European Food Safety Authority

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SCIENTIFIC OPINION

Safety and efficacy of chromium methionine (Availa®Cr) as feed additive for all species[1]

Scientific Opinion of the Panel on Additives and Products or Substances used in Animal Feed

(Question No EFSA-Q-2006-066)

Adopted on 1 April 2009

PANEL MEMBERS

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SUMMARY

Following a request from the European Commission, the European Food Safety Authority was asked to deliver a scientific opinion on the safety and efficacy of chromium methionine (Cr-Met) as feed additive for all species.

Currently, trivalent chromium (Cr(III)) is not authorised as a feed additive in the EU. Therefore, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) considered it necessary to first perform an assessment of Cr(III) as a trace element in animal nutrition and the consequences of its use for consumers of animal products.

General assessment of chromium(III) as trace element in animal nutrition (Part I)

Dietary Cr is poorly absorbed upon ingestion by animals. There is limited evidence that organic forms of Cr(III) are better absorbed than inorganic forms and may have a higher bioavailability. Chromium(III) is found in animal tissues (including liver, kidney and muscle). Chromium(III) potentiates insulin-dependent glucose entry into the cells. Other biological effects are less well established (e.g. on immune response and lipid metabolism).

No symptoms of Cr deficiency in animals have been demonstrated in experimental conditions or observed in the field. The FEEDAP Panel considers that there is no evidence of essentiality

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of Cr(III) as trace element in animal nutrition and consequently, no Cr(III) requirements could be established.

Plant sources make only a limited contribution to the total Cr in compound feedingstuffs; the greater part comes from mineral sources. Concerning foods of animal origin, the highest levels of Cr are found in offals, followed by muscle tissue, fish flesh and eggs. Other animal products contain very little Cr.

The available data did not allow a consistent pattern of tissue deposition to be identified in farm animals given feed supplemented with Cr sources. The FEEDAP Panel notes that the assessment of data on Cr concentration in feeds, foods and biological samples need to be interpreted with caution due to the uncertainties associated with the analytical procedures.

Conditions adversely affecting health/welfare and/or metabolic challenges generally appear to favour the occurrence of beneficial zootechnical effects of supplemental Cr(III). However, the responses are highly inconsistent, possibly due to the wide range of natural Cr background in feed, the sources and levels of supplementary Cr and the presence of other dietary factors (e.g. phytate, ascorbic acid, organic acids, other trace elements).

There is no evidence of adverse effects on farm animals arisen from background levels of total Cr in unsupplemented feed. Most of the recorded inconsistencies and/or species differences in adverse response to supplementary Cr(III) may be due to the source, to the (mostly unknown) Cr(III) dietary background, to other dietary factors influencing Cr(III) bioavailability or to analytical uncertainties. Very few studies specifically investigated safety in target species. In view of the limited available data, the FEEDAP Panel is not in a position to define maximum tolerable levels of Cr(III) in feed for farm animals.

The toxicology of Cr(III) is not yet fully clarified. Available data clearly indicate that Cr(III) is much less toxic than Cr(VI), an established animal and human carcinogen. However, Cr(III) is the likely ultimate intracellular toxic form of Cr(VI). The FEEDAP Panel notes that the most recent available literature and the carcinogenicity studies in rats and mice indicate that Cr(III) may be a genotoxic compound under *in vivo* conditions. Considering the concerns related to consumer safety for Cr(III), the FEEDAP Panel considers it prudent to avoid any additional exposure of the consumers resulting from the use of supplementary Cr in animal nutrition.

The consumer background dietary intake of Cr(III) is not expected to exceed 0.3 mg day^{-1} and is likely to be no more than 0.1 mg day^{-1}. The mean contribution of foodstuffs of animal origin from unsupplemented Cr(III) animals to the background dietary intake of Cr for the adult population has been estimated at approximately 25 %. No reliable data have been found concerning the additional consumer's exposure resulting from the use of supplementary Cr in animal nutrition.

Occupational exposure to Cr(III) may elicit allergic dermatitis and may lead to a significant increase of micronuclei in industrial workers. The FEEDAP Panel concludes that, due to concerns related to allergenicity and potential genotoxicity, any occupational exposure to Cr(III) in the feed industry should be kept to a minimum.

Chromium, as a natural element, is ubiquitous in the environment, occurring in a number of oxidation states. Chromium(III) is the predominantly naturally occurring form. The predicted no effect concentrations for Cr(III) have been determined to be 2.8 mg kg^{-1}, 4.7 μg L^{-1} and 31 mg kg^{-1} wet weight for soil, water and sediment respectively.

Specific assessment of chromium-methionine (Part II)

The additive chromium-methionine (Availa®Cr) contains 3 % of Cr(III) in the form of a chloride salt of a hexacoordinate chelate complex of a single trivalent chromium atom with three molecules of the amino acid methionine. It is intended to be used as a nutritional additive for all species at an inclusion level of 0.4–1.6 mg Cr kg^{-1} complete feedingstuffs.

The FEEDAP Panel considers Cr-Met as a source of available Cr(III). This conclusion is based on increased glucose clearance rate in pigs and cattle for fattening, on reduced plasma glucose and insulin in horses and reduced plasma glucose in dairy cows. No conclusion on the effects of Cr-Met on zootechnical parameters could be derived.

Since data on target animals safety was provided only for pigs, and since in one efficacy study with cows there was a suggestion of negative effects on milk production at near-use levels, the FEEDAP Panel cannot conclude on the safety of Cr-Met for all species/categories.

Considering the availability of data on tissue deposition only for bovines and the inadequacies of the studies performed, the FEEDAP Panel cannot conclude on tissue deposition in target species administered with Cr-Met and, consequently, is not in a position to perform a consumer exposure assessment.

Because of the inadequate data on the genotoxicity of Cr(III) from Cr-Met and on tissue deposition, the FEEDAP Panel cannot conclude on whether the use of Cr-Met in farm animals feeds would result in any greater or lesser concern for consumer safety identified in Part I.

