-year dietary study period. The weights of hearts, kidneys and livers were not significantly different among groups. However, the weight of epididymal fat pad was statistically significantly less at 12 months in Group 2. Almost from the initiation of the dietary intervention, the SBP figures of Group 2 were statistically significantly lower compared to Group 1 (control). Hepatic TBARS formation, an estimate of lipid peroxidation was significantly lower after 1 year in Group 2 and HbA1C was also statistically significantly lower in Group 2. It was concluded that prolonged supplementation with a combination of agents including GSE in addition to chromium nicotinate can markedly lower SBP in normotensive rats, lessen oxidative damage to fats, and lower HbA1C without showing signs of toxicity.

Norseth concluded that evidence available at that time indicated that the trivalent chromium compounds do not cause cancer although high concentrations in some in vitro systems have shown genotoxicity (Norseth, 1981). The review indicated that trivalent chromium may cause mutation and cellular transformation in vitro at high concentrations. The review also outlined that there was at that time no epidemiological evidence for trivalent chromium to be carcinogenic. It was also indicated that this epidemiological evidence is supported by animal experiments and by in vitro results and biochemical considerations. When tested in systems which require that chromium passes biological membranes to exert an effect, reduction of the
hexavalent form to the trivalent form reduces or abolishes the genotoxic effect. Similarly, trivalent chromium which is without effect in such systems, has genotoxic effects when oxidized to the hexavalent form in the system.

3.2.6. Human studies

There have been a number of clinical investigations involving dietary supplementation of healthy subjects, obese women, hypercholesterolemic subjects or patients with cardiovascular disease with chromium nicotinate at levels from 200 up to 800 μg/day for periods of in most cases about 2 months. Generally these studies were focussed on possible beneficial effects of chromium nicotinate intake, studying effects on body weight, routine blood chemistry, fat and non-fat body mass, insulin response to an oral glucose load, plasma insulin, 12 hour fasting insulin, glucose, total cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol and one hour post–challenge insulin and glucose values and antidepressant therapy of dysthmic disorder (Crawford et al., 1999; Grant et al., 1997; Lefavi et al., 1993; McLeod et al., 1999; Mertz, 1993; Preuss et al., 1999; Preuss et al., 2000a; Thomas and Gropper, 1996; Wilson and Gondy, 1995). Although in these studies generally no adverse effects were reported, these studies were not designed to study the safety of chromium nicotinate.

3.2.7. Other studies

There are a number of other non-clinical studies evaluating various mechanisms of action of chromium nicotinate in rats and poultry. There is no evidence of toxicity in any of these studies and endpoints studied included effects on sugar-induced hypertension in rats (Preuss et al., 1995), effects on blood pressure and lipid peroxidation in hypertensive rats (Preuss et al., 1997), effects on cardiovascular parameters in normotensive and hypertensive rats (Preuss et al., 2000b) beneficial effects in a hamster atherosclerosis model (Vinson et al., 2002), effects on carbohydrate metabolism on poults (Rosebrough and Steele, 1981), effects on performance (weight gain), blood chemistry of growing turkeys (Chen et al., 2001) or effects of niacin-bound chromium, Maitake mushroom fraction SX and (-)hydroxycitric acid on the metabolic syndrome in aged diabetic Zucker fatty rats (Talpur et al., 2003). However these studies were of limited value to evaluate the safety of chromium nicotinate.

4. Discussion

Studies in rodents reveal a low (<1%) bioavailability of trivalent chromium from various sources, including chromium nicotinate. Some studies reveal that there can be differences in the bioavailability and tissue levels of chromium resulting from intake of different forms of chromium compounds (Olin et al., 1994; US Patent 5,194,615; 1993), but these differences are small and the overall bioavailability of chromium from all these sources is low. In general, the low bioavailability of chromium found in rodents is comparable to that noted in humans, which was also reported to be low (0.5 to 2.0%) (Anderson and Kozlovsy, 1985; SCF, 2003). The Panel concludes that chromium bioavailability from chromium nicotinate is low and is comparable to the bioavailability of trivalent chromium from other sources.
Mixture of chromium di- and tri-nicotinate as a source of chromium

Trivalent chromium


The SCF issued an opinion on the UL of trivalent chromium (SCF, 2003) and concluded that limited data from subchronic, chronic and reproductive toxicity studies on soluble trivalent chromium salts and the available human data do not give clear information on the dose-response relationships and that therefore a UL could not be derived.

