SCIENTIFIC OPINION

Chromium picolinate, zinc picolinate and zinc picolinate dihydrate added for nutritional purposes in food supplements

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


Adopted on 4 June 2009

PANEL MEMBERS


SUMMARY

Following a request from the European Commission to the European Food Safety Authority, the Scientific Panel on Additives and Nutrient Sources added to Food was asked to provide a scientific opinion on the safety of chromium picolinate (anhydrous), zinc picolinate and zinc picolinate dihydrate added for nutritional purposes as sources of chromium and zinc in food supplements, and on the bioavailability of chromium and zinc from these sources.

The present opinion deals with the bioavailability of chromium from chromium picolinate, and of zinc from zinc picolinate and zinc picolinate dihydrate, respectively, and with the safety of these sources. The safety of chromium and zinc themselves, in terms of amounts that may be consumed, is outside the remit of this Panel.

Zinc picolinate and chromium picolinate are proposed to be used as sources of zinc and chromium, respectively, in food supplements. The majority of studies reveal a slightly greater bioavailability of zinc from zinc picolinate than from other zinc compounds, although some studies showed similar levels of absorption, and a few investigators claim a somewhat lower absorption of zinc from zinc picolinate than from other zinc compounds. Based on studies on chromium intervention, it appears that chromium from chromium picolinate is also equally or

1 For citation purposes: Scientific Opinion of the Panel on Food Additives and Nutrient Sources added to Food on chromium picolinate, zinc picolinate and zinc picolinate dihydrate added for nutritional purposes in food supplements following a request from the European Commission. The EFSA Journal (2009) 1113, 1-41.

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Chromium picolinate, zinc picolinate and zinc picolinate dihydrate added for nutritional purposes in food supplements

slightly better absorbed than chromium from other chromium compounds, although direct studies on the bioavailability are limited and generally bioavailability of inorganic chromium(III) from food sources and food supplements is very low (0.1-2%).

The Panel concludes that chromium from chromium picolinate, and zinc from zinc picolinate and from zinc picolinate dihydrate are bioavailable.

Data on the toxicity of zinc picolinate are limited. The toxicity data on chromium picolinate and picolinic acid are therefore also used for the risk assessment of zinc picolinate.

Chromium picolinate (monohydrate) did not produce significant indications of toxicity in 13-week studies reported by the National Toxicology Program (NTP) in 2008 in rats and mice at dose levels up to 50,000 mg/kg diet, equivalent to 4 240 mg/kg body weight/day. This No-Observed-Adverse Effect Level (NOAEL) corresponds to 3 616 mg picolinic acid/kg bw/day. This could indicate that picolinic acid is of low toxicity.

Based on the available data on reproductive and developmental toxicity, the Panel concluded that at present there are no indications that chromium picolinate or picolinic acid (at dose levels up to 200 mg chromium picolinate or 174 mg picolinic acid/kg bw/day) cause developmental effects in mice.

From the long-term carcinogenicity studies performed by NTP in 2008 in mice and rats, it was concluded that under the conditions of these 2-year feeding studies there was equivocal evidence of carcinogenic activity of chromium picolinate in male rats based on an increase in the incidence of preputial gland adenomas. It was also concluded that there was no evidence of carcinogenic activity due to chromium picolinate in female rats or in male or female mice. Preputial gland adenomas in rats are rather common in the strain of rats used. The incidences of these adenomas were not dose-related, and did not occur consistently across species and sexes (female rats have similar/corresponding tissue). Therefore, the Panel concluded that this lesion is not of toxicological relevance and that a (NOAEL) could be established. The NOAEL in the rat study was 2400 mg chromium picolinate/kg bw/day, which was equal to 2100 mg picolinic acid/kg bw/day.

Based on the data available, a final conclusion on the genotoxic potential of picolinic acid could not be drawn. The Panel noted however that in the long term NTP study with chromium picolinate no treatment related malignant lesions were observed.

According to the exposure assessment, at the 97.5th percentile the total potential zinc exposure (from food and anticipated exposure from zinc picolinate at the highest proposed level) is 70.5 mg/day for adults and 65.9 mg/day for children (3-17 years), which is above the Tolerable Upper Intake Level (UL) for zinc.

If all zinc would be applied up to the UL for zinc (25 mg/day), as established by the Scientific Committee on Food (SCF), by zinc picolinate, the potential exposure to picolinate would correspond to approximately 94 mg picolinate/day or 1.57 mg picolinic acid/kg bw/day for a 60 kg person. The Panel considers an exposure estimate based on the UL as a conservative estimate. If the NOAEL derived from the long-term NTP of 2 100 mg picolinic acid/ kg bw/day is taken there would be a Margin of Safety of approximately 1 340.

The exposure assessment revealed that at the 97.5th percentile the total exposure to chromium from food intake and from supplements at the highest proposed use level (600 µg chromium/day), would be 668-770 µg/day for adults and 726 µg/day (taken the adult dose) for children (3-17 years). If this dose was to be provided completely by chromium picolinate, this would lead to an exposure to picolinate of up to approximately 4.3 mg picolinate/day. However, the World Health Organization in 1996 considered that supplementation of
Chromium should not exceed 250 μg/day. If a dose of 250 μg chromium/day would be provided as chromium picolinate, the potential exposure would correspond to approximately 1.8 mg picolinate/day or 30 μg picolinate/kg bw/day for a 60 kg person.

This leads to a combined estimated conservative exposure to picolinate from the proposed uses of zinc and chromium picolinate of 93.8 mg picolinate/day plus 1.8 mg picolinate/day, resulting in approximately 96 mg picolinate/day which amounts to 1.6 mg picolinate/kg bw/day for a 60 kg person. Based on the NOAEL derived from the long-term NTP study of 2 100 mg picolinic acid/kg bw/day there would be a Margin of Safety of approximately 1 310.

The Panel also noted that, although the safety of zinc itself, in terms of amounts that may be consumed, is outside the remit of this Panel, at the 97.5th percentile the dietary zinc intake from total food only is already close to the UL, as established by the SCF.

Based on the available, albeit limited toxicological database, the Panel concluded that at the anticipated use and use levels of zinc picolinate and zinc picolinate dihydrate, as a source of zinc, when added for nutritional purposes in food supplements, are not of safety concern as long as the UL for zinc is not exceeded.

The Panel also concluded that the use of chromium (III) picolinate, as a source of chromium, is of no safety concern provided that the amount of supplemental chromium does not exceed the level of 250 μg/day, the value set by the World Health Organization.

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 these recent reviews and evaluations.

Key words:
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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

The European Community Legislation lists nutritional substances that may be used for nutritional purposes in certain categories of foods as sources of certain nutrients. The Commission has received requests for the evaluation of zinc and chromium picolinate added for nutritional purposes to food supplements. The relevant Community Legislative Directives are:


TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

In accordance with article 29 (1) (a) of Regulation (EC) No 178/2002, the European Commission asks the European Food Safety Authority to provide scientific opinions based on its consideration of the safety and bioavailability of zinc or chromium picolinate for nutritional purposes in food supplements.

ACKNOWLEDGEMENTS

ASSESSMENT

1. Introduction

The present opinion deals only with the safety and bioavailability of chromium picolinate, zinc picolinate and zinc picolinate dihydrate, as particular sources of chromium and zinc, intended to be used in food supplements. The safety of chromium and zinc themselves, in terms of amounts that may be consumed, is outside the remit of this Panel.

2. Technical data

2.1. Chemistry

Zinc picolinate and zinc picolinate dihydrate

Zinc picolinate is slightly soluble in water. According to IUPAC nomenclature, the chemical name is zinc dipicolinate or zinc picolinate. The chemical formula for the anhydrous form is C_{12}H_{8}N_{2}O_{4}Zn, and for the dihydrate is C_{12}H_{8}N_{2}O_{4}Zn.2H_{2}O. The CAS Registry Number for the anhydrous form is 17949-65-4, and the molecular weight of zinc picolinate is 309.59 g/mol (and for its dihydrate 345.58 g/mol). Picolinate in zinc picolinate is 2-picolinate.

![Structure of zinc picolinate](image)

Figure 1. Structure of zinc picolinate

One gram of zinc picolinate provides 0.21 gram zinc and 0.79 gram picolinic acid.

