

## SCIENTIFIC OPINION

### **Chromium(III) lactate trihydrate as a source of chromium added for nutritional purposes to food supplements<sup>1</sup>**

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

(Question No EFSA-Q-2006-307)

**Adopted on 2 June 2009**

#### **PANEL MEMBERS**

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#### **SUMMARY**

Following a request from the European Commission to the European Food Safety Authority (EFSA), the Scientific Panel on Food Additives and Nutrient Sources added to Food (ANS) was asked to deliver a scientific opinion on the safety of chromium(III) lactate trihydrate, as a source of chromium, added for nutritional purposes to food supplements and on the bioavailability of chromium from this source.

The Scientific Committee for Food (SCF) has previously given an opinion on the Tolerable Upper Intake Level (UL) of chromium.

The present opinion deals only with the safety of a particular source of chromium, chromium(III) lactate trihydrate, intended to be used in food supplements and with the bioavailability of the nutrient cation from this source. The safety of chromium itself, in terms of the amounts that may be consumed, is outside the remit of this Panel.

Insufficient evidence was provided by the petitioner supporting the bioavailability of chromium from chromium(III) lactate. Although chromium(III) lactate is described by the petitioner as freely soluble in water, chromium(III) lactate exists as a weak complex that is

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<sup>1</sup> For citation purposes: Scientific Opinion of the Panel on Food Additives and Nutrient Sources added to Food on chromium(III) lactate trihydrate as a source of chromium added for nutritional purposes to food supplements, following a request from the European Commission. *The EFSA Journal* (2009) 1112, 1-7

insoluble at pH >5; this may influence the bioavailability of chromium(III) in the gastrointestinal tract. Absorption of trivalent chromium from food lies in the range of 0.5–2%, depending, among other factors, on the chemical properties of the ingested source and on the presence of other dietary components in the diet. The EFSA Scientific Panel on Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC) recently noted that absorption of chromium doubled when supplied as chromium amino acid chelate, rather than inorganic chromium. Following absorption, trivalent chromium binds to plasma proteins such as transferrin and is transported to the liver where it is sequestered; uptake by the spleen, soft tissue, and bone also occurs. Urine is the main excretory route for absorbed chromium, with small amounts being excreted in perspiration and bile. However, the data provided by the petitioner did not show any significantly increased levels of chromium in the livers and kidneys of chromium(III) lactate fed pigs and rats.

Only limited toxicological studies appear to have been carried out on chromium(III) lactate. The petitioner reports an unpublished commissioned study in pigs where the effect of chromium(III) lactate in feeding mixtures containing 0.15 mg chromium(III)/kg feed (corresponding to 4-7 µg chromium/kg bw/day) was evaluated on body weight, food intake, liver and kidney chromium contents and general physical condition. Small increases in body weight gain (2.4-4.4 %, depending on the age of the animals) were noted for the pigs fed the chromium (III) feed.

Another study was performed in dairy cows suffering from ketosis. The animals were exposed to 5 mg chromium(III) lactate/day mixed in the feed from one month prior to parturition to three months after parturition. No effect on blood chemistry and milk chemistry were observed except for a small but statistically significant increase in gamma glutamyl transferase activity at four and eight weeks after parturition in primiparous but not in pluriparous animals.

A study performed in rats fed with milk containing chromium(III) lactate corresponding to concentrations of 0.25 to 100 µg chromium per mL milk. The daily consumption of milk was reported to be 25 mL/animal; this would provide the rats with an exposure corresponding to 6.2-2500 µg chromium/day. This study reported that the administration of chromium(III) lactate did not have any observable adverse effects on growth, general health and reproduction.

A limited study commissioned by the petitioner was carried out in female rats (280 g/bw; no information about strain; five animals per group) treated by gavage for 30 days with chromium(III) lactate. In the dossier provided by the petitioner, it is stated that the animals were exposed to 2.83, 5.66 or 14.15 µg chromium/kg bw daily, which, as calculated by the Panel, corresponds to 20, 41 and 102 µg chromium(III) lactate trihydrate/kg bw/day. A statistically significant decrease between the control group and the chromium(III) lactate-treated groups was established for urea and plasma creatinine. However, the changes in plasma urea content were not dose-dependent. A small but statistically significant increase in plasma lactate dehydrogenase (LDH) activity was reported for the highest dosage group as compared to the control animals but this occurred in the absence of any detectable changes in serum biomarkers for hepatic injury. Weight gains in the 102 µg chromium(III) lactate/kg group were significantly lower than in the control group.