No specific data on user safety for Cr-Met has been submitted. However, the literature available indicates potential concerns for Cr(III) occupational exposure. Considering the lack of specific information, the FEEDAP Panel considers such concerns to be also relevant to those handling the product.

Cr-Met contains Cr(III), which is relatively abundant in the environment. Although trigger values for soil are reached in some applications, predicted environment concentrations would not reach the estimated effect concentrations in soil, water and sediment. The FEEDAP Panel concludes that the contribution of Cr-Met in excretions of terrestrial animals to the natural levels of Cr in soil and the aquatic environment would not pose a risk to the environment.

Key words: nutritional additive, trace element, chromium, trivalent chromium, chromium-methionine, Availa®Cr, analytical methods, essentiality, deficiency, animal requirements, availability, efficacy, safety, toxicity, environment

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BACKGROUND

Regulation (EC) No 1831/2003[2] establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 4(1) of that Regulation lays down that any person seeking authorisation for a feed additive or for a new use of a feed additive shall submit an application in accordance with Article 7.

The European Commission received a request from the company Zinpro Animal Nutrition Inc.,[3] for the authorisation of chromium methionine (Availa®Cr), to be used as a feed additive for all species (category: nutritional additives; functional group: compounds of trace elements) under the conditions mentioned in Table 1.

According to Article 7(1) of Regulation (EC) No 1831/2003, the Commission forwarded the application to the European Food Safety Authority (EFSA) as an application under Article 4.1 (authorisation of a feed additive or new use of a feed additive). EFSA received directly from the applicant the technical dossier in support of this application.[4] According to Article 8 of that Regulation, EFSA, after verifying the particulars and documents submitted by the applicant, shall undertake an assessment in order to determine whether the feed additive complies with the conditions laid down in Article 5. The particulars and documents in support of the application were considered valid by EFSA as of 25 October 2006.

The trace element chromium has not been previously authorised as a feed additive at Community level, being this one the first application for such a product.

TERMS OF REFERENCE

According to Article 8 of Regulation (EC) No 1831/2003 EFSA shall determine whether the feed additive complies with the conditions laid down in Article 5. Therefore, EFSA shall deliver an opinion on the efficacy and the safety for the target animals, consumer, user and the environment, of chromium methionine (Availa®Cr), when used under the conditions described in Table 1.

ACKNOWLEDGEMENTS

The European Food Safety Authority wishes to thank the members of the Working Group on Trace Elements as well as Andrew Renwick and Francesco Cubadda for the preparation of this opinion.

[2] OJ L 268, 18.10.2003, p.29
[3] Gerard Doustraat 4a. 5831 CC Boxmeer. The Netherlands
[4] Dossier reference: FAD-2006-0013

Table 1. **Register entry as proposed by the applicant**

Additive	Availa®Cr
Registration number/EC No/No (if appropriate)	-
Category of additive	Nutritional additive
Functional group of additive	3(b)

Description			
Composition, description	**Chemical formula**	**Purity criteria (if appropriate)**	**Method of analysis (if appropriate)**
Chromium methionine HCl	$[CH_3S(CH2)_2CHNH_2COO]_3$ Cr•HCl	3 % chromium	Cr assay using ICP

Trade name (if appropriate)	Availa®Cr
Name of the holder of authorisation (if appropriate)	Zinpro Animal Nutrition Inc., The Netherlands

Conditions of use

Species or category of animal	**Maximum Age**	**Minimum content** (mg kg^{-1} of complete feedingstuffs)	**Maximum content** (mg kg^{-1} of complete feedingstuffs)	**Withdrawal period (if appropriate)**
Piglets (suckling and weaning period)	until approx. 35 kg	-	13.3	
Pigs for fattening	-	-	13.3	
Sows (for reproduction and in order to have benefit in piglets)	-	-	13.3	
Poultry (all categories: chicken, laying hens, turkeys and others)	-	-	13.3	
Calves for fattening	-	-	52.8	
Calves for rearing	-	-	52.8	
Cattle for fattening	-	-	23.5	
Dairy cows and cows for reproduction	-	-	23.5	
Lambs for rearing	-	-	52.8	
Lambs for fattening	-	-	52.8	
Goats: kids for rearing	-	-	52.8	
Goats: kids for fattening	-	-	52.8	
Horses (all diets)	-	-	23.5	
All other species and categories	-	-	26.7	

Other provisions and additional requirements for the labelling	
Specific conditions or restrictions for use (if appropriate)	-

Specific conditions or restrictions for handling (if appropriate)	Use face mask and rubber gloves and use goggles in dusty conditions (see MSDS)
Post market monitoring (if appropriate)	Tracking & tracing will be in full place, all remarks during the use of Availa®Cr will be noticed and recorded
Specific conditions for use in complementary feedingstuffs (if appropriate)	Availa®Cr can be mixed in feeds, dosage to be calculated based on the table above

Maximum Residue Limit (MRL) (if appropriate)			
Marker residue	**Species or category of animal**	**Target tissue(s) or food products**	**Maximum content in tissues**
Non applicable	Non applicable	Non applicable	Non applicable

ASSESSMENT

Availa®Cr contains 3 % trivalent chromium in a complexed form (hexacoordinate chelate complex of single trivalent chromium atom with three molecules of the amino acid methionine as the chloride salt). The additive is intended to be used as a source of the trace element chromium(III) in animal nutrition. The applicant is seeking authorisation to use Availa®Cr in feed for all animal species and categories without withdrawal period at maximum contents between 0.4 and 1.6 mg added chromium kg^{-1} complete feedingstuff.