Chromium picolinate

Chromium picolinate is slightly soluble in water (100 \( \mu \)g/mL). According to IUPAC nomenclature, the chemical name is chromium tripicolinate. The chemical formula is C_{18}H_{12}N_{3}O_{6}Cr. The CAS Registry Number for chromium picolinate is 14639-25-9, and the molecular weight is 418.31 g/mol. Chromium picolinate is a stable complex of trivalent chromium Cr(III). Picolinate in chromium picolinate is 2-picolinate.
Chromium picolinate, zinc picolinate and zinc picolinate dihydrate added for nutritional purposes in food supplements

Figure 2. **Structure of chromium picolinate**

One gram of chromium picolinate provides 0.12 gram chromium and 0.88 gram picolinic acid.

### 2.2. Specifications

**Zinc Picolinate**

The food-grade specifications provided by the petitioner for anhydrous zinc picolinate are listed below:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zinc</td>
<td>200-210 mg Zn/g</td>
</tr>
<tr>
<td>Loss on drying (moisture)</td>
<td>&lt; 2.50 %</td>
</tr>
<tr>
<td>Chloride</td>
<td>≤ 0.060 %</td>
</tr>
<tr>
<td>Sulphate</td>
<td>≤ 0.20 %</td>
</tr>
<tr>
<td>Appearance</td>
<td>white free-flowing powder</td>
</tr>
<tr>
<td>Heavy metals (as lead)</td>
<td>≤ 10 mg/kg</td>
</tr>
<tr>
<td>Powder fineness</td>
<td>90 % through 60 mesh</td>
</tr>
</tbody>
</table>

Microbiological specifications were also provided by the petitioners.

One petitioner for zinc picolinate indicated that compliance with the food-grade specification was demonstrated by analyses of five lots or batches of zinc picolinate.
Chromium picolinate

The food-grade specifications proposed by the petitioner for anhydrous chromium picolinate are listed below:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromium</td>
<td>121.8 to 126.8 mg/g</td>
</tr>
<tr>
<td>Loss on drying (moisture)</td>
<td>&lt; 4.5 %</td>
</tr>
<tr>
<td>Powder fineness</td>
<td>99 % through 80 mesh</td>
</tr>
<tr>
<td>Chloride</td>
<td>≤ 0.060 %</td>
</tr>
<tr>
<td>Sulphate</td>
<td>≤ 0.20 %</td>
</tr>
<tr>
<td>Appearance</td>
<td>white free-flowing powder</td>
</tr>
<tr>
<td>Heavy metals (as lead)</td>
<td>≤ 2-10 mg/kg</td>
</tr>
</tbody>
</table>

Microbiological specifications were also provided by the petitioners.

The appliance with the food-grade specifications was demonstrated by analyses of five lots of batches of chromium picolinate. The Panel notes that according to Commission Regulation (EC) No 629/2008 the maximum levels of lead, mercury and cadmium, in food supplements as sold, should be 3.0 mg/kg, 0.1 mg/kg and 1.0 mg/kg, respectively.

2.3. Manufacturing process

Zinc picolinate is reported by one petitioner to be synthesised by adding a picolinate source to a supersaturated solution of zinc oxide in warm deionised water, followed by stirring with gentle heating until crystals begin to form. The solution is then cooled. The supernatant is discarded and the zinc picolinate crystals are dried, milled and packaged. The procedure applied by another petitioner was claimed to be confidential, but the information on the manufacturing process was available to the Panel.

The manufacturing process for chromium picolinate was claimed to be confidential but the information on the manufacturing process was available to the Panel. The starting chromium product is controlled for the presence of chromium(VI) and for other contaminants. No chromium(VI) was detected. The other contaminants were in accordance with the specifications of the product.

2.4. Methods of analysis in food

The proposed analytical methodology for the quantification of total zinc in foods was adapted from the Official Association of Analytical Chemists (AOAC) methods (AOAC, 2000).

The proposed analytical method for the quantification of total chromium in food, resulting from the proposed uses of chromium picolinate, is the AOAC Official Method 990.08 (Inductively Coupled Plasma - Atomic Emission Spectrometric method). Liquid

2 According the US Pharmacopoeia
Chromium picolinate, zinc picolinate and zinc picolinate dihydrate added for nutritional purposes in food supplements

Chromatography - Mass Spectrometry (LC-MS) and High Performance Liquid Chromatography (HPLC) were also applied.

No information was given on the analysis of picolinate in food.

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

Stability studies for zinc picolinate were performed using the above mentioned analytical methods, and thus quantifying only the level of zinc and not the stability of zinc picolinate. Stability studies for the chromium picolinate were performed using the above mentioned analytical methods, and thus quantifying only the level of chromium and not the stability of chromium picolinate.

2.6. Case of need and proposed uses

Zinc picolinate and chromium picolinate are to be used as sources of zinc and chromium in food supplements.

One petitioner indicated that according to the EVM UK Expert Committee on Vitamins and Minerals (EVM) (EVM, 2003), supplements provide up to 50 mg zinc/day. The other petitioner proposed a supplement intake of 53 mg zinc picolinate dihydrate/day, which would equate to 10 mg zinc, 37.5 mg picolinate and 5.5 mg water.

One petitioner discussed the use of chromium picolinate in multi-vitamin and multi-mineral food supplements, and in products for chromium supplementation without further specification. According to this petitioner, typical amounts of chromium picolinate used in food supplements ranged from 150-1000 μg/day, which corresponds to 20-120 μg chromium/day and approximately 128-856 μg picolinic acid/day.

2.7. Existing authorisations and evaluations

Zinc and chromium have been evaluated by several authorities in Europe and the USA.

**Zinc**

The European Population Reference Intake (PRI) for zinc for adult males and females, is 9.5 mg/day and 7.0 mg/day, respectively (SCF, 1993). In the US, recent guidelines recommend daily intakes of 11 mg/day and 8 mg/day for adult men and women respectively (IOM, 2001).

The Scientific Committee on Food (SCF) has established a Tolerable Upper Intake Level (UL) for zinc of 25 mg/day for adults including pregnant and lactating women, and the UL for children and juveniles ranged from 7 mg/day (1-3 years old) to 22 mg/day (for 15-17 years old) (SCF, 2003a). The Panel noted that this UL is related to the total zinc intake.

The EVM established a safe upper level of 25 mg/day for supplemental zinc (EVM, 2003), whereas the IOM’s Tolerable Upper Intake Level of 40 mg/day applied to dietary and supplemental zinc intake (IOM, 2001).

Other sources of zinc, such as zinc salts of acetate, chloride, citrate, gluconate, lactate, oxide, carbonate and sulphate, have already been approved for the use in the manufacture of food.

Chromium

For chromium, the SCF stated that since data on essentiality and metabolism of chromium were sparse, the Committee was not able to specify any requirements (SCF, 1993). The SCF stated also that the evaluation was not applicable to chromium picolinate.

The UK Committee on Medical Aspects of Food Policy (COMA) calculated a theoretical requirement for adults from balance studies of 23 µg chromium/day by using regression equations, and concluded that a safe and adequate level of intake lies above 25 µg chromium for adults and between 0.1 and 1.0 µg chromium/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 chromium/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).

In 2003, the SCF was not able to derive an UL for chromium because available human data and the data from studies on subchronic, chronic and reproductive toxicity in experimental animals of soluble trivalent chromium salts did not provide clear information on the dose response relationships (SCF, 2003b). Also the US Food and Nutrition Board concluded that the data from animal and human studies were insufficient to establish an UL for soluble chromium (III) salts (IOM, 2001). The EVM also 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) would be expected to be without adverse health effects (EVM, 2003).

The World Health Organisation (WHO) considered that supplementation of chromium should not exceed the level of 250 µg/day (WHO, 1996).

One petitioner proposed to use chromium picolinate in food supplements in dosed forms, as regulated and labelled in accordance with their respective legislation, and also indicated that according to the EVM (2003) supplements provide up to 600 µg chromium/day.

Picolinate

In 1999, the SCF concluded that an evaluation of the acceptability of chromium picolinate as a nutrient source of chromium in PARNUTs was not possible unless data on bioavailability in humans were provided (SCF, 1999).

In Germany, all special permissions for the use of chromium picolinate in food supplements were withdrawn in 2001 due to recent investigations which do not exclude adverse effects on human health (BMVEL, 2001; BgVV, 2002).