Limited information is available on the reproductive and developmental toxicity of chromium(III) lactate in animals. One study performed in dairy cows suffering from ketosis and exposed to 5 mg chromium(III) lactate/day/animal mixed in the feed from one month

prior to parturition to three months after parturition showed no adverse effect on reproduction parameters.

No information is available on the genotoxic potential or carcinogenicity of chromium(III) lactate.

The limited amount of toxicological data available on chromium(III) lactate is underpinned by the data available on other chromium(III) compounds such as chromium(III) chloride and chromium(III) sulphate, which are included in Annex II of the Food Supplements Directive 2002/46/EC as approved chromium compounds. Overall, the conclusion of the SCF on trivalent chromium was that the limited available data from studies on subchronic, chronic, and reproductive toxicity on soluble trivalent chromium salts and the available human data do not give clear information on the dose-response relationship.

The Panel noted that the SCF was unable to derive a UL for chromium(III), because of the deficiencies in the database. The Panel noted that the UK Expert Group on Vitamins and Minerals (EVM) similarly concluded that overall there were insufficient data from human and animals studies to derive a safe upper level for chromium, but that in the opinion of the EVM a total daily intake of about 0.15 mg chromium(III)/kg bw/day (approximately 10 mg/day) would be expected to be without adverse health effects. The US Food and Nutrition Board (FNB) also concluded that the data from animal and human studies are insufficient to establish a UL for soluble chromium(III) salts, while the World Health Organization (WHO) considered that supplementation of chromium should not exceed 250 µg/day.

Although no UL has been defined by the SCF for trivalent chromium, based on the available albeit limited toxicological database, the Panel noted that the WHO concluded that supplementation of chromium should not exceed 250 µg/day. This level is above the maximum use level of 200 µg/day proposed by the petitioner and would be expected to be without adverse health effects. The Panel noted that based on mean and 97.5<sup>th</sup> percentile European dietary chromium(III) intakes in the ranges of 60–160 µg/day and 126–170 µg/day, respectively, the consumption of an additional food supplement containing 200 µg chromium(III)/day would result in a total daily chromium intake between 260 and 360 µg chromium(III)/day in adults at the average level of dietary exposure and between 326 and 370 µg/day for adults at the high level of dietary exposure, if no other food supplements containing chromium(III) were taken. In children (3 to 17 years old), based on mean and 97.5<sup>th</sup> percentile dietary chromium(III) intakes in the ranges of 63–69 µg/day and 107–119 µg/day, respectively, the consumption of an additional food supplement containing 200 µg chromium(III)/day would result in a total daily chromium intake between 263 and 269 µg chromium(III)/day at the average level of dietary exposure and between 307 and 319 µg/day at the high level of dietary exposure, if no other food supplements containing chromium(III) were taken.

For information, in order to have an intake of 250 µg of chromium from chromium(III) lactate, the anticipated daily intake of lactate would be 1.28 mg.

The Panel concludes that because of the complex chemistry of lactate in aqueous solutions and limited solubility at pH >5, the bioavailability of from lactate is low and likely to be similar to other organic chromium-containing compounds.

Information on the toxicity of lactate is very limited but there was some evidence of chromium compound-related effects in a rat study at the top dose group. The Panel concludes that this study is poorly presented, has not been peer-reviewed and is based on an insufficient number of animals per dose group. The Panel considers that the limited changes reported by

the petitioner are not of toxicological significance. The Panel therefore concludes that the use of chromium(III) lactate, at the proposed use levels, is of no safety concern provided that the maximum level of chromium supplementation identified by the WHO is not exceeded.

The Panel concludes that the exposure to lactate arising from the use of chromium(III) lactate in food supplements, at the proposed use levels, is of no safety concern.

The Panel also 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:**

Food supplements, foods, chromium(III) lactate trihydrate, CAS Registry Number 19751-95-2

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## **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(III) lactate trihydrate added for nutritional purposes to foodstuffs. The relevant Community legislative measure is:

Directive 2002/46/EC of the European Parliament and of the Council on the approximation of the laws of the Member States relating to food supplements<sup>2</sup>.

## **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(III) lactate trihydrate added for nutritional purposes in food supplements.

## **ACKNOWLEDGEMENTS**

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

## ASSESSMENT

### 1. Introduction

The present opinion deals only with the safety of chromium(III) lactate trihydrate, as a source of chromium, added for nutritional purposes to food supplements and with the bioavailability of the nutrient cation from this source. The safety of chromium itself, in terms of the amounts that may be consumed, is outside the remit of this Panel.