In the EU, chromium is authorised to be added to food in its trivalent oxidation state as chromium chloride and its hexahydrate and as chromium sulphate and its hexahydrate; maximum amounts to be added to food as food supplements are expected to be set in 2009 (Regulation (EC) No 1925/2006).[5] In October 2008, the RDA 40 µg for Cr(III) in the EU was set (Commission Directive 2008/100/EC).[6]

Trivalent chromium is not authorised as a feed additive in the EU. Total chromium was targeted in feed monitoring plans as an undesirable substance in some EU countries (e.g. Italy). Therefore, the FEEDAP Panel considered it essential to make a general review of chromium(III) as trace element in animal nutrition before assessing the specific application for Availa®Cr.*

1. Part I: General assessment of chromium(III) as trace element in animal nutrition

1.1. Chromium(III) as trace element

Chromium (Cr) is a metallic element which can exist in a variety of oxidation states, but others than 0, +2, +3 and +6 are uncommon. Biologically, trivalent (III) and hexavalent (VI) Cr are the most important. Cr(III) is ubiquitous in nature, occurring in air, water, soil and biological materials, while Cr(VI) compounds are generally believed to be man-made and do not occur naturally in the environment. Feed and food contain Cr in both inorganic forms and organic complexes. However, the precise speciation of dietary Cr compounds (according to oxidation states and ligands) is not known (Metze et al., 2005). Chromium(VI) is well-known to be a strongly oxidising agent and a potent carcinogen (Hamilton and Wetterhahn, 1986).

The biological role of Cr(III) as a trace element for animals was suggested already in the late 50's of the former century (Schwartz and Mertz, 1959). Chromium(III) had been considered as an essential trace element since the seventies when a significant improvement in glucose tolerance in two patients on long-term total parenteral nutrition (TPN) was reported after the addition of Cr-chloride to the TPN-solution (Jeejeebhoy et al., 1977; Freund et al., 1979). The 'essentiality' status of Cr(III) in humans was subsequently questioned (Anonymous, 1988). In 1996, the World Health Organisation classified Cr(III) as an essential trace element (WHO, 1996). Later on, the demonstration of Cr(III) essentiality, based almost entirely on case reports of five patients given Cr(III) via TPN (Jeejeebhoy et al., 1977; Freund et al., 1979; Brown et al., 1986; Verhage et al., 1996; Tsuda et al., 1998), was critically reviewed by Stearns (2000) because of the general health status of patients involved with TPN and other uncertainties. More recently, the essentiality of Cr(III) has been seriously questioned based on the current criteria required for essential elements; the current best classification of Cr(III) would be that of a nutritionally or pharmacologically beneficial element (Nielsen, 2007).

[5] OJ L 404, 30.12.2006, p.26
[6] OJ L 285, 28.10.2008, p. 9
* In compliance with the terms of Article 18 of Regulation (EC) No 1831/2003, Part II of this opinion will be made available at a later stage.

1.2. Kinetics of chromium(III)

1.2.1. Absorption and transport

It is generally known that Cr(III), contrary to Cr(VI), enters the cells with a very low efficiency. Cr(III) is absorbed in the upper small intestine (Chen et al., 1973). Dowling et al. (1989) reported that inorganic Cr(III) was absorbed by a non-saturable passive diffusion process. *In vitro* studies indicate that absorption of dietary Cr from unsupplemented human diets ranges from 0.4 to 1.6 % (Garcia et al., 2001). The dialyzable Cr fraction was estimated to be about 4.6 %, this being considered an indicator of maximum Cr absorption (Velasco-Reynold et al., 2008).

The average absorption rate of Cr naturally contained in food, estimated on the basis of metabolic balance studies or on urinary excretion, is considered to be in a range of 0.4 to 2.5 % (Anderson and Kozlovsky, 1985; EC, 2003a). The estimated absorption rate was inversely proportional to the level of Cr intake (Anderson and Kozlovsky, 1985).

In rats fed diets supplemented with Cr-chloride, chromium showed an apparent absorption rate of up to 0.9 %, while that for organic Cr sources, such as Cr-nicotinate, Cr-picolinate, Cr-complex of dinicotinic acid-diglycine-cysteine-glutamic acid, was 1.3, 1.1 and 0.6 %, respectively (Anderson et al., 1996). The absortion rate of Cr-chloride in man ranges from 0.1 to 0.4 %, whereas that of Cr-picolinate is 2.8 % (EC, 2003a). The Cr absorption rate from Cr-brewer yeast in humans was estimated to be in a range of 5 to 10 % (Mertz and Cornatzer, 1971). The absorption rate of Cr(III) from Cr-polynicotinate, Cr-nicotinate-glycinate and Cr-picolinate was several times higher than that from Cr-chloride, as indirectly estimated from urinary excretion of Cr in human volunteers (DiSilvestro and Dy, 2007). Chromium(III) sources in the form of propionate or amino acid chelates are also suggested to have a higher absorption rate than inorganic Cr(III) (Ohh and Lee, 2005).

There are many dietary factors affecting Cr(III) absorption. Starch, simple sugars, ascorbic acid, oxalate, nicotinic acid and organic acids were shown to increase Cr(III) absorption rate (Chen et al., 1973; Dowling et al., 1989; Kozlovsky et al., 1986; Offenbacher, 1994; Samanta et al., 2008; Seaborn and Stoecker, 1989; Urberg and Zemel, 1987). Some amino acids and histamine were also reported to result in a higher Cr absorption rate (Mertz et al., 1965). Habitual consumption of acetylsalicylic acid derivatives enhanced Cr absorption (Davis et al., 1995), while higher phytate, calcium, manganese, titanium, zinc, vanadium and iron inhibited Cr absorption (Chen et al., 1973; Hill, 1975; Mertz, 1970).