Previously, the Food Standard Agency (FSA) advised people not to take chromium picolinate supplements, following the 2003 report of the EVM (EVM, 2003). However, the Panel noted that following a review of new research in 2004 by the Committee on Mutagenicity (COM), the Agency has decided that it was no longer necessary to advise people to avoid chromium picolinate (COM, 2004).
In the USA, chromium picolinate matching the present specifications has been affirmed as Generally Recognised as Safe (GRAS) as a nutrient supplement in food, for use in functional foods and beverages at a daily intake of up to 600 µg of trivalent chromium.

### 2.8. Exposure

**Zinc from zinc picolinate and diet**

Zinc is widely distributed in foods. Good food sources of zinc include red meat, whole wheat, raisins, unrefined cereals (high zinc content, low availability), whereas milk, fruit and vegetables are low in zinc (Sandstead and Smith Jr, 1996).

In adults, the daily average zinc intake in European adults is usually between 7.5 and 12.1 mg/person/day, and the 97.5th percentile of zinc intake varies from 15 to 20.5 mg/person/day (SCF, 2003b; Leblanc et al, 2005). For children, the daily average zinc intake in France varies from 7.7 to 9 mg/person/day, and the 97.5th percentile from 12.5 to 15.9 mg/person/day (AFSSA, 2009).

One petitioner proposed a supplement intake of 53 mg zinc picolinate dihydrate/day, which would equate to 10 mg zinc, 37.5 mg picolinate and 5.5 mg water. Another petitioner proposes level of up to 50 mg zinc/day. The Panel calculated an anticipated level of exposure to zinc picolinate based on the provision of 50 mg zinc /day. This would lead to a potential dietary exposure to 187.7 mg picolinate/day.

For adults, the total exposure to zinc from food intake and from supplements at the highest proposed use level would be 58-62 mg/day at the mean and 65-71 mg/day at the 97.5th percentile. For children of 3-14 years, the total exposure to zinc from food intake and from supplements at the highest proposed use level would be 58-59 mg/day at the mean and 63-66 mg/day at the 97.5 percentile assuming they are taking the adult dose (see Table 1).

### Table 1. Summary information on zinc intake and anticipated exposure to zinc from zinc picolinate

<table>
<thead>
<tr>
<th>Nutrient: Zinc</th>
<th>Amount (mg/day)</th>
<th>High intake (95th or 97.5th) (mg/day)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Population reference intake / adults</td>
<td>9.5 (males)</td>
<td>7 (females)</td>
<td>SCF, 1993</td>
</tr>
<tr>
<td>Tolerable Upper Intake Level / adults, including pregnant and lactating women</td>
<td>25</td>
<td>25</td>
<td>SCF, 2003b</td>
</tr>
<tr>
<td>Tolerable Upper Intake Level / children (1-3 years, 4-6 years, 7-10 years, 11-14 years, 15-17 years)</td>
<td>7, 10, 13, 18, 22</td>
<td>7, 20, 25, 28, 32</td>
<td>SCF, 2003b</td>
</tr>
<tr>
<td>Intake range from food in Europe for adults</td>
<td>7.5-12.1 (average)</td>
<td>15-20.5</td>
<td>SCF, 2003b; Leblanc et al, 2005</td>
</tr>
<tr>
<td>Intake range from food in Europe for children (3-17 years)</td>
<td>7.7-9 (average)</td>
<td>12.5-15.9</td>
<td>AFSSA, 2009</td>
</tr>
<tr>
<td>Highest amount (range) of zinc to be used in food supplements</td>
<td>50 (10-50)</td>
<td></td>
<td>Technical dossier</td>
</tr>
</tbody>
</table>

Source: Zinc picolinate

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Total anticipated exposure to zinc from supplement and food intake\(^1\) for adults.

<table>
<thead>
<tr>
<th>Amount (µg/day)</th>
<th>High intake (95th or 97.5th) (µg/day)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>57.5-62.1</td>
<td>65-70.5</td>
<td>Calculation by Panel</td>
</tr>
</tbody>
</table>

Total anticipated exposure to zinc from supplement and food intake\(^2\) for children (3-17 years).

<table>
<thead>
<tr>
<th>Amount (µg/day)</th>
<th>High intake (95th or 97.5th) (µg/day)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>57.7-59</td>
<td>62.5-65.9</td>
<td>Calculation by Panel</td>
</tr>
</tbody>
</table>

\(^1\) Calculation based on proposed use level of 50 mg/day plus average dietary intake of 7.5-12.1 mg/day and high dietary intake of 15-20.5 mg/day for adults

\(^2\) Calculation based on proposed use level of 50 mg/day plus average dietary intake of 7.7-9 mg/day and high dietary intake of 12.5-15.9 mg/day for children

**Chromium from chromium picolinate and diet**

Trivalent chromium occurs naturally in the food supply. The chromium content of foods is included in only few food composition databases. Most foods contain very little chromium (under 1 µg/kg) while some foods have higher contents, e.g. tea leaves (33 µg /kg), cacao powder (17 µg /kg) and smoked pork, mussels and coffee (2-3 µg /kg). The exposure to chromium is available from total diets or duplicate portion techniques. The Total Diet Study (TDS) in the UK in 1997 reported that the mean chromium exposure of adults was 100 µg/day and 170 µg/day at the 97.5th percentile (Ysart et al., 2000), while the French TDS of 2001 indicated a mean exposure of 77 µg/day for adults > 15 years, and 68 µg/day for children of 3-14 years, and at the 97.5th percentile, of 126 µg/day for adults and 124 µg/day for children (Leblanc et al., 2005). In duplicate diets in Germany, Sweden and Spain, mean intakes of adults varied from 53 to 160 µg/day (SCF, 2003b; Van Cauwenbergh et al., 1996). The higher end of the range of duplicate studies might not necessarily be representative of the population mean but rather represent a high percentile intake.

One petitioner proposed to use chromium picolinate in food supplements in dosed forms, as regulated and labelled in accordance with their respective legislation, and also indicated that according to the EVM (2003) supplements provide up to 600 µg chromium/day. This would correspond to a potential dietary exposure of about 4300 µg picolinic acid/day assuming that all chromium supplements are based on chromium picolinate. The other petitioner stated that typical amounts of chromium picolinate used in food supplements range from 150-1000 µg/day. This would correspond to a potential dietary exposure to 20-120 µg chromium/day and approximately 128-856 µg picolinic acid/day.

Table 2  **Summary information on chromium intake and anticipated exposure to chromium from chromium picolinate**

<table>
<thead>
<tr>
<th>Nutrient: Chromium</th>
<th>Amount (µg/day)</th>
<th>High intake (95th or 97.5th) (µg/day)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tolerable Upper Intake Level</td>
<td>61 to 160 (average)</td>
<td>68-170</td>
<td>SCF, 2003b, Ysart et al., 2000, Leblanc et al., 2005</td>
</tr>
<tr>
<td>Intake range from food in Europe for adults</td>
<td>68 (average)</td>
<td>126</td>
<td>Leblanc et al., 2005</td>
</tr>
<tr>
<td>Intake range from food in Europe for children (3-14 years)</td>
<td>600 (20-600)</td>
<td>Technical dossier EVM 2003</td>
<td></td>
</tr>
</tbody>
</table>

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The total exposure to chromium from food intake and from supplements at the highest proposed use level of adults would be 661-760 µg/day at the mean, and 668-770 µg/day at the 97.5th percentile. For children of 3-14 years, the total exposure to chromium from food intake and from supplements at the highest proposed use level would be 668 µg/day at the mean, and 726 µg/day at the 97.5th percentile, assuming they are taking the adult dose (see Table 2).

**Picolinate from zinc and chromium picolinate and from the diet**

Picolinic acid, a pyridine compound structurally similar to nicotinic acid, is not typically found in foods. However, it is a metabolite of tryptophan and is synthesized *in vivo* via the kynurenine pathway, thus occurring naturally in the body (Reading and Wecker, 1996). The estimated urinary output of picolinate by adults is 14 mg/day (Evans, 1993).