### 2. Technical data

#### 2.1. Chemistry

##### *Chromium(III) lactate trihydrate*

The molecular formula of chromium(III) lactate trihydrate is  $\text{Cr}(\text{C}_3\text{H}_5\text{O}_3)_3 \cdot 3\text{H}_2\text{O}$ , its molecular weight is 373.2 g/mol and the CAS Registry Number for the anhydrous form is 19751-95-2 (Technical dossier, 2005).

A synonym proposed by the petitioner is chromium(III) 2-hydroxypropionate.

Chromium(III) lactate is described by the petitioner as freely soluble in water. However, based on the Pearson acid base concept (Pearson, 1963), also known as the HSAB concept, chromium(III) is a hard acid that readily forms complexes with O-donor ligands such as lactate. A recent study by Hamada *et al.* (2005) has shown that chromium(III) lactate has a pKa of 3.66 and exists as a weak complex that precipitates in aqueous solutions at pH >5.

#### 2.2. Specifications

The petitioner indicates that chromium(III) lactate trihydrate is a green powder with a purity not less than 98.0%; the remaining 2% being accounted for by moisture in the material. The petitioner also states that the content of chromium(III) is 136 g/kg of the source and limits for impurities are as follows: heavy metals (as lead) not more than 10 mg/kg and sulphates not more than 0.1%.

The Panel notes that according to Commission Regulation (EC) No 629/2008 (EC, 2008) the maximum levels of lead, mercury and cadmium in food supplements as sold should be 3 mg/kg, 0.1 mg/kg and 1 mg/kg, respectively.

#### 2.3. Manufacturing processes

The petitioner described the manufacturing process of a 0.1% premix of chromium(III) lactate in lactose. Chromium(III) lactate is manufactured by reaction of a saturated solution of calcium lactate with a saturated solution of chromium(III) sulphate at 60°C. This reaction yields chromium(III) lactate, and calcium sulphate that is precipitated and separated by filtration. The solution of chromium(III) lactate is applied onto a support (food lactose). The compound is dried at a temperature up to 102°C in a cabinet drier.



#### 2.4. Methods of analysis in food

The petitioner indicated that the content of chromium in chromium lactate and in the chromium lactate premix is determined through a spectrophotometric method after the previous mineralization of the sample. Chromium(III) is oxidized with perchloric acid to chromium(VI) which, after a reaction with diphenylcarbazide, gives a chromophore complex that is quantified by UV-VIS absorption spectroscopy.

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

No information is provided by the petitioner relating to the reaction and fate in foods to which the source is added. The petitioner states that chromium(III) lactate under recommended storage conditions (closed and dry) is stable.

#### 2.6. Case of need and proposed uses

The proposed use of chromium(III) lactate is in supplements for chromium supplementation and will be provided as a 0.1% premix in lactose vehicle. The petitioner proposes a maximum daily intake of 1435 µg chromium(III) lactate trihydrate to provide up to a maximum daily intake of 200 µg chromium per person. The petitioner further recommended a maximum single dose of chromium(III) lactate trihydrate of 718 µg (to provide 100 µg chromium).

#### 2.7. Information on existing authorisations and evaluations

Chromium(III) chloride and chromium(III) sulphate are included in Annex II of the Food Supplements Directive 2002/46/EC (EC, 2002) as approved chromium compounds and also within Annex Category 2 of Directive 2001/15/EC (EC, 2001) as substances that may be added for specific nutritional purposes to Food for Particular Nutritional Uses (PARNUTS).

The societies for Nutrition of Germany (DGE), Austria (ÖGE) and Switzerland (SGE) jointly established an Adequate Intake (AI) of 30-100 µg chromium/day for adults (D-A-CH, 2000). The SCF has previously given an opinion on the UL of chromium(III) (SCF, 2003). The SCF considered that the data from studies in experimental animals on the subchronic, chronic and reproductive toxicity of soluble trivalent chromium salts and the available human data did not provide clear information on the dose-response relationships, and therefore the SCF was not able to derive a UL for trivalent chromium. The UK Expert Group on Vitamins and Minerals (EVM) similarly concluded that overall there were insufficient data from human and animal studies to derive a safe upper level for chromium. However, in the opinion of the EVM a total daily intake of about 150 µg chromium(III)/kg bw/day (approximately 10 mg/day for a person weighing 67 kg) would be expected to be without adverse health effects (EVM, 2003). The US FNB also concluded that the data from animal and human studies were insufficient to establish an UL for soluble chromium(III) salts (FNB, 2001), while the WHO considered that supplementation of chromium should not exceed 250 µg/day (WHO, 1996).