More than 99 % of Cr(III) absorbed in the blood appears in plasma. Iron-binding proteins are involved in chromium binding, transport and storage (Feng et al., 2003). Both *in vitro* and i*n vivo* studies with Cr-chloride on rats showed that approximately 90 % of Cr(III) in the serum was associated with β-globulin fraction and 80 % of all Cr was bound to transferrin. Other proteins such as albumin, γ-globulins and lipoproteins in plasma can also bind Cr(III) when excessive amounts are given (Hopkins and Schwarz, 1964; Brock, 1985). When the saturation of transferrin with iron increases over 50 % in blood, iron may competes with Cr(III) binding and thus affects the Cr(III) transport (Sargent et al., 1979; Lim et al., 1983). The transport of Cr(III) via the cell membrane of target tissues provided by transferrin is insulin-sensitive (Clodfelder and Vincent, 2005).

1.2.2. Distribution and excretion

Using the plasma disappearance curve after administration of $^{51}CrCl_3$ to rats, a three compartment model with three half-lives of 0.5, 5.9 and 83.4 days was suggested (Mertz et al., 1965). Subsequent studies reported similar results in man and rat (Hopkins, 1965; Onkelinx,

1977; Jain et al., 1981). Chromium(III) was mainly accumulated in liver, moderately accumulated in kidneys, spleen and muscle. It is also found in many other organs, such as heart, pancreas, lung, bone and brain (Feng et al., 1988; Feng, 2007). It has been shown that some tissues, such as bone, testes and epididymides, retain chromium longer than the heart, lungs, pancreas or brain (Borel and Anderson, 1984). No equilibrium between tissue deposits of Cr and plasma Cr level exists (NRC, 1997).

Cr(III) is mostly excreted via urine, with only small amounts being eliminated through the bile and perspiration (Feng, 2007). The information on renal handling of Cr(III) is rather scarce. Former studies suggested that 5 to 40 % of plasma Cr(III) were ultrafiltrable and that 70 to 95 % of Cr(III) filtered in glomeruli was reabsorbed by renal tubules (Donaldson and Rennert, 1981). However, based on equal amounts of Cr(III) filtered and excreted, it was later suggested that the predominant mechanism controlling Cr(III) excretion would be glomerular fitration (Donaldson et al., 1984). In a Cr-loaded transferrin transportation study on rat, the single ^{51}Cr-containing species was identified as Low Molecular Weight Cr-binding Substance in urine. Cr-transferrin and urinary chromium were not found in direct equilibrium, thus indicating that intermediates are involved in the transport of Cr(III) (Clodfelder and Vincent, 2005).

Urinary Cr(III) excretion can be increased in humans by high sugar intake, exercise, physical trauma, pregnancy and lactation (Anderson, 1989; Rubin et al., 1998). Insulin treatment has been also shown to increase urinary excretion of Cr(III) in rats (Clodfelder et al., 2001).

1.3. Biological effects of chromium(III)

1.3.1. Mode of action of chromium(III)

Chromium(III) is a well-known factor potentiating insulin-dependent glucose entry into the cells. Currently, the predominant hypothesis on the Cr(III) action is the chromodulin-mediated role on the insulin-activated glucose uptake by cells. In this model, the Cr(III) action on insulin receptors is carried out by a Cr-oligopeptide complex with a molecular weight of about 1500 Da, (Yamamoto et al, 1989), often referred to as chromodulin. Chromodulin, which is capable of binding four equivalents of Cr(III), occurs in its apoform in both cytosol and nucleus of insulin-sensitive cells. In a first step, insulin activates both insulin and transferrin receptors, which results in the transfer of Cr-binding transferrin into the cells with the subsequent release of metal ions. Four equivalents of Cr(III) bind to apochromodulin and change it to the active holo-form of chromodulin, which in turn binds to the active site of the insulin receptor, further activating its kinase activity. In such a way, the Cr(III) ions are thought to activate the insulin receptors and amplify the insulin signal. The result is the induction of the cascade of intracellular signals stimulating the expression of the glucose transporter, Glut 4, and the increase of glucose uptake by cells (Chen et al., 2005). When the insulin level in blood drops, holochromodulin, with bound Cr ions, is released from the cell membrane into the bloodstream and, subsequently, Cr is excreted in the form of chromodulin by the kidney (Vincent, 2001; Clodfelder and Vincent, 2005).

Nevertheless, to this date the attempts to characterise chromodulin have not been successful and that is why the experimental results with Cr(III) supplementation could also be interpreted as non-specific bindings of Cr(III) to carboxylate-rich stretches of amino acid sequences. The finding of nine different types of high molecular weight Cr-containing proteins in rat liver, with masses ranging from 4 to 97 kDa (Feng et al., 2003), also supports the view of rather non-specific bindings of Cr(III) by proteins. Recently, a novel Cr(III) binding protein (15.6 kDa) was isolated from bovine liver tissue after Cr(VI) exposure, further suggesting on non-specific process of Cr(III) binding by proteins (Peterson et al., 2008). In addition, the effect of

chromodulin lacks the experimental evidence of specificity in stimulating tyrosine kinase activity. It has been shown that stimulation of the kinase activity of the insulin-activated insulin receptor can be done by several metals, such as zinc, divalent manganese, cadmium and some drugs (Stearns, 2007). In the case of zinc, it has been demonstrated that the increased phosphorylation state of tyrosine kinases is caused by the inhibition of protein tyrosine phosphatases (Haase and Maret, 2005).

Based on demonstrated oxidation of Cr(III) *in vitro* in the presence of hydrogen peroxide, chlorite (ClO^-), glucose oxidase and xanthine oxidase (Levina et al. 2003), Levina et al. (2007) proposed another role for chromodulin in the stimulation of glucose cell entry. They suggest that the inhibition of phosphatases by Cr(V) and Cr(VI) as products of potential trivalent chromium oxidation *in vivo* may play a key role in insulin-dependent glucose entry into the cells.