**Table 3** Summary information on picolinate intake and anticipated cumulative exposure to picolinate from chromium and zinc picolinate

<table>
<thead>
<tr>
<th>Source: Picolinate</th>
<th>Amount (mg/day)</th>
<th>High intake (95th or 97.5th) (µg/day)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Population Reference Intake / adults</td>
<td>-</td>
<td>-</td>
<td>Technical dossier</td>
</tr>
<tr>
<td>Tolerable Upper Intake Level</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Intake range from food in Europe for adults</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Intake range from food in Europe for children (3-14 years)</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Highest amount (range) of picolinate from zinc picolinate as indicated by the petitioner</td>
<td>187.7 (37.5-187.6)</td>
<td></td>
<td>Technical dossier</td>
</tr>
<tr>
<td>Highest amount (range) of picolinate from chromium picolinate as indicated by the petitioner</td>
<td>4.3 (0.1-4.3)</td>
<td></td>
<td>Technical dossier</td>
</tr>
<tr>
<td>Total anticipated exposure to picolinate from supplement and food intake for adults</td>
<td>191.9 (37.6-191.9)</td>
<td></td>
<td>Calculation by Panel</td>
</tr>
</tbody>
</table>

The total anticipated cumulative exposure to picolinate from food intake and supplements for adults or children from a daily consumption of zinc picolinate and chromium picolinate at the highest proposed range use levels, as indicated by petitioners, would be approximately 192 mg/day ranging from 37.6 to 192 mg picolinate/day depending on the dose in the supplements.
3. Biological and toxicological data

3.1. Bioavailability, absorption, distribution, metabolism and excretion

Few toxicokinetic studies are available with zinc picolinate, chromium picolinate or picolinic acid.

Picolinic acid is an endogenous metabolite of tryptophan and has a high-affinity metal-binding capacity (IOM, 2001). Cations readily complex with picolinate in the small intestine. The pancreas secretes picolinic acid into the small intestine, with increasing levels being excreted with increasing levels of pyridoxine supplementation (Evans and Johnson, 1981; Evans, 1983). Previous studies have identified picolinic acid in pancreas preparations used as digestive aids in pancreatic insufficiency (PDR, 2004). Picolinic acid has a high metal binding affinity (FNB, 2001), and like citric acid, has been reported to be a zinc-binding ligand influencing intestinal zinc absorption (Evans, 1980; Evans and Johnson, 1980b; Evans and Johnson, 1981; Hurley and Lönnerdal, 1980; Seal and Heaton, 1985; Seal, 1988; Roth and Kirchgessner, 1983; Johnson et al., 1988; Johnson and Evans, 1982; Evans, 1992). It has been stated that at a low pH in the stomach (pH 1-2), 95-100% of the ingested amount of zinc (di)picolinate would still be complexed, either as zinc monopicolinate or zinc (di-)picolinate. For chromium picolinate, it has been suggested that it is absorbed intact from the gastrointestinal tract and once it reaches the cell, the metal ion may dissociate from the picolinate moiety, leaving the picolinate ion systemically available (Berner et al., 2004).

**Zinc picolinate**

The bioavailability of zinc in the diet is dependent upon a number of factors. Dietary zinc is readily available for absorption at gastric pH (i.e. low pH). At higher pH values zinc tends to bind to organic components such as low molecular weight ligands (e.g. amino acids or some organic acids) that may potentially increase the solubility of zinc and facilitate its absorption, or alternatively zinc may form poorly soluble complexes with organic substances that may reduce absorption (EVM, 2003).

The bioavailability of zinc is affected by the availability of picolinic acid, as a metal chelating agent, to bind to zinc in the small intestine aiding the absorption of metal cations (e.g. zinc, copper, iron) (IOM, 2001). Mechanisms of zinc absorption mediated by endogenous picolinic acid have been proposed. During the metabolism of tryptophan in the exocrine cells of the pancreas, picolinic acid is produced and excreted into the lumen of the intestine. In the lumen, picolinic acid forms a complex with zinc that facilitates the passage of zinc through the luminal membrane, across the absorptive cell and through the basolateral membrane of the cell. Alternatively, at the basolateral membrane, receptor ligands may coordinate zinc which is then transferred to transferrin. In humans and animals consuming a diet that contains physiological levels of zinc, the quantity of zinc transported across the absorptive cells is directly related to the availability of picolinic acid. The availability of picolinic acid depends upon the level of dietary tryptophan, pyridoxine and cations that compete with zinc for coordination with picolinic acid. Inborn errors of metabolism that affect the conversion of tryptophan to picolinic acid also cause impaired zinc absorption (Evans, 1980).

Various studies reported a somewhat increased bioavailability of zinc from zinc picolinate than from other zinc sources, reflected in higher serum concentrations and/or urinary excretion (Evans, 1980; Evans and Johnson, 1981; Evans and Johnson, 1985b; Hurley and Lönnerdal, 1980; Johnson et al., 1988; Johnson and Evans, 1982; Roth and Kirchgessner, 1983; Seal, 1988; Seal and Heaton, 1985). However, other investigators reported that...
picolinic acid had no effect on zinc absorption (Flagstad, 1981; Hill et al., 1986; Ivan and Lammand, 1981; Luh and Song, 1988; Oestreicher and Cousins, 1982; Rebello et al., 1982), and in some cases, slightly reduced zinc absorption due to picolinate has also been reported (Hill et al., 1987b,c; Turnbull et al., 1990).

**Human data**

Human clinical and volunteer studies were performed by several investigators (Krieger, 1980; Barrie et al., 1987; Sakai et al., 2002; Johnson et al., 1988; Ljuin, 1998; Canfield et al., 1982). It was generally concluded that zinc from zinc picolinate, was bioavailable and also that it was slightly more bioavailable than zinc from other zinc compounds (Barrie et al., 1987; Ljuin, 1998; Canfield et al., 1982).

**Animal data**

The positive effect of picolinic acid (either alone or in the form of zinc dipicolinate), pyridoxine and tryptophan on the absorption and retention of zinc has been demonstrated in a number of animal studies, predominantly performed in rats (Evans and Johnson, 1980a,b,c,d; 1981, 1982, 1985; Johnson and Canfield, 1986; Johnson and Evans, 1982; Kashiwabara, 1986; Krieger, 1982; Schwarz et al., 1983a,b; Seal, 1988; Seal and Heaton, 1983; 1985; Seal and Uanson, 1985; Seal, 1988; Hill et al., 1986,1987a, Roth and Kirchgesser, 1983,1985; Hill et al., 1987b,c; Turnbull et al., 1990).

**Chromium picolinate**

Trivalent chromium is primarily utilised in the maintenance of carbohydrate and lipid metabolism. Chromium deficiency in humans results in symptoms similar to those observed in non-insulin-dependent diabetes mellitus (NIDDM) or type 2 diabetes, including elevated blood levels of glucose, insulin, cholesterol, triglycerides, and high-density lipoprotein (HDL) cholesterol, and in more severe cases, nerve and brain disorders (Anderson, 1996, 1997a).

Studies on rats found that the ranking of the relative absorption and retaining of chromium was chromium nicotate > chromium picolinate > chromium chloride. The concentration of chromium picolinate in the kidney of rats was found to be higher than that in the other organs (Lamson and Plaza et al., 2002).

Treatment with $^3$H-labelled chromium picolinate resulted in a relative great portion of the label appearing in the faeces and urine (70 to 100 % of the label excreted daily), and chromatographic analysis of the urine revealed that the excreted $^3$H corresponded to a derivative of picolinic acid. As reported by Hepburn and Vincent (2002), although the large majority of the $^3$H was lost in the waste products, examination of the tissues showed the greatest amount to be present in the liver, followed by the epididymal fat, kidneys, testes, blood, heart and spleen. Chromatographic analyses of hepatocyte subcellular fractions could not be performed due to the insufficient quantities of $^3$H present in the organs in the in vivo experiment. Hepburn and Vincent (2002), concluded on both the in vivo and in vitro data that the complex chromium picolinate has a lifetime of less than 1 day in vivo, and that the chromium ions and picolinate derived from it have different metabolic fates. While picolinate was cleared rapidly in the urine and faeces, the chromium ions were largely retained in cells over the 14-day period of investigation.
The Panel noted that the bioavailability of inorganic chromium(III) from food sources and food supplements is generally very low (0.1-2%)(SCF, 2003) and that the bioavailability of chromium from chromium picolinate may be higher because complex formation may influence the chromium bioavailability.

Human data

Contrary to the data on bioavailability on zinc picolinate, most information of the bioavailability of chromium from chromium picolinate is based on indirect evidence. Nevertheless, the data on the bioavailability of zinc can be considered as supportive to the data on bioavailability of chromium.