L-lactic acid is a permitted food additive (*quantum satis*) with the number E270.



## 2.8. Exposure

Currently, trivalent chromium (Cr(III)) is used in food supplements in a number of countries in the European Union. Exposure to chromium(III) also commonly occurs via food, with the highest levels being found in meat and meat products, oils and fats, breads and cereals, fish, pulses and spices (EVM, 2003; SCF, 2003). No exposure data are available for chromium lactate.

The SCF report provides information on average chromium dietary intakes of 60 to 160 µg/day in adults in some European countries (SCF, 2003). The 97.5<sup>th</sup> percentile dietary intake values of chromium were reported to range from 126 to 170 µg/day (Leblanc *et al.*, 2005; SCF, 2003). Data from the French Total Diet Study provided average intakes of chromium of 63 µg/day for children aged 3-10 years and 69 µg/day for 11-17 year olds, and high percentile intakes of 107 µg/day for 3-10 year olds and 119 µg/day for 11-17 year olds.

In an adult population (over 18 years old), assuming mean and 97.5<sup>th</sup> percentile European dietary chromium(III) intakes in the ranges of 60–160 µg/day and 126-170 µg/day, respectively, consumption of an additional food supplement containing 200 µg chromium(III)/day (the highest proposed use level by the petitioner) would result in a total anticipated daily chromium intake between 260 and 360 µg chromium(III)/day at the average level of dietary exposure and between 326 and 370 µg/day at the high level of dietary exposure, if no other food supplements containing chromium(III) were taken (Table 1).

In children aged 3 to 17 years old, assuming mean and 97.5<sup>th</sup> percentile dietary chromium(III) intakes in the ranges of 63–69 µg/day and 107-119 µg/day, respectively, consumption of an additional food supplement containing 200 µg chromium(III)/day (the highest proposed use level by the petitioner) would result in a total anticipated daily chromium intake between 263 and 269 µg chromium(III)/day at the average level of dietary exposure and between 307 and 319 µg/day at the high level of dietary exposure, if no other food supplements containing chromium(III) were taken (Table 1).

Table 1. **Summary information on Chromium intake and anticipated potential exposure to chromium from chromium(III) lactate.**

| Nutrient: Chromium   | Amount (µg/day)      | Average intake (µg/day) | High intake (µg/day) | References                              |
|--|----------------------|-------------------------|----------------------|---|
| Recommended Daily Intake (RDI)   | 30 to 100 for adults |                         |                      | D-A-CH, 2000                            |
| Maximum level of supplementation   | 250                  |                         |                      | WHO, 1996                               |
| Intake range from food in Europe for adults  |                      | 60-160                  | 126-170              | Leblanc <i>et al.</i> , 2005; SCF, 2003 |
| Intake range from food in Europe for children (3-17 years old)   |                      | 63-69                   | 107-119              | Leblanc <i>et al.</i> , 2005            |
| Maximum daily intake of chromium(III) from food supplements containing chromium(III) lactate trihydrate, as proposed by petitioner | 200                  |                         |                      | Technical dossier, 2005                 |
| <b>Source:</b><br><b>Chromium Lactate</b>  |                      |                         |                      |   |

|  |  |         |         |                      |
|--|--|---------|---------|----------------------|
| Total anticipated exposure to chromium from food supplements and dietary intake for adults <sup>1</sup>                    |  | 260-360 | 326-370 | Calculation by Panel |
| Total anticipated exposure to chromium from food supplements and dietary intake for children (3-17 years old) <sup>2</sup> |  | 263-269 | 307-319 | Calculation by Panel |

<sup>1</sup>calculation based on proposed use level of 200 µg/day plus average dietary intake of 60-160 µg/day and high dietary intake of 126-170 µg/day for adults

<sup>2</sup>calculation based on proposed use level of 200 µg/day plus average dietary intake of 63-69 µg/day and high dietary intake of 107-119 µg/day for children

To provide a maximum daily dose of 250 µg chromium, the exposure to lactate would be 1.28 mg from 1.80 mg of chromium(III) lactate trihydrate<sup>2</sup>. The exposure from supplements to lactate is 1245 µg/day from 1435 µg chromium(III) lactate trihydrate in food supplements. Lactic acid is a normal constituent of foods and is readily metabolised. Normal blood lactate levels range from 45 to 200 mg/L. Assuming a daily consumption of 100 g meat, human exposure to lactic acid from this source will range from 100 to 400 mg/day (Choe *et al.*, 2008).