Besides the effects on blood glucose clearance, Cr(III) via insulin action is thought to participate also in the protein metabolism by stimulating the amino acids uptake by cells (Evans and Bowman, 1992). The increased delivery of Cr(III) into the cells increases the entry of blood glucose into the cells, which may be associated with some changes in lipid profile of plasma too. Several studies on ruminants (Bunting et al., 2000; Kegley et al., 2000; McNamara and Valdez, 2005), poultry (Debski et al., 2004) and rats (Lai et al., 2006; Bennett et al., 2006) indicate that high levels of dietary Cr(III) may result in decreased total blood cholesterol, LDL-cholesterol, triglycerides and non-esterified fatty acids concentrations in serum while the level of serum HDL-cholesterol can be increased.

Chromium(III) supplementation in farm animals tended to decrease serum cortisol levels in several species (Lindemann, 2007). Nevertheless, there is still no clear-cut evidence for the association between Cr(III) and cortisol as indicator of stress in animals (Pechova and Pavlata, 2007). Some authors have suggested also the possible role of Cr(III) in supporting animal immunocompetence, but to this date the evidence appears to be sparse and rather inconsistent (Shrivastava et al., 2002).

In male rats, oral Cr(III) at dose levels $\geq$ 1 mg kg^{-1} bw was shown to elicit a significant, dose-related trend towards reduced serum leptin (Bennett et al., 2006). Cr(III) supplementation was shown to reduce the plasma level of leptin also in humans (Inanc et al., 2006). Thus, if such effects are confirmed in other species as well, Cr(III) biological activity could have a broader range than that previously recognised.

1.3.2. Evaluation of evidence of chromium deficiency and essentiality

Studies on rats using yeast- or sucrose-based diets to induce dietary Cr deficiency have shown very inconsistent results in terms of impaired glucose tolerance, elevated circulating insulin level, insulin insensitivity and fasting glycemia (Mertz and Roginski, 1969; Jain et al., 1981; Donaldson et al., 1985; Flatt et al., 1989; Striffler et al., 1998). However, there is no actual proof that the diets used in those studies, especially in the ones carried out before 1980, were really low in Cr(III). In addition, the effects of some other dietary factors cannot be excluded (Vincent and Stallings, 2007).

To date no experimental evidence of Cr deficiency has been demonstrated. Symptoms of Cr deficiency in farm animals have not been recognised in field conditions. Consequently, no Cr(III) requirements could be established so far.

In human patients on long-term TPN, the signs of glucose intolerance, hyperglycemia and increasing insulin insensitivity were observed and interpreted as Cr deficiency. The inclusion of Cr(III) in the form of Cr-chloride to TPN resulted in an amelioration of diabetes symptoms in those patients (Jeejeebhoy et al., 1977; Freund et al., 1979; Brown et al., 1986; Verhage et

al., 1996). In a study applying short-term Cr(III) supplementation, only two out of five patients on TPN showed a benefit induced by Cr(III) addition (Wongseelashote et al., 2004). The interpretation of the above findings as evidence of Cr(III) deficiency and essentiality in humans has been questioned due to the severe health problems of subjects involved in those studies, the uncertainties about Cr background in TPN solutions (Anonymous, 1988; Stearns, 2000) and the low rate of patients reacting positively to Cr(III) administration (Stearns, 2007).

The FEEDAP Panel follows the definition of Stearns (2000) for the essentiality of a trace element: 'it must be naturally present in the body and have a demonstrated function. A deficiency of the element will result in a disease or some impairment of function. Symptoms of the disease or function impairment induced by deficiency should be reduced by the return of physiologically relevant amounts of the substance to the diet.'

Despite the extensive research on Cr(III) in man and animals, the FEEDAP Panel considers that at present there is no conclusive evidence supporting essentiality or non-essentiality of Cr(III) as trace element. Following similar considerations, Nielsen (2007) concluded that 'the best classification for chromium is that of a nutritionally or pharmacologically beneficial element.'

1.4. Occurrence of chromium in feedingstuffs and food of animal origin

1.4.1. Issues in the analytical determination of chromium in biological matrixes

The determination of Cr in biological matrices at naturally-occurring concentrations, i.e. 0.1-200 ng g^{-1}, is rather demanding due to the risk of sample contamination and the need for analytical techniques with a high detection power (Veillon and Patterson, 1999; Cubadda et al., 2003). Furthermore, such techniques, e.g. graphite furnace atomic absorption spectrometry or inductively coupled plasma mass spectrometry, are prone to serious interferences. The technical problems encountered in the analytical methods for the determination of Cr have been compiled by the US Committee on Minerals and Toxic Substances in Diets and Water for Animals (NRC, 2005). During the collection and preparation of samples, it is essential to minimise contamination from dust and stainless steel material; reagents used in the analyses must be of the highest purity; losses of Cr can occur during heating or acid digestion of samples in open systems. Therefore, the use of laboratory, controlled conditions, the avoidance of interferences and appropriate quality control measures are needed to achieve reliable results. The increasing awareness of analytical quality assurance issues has certainly improved the reliability of Cr determinations performed by laboratories; still, the range of Cr concentrations found in biological matrixes reported in the literature is extremely wide and largely exceeds that accounted for by the biological variability (Veillon and Patterson, 1999; Engman and Jorhem, 1998).

Speciation of Cr is most often limited to the quantification of the two main oxidation states (III and VI) in water and other liquid matrices. Chromium speciation in food and feed is analytically challenging because of the extraction procedure, which is problematic concerning recovery as well as species conversion. Accordingly, very few attempts have been made in this field and no established methods exist (Metze et al., 2005).

The following data refer to total chromium. Quantitative figures can only be considered taking into account those analytical caveats.

1.4.2. Occurrence of chromium in feedingstuffs

Chromium(III) and Cr(VI) are taken up by plants: Cr(VI) is taken up actively by a sulphate carrier and immediately converted to Cr(III) in roots; in contrast, Cr(III) is taken up passively, being retained by the ion-exchange sites of the cell walls (Scoccianti et al., 2006). Chromium concentration in plants associated with the root is greater than in the leaf, which in turn is greater than in the fruit (Cary and Kubota, 1990); the Cr content in whole cereals is mostly concentrated in pericarps (Plessi and Monzani, 1990).