Several randomised, placebo-controlled clinical trials have demonstrated significant improvements in blood glucose and insulin measurements following consumption of 200 to 1000 µg chromium/day, given as chromium picolinate for periods varying from 4 weeks to 6 months (Anderson et al., 1997a, Anderson, 1998; Evans, 1989; Feng et al., 2002; Ghosh et al., 2002; Houweling et al., 2003; Jovanovich et al., 1999). In contrast, Lee and Reasner (1994) reported no significant improvements in blood glucose parameters among type 2 diabetic subjects who consumed 200 µg chromium/day from chromium picolinate for a period of 4 months. In all studies chromium picolinate was well tolerated and no adverse effects were reported. Similar improvements of blood glucose parameters were observed in type 1 and type 2 diabetics that consumed 200 to 1000 µg chromium/day from chromium picolinate for a period of 10 days to 10 months (1999; Bahadori et al., 1999; Cheng et al., 1999; Morris et al., 2000; Rabinovitz et al., 2004; Ravina et al., 1995a,b).

A number of reviews evaluating studies on the role of chromium supplementation in diabetes have concluded that dietary supplementation with chromium picolinate may improve glucose homeostasis in diabetic subjects, while having no effect in individuals with normal glucose tolerance (Anderson, 1998b; Cefalu and Hu, 2004; Chowdhury et al., 2003; Ryan et al., 2003). Some authors noted that chromium picolinate is more effective than chromium chloride on parameters related to glucose homeostasis.

Clinical studies of chromium picolinate in healthy subjects have demonstrated no adverse effects on glucose tolerance and no chromium picolinate-induced hypoglycaemia was observed (Anderson et al., 1997a; Grant et al., 1997; Pasman et al., 1997; Anderson, 2007, Boyd et al., 1998; Walker et al., 1998; Joseph et al., 1999; Amato et al., 2000; Volpe et al., 2001; Althuis et al., 2002).

Animal data

A cooperative research study involving 353 litters was conducted at three stations to determine the effects of graded levels of supplemental chromium tripicolinate on the reproductive performance of sows and the pre-weaning performance of their pigs. Primiparous and multiparous sows were fed fortified corn-soybean meal diets with supplemental levels of 0, 200, 600 or 1000 µg chromium (as-fed basis)/kg diet. Each station used three of the supplemental chromium levels, with two of those levels being 0 and 200 µg chromium/kg diet. Station effects were observed for sow gestation weight gain, lactation weight change, lactation feed intake, litter size at birth, weaning, and pig weight at birth and weaning. Tissues were obtained from a subset of sows from one station after they had completed three parities on the study. The content of chromium in the adrenal gland (16.4, 20.0, 34.0 and 48.4 µg chromium/kg), kidney (35.8, 56.4, 132.6 and 176.0 µg chromium/kg), and liver (22.8, 37.4, 87.6 and 92.2 µg chromium/kg) was increased linearly (P=0.001 to
Chromium picolinate, zinc picolinate and zinc picolinate dihydrate added for nutritional purposes in food supplements

0.005) by increasing chromium tripicolinate supplementation. Additionally, supplementation of chromium at 1000 µg/kg was not detrimental to sow performance, even when fed continuously for three parities (Lindemann et al., 2004).

Chromium from chromium picolinate is equally or slightly better bioavailable than chromium from other chromium compounds and that zinc from zinc picolinate and from zinc picolinate dihydrate is also bioavailable.

3.2. Toxicological data

3.2.1. Acute toxicity of zinc and chromium picolinate and other picolimates

The intraperitoneal LD$_{50}$ of zinc picolinate determined in 5-week old ICR mice was found to be 89-92 mg/kg bw for males and 92-95 mg/kg bw for females (Kojima et al., 2002). No oral acute toxicity data were available.

3.2.2. Short-term toxicity

Information on the short-term toxicity of zinc picolinate or picolinic acid is not available.

3.2.2.1. Short-term toxicity of chromium picolinate

Male and female Sprague-Dawley rats were divided into 11 groups (5 rats/sex/group) and were administered diets supplemented with one of nine chromium compounds for a period of three weeks (Anderson et al., 1996). One group received chromium picolinate, which provided 5 mg chromium/kg diet (approximately 0.5 mg chromium tripicolinate/kg bw/day). No significant changes in whole body or tissue weights, or in blood chemistry were reported to occur following chromium picolinate supplementation.

Studies in rats also offer supportive evidence for the efficacy of chromium picolinate in treating diabetes. Chromium picolinate was shown to increase insulin sensitivity and prevent or reverse diabetes-induced functional alterations in the kidneys and liver following oral administration of doses in drinking water or food ranging from 0.08 to 100 mg/kg bw/day (0.018 to 12.4 mg Cr/kg bw/day) for periods of 14 to 90 days (Cefalu et al., 2002; Kim et al., 2002, 2004; Shinde and Goyal, 2003). Shinde and Goyal (2003) reported no significant pathological lesions in the liver and kidney following 8 µg/mL chromium picolinate supplementation in drinking water for 6 weeks and also no significant adverse effects were reported in the remainder of the studies (Cefalu et al., 2002; Kim et al., 2002, 2004).

Six groups of Fisher F-344 rats (n=10/sex/group) and B6C3F1 mice (n=10/sex/group) were given 0, 80, 240, 2000, 10,000 or 50,000 mg/kg diet chromium(III) picolinate monohydrate in the diet (equivalent to average daily doses of approximately 0, 7, 20, 160, 800, 4240 mg/kg bw/day for male rats, and 0, 6, 20, 160, 780, 4250 mg/kg bw for female rats; 0, 17, 50, 450, 2300, 11900 mg/kg bw for male mice and 0, 14, 40, 370, 1775 and 9140 mg/kg bw in female mice) for 13 weeks. Chromium(III) picolinate monohydrate had no effect on survival, body weight gain or feed consumption. No differences in haematology parameters, gross or microscopic lesions or organ weights were observed that were due to chromium(III) picolinate monohydrate administration in rats and mice (NTP 2008; Rhodes et al., 2005).
Five groups of Harlan Sprague Dawley rats (n=8/group, 4 weeks of age, gender not specified) were given diets containing 0, 5, 25, 50 or 100 mg of chromium/kg diet as chromium picolinate or chromium chloride; the amounts of feed consumed were not specified; the indication provided by the authors was the following: 15 mg/kg feed/day equivalent to 0.75 mg/kg bw/day as chromium picolinate for 20 weeks. Weights of heart, liver, kidney, spleen, pancreas, testes and epididymal fat pad were not altered by dietary chromium. Haematocrit was not changed. Haematological values, serum glucose, cholesterol, triglycerides blood urea nitrogen and total protein concentrations, as well as the lactate dehydrogenase, alanine aminotransferase and aspartate aminotransferase were not changed compared to control groups after 11, 17 and 24 weeks (sacrifice at 24 weeks). Liver chromium concentrations increased with increasing supplemental chromium picolinate (Anderson et al., 1997b).

3.3. Reproductive and developmental toxicity

Few data are available on chromium picolinate, picolinic acid and zinc picolinate. A cooperative research study involving 353 litters was conducted at three stations to determine the effects of graded levels of supplemental chromium tripicolinate on the reproductive performance of sows and the pre-weaning performance of their pigs. Primiparous and multiparous sows were fed fortified corn-soybean meal diets with supplemental levels of 0, 200, 600 or 1000 µg chromium (as-fed basis)/kg diet. Each station used three of the supplemental chromium levels, with two of those levels being 0 and 200 µg chromium/kg bw). Station effects were observed for sow gestation weight gain, lactation weight change, lactation feed intake, litter size at birth, weaning, and pig weight at birth and weaning. Supplemental chromium increased the number of pigs born alive per litter (9.49, 9.82, 10.92 and 10.07; quadratic, P=0.05) and sow lactation weight change (- 0.2, 0.8, -4.1 and 3.9 kg; linear, P=0.01), but decreased individual birth weight of total pigs born (1.61, 1.576, 1.47 and 1.56 kg; quadratic, P= 0.10). Tissues were obtained from a subset of sows from one station after they had completed three parities on the study. Additionally, supplementation of chromium at 1000 µg/kg diet was not detrimental to sow performance, even when fed continuously for three parities (Lindemann et al., 2004).