### 3. Biological and toxicological data

#### 3.1. Absorption, distribution, metabolism and excretion

Although chromium(III) lactate is described by the petitioner as freely soluble in water, chromium(III) lactate exists as a weak complex that may influence its bioavailability in the gastrointestinal tract. With a pKa of 3.66, chromium(III) lactate will not be ionised at acidic pH and could be absorbed from the stomach. However, at pH >5 chromium(III) lactate will not only be fully ionised but has been reported to precipitate in aqueous solutions (Hamada *et al.*, 2005). The petitioner did not present any data on the bioavailability of chromium(III) from chromium(III) lactate but claims that 50 µg chromium(III) in the form of lactate corresponds to the metabolic equivalent of 140-150 µg chromium(III) in the form of picolinate or nicotinate. However, the Panel noted that no evidence supporting this contention was provided by the petitioner and that the bioavailability of inorganic chromium(III) from food sources and supplements is generally very low (0.1-2 %) (SCF, 2003). Instead, the petitioner reports an unpublished commissioned study in pigs fed chromium(III) lactate in feeding mixtures containing 0.15 mg chromium(III)/kg; the effects on body weight, food intake, liver and kidney chromium content and general physical condition were evaluated. Based on pig feed intake data obtained from the Danish Pig Producers ([www.dansksvineproduktion.dk/](http://www.dansksvineproduktion.dk/)), the Panel estimated the corresponding daily exposure to range from 4-7 µg chromium/kg bw/day. There were no statistically significant changes in liver and kidney chromium contents. Another study commissioned by the petitioner did not show any statistically significant changes in chromium content in the livers and kidneys of female rats (no information about strain) treated by gavage for 30 days with 20, 41 or 102 µg chromium(III) lactate/kg bw. Another study was performed in rats fed with milk containing chromium lactate, corresponding to concentrations of 0.25 to 100 µg chromium/mL milk for up to 90 days (Conn *et al.*, 1932). In that study, the average daily consumption of milk was reported to be 25 mL/animal; this would provide the rats with an exposure corresponding to

<sup>2</sup> The calculation of the amount of lactate provided by the source takes into account the three moles of H<sub>2</sub>O released per mole chromium(III) lactate trihydrate.

6.2-2500 µg chromium/day. The rats were then killed, and the alimentary tract was washed out and, together with the body, was dried and ashed, and the ash analysed. The results show that with daily doses of 250 and 2500 µg chromium/day, only 0.03 and 0.003 %, respectively, of the chromium consumed was retained. No detectable retention was observed at lower doses of chromium(III) lactate. The Panel therefore concludes that because of the complex chemistry of chromium(III) lactate in aqueous solution and limited solubility at pH >5, the bioavailability of chromium(III) from chromium(III) lactate is low and likely to be similar to that from other organic chromium-containing compounds. No specific toxicokinetic studies on chromium(III) lactate were provided by the petitioner. Most dietary chromium (> 98%) is not absorbed and is excreted via the faeces. Dietary factors such as starch, ascorbic acid, minerals, oxalate, and amino acid intake can have a significant influence on chromium absorption, and carbohydrate intake has been shown to influence chromium urinary excretion and tissue concentration (Lamson and Plaza, 2002).

Following limited absorption, trivalent chromium binds to plasma proteins such as transferrin and is transported to the liver. In humans, chromium concentrates in the liver, spleen, soft tissue, and bone; a similar pattern is seen in rats with incorporation in the kidneys and testes in addition to the liver spleen and bone (FNB, 2001). However, evidence for the bioavailability of chromium from chromium(III) lactate in pigs and rats is limited. Mertz *et al.* (1965) proposed a three-compartment model with half-lives of 0.5, 5.9, and 83 days based on studies of radiolabelled chromium ( $^{51}\text{CrCl}_3$ ) in rats. Urine is the main route of elimination for absorbed chromium in both animals and humans, with small amounts being excreted in perspiration and bile. Urinary chromium excretion reflects the dietary chromium intake in a dose-dependent manner (Kumpulainen, 1992; Uusitupa *et al.*, 1992).

As indicated above, chromium binds to transferrin (Peterson, 1967), and interactions between iron and chromium are therefore possible, resulting in impairment of iron storage and metabolism. These interactions have been studied in animals fed a range of trivalent chromium compounds (inorganic and organic) in the diet at relatively high levels (Anderson *et al.*, 1993; Anderson *et al.*, 1996).

## **3.2. Toxicological data**

### **3.2.1. Acute toxicity**

No information is available on the acute toxicity of chromium(III) lactate in animals.