Selected data on total Cr content in feed are summarised in Appendix A (Table A.1). The available literature on feedingstuffs is limited and shows that Cr content is highly variable. In addition to natural environmental sources of Cr, the technological processing of feed materials may contribute to feedingstuffs contamination (EC, 2003b). However, part of this variability could also result from analytical shortcomings due to the difficulty of obtaining reliable results for Cr determination in complex matrices. In feed raw materials, high Cr values have been detected in some feed grade phosphates and mineral mixtures. Total Cr measured in compound animal feed was in the range of 0.2 to 7 mg kg^{-1}; in mineral mixtures, Cr values are usually considerably higher, ranging from 1.5 to 220 mg kg^{-1} (Appendix A: Table A.2).

The above data refer to total Cr, but it can be assumed that the only naturally-occurring form of Cr in feed is Cr(III). Soluble Cr(VI) compounds are unstable and readily reduced to Cr(III) in the presence of electron donors such as organic matter or reducing inorganic compounds, especially at an acidic pH (Metze et al., 2005). Notwithstanding this, two reports on the detection of Cr(VI) in animal feed and milk, respectively, at levels of about one tenth of total Cr, are available in the literature (Soares et al., 1994; Lameiras et al., 1998). However, those results have been obtained without a selective species detection method (i.e. an analytical method able to separately measure the extracted Cr species) and thus await confirmation from further studies.

1.4.3. Occurrence of chromium in food of animal origin

Chromium is generally present in tissues, animal products, plasma and urine in the µg kg^{-1} or µg L^{-1} range (NRC, 2005). In muscle, Cr levels normally range from some µg kg^{-1} to a few tens of µg kg^{-1}, even though concentrations as high as 154 µg kg^{-1} have been reported (Appendix A: Table A.3). Differences in the Cr content of edible tissues between the animal species cannot be assessed from the available data due to the limited database and the high data variability. Chromium concentration in fish flesh is generally similar to that of terrestrial animals, whilst higher levels are found in bivalve molluscs (Appendix A: Table A.3). Based on the limited data available, Cr levels in eggs seem to be comparable to those in meat, whereas other animal products such as honey and milk contain very little Cr. In dairy products, the Cr levels usually found are generally higher compared to milk due to the lower water content and processing changes.

1.4.4. Influence of supplemental chromium(III) on deposition in animal tissues and products

In animals fed diets supplemented with Cr(III), the resulting contents of Cr in tissues are generally considered to vary according to the form of the dietary supplementation, reflecting the differences in bioavailability of the various sources of Cr(III) (Lindemann, 2002; Ohh and Lee, 2005). It has been shown that the mean bioavailability in terms of tissue deposition for Cr-propionate, Cr-methionine and Cr-yeast in pigs, expressed in relative values to Cr-picolinate, is 13.1, 50.5 and 22.8 %, respectively (Lindemann et al., 2008); nevertheless, these values were

obtained with a supplementation dose of 5 mg Cr(III) kg^{-1} feed, which is 25 times higher than that currently used as inclusion level for pigs in North America. However, a recent work of Kottwitz et al. (2008) demonstrated that in rats the final whole body retention of Cr from Cr-chloride was more than twice that from Cr-picolinate despite the considerable higher absorption of Cr from Cr-picolinate compared to that from Cr-chloride. Those authors found that due to the absorption of Cr-picolinate in the form of intact molecule, its main part was directly excreted by kidney before the degradation in the liver can occur.

To date, no information on Cr speciation in tissues of animals supplemented with various Cr(III) sources is available.

Five tables in Appendix B present the available data on Cr levels in animal tissues, published in peer-reviewed journals. In general, the measured values show very large variations, not only among species but also within species. As illustrated, even the Cr levels in tissues from unsupplemented groups show a very large scatter, sometimes even of order of magnitudes. This is mainly due to difficulties in the methods of Cr analysis (see Section 1.4.1).

In cattle for fattening, the available data do not show any significant effects of Cr(III) supplemented on Cr levels in muscle, liver and kidney (Appendix B: Table B.1; Chang et al., 1992; Spears et al., 2004). Only one available study on milk deposition of Cr (Hayirli et al. 2001) indicates no response in milk Cr concentration to Cr(III) supplemented at three doses (0.22, 0.39 and 0.83 mg Cr kg^{-1} complete feed) in dairy cows.

In pigs, the studies indicate some differences in Cr tissue deposition according to the source of Cr(III) supplemented; for Cr-picolinate, the dose-response pattern could also be observed (Lindemann et al., 2004). While Cr levels in muscle do not appear to change with the supplementation of several inorganic or organic Cr(III) sources at doses of 0.2 mg kg^{-1} complete feed (for Cr-picolinate, even at 0.3 mg kg^{-1}), the Cr contents of liver and kidney were enhanced by some of them (Cr-acetate, Cr-oxalate and Cr-picolinate) (Appendix B: Table B.2; Ward, 1995; Anderson et al., 1997a). Nonetheless, Cr(III)-nanoparticles, already at doses of 0.2 mg Cr(III) kg^{-1}, significantly increased Cr concentration in all tissues investigated (Wang and Xu, 2004). The validity of available pig data is limited due to the severe discrepancy in the results of Cr distribution between muscle and other tissues of unsupplemented animals.

The data from poultry studies (Appendix B: Table B.3) are inconsistent due to the extremely wide range of measured values already found in tissues of unsupplemented birds. Nevertheless, a dose-response pattern of Cr deposition appears in muscle, liver and kidney of turkeys and Japanese quail given feed with rising levels of Cr-chloride and Cr-picolinate (Anderson et al., 1989; Sahin et al., 2002; Uyanik et al, 2005). Data on Cr deposition in eggs from laying hens (Dębski et al., 2001; Piva et al., 2003) and from laying turkeys (Anderson et al., 1989) supplemented with Cr-chloride, Cr-yeast or Cr-aminoniacinate show rather inconsistent results.