From gestation days (GD) 6-17, pregnant CD-1 mice were fed diets containing either 200 mg mg/kg bw/day chromium picolinate or chromium chloride, 174 mg/kg bw/day picolinic acid, or the diet only to determine if these chemicals could cause developmental toxicity (Bailey et al., 2006). A dose level of 200 mg chromium picolinate/kg bw/day would result in a daily dose of 174 mg picolinic acid/kg bw/day. The incidence of the bifurcated cervical arches was significantly increased in foetuses from the chromium picolinate group, as compared to the diet-only group, whereas foetuses in the picolinic acid-treated group had an incidence double that of the control group; however, this increase was not statistically significant. Foetuses in the chromium chloride group did not differ from the controls in any variable amount. No maternal toxicity was observed in any of the treatment groups. Bailey et al. (2006) reported that chromium picolinate-treated dams (200 mg/kg bw/day from GD 6-17) produced a significantly increased incidence of cervical arch defects in the offspring, but this finding was not reproduced in the study of Bailey et al. (2008) due to the high incidence of cervical arch defects in the control offspring group in comparison with historical control data (Bailey et al., 2008a). Bailey et al. (2006) had proposed that possibly picolinic acid or the combination of picolinic acid and chromium was responsible for the increased incidences of cervical arch defects as this was not observed in the offspring of CrCl3-treated dams. They concluded that high maternal oral exposures to chromium picolinate can cause morphological defects in
developing offspring of mice, whereas Bailey et al. (2008a) concluded that maternal exposure to either chromium picolinate and chromium chloride, at dosages employed, did not appear to cause deleterious effects to the developing offspring in mice. The Panel noted that the studies on developmental effects by Bailey et al. (2006; 2008a) were performed in line with the OECD guidelines.

Bailey et al. (2008b) investigated the effects of maternal exposure of chromium picolinate and picolinic acid during gestation and lactation of CD-1 mice on neurological development of the offspring. Mated female mice were fed diets from implantation through weaning that were either untreated or contained chromium picolinate (200 mg/kg bw/day) or picolinic acid (174 mg/kg bw/day). A comprehensive battery of postnatal tests was administered, including a modified Fox battery, straight-channel swim, open-field activity, and odor-discrimination tests. Pups exposed to picolinic acid tended to weigh less than either control or chromium picolinate-exposed pups, although the difference was not significant. The results indicate that there were no significant effects in offspring with regard to neurological development and learning ability from supplementation of the dams, with either chromium picolinate or picolinic acid.

As all, but one, developmental/reproduction studies did not reveal any significant adverse effects, the Panel concluded that at present there are no indications that chromium picolinate or picolinic acid (at dose levels up to 200 mg chromium picolinate or 174 mg picolinic acid/kg bw/day) cause developmental effects in mice.

### 3.4. Chronic toxicity and carcinogenicity

No data are available on zinc picolinate and picolinic acid.

A long-term toxicity and carcinogenicity study (2-year duration) with chromium picolinate was performed by the National Toxicology Program (NTP). Fisher-344 rats (n=50/sex/group) and B6C3F1 mice (n=50/sex/group) with dose levels of 0, 2000, 10,000 or 50,000 mg chromium picolinate/kg feed (equivalent to average daily dose of approximately 0, 90, 460, and 2400 mg/kg bw/day for male rats; 0, 100, 510, 2630 mg/kg bw/day for female rats; 0, 250, 1200, 6565 mg/kg bw/day for male mice and 240, 1200 and 6100 mg/kg bw/day for female mice) (NTP, 2008). The incidence of preputial gland adenoma was increased in male rats exposed to 460 and 2400 mg chromium picolinate/kg bw/day, at incidences of 7/50 and 4/50, respectively, whereas the low dose and the control groups revealed an incidence of 1 out of 50. The increased incidence was not dose related. Female rats did not show any treatment related non-neoplastic lesions. In mice no neoplasms or non-neoplastic lesions were attributed to exposure to chromium picolinate (monohydrate). The NTP concluded that under the conditions of these 2-year feeding studies, there was equivocal evidence of carcinogenic activity of chromium picolinate in male rats based on an increase in the incidence of preputial gland adenomas. There was no evidence of carcinogenic activity due to chromium picolinate in female rats or in male or female mice (NTP, 2008; Stout et al., 2009). Preputial gland adenomas in rats are rather common in the strain of rats used. The incidences of these adenomas were not dose related and did not occur consistent across species, and not across sexes (female rats have similar/corresponding tissue). Therefore the Panel concluded that this lesion is not of toxicological relevance and that a No-Observed-Adverse Effect Level (NOAEL) could be established. The NOAEL in the rat study was 2400 mg chromium picolinate/kg bw/day, which corresponds to 2100 mg picolinic acid/kg bw/day and to 288 mg chromium/kg bw/day.
3.5. Genotoxicity

No data on zinc picolinate were submitted by the petitioner.

Chromium picolinate

The Ames mutation assays with (2-5) strains of *Salmonella typhimurium* conducted with chromium picolinate (anhydrous) revealed negative effects (NTP, 2008; Esber et al., 1997; Juturu and Komorowski, 2003).

Stearns et al. (1995) investigated the potential genotoxicity of chelated chromium(III) picolinate in Chinese hamster ovary (CHO) AA8 cells. These cells were cultured as adherent monolayers and then treated for 24 hours with stock solutions of aqueous chromium picolinate (0.025, 0.050, 0.10, 0.50 and 1 mM chromium(III)picolinate). Chromium picolinate was non-toxic up to 0.50 mM with ≥ 86 % cell viability. Chromium picolinate was dose dependently clastogenic from 50 μM to 1 mM. Higher doses were not investigated because of severe cytotoxicity. Treatment with picolinic acid alone produced ≥ 91 % survival up to a 2 mM concentration, with picolinic acid being more toxic than nicotinic acid above concentrations of 2 mM. Picolinic acid showed dose-dependent chromosome damage up to 2 mM. However, no chromosome damage was observed at or below doses of 1 mM free picolinic acid. The data suggest that the picolinic acid rather than the Cr(III) is active, because chromium chloride and chromium nicotinate were not clastogenic at equivalent doses.

Komorowski et al. (2008) investigated the cytogenetic effects of chromium picolinate in the bone marrow of Sprague-Dawley rats. The study was performed according to OECD guideline 475 (1997) (with a minor deviation concerning the number of cells counted for the determination of the mitotic index). The rats received a single oral dose of 33, 250 or 2000 mg/kg bw chromium picolinate and were sacrificed after 18 or 42 hours. Chromium picolinate did not induce chromosomal aberrations in cells derived from male or female rats at any of the concentrations tested in comparison to the control (Komorowski et al., 2008).

Hininger et al. (2007) used human HaCaT keratinocytes to determine the cytotoxic and genotoxic effects of chromium picolinate and other Cr(III) complexes. Chromium picolinate did not result in any DNA damage in the Comet assay at a concentration of 120 μM which was non-cytotoxic in the MTT-test, while the tail extent was statistically significantly increased in the Comet assay (250 % compared to control) at a concentration of 6 mM which was the LC50 in the MTT-Test (Hininger et al., 2007).

Stearns et al. (2002) investigated the mutagenicity of chromium picolinate in CHO cells, using solutions of chromium picolinate up to 1 mM and of picolinic acid up to 3 mM. Quantification of HPRT mutations was used to determine genotoxicity. Chromium (III) picolinate was found positive for HPRT mutations. The HPRT mutations were increased up to 40-fold compared to control. Picolinic acid was more cytotoxic than the corresponding chromium picolinate complex. A concentration of 3 mM of picolinic acid at which the 48-hour cell viability was 27 % in comparison to the control produced no mutations. Therefore, the Panel concluded that chromium picolinate was positive while picolinic acid tested negative in this assay.

Juturu et al. (2004) performed a chromosomal aberration assay with CHO cells, at concentrations of 96.5, 192.5, 385 or 770 μg/mL chromium picolinate (4 hours in the presence of metabolic activation and 4 and 20 hours in the absence of metabolic activation), revealing negative results. Slesinksi et al. (2004) performed a HPRT assay in CHO cells
exposed to chromium picolinate concentration ranges from 15.6 to 500 µg/mL for 5- and 48-hour periods. This study also showed no mutagenic effects.