The US Food and Nutrition Board also concluded that the data from animal and human studies are insufficient to establish a UL for soluble chromium (III) salts (IOM, 2001).

The EVM also concluded that overall there are insufficient data from human and animal studies to derive a safe upper level for chromium. However, in the opinion of the EVM a total daily intake of about 0.15 mg trivalent chromium/kg bw/day (or 10 mg/person/day) would be expected to be without adverse health effects (EVM, 2003).

The WHO considered that supplementation of chromium should not exceed 250 μg/day (WHO, 1996). The SCF opinion (SCF, 2003) indicates that in the UK a total diet study has shown that the highest concentration of chromium has been found in meat products (230 μg/kg), followed by oils and fats (170 μg/kg), bread (150 μg/kg), nuts and miscellaneous cereals (140 μg/kg), fish, sugar and preserves (130 μg/kg). The opinion also indicates that a number of multivitamin and mineral food supplements contain up to 100 μg chromium in a daily serving unit (EVM, 2002). In the US relatively high concentrations of chromium have been found in seafood (120-470 μg/kg) followed by meat and fish (110-230 μg/kg), grains and cereals (40-220 μg/kg), fresh fruits (90-190 μg/kg) and fresh vegetables (30-149 μg/kg) (SCF, 2003). This indicates that an intake of 100 to 200 μg/day from food supplements or from foods for particular nutritional uses would be in the same order of magnitude as what could be present in the diet.

The Panel notes that recent reviews and evaluations of chromium (III) (including Eastmond et al., 2008; Levina and Lay, 2008) point at conflicting outcomes of genotoxicity assays and report diverging views and conclusions on the consequences of this genotoxicity issue for the ultimate safety assessment of chromium (III). The Panel is aware that given this situation, the safety of chromium (III) might need to be re-evaluated in light of these recent reviews and evaluations.

Nicotinate

The safety of nicotinate (nicotinic acid) has been evaluated by various authorities including the SCF (2002) and the EVM (2003).

The SCF established a UL for nicotinic acid of 10 mg/day, based on the available data indicating occasional flushing, the limiting adverse effect, at 30 mg per day. An uncertainty factor (UF) of 3 was applied to allow for the fact that a slight effect was reported, and that the study was performed in a small number of subjects, but taking into account the steep dose-response relationship (SCF, 2002).

The EVM has not established a UL for nicotinic acid, due to insufficient data. For guidance purposes, based on a LOAEL of 50 mg/day due to the absence of a NOAEL, an uncertainty factor of 3 is used. Thus, a dose of 17 mg/day, for supplementation only, would not be
expected to have any significant adverse effects. The dose is equivalent to 0.28 mg/kg bw/day in a 60 kg adult.

**Chromium nicotinate**

There have been an extensive number of studies in humans and animals to evaluate chromium polynicotinates with no reports of toxicity or adverse effects. However these studies were not designed to investigate the safety of chromium nicotinate.

The petitioner indicates that use levels foreseen will provide 200 μg chromium (which can be calculated to be equivalent to 2 mg chromium nicotinate) daily.

A dose of 2 mg nicotinate would provide 200 μg trivalent chromium/day and 1.1 mg nicotinate /day. This amount of chromium would be below the level of 250 μg chromium/day considered by the WHO as a value for supplementation that should not be exceeded. The dose of 1.1 mg nicotinate/day would amount to 11% of the UL for nicotinate established by the SCF (2002).

The Panel notes that the simultaneous use of nicotinate as a source of chromium (III) in both foods intended for particular uses and in food supplements, both at use levels up to 200 μg/day could amount to use levels of 400 μg/day. This would be equivalent to 4 mg chromium nicotinate daily, containing 2.2 mg nicotinate/day. This amount of chromium would be above the level of 250 μg chromium/day considered by the WHO as a value for supplementation that should not be exceeded. The dose of 2.2 mg nicotinate/day would amount to 22% of the UL for nicotinate established by the SCF (2002).

**CONCLUSIONS**

The present opinion deals only with the safety of chromium nicotinate as a particular source of chromium to be used in foods intended for particular nutritional uses and in food supplements and with the bioavailability of chromium from this source.

The Panel concludes that chromium bioavailability from chromium nicotinate is low and is comparable to the bioavailability of trivalent chromium from other sources.