### **3.2.2. Subchronic and chronic toxicity studies**

As noted by the SCF (2003) and the EVM (2003) there are limited data from subchronic and chronic studies on the toxicity of soluble trivalent chromium salts. Only limited toxicological studies appear to have been carried out on chromium(III) lactate. The petitioner reports an unpublished commissioned study in pigs fed chromium(III) lactate in feeding mixtures containing 0.15 mg chromium(III)/kg; the effects on body weight, food intake, liver and kidney chromium content and general physical condition were evaluated. The Panel estimated the corresponding daily intake to range from 4-7 µg chromium/kg bw/day. Small increases in body weight gains (2.4-4.4%, depending on the age of the animals) in animals fed the chromium(III) lactate feed as compared to controls were noted.

A study performed in rats fed with milk containing chromium(III) lactate reported that the daily exposure to 6.2-2500 µg chromium/day for up to 90 days did not have any adverse effect on growth, general health and reproduction (Conn *et al.*, 1932).

A study commissioned by the petitioner in 2007 was carried out in female rats (280 g bw; no information about strain) treated by gavage for 30 days with chromium(III) lactate. A poorly presented and non-peer-reviewed report of this study was provided by the petitioner. It is stated that the rats (5 animals per group) were exposed to 2.83, 5.66 or 14.15 µg chromium/kg bw daily, which is equivalent to 20, 41 and 102 µg chromium(III) lactate/kg bw/day. Examination of blood parameters (number of leukocytes, number of erythrocytes, amount of haemoglobin, haematocrit, average colour concentration, number of thrombocytes) revealed no statistically significant differences in the parameters measured between the different treatment groups. A statistically significant decrease in plasma urea was observed between the control group and the 41 and 102 µg chromium(III) lactate/kg bw/day groups (ANOVA followed by Tukey-HSD post-hoc test,  $p < 0.05$ ); no statistically significant difference was found between the control and the 20 µg chromium(III) lactate/kg bw/day group. Plasma creatinine exhibited similar decreases between the 41 and 102 µg chromium(III) lactate/kg bw/day groups and the control group ( $p < 0.05$ ). A statistically significant increase in plasma lactate dehydrogenase (LDH) activity (to 150%) was observed in the 102 µg chromium(III) lactate/kg bw/day group compared to the control group ( $p < 0.05$ ). No statistically significant differences between individual groups were found for calcium, phosphorus, chlorides, alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), cholesterol, triglycerides, total protein, glucose, magnesium, and albumin. Weight gains in the 102 µg chromium(III) lactate/kg bw/day group were significantly lower than in the control group ( $p < 0.05$ ). According to the petitioner, histopathological examinations of the liver and kidney revealed no differences between controls and the treated groups.

The Panel considers the increased LDH activity in the treated groups to be within the normal range. Furthermore, the Panel notes that such findings were not observed in other studies. The Panel also considers that the decreases in plasma urea and creatinine are not indicative of any damage to the kidneys.

### 3.2.3. Reproduction and developmental studies

Limited information is available on the reproductive and developmental toxicity of chromium(III) lactate in animals. One study (Pechova *et al.*, 2003) was performed in dairy cows suffering from ketosis. The animals were exposed to 5 mg chromium(III) lactate/day/animal mixed in the feed from one month prior to parturition to three months after parturition. No effects on reproduction parameters were reported. No effect on blood chemistry and milk chemistry were observed except for a small but statistically significant increase in gamma glutamyl transferase activity at four and eight weeks after parturition in primiparous but not in pluriparous animals and a significant increase in milk fat content after 100 days of lactation in pluriparous but not primiparous animals.

The SCF (2003) report that chromium chloride given in drinking water at levels of 2000 or 5000 mg/L *ad libitum* for 12 weeks to Swiss mice reduced body weights (male only) and fertility (Elbetieha and AlHamood, 1997). Significant changes in the weights of reproductive organs compared to controls were noted. The SCF noted that the authors of this study did not report the actual intake of chromium chloride but oral doses for chromium(III) of approximately 500 or 1250 mg/kg bw/day for females and 250 or 1250 mg/kg bw/day for

males were estimated by the EVM (EVM, 2003). The SCF (2003) also reported that the weight of reproductive organs of male Sprague Dawley rats exposed to chromium(III) chloride ( $\text{CrCl}_3$ ) in drinking water (1000 mg/L for 12 weeks, equivalent to about 50 mg  $\text{CrCl}_3$ /kg bw/day or about 16.5 mg chromium(III)/kg bw/day) was significantly reduced compared to controls (Bataineh *et al.*, 1997).

### 3.2.4. Genotoxicity

No information is available on the genotoxicity of chromium(III) lactate in animals.

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. Carcinogenicity

No information is available on the carcinogenicity of chromium(III) lactate in animals.

In a recent study in F344/N rats and B6C3F1 mice of both sexes, animals were exposed for 2 years (in feed) to chromium(III) picolinate monohydrate at very high concentrations equivalent to 0, 10.7; 54.9 and 286.2 mg chromium(III)/kg bw/day. (Stout *et al.*, 2009). The data showed very little evidence of adverse effects; in male rats, there was equivocal evidence of carcinogenic activity based on an increased number of preputial gland adenomas (only in the 54.9 mg Cr(III)/kg bw/day group). However, there was no evidence of carcinogenic activity in female rats or in male or female mice.

According to the International Agency for Research on Cancer (IARC) “metallic chromium and chromium(III) compounds are not classifiable as to their carcinogenicity to humans” (Group 3) (IARC, 1990). In recent chronic toxicity and carcinogenicity studies, rats and mice (Stout *et al.*, 2009) were exposed for 2 years (in feed) to chromium(III) picolinate monohydrate at very high concentrations equivalent to 0, 10.7; 54.9 and 286.2 mg chromium(III)/kg bw/day. The data showed very little evidence of adverse effects; in male rats, there was equivocal evidence of carcinogenic activity based on increased preputial gland adenomas (only in the 54.9 mg chromium(III)/kg bw/day dose group). There was no evidence of carcinogenic activity in female rats or in male and female mice.

## 4. Discussion

No evidence was provided by the petitioner supporting the bioavailability of chromium from chromium(III) lactate. Although chromium(III) lactate is described by the petitioner as freely soluble in water, chromium(III) lactate exists as a weak complex that may influence the bioavailability of chromium(III) in the gastrointestinal tract. Absorption of trivalent chromium from food lies in the range of 0.1–2 %, depending, among other factors, on the chemical properties of the source compound and on the presence of other dietary components



in the diet (SCF, 2003). Although there are reports in the literature that chromium from organic sources such as chromium-enriched yeast is more bioavailable than chromium from inorganic chromium compounds such as chromium chloride, overall the available data do not permit a definite conclusion on this issue. The EFSA Scientific Panel on Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC) recently noted that absorption of chromium doubled when supplied as chromium amino acid chelate, in comparison with inorganic chromium (EFSA, 2008). The Panel concludes that because of the complex chemistry of chromium(III) lactate in aqueous solutions and its limited solubility at pH >5, the bioavailability of chromium(III) from chromium(III) lactate is low and likely to be similar to that from other organic chromium-containing compounds.

Following absorption, trivalent chromium binds to plasma proteins such as transferrin and is transported to the liver where it is sequestered; uptake by the spleen, soft tissue, and bone also occurs. Urine is the main excretory route for absorbed chromium, with small amounts being excreted in perspiration and bile.

Only limited toxicological studies appear to have been carried out on chromium(III) lactate. In a study in pigs, where the effect of chromium(III) lactate in feeding mixtures containing 0.15 mg chromium(III)/kg feed was evaluated, no adverse effects were noted. In another study performed in dairy cows suffering from ketosis and exposed to 5 mg chromium(III) lactate /day mixed in the feed, from one month prior to parturition to three months after parturition, a small but statistically significant increase in gamma glutamyl transferase activity was observed at four and eight weeks after parturition in some animals.

A study performed on a limited number of rats fed with milk containing chromium(III) lactate to provide an exposure corresponding to 6.2-2500 µg chromium/animal/day reported no observable adverse effects on growth, general health and reproduction.

A study was carried out in female rats treated by gavage for 30 days with 20, 41 and 102 µg chromium(III) lactate/kg bw/day. The Panel notes that the study is poorly presented, it was not performed according to the OECD Test Guidelines for 28-day studies and therefore it has not been used for the safety assessment of chromium(III) lactate.

The only information available on the reproductive and developmental toxicity of chromium(III) lactate in animals comes from a single study performed in dairy cows suffering from ketosis that showed no adverse effect on reproduction parameters.

No information is available on the genotoxic potential or carcinogenicity of chromium(III) lactate.

The Panel noted that the SCF was unable to derive a UL for chromium(III), because of the deficiencies in the database. The Panel also noted that the EVM similarly concluded that overall there were insufficient data from human and animals studies to derive a safe upper level for chromium, but that in the opinion of the EVM a total daily intake of about 0.15 mg chromium(III)/kg bw/day (approximately 10 mg/person) would be expected to be without adverse health effects (SCF, 2003). The US FNB also concluded that the data from animal and human studies are insufficient to establish a UL for soluble chromium(III) salts (FNB, 2001), while the WHO considered that supplementation of chromium should not exceed 250 µg/day (WHO, 1996).

In an adult population (over 18 years old), assuming mean and 97.5<sup>th</sup> percentile European dietary chromium(III) intakes in the ranges of 60–160 µg/day and 126-170 µg/day, respectively, consumption of an additional food supplement containing 200 µg chromium(III)/day (the highest proposed use level by the petitioner) would result in a total

anticipated daily chromium intake between 260 and 360 µg chromium(III)/day at the average level of dietary exposure and between 326 and 370 µg/day at the high level of dietary exposure, if no other food supplements containing chromium(III) were taken.

In children aged 3 to 17 years old, assuming mean and 97.5<sup>th</sup> percentile dietary chromium(III) intakes in the ranges of 63–69 µg/day and 107–119 µg/day, respectively, consumption of an additional food supplement containing 200 µg chromium(III)/day (the highest proposed use level by the petitioner) would result in a total anticipated daily chromium intake between 263 and 269 µg chromium(III)/day at the average level of dietary exposure and between 307 and 319 µg/day at the high level of dietary exposure, if no other food supplements containing chromium(III) were taken.

The Panel noted that the amount of chromium(III) added to supplements from chromium(III) lactate as indicated by the petitioner does not exceed the maximum level of supplementation of 250 µg identified by the WHO.

The Panel notes that there are no available data on exposure to chromium lactate intake from the diet.

In order to have an intake of 200 µg of chromium from chromium(III) lactate, the anticipated daily intake of lactate would be 1.232 mg and given that lactate is a natural constituent of food, an endogenous metabolite and a permitted food additive (*quantum satis*) it is considered to be 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 notes that additional relevant *in vivo* studies have shown that exposure to chromium(III) chloride and chromium(III) nitrate induced DNA deletions in mice and yeast respectively (Kirpnick-Sobol *et al.*, 2006) and that it was recently reported that occupational exposure to chromium(III) can lead to DNA damage to human peripheral lymphocyte as evidenced by the Comet assay (Zhang *et al.*, 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.

## CONCLUSIONS

The present opinion deals only with the safety of chromium(III) lactate, as a source of chromium, added for nutritional purposes to food supplements and with the bioavailability of chromium from this source. The safety of chromium itself, in terms of the amounts that may be consumed, is outside the remit of this Panel.

The Panel concurs with the view of the SCF that overall, the bioavailability of chromium is low and therefore the bioavailability of chromium from chromium(III) lactate likely to be similar to that of chromium from dietary sources (0.5–2%).

The Panel concludes that the use of chromium(III) lactate as a source of chromium(III) in food supplements would not be of safety concern at the proposed use level. This amount would result in an exposure to approximately 1.23 mg of lactate which is not of safety concern.

The Panel also 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.

#### **DOCUMENTATION PROVIDED TO EFSA**

1. Technical dossier, 2005. Dossier for Safety Evaluation of Chromium(III) lactate for Use in the Manufacture of Food Supplements. April 2005. Submitted by Agrobac, Czech Republic.

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## GLOSSARY / ABBREVIATIONS

|         |  |
|---------|--|
| AFC     | Scientific Panel on Additives, Flavourings, Processing Aids and Materials in Contact with Food |
| AI      | Adequate Intake  |
| ALP     | Alkaline Phosphatase   |
| ALT     | Alanine Transaminase   |
| ANOVA   | Analysis of Variance   |
| ANS     | Scientific Panel on Food Additives and Nutrient Sources added to Food                          |
| AST     | Aspartate Transaminase   |
| CAS     | Chemical Abstracts Service   |
| EC      | European Commission  |
| EFSA    | European Food Safety Authority   |
| EVM     | UK Expert Group on Vitamins and Minerals   |
| FNB     | US Food and Nutrition Board  |
| HSAB    | Hard and Soft Acids and Bases  |
| HSD     | Honestly Significant Difference  |
| IARC    | International Agency for Research on Cancer  |
| LDH     | Lactate Dehydrogenase  |
| OECD    | Organisation for Economic Co-operation and Development   |
| PARNUTS | Foods for Particular Nutritional Uses  |
| RDI     | Recommended Daily Intake   |
| SCF     | Scientific Committee for Food  |
| UL      | Tolerable Upper Intake Level   |
| UV-VIS  | Ultraviolet-visible spectroscopy   |
| WHO     | World Health Organization  |