Andersson et al. (2007) evaluated the potential genotoxicity of chromium picolinate in *in vitro* and *in vivo* assays. Mice were given a single intraperitoneal injection (up to 3 mg/kg bw) followed by evaluating the frequency of micronucleated polychromatic erythrocytes in peripheral blood and DNA damage in lymphocytes and hepatocytes. The frequency of micronucleated polychromatic erythrocytes (MNPCE) was evaluated after 42 hours in peripheral blood, and DNA damage in lymphocytes and hepatocytes was evaluated after 16 hours. Using the Comet assay, DNA damage was also monitored in extended-term cultures of human lymphocytes and in L5178Y mouse lymphoma cells that had been exposed for 3 hours to 500 µM chromium picolinate under different exposure conditions. A slight but significant chromium picolinate-induced increase in DNA damage (P<0.001) was observed in human lymphocytes, but only when these cells were exposed in the absence of serum. In all other experiments chromium picolinate was found to be without genotoxic effects, both *in vitro* and *in vivo*. The study results suggest that a high concentration of chromium picolinate might cause DNA damage, but only under non-physiological conditions (Andersson et al., 2007).

In the *Drosophila melanogaster* mutagenicity assay, chromium picolinate was given as a component of the standard diet in concentrations of 10.4 to 260 µg/kg, expressed as chromium, equivalent to 84 to 2107 µg/kg chromium picolinate. No significant differences in survival, behaviour or fertility in adult *Drosophila* were found. Larvae exposed to a similar concentration range were reported to undergo developmental delays and decreased pupation success. Chromium tripicolinate at levels of 260 µg chromium/kg food or less, showed in X-linked analysis indications that the supplement greatly enhanced the rate of appearance of lethal mutations and dominant female sterility (Hepburn et al., 2003).

In the standard screenings assays conducted by the NTP, Chromium tripicolinate monohydrate was negative in *Salmonella typhimurium* strain TA 98 or TA 100 or *Escherichia coli* strain WP2uvr/pKM101, when tested with or without exogenous metabolic activation (S9). In an *in vivo* micronucleus assay, male rats were given orally chromium tripicolinate doses of 0, 156, 312, 625, 1250, 2500 mg/kg bw, and mice chromium tripicolinate monohydrate at doses of 0, 80, 240, 2000, 10000, 50000 mg/kg bw for 13 weeks. Chromium tripicolinate (anhydrous) did not produce chromosomal damage as determined by the absence of micronuclei in the *in vivo* rat micronucleus assay. Chromium tripicolinate monohydrate was negative in the male mice. The weak increases in the presence of micronuclei in female mice were considered equivocal findings as the anhydrous form did not show this effect at all (NTP, 2008).

The evaluation of COM (2004) led to the overall conclusion that the balance of the data on the *in vitro* genotoxicity assays suggest that chromium picolinate is negative. Arguments for this conclusion are presented in the discussion section. The Panel notes that recent reviews and evaluations of chromium (III) (Eastwood 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 further noted that there are additional studies on the *in vivo* genotoxicity of chromium (III) compounds (Kirpnick-Sobol et al., 2006; Zhang et al., 2008) which were not covered by these reviews. 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.
Picolinic acid

Bowden et al. (1976) have tested solutions of picolinic acid (up to 500 μg/plate), in both the presence and absence of S9 mix, with five Salmonella typhimurium strains in the Ames mutagenesis assay. This assay produced negative results for picolinic acid. However, the study has limited validity since the determination of the maximum amount of the test substance was not explained and the results were not reported in detail for all concentrations.

Picolinic acid, at a concentration of 2 mM, did not stimulate unscheduled DNA synthesis in UV-irradiated human lymphocytes (Sims et al., 1982).

Stearns et al., (1995) investigated the potential genotoxicity of picolinic acid using CHO AA8 cells. These cells were cultured as adherent monolayers and then treated for 24 hours with 1, 1.5 and 2 mM picolinic acid. The stock solution of picolinic acid has been adjusted to pH 7.3 to 7.4 with NaOH, however, the pH of the cell culture medium during exposure to the test substance has not been reported. Treatment with picolinic acid concentrations up to 2 mM produced survival rates ≥ 91%. Picolinic acid showed dose-dependent chromosomal damage at 1.5 and 2 mM. However, no chromosome damage was observed at or below concentrations of 1 mM free picolinic acid. The Panel noted that it was not clear if gaps were included or excluded from the evaluations. Therefore, the Panel considered that this study is of limited validity.

More recently, Stearns et al. (2002) investigated the mutagenicity of picolinic acid in CHO cells using the following concentrations: 0, 0.375, 0.75, 1.50, 2.25 and 3 mM. Quantification of HPRT mutations was used to determine genotoxicity. For the 3 mM picolinic acid dose, the 48-hour cell viability was 27% in comparison to the control. However, this cytotoxic concentration produced no mutations.

San (2000) performed a mutagenesis assay in mouse lymphoma L5178YTK +/- cells with picolinic acid at concentrations of 150, 500, 1000, 1500, 2000 μg/mL (equivalent to 1, 4, 8, 12, 16 mM) in the presence of exogenous activation from Aroclor-induced rat liver S9, and at concentrations of 750, 1000, 1500, 3000, 5000 µg/mL (equivalent to 6, 8, 12, 24, 40 mM) in the absence of exogenous activation. The study was conducted in compliance with OECD guideline 476 (1997) and according to GLP. While the authors of the study report concluded that picolinic acid produced a positive, dose-dependent response in both the presence and absence of exogenous metabolic activation, the Panel considered the result in the presence of metabolic activation equivocal, because it was accompanied by cytotoxicity (total growth less than 20%).

The Panel noted that the positive findings of the in vitro MLTK assay might also have been due to changes in pH, however, since the pH has not been reported in this study, a final conclusion on the genotoxic potential of picolinic acid could not be drawn based on the data available.

3.6. Human data

Zinc picolinate

No adverse effects were reported in a double-blind, placebo-controlled trial on the efficacy of zinc picolinate in 73 patients with idiopathic zinc deficiency taste disorders (Sakai et al., 2002). Patients in the zinc treatment group (n=37) were provided with 29 mg of zinc picolinate in capsules taken orally three times a day for three months. No improvement or
significant difference in subjective symptoms or whole-mouth taste sensation was reported in zinc supplemented subjects compared to the placebo group. Serum zinc levels were reported to be significantly higher in the zinc picolinate supplement group compared with the control group (Sakai et al., 2002).

Chromium picolinate

There are several case studies, clinical studies and volunteer studies performed with chromium picolinate reporting different kind of effects, but these are not suitable for safety assessment (Wasser et al., 1997a,b; Mennen, 1997; Michenfelder et al., 1997; Cerulli et al., 1998; Martin and Fuller, 1998; Kato et al., 1998; Campbell et al., 1997; Pittler et al., 2003; Jeejeebhoy 1999; Anderson, 1997; Yeh et al., 2003; Finch et al., 2009; IOM, 2004). Points of concern, in a few case studies, were possible impairment of the kidney function and rhabdomyolysis, although due to pivotal confounding factors these studies were not conclusive.

4. Discussion

Zinc picolinate, zinc picolinate dihydrate and chromium picolinate are proposed to be used as sources of zinc and chromium, respectively, in food supplements. The majority of studies reveal a greater bioavailability of zinc from zinc picolinate than from other zinc compounds, although some studies showed similar levels of absorption, and a few investigators claimed a somewhat lower absorption of zinc from zinc picolinate than from other zinc compounds. Based on chromium intervention studies the Panel concludes that chromium from chromium picolinate is equally or slightly better bioavailable than chromium from other chromium compounds, although direct studies on the bioavailability are limited and generally bioavailability of inorganic chromium(III) from food sources and food supplements is very low (0.1-2%)(SCF, 2003).

The Panel concludes that zinc from zinc picolinate and from zinc picolinate dihydrate is bioavailable.

Data on the toxicity of zinc picolinate are limited. The toxicity data on chromium picolinate and picolinic acid are therefore also used for the risk assessment of zinc picolinate.

As all, but one, developmental/reproduction studies did not reveal any significant adverse effects, the Panel concluded that at present there are no indications that chromium picolinate or picolinic acid (at dose levels up to 200 mg chromium picolinate or 174 mg picolinic acid/kg bw/day) cause developmental effects in mice.

Chromium picolinate (monohydrate) did not produce significant indications of toxicity in a 13-week study in rats and mice at dose levels up to 50,000 mg/kg diet, equal to 4240 mg/kg bw/day for male rats, 4250 mg/kg bw/day for female rats, 11900 mg/kg bw/day for male mice and 9140 mg/kg bw/day for female mice (NTP, 2008). This NOAEL in male rats of 4240 mg chromium picolinate/kg bw/day would correspond to 3731 mg picolinic acid/kg bw/day, indicating that picolinic acid is of low toxicity.