The Panel notes that the simultaneous use of nicotinate as a source of chromium (III) in both foods intended for particular uses and in food supplements, both at use levels up to 200 μg/day could amount to use levels of 400 μg/day. This would be equivalent to 4 mg chromium nicotinate daily, containing 2.2 mg nicotinate/day. This amount of chromium would be above the level of 250 μg chromium/day considered by the WHO as a value for supplementation that should not be exceeded. The dose of 2.2 mg nicotinate/day would amount to 22% of the UL for nicotinate established by the SCF (2002). Although the amount of nicotinate that would be consumed as a result of these proposed uses would be safe, the Panel could not conclude that these uses of chromium (III) nicotinate are of no safety concern.

The Panel notes that recent reviews and evaluations of chromium (III)( Eastmond *et al.*, 2008; Levina and Lay, 2008) point at conflicting outcomes of genotoxicity assays and report diverging views and conclusions on the consequences of this genotoxicity issue for the ultimate safety assessment of chromium (III). The Panel is aware that given this situation, the safety of chromium (III) might need to be re-evaluated in light of the recent evaluations and reviews.
Mixture of chromium di- and tri-nicotinate as a source of chromium

DOCUMENTATION PROVIDED TO EFSA

REFERENCES


Mixture of chromium di- and tri-nicotinate as a source of chromium


Mixture of chromium di- and tri-nicotinate as a source of chromium


SCF, (Scientific Committee on Food), 2003. Opinion of the Scientific Committee on Food on the Tolerable Upper Intake Level of Trivalent Chromium.


## Glossary / Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADI</td>
<td>Acceptable Daily Intake</td>
</tr>
<tr>
<td>AI</td>
<td>Adequate Intakes</td>
</tr>
<tr>
<td>ANS</td>
<td>Panel on Food Additives and Nutrient Sources added to Foods</td>
</tr>
<tr>
<td>bw</td>
<td>body weight</td>
</tr>
<tr>
<td>CAS</td>
<td>Chemical Abstracts Service</td>
</tr>
<tr>
<td>CFU</td>
<td>Colony Forming Units</td>
</tr>
<tr>
<td>EC</td>
<td>European Commission</td>
</tr>
<tr>
<td>EFSA</td>
<td>European Food Safety Authority</td>
</tr>
<tr>
<td>ESADDI</td>
<td>Estimated Safe and Adequate Daily Dietary Intake</td>
</tr>
<tr>
<td>EVM</td>
<td>Expert group on Vitamins and Minerals</td>
</tr>
<tr>
<td>FDA</td>
<td>U.S. Food and Drug Administration</td>
</tr>
<tr>
<td>GI</td>
<td>Gastrointestinal</td>
</tr>
<tr>
<td>GRAS</td>
<td>Generally Recognised As Safe</td>
</tr>
<tr>
<td>GSE</td>
<td>Grape Seed Extract</td>
</tr>
<tr>
<td>HDL</td>
<td>High-Density Lipoprotein</td>
</tr>
<tr>
<td>HPLC</td>
<td>High Performance Liquid Chromatography</td>
</tr>
<tr>
<td>ICP</td>
<td>Inductively Coupled Plasma spectroscopy</td>
</tr>
<tr>
<td>LD₅₀</td>
<td>Lethal Dose, 50%</td>
</tr>
<tr>
<td>LDL</td>
<td>Low-Density Lipoprotein</td>
</tr>
<tr>
<td>LOAEL</td>
<td>Lowest Observed Adverse Effect Level</td>
</tr>
<tr>
<td>NHPD</td>
<td>Natural Health Products Directorate</td>
</tr>
<tr>
<td>NOAEL</td>
<td>No Observable Adverse Effect Level</td>
</tr>
<tr>
<td>PARNUTS</td>
<td>Foods for Particular Nutritional Uses</td>
</tr>
<tr>
<td>RDI</td>
<td>Recommended Daily Intake</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive Oxygen Species</td>
</tr>
<tr>
<td>SBP</td>
<td>Systolic Blood Pressure</td>
</tr>
<tr>
<td>SCF</td>
<td>Scientific Committee on Food</td>
</tr>
<tr>
<td>TBARS</td>
<td>Thiobarbituric Acid Reactive Substances</td>
</tr>
<tr>
<td>TDS</td>
<td>Total Diet Study</td>
</tr>
<tr>
<td>UF</td>
<td>Uncertainty Factor</td>
</tr>
<tr>
<td>UL</td>
<td>Tolerable Upper Intake Level</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organisation</td>
</tr>
</tbody>
</table>