Experiments on minor species such as rabbits (Appendix B: Table B.4; Sahin et al., 2001) fed diets supplemented with Cr(III) in the form of Cr-chloride do not show any significantly increased Cr deposition in edible tissues resulting from additional dietary chromium. The only available study on fish shows a dose-dependent response in Cr liver content of rainbow trouts given rising doses (0.4, 0.8 and 1.2 mg kg^{-1} feed) of Cr-picolinate (Kucukbay et al., 2006). No data on fish flesh were available.

The studies on laboratory animals such as rats and mice supplemented with both inorganic and organic Cr(III) forms (Appendix B: Table B.5; Anderson et al., 1996; Anderson et al., 1997b; Zha et al., 2007) demonstrated that the primary tissues showing increased Cr deposition were kidney and liver, as a response to all given Cr(III) forms, while the levels of Cr in muscle were only enhanced by some organic Cr(III) sources and Cr(III)-nanoparticles.

1.4.5. Conclusions

Despite the advances in the analytical techniques, many uncertainties surrounding the determination of total Cr remain. In general, the resulting analysed Cr values in biological samples show sometimes very wide scatter which apparently may be ascribed largely to the analytical inadequacies.

Plants sources make only a limited contribution to the total Cr in compound feedingstuffs; the greater part comes from mineral sources.

Concerning foods of animal origin, the higher levels of Cr are found in offals, followed by muscle tissue, fish flesh and eggs. Other animal products contain very little Cr.

The available data did not allow a consistent pattern of tissue deposition to be identified in farm animals given feed supplemented with Cr sources.

1.5. Chromium(III) dietary supplementation in farm animals

Many studies dealing with the zootechnical effects of dietary supplementation of various sources of Cr(III) in farm animals have been published in the last decades. Mainly the effects of supplemented Cr-chloride and organic sources of Cr(III), such as Cr-picolinate, Cr-nicotinate, Cr-propionate, Cr-yeast or amino acid chelates of Cr(III), were studied.

In 1997, the US National Research Council (NRC) could not give specific recommendations concerning the Cr(III) source and supplementation levels for cattle, poultry and swine (NRC, 1997). Currently, two additives based on trivalent chromium in the form of chromium picolinate or chromium propionate are recognised by the Association of American Feed Control Officials (AAFCO),[7] being restricted for use in swine i doses of up to 0.2 mg Cr(III) kg^{-1} diet. In 2005, chromium L-methionine complex was adopted by AAFCO to supply not more than 0.4 mg Cr(III) kg^{-1} diet for swine, but in 2007 it was removed from AAFCO list.[8] In August 2007, the Food and Drug Administration's (FDA) Center for Veterinary Medicine (CVM) signed a Memorandum of Understanding with AAFCO that allows FDA to formally recognise the Association's list of feed ingredients and defines the role that FDA can play in deciding on the suitability of feed ingredients offered for addition to the list.[9] In Canada, chromium(III) in the form of chromium yeast is currently registered as a feed additive for first lactation dairy cattle only at a maximum supplementation Cr(III) level of 0.4 mg kg^{-1} feedingstuff, while chromium propionate is temporary approved for growing and finishing pigs at a level that would not supply more than 0.2 mg Cr(III) kg^{-1} complete swine feed.[10]

In cattle, Cr(III) supplementation in the form of Cr yeast at doses up to 1 mg Cr kg^{-1} feed resulted in reduced morbidity of feeder-calves and improved the immune status of dairy cows. Improved growth rate could be observed only in animals showing poor performance due to various stress factors (Mowat et al., 1993; Moonsie-Shageer and Mowat, 1993; Mowat, 1997). In general, no significant effects on performance or carcass traits in calves supplemented with Cr yeast (0.2 mg Cr kg^{-1}) or Cr-picolinate (0.8 mg Cr kg^{-1}) were observed (Chang et al., 1992; Swanson et al., 2000; Bedsong et al., 2001; Stahlhut et al., 2006). In herds with recognised history of placenta retention, the diet enrichment with Cr-picolinate (3.5 mg Cr day^{-1}) significantly reduced the incidence of this disorder (Villalobos et al., 1997). In summary, it has been proposed that the

[7] Official Publications Ref. 57.155 and 57.160
[8] AAFCO 97th Annual Meeting, August 1-4, 2007. p. 22., Ref. T57.164
[9] http://www.fda.gov/OHRMS/DOCKETS/98fr/fda225-07-7001-mou0001.pdf
[10] Canadian Food Inspection Agency, Animal Feed Division, Reg. # 990534, Reg. # 982488T and Reg.# 982489T

zootechnical effects of Cr(III) supplements would mostly appear only in animals under stress conditions such as regrouping, initiation of lactation, transportation or immune challenge (Lindemann, 2007; Pechova and Pavlata, 2007).

In pigs, Cr(III) supplementation of growing animals showed very inconsistent response in terms of improvement of feed efficiency and carcass composition (lean body mass, muscling and fat depth), as given by Lindemann (2007) reviewing 18 experiments using Cr-picolinate, Cr-propionate and Cr-yeast at doses up to 0.4 mg Cr kg^{-1} complete feed. Nevertheless, the uniformly consistent effects such as increased litter size and reduced number of stillborn piglets occur when sows are supplemented with Cr-picolinate or Cr-methionine at dose of 0.2 mg Cr kg^{-1} complete feed (Lindemann et al., 1995, 2004; Hagen et al., 2000; Perez-Mendoza et al., 2003).

In poultry, studies using supplementation with Cr-picolinate, Cr-yeast and Cr-nicotinate at doses up to 0.8 mg Cr kg^{-1}feed did not show consistent effects on performance and carcass traits in chickens or turkeys for fattening (Debski et al., 2004; Holoubek et al., 1997; Hossain et al., 1998; Kim et al., 1996; Lee et al., 2003; Lien et al., 1999; Wang et al., 2003; Ward et al., 1993). Similarly to cattle, birds under stress conditions tend to show reduced mortality. As reviewed by Lindemann (2007), the only relatively consistent effects of Cr(III) supplements occur in serum lipid profile and reduced egg cholesterol (Amatya et al., 2004; Dębski et al., 2001; Lien et al., 1996; Lien et al., 2003, 2004; Samanta et al., 2008).

Limited available data from fish (trout, *Oncorhynchus mykiss*; hybrid tilapia, *Oreochromis niloticus* X, *Oreochromis aureus*; gilthead seabream, *Sparus aurata* L.) did not show any significant effects of Cr-picolinate or Cr yeast (doses up to 2 mg Cr kg^{-1} feed) on performance parameters in tilapia and gilthead seabream (Pan et al., 2003; Gatta et al., 2001). In rainbow trout, feed supplementation with 1.6 mg Cr kg^{-1} feed in the form of Cr-picolinate reduced serum cholesterol level (Kucukbay et al., 2006).

1.5.1. Conclusions

The available data do not allow the establishment of Cr(III) requirements in animal nutrition.

Conditions affecting health/welfare and/or metabolic challenges generally appear to favour the occurrence of beneficial effects of supplemental Cr(III) such as reduced mortality. However, the majority of the zootechnical parameters measured in animals supplemented with Cr(III) show very inconsistent results, with a few studies suggesting negative effects in terms of compromised production parameters at relatively low supplementation dose (see Section 1.6).

The inconsistencies seen between the studies might be ascribed to (i) the wide range of natural Cr background in feed components, its different forms, sources and levels, and consequently different bioavailability of Cr, (ii) other dietary factors (e.g. phytate, ascorbic acid, organic acids, and other trace elements) and (iii) the health condition and physiological state of the animals.

1.6. Safety for farm animals

Episodes of acute toxicity in food-producing species are seldom encountered, mainly because of the low solubility and bioavailability of Cr compounds, including the oxides which are among the most common sources of Cr in the environment. Chromium oxide (Cr_2O_3) has been used for decades as a digestibility marker in cattle, sheep and pigs at levels up to 3000 mg Cr kg^{-1} feed without signs of acute toxicity.

The maximum tolerable levels for Cr(III) was given at 3000 mg kg^{-1} from Cr-oxide for all species by the US NRC (2005). For more soluble forms of Cr(III), the maximum tolerable level was given at 500 mg kg^{-1} for poultry and 100 mg kg^{-1} for mammals.

1.6.1. Cattle

After accidental poisoning of veal/calves with an unknown quantity of Cr_2O_3 from dyestuffs, lethargy, increased respiratory and cardiac frequencies, diarrhoea and abdominal distress, paresis and paralysis were observed as symptoms, besides high mortality. At necropsy, catarrhal enteritis, greenish faeces, liver and kidney degeneration, and cerebral oedema were found. (Farina et al., 1991).

The average daily weight gain in steers was not affected by the daily exposure to a basal diet containing 0.66 mg total Cr kg^{-1} feed DM (fortified with 0.4 mg Cr(III) kg^{-1} as Cr-chloride, yeast or nicotinate) for 56 days (Kegley and Spears, 1995). By contrast, feedlot steers fed a basal diet (1.3 mg kg^{-1} Cr DM) supplemented with 0.2 or 0.4 mg kg^{-1} Cr DM from Cr yeast for 196 days resulted in a significant reduction of weight gain at the highest level of supplementation (Pollard et al., 2002).

In dairy cows supplemented with Cr(III) from Cr-Met at levels of 0.03, 0.06 and 0.12 mg kg^{-1} $bw^{0.75}$ (doses corresponding in this experiment to 0.22, 0.39 and 0.83 mg Cr kg^{-1} complete feed, respectively) from 28 days before expected calving to 28 days after calving, a reduced milk production was found at the highest supplementation level (Hayirli et al., 2001). In another study, milk yield was not affected by the addition of 6.25 mg Cr day^{-1} as Cr-Met (Bryan et al., 2004), a dosage comparable to the middle dosage used in the formerly described study.

1.6.2. Pigs

There are conflicting reports about the levels of Cr dietary supplementation capable of negatively affecting growth performance. While a number of authors reported no or positive effects on those parameters, negative effects on weight gain of barrows, after supplementing the diet with as low as 0.2 mg Cr (from Cr-picolinate or Cr-propionate) kg^{-1} diet, were described in two studies (Boleman et al., 1995, Matthews et al., 2001). However, in crossbred pigs of each sex administered either a basal diet containing 2075 mg Cr kg^{-1} or a total of 5000 mg Cr kg^{-1} from different sources, and namely Cr-picolinate, Cr-propionate, Cr-methionine or Cr-yeast, for 75 days, average daily gain remained unaffected or even showed a limited but statistically significant increase (Cr-yeast, $P < 0.025$); among the tested serum clinical chemistry parameters, only K, CO_2 and alkaline phosphatase values were significantly different from the control values, with differences related to the source of Cr supplementation (Lindemann et al., 2008) but within what is considered as a normal range for this species (Merck, 2005).

1.6.3. Horses

The addition of 0.4 mg Cr (as Cr-picolinate) kg^{-1} diet resulted after 112 days in a significant reduction of weight gain of yearling horses compared to the unsupplemented group (Ott and Kivipelto, 1999).

1.6.4. Avian species

Chickens appear more tolerant to soluble forms of Cr(III) compared to mammalian species (NRC, 2005).

Piva et al. (2003) could not show adverse effects in laying hens fed with a basal diet containing 3.4 mg Cr kg^{-1} feed after a five-week supplementation with 24.1 mg Cr kg^{-1} feed from Cr-chloride, 36.3 mg Cr kg^{