No short-term or long-term toxicity studies on picolinic acid were available.

From the long-term carcinogenicity studies performed by NTP (2008), it was concluded that there was equivocal evidence of carcinogenic activity of chromium picolinate in male rats, and that there was no evidence of carcinogenic activity of chromium picolinate in female rats.
Chromium picolinate was negative in bacterial mutagenicity tests (NTP, 2008; Esber et al., 1997; Juturu and Komorowski, 2002) while clastogenic effects were reported in some assays in CHO cells (Stearns et al., 1995) but not in others (Slesinski et al., 2004). However, chromium tripicolinate (anhydrous) was negative in an in vivo mouse micronucleus assay. Coryell and Stearns (2006) discussed these conflicting results and considered that these could be due to the use of dimethyl sulfoxide, as a solvent of chromium picolinate, which could quench reactive oxygen species produced by chromium picolinate, resulting in no damage observed.

Picolinic acid was negative in an Ames tests (Bowden et al., 1976), which was however of limited validity, and did not induce unscheduled DNA synthesis in UV-irradiated human lymphocytes (Sims et al., 1982). In a chromosome aberration test, no genotoxic effects were seen at non-cytotoxic concentrations up to 1 mM picolinic acid (Stearns et al., 1995). In contrast, other investigators (San, 2000) observed concentration-dependent effects in mouse lymphoma cells both with and without metabolic activation. However, the Panel considered the result in the presence of metabolic activation equivocal. The Panel noted however that in the long term NTP study with chromium picolinate no treatment related malignant lesions were observed.

The Panel also noted that the Committee on Mutagenicity of Chemicals in Food of the UK has evaluated the genotoxicity of chromium picolinate (COM, 2004). The conclusions were as follows:

1) The evaluation of the mutagenicity of chromium picolinate is complex and the available data conflicting. Chromium picolinate has given positive results in some in vitro mutagenicity tests. The mechanism by which this occurs is unclear. However, in these studies the test material had been synthesised in the laboratory concerned and an adequate specification was not available. Replication of the tests using commercial grade material in tests conducted to international accepted protocols gave negative results. The Committee (COM, 2004) expressed some reservations regarding the conduct of these studies (possible limitations in sensitivity) and in particular regarding the repeat in vitro chromosome aberration study in CHO cells. However, overall it can be concluded that the balance of data suggest that chromium picolinate should be regarded as not being mutagenic in vitro;

2) The available in vivo tests in mammals with chromium picolinate were negative. In view of the negative in vitro results with commercial grade chromium picolinate, there is no further requirement for in vivo testing at the current time.”

The Panel notes that recent reviews and evaluations of chromium (III) (Eastwood 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 further noted that there are additional studies on the in vivo genotoxicity of chromium (III) compounds (Kirpnick-Sobol et al.,
2006; Zhang et al., 2008) which were not covered by these reviews. 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.

According to the exposure assessment, at the 97.5th percentile, the potential exposure to zinc (from food and anticipated exposure from zinc picolinate at the highest proposed level (50 mg/day)) is approximately 71 mg/day for adults and 66 mg/day for children (3-17 years). The Panel noted that this level is higher than the UL of 25 mg Zn/day for adults and for ranges from 7-22 mg Zn/day children.

The Panel noted that according to the EVM (2003), the highest zinc content on the market is 50 mg/capsule. Assuming that all zinc supplements contain zinc picolinate, this would lead to a potential dietary exposure to 187.7 mg picolinic acid/day. If all zinc would be applied up to the UL for zinc (25 mg/day), as established by the SCF, the potential exposure would correspond to approximately 94 mg picolinate/day or 1.57 mg picolinate/kg bw/day. The Panel considers an exposure estimate based on the UL as a conservative estimate. If the NOAEL derived from the long-term NTP of 2100 mg picolinic acid/kg bw/day is taken, there would be a Margin of Safety of approximately 1340.

The exposure assessment revealed that at the 97.5th percentile the total exposure to chromium, from food intake and from supplements at the highest proposed use level (600 µg chromium/day), would be 668-770 µg/day for adults and 726 µg/day (taken the adult dose) for children (3-17 years). This would lead to an exposure to picolinate of up to approximately 4.3 mg/day. However, WHO considered that supplementation of chromium should not exceed 250 µg/day (WHO, 1996). If such a dose would be provided as chromium picolinate, the potential exposure would correspond to approximately 1.8 mg picolinate or 30 µg picolinate/kg bw/day for a 60 kg person.

The Panel concluded that the uses of chromium (III) picolinate are of no safety concern provided that the amount of total chromium does not exceed 250 µg/day.

This leads to a combined estimated conservative exposure to picolinate, from the proposed uses of zinc and chromium picolinate of 94 mg picolinic acid/day plus 1.8 mg picolinate/day, resulting in approximately 96 mg picolinic acid/day which amounts to 1.6 mg/kg bw/day for a 60 kg person. Based on the NOAEL derived from the long-term NTP study of 2100 mg picolinic acid/kg bw/day, there would be a Margin of Safety of approximately 1310.

**CONCLUSIONS**

The present opinion deals with bioavailability of chromium and zinc from chromium picolinate, zinc picolinate and zinc picolinate dihydrate, respectively, and the safety of these sources. The safety of chromium and zinc themselves, in terms of amounts that may be consumed, is outside the remit of this Panel.

The Panel concludes that chromium from chromium picolinate is equally or slightly better bioavailable than chromium from other chromium compounds.

The Panel concludes that zinc from zinc picolinate and from zinc picolinate dihydrate is bioavailable.

Based on the available, albeit limited toxicological database, the Panel concluded that the use of zinc picolinate as a source of zinc, when added for nutritional purposes in food supplements, is not of safety concern, as long as the UL for zinc is not exceeded.

The Panel noted that, although the safety of zinc itself, in terms of amounts that may be consumed, is outside the remit of this Panel, at the 97.5th percentile the dietary zinc intake from total food only is already close to the UL as established by the SCF. However for children less than 10 years old, the anticipated total mean exposure may exceed the respective UL.

The Panel also concluded that the use of chromium (III) picolinate, as a source of chromium, is of no safety concern provided the use does not lead to supplemental intake of chromium higher than 250μg/day.

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) (Eastwood et al., 2008; Levina and Lay, 2008). 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.

**DOCUMENTATION PROVIDED TO EFSA**


2. Application for the approval of Chromax® Chromium Picolinate as a source of Chromium for use in the manufacture of food supplements, PARNUTS products and fortified foods in the EU. March 2005. Submitted by CANTOX.

3. Application for the derogation of Zinmax® Zinc Picolinate as a source of Zinc for use in the manufacture of food supplements, PARNUTS products and fortified foods in the EU. April 2005. Submitted by CANTOX.


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Kashiwabara N, 1986. Studies on zinc deficiency and zinc absorption in animals. Reports of Research Laboratory 82, 137-225.


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Glossary / Abbreviations

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<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>ANS Panel</td>
<td>Scientific Panel on Food Additives and Nutrient Sources added to Food</td>
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<td>CAS</td>
<td>Chemical Abstract Service</td>
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<td>CHO</td>
<td>Chinese Hamster Ovaries</td>
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<td>COM</td>
<td>Committee on Mutagenicity of Chemicals in Food</td>
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<td>EC</td>
<td>European Commission</td>
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<td>EFSA</td>
<td>European Food Safety Authority</td>
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<td>EVM</td>
<td>UK Expert Group on Vitamins and Minerals</td>
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<td>GD</td>
<td>Gestation Days</td>
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<td>IOM</td>
<td>Institute of Medicine</td>
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<td>NIDDM</td>
<td>Non-Insulin-Dependent Diabetes Mellitus</td>
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<tr>
<td>NOAEL</td>
<td>No-Observed-Adverse Effect Level</td>
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<td>NTP</td>
<td>National Toxicology Program</td>
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<td>PARNUT</td>
<td>Foods for Particular Nutritional Uses</td>
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<td>PRI</td>
<td>Population Reference Intake</td>
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<td>SCF</td>
<td>Scientific Committee on Food</td>
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<td>UL</td>
<td>Tolerable Upper Intake Level</td>
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<td>TDS</td>
<td>Total Diet Study</td>
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<td>WHO</td>
<td>World Health Organisation</td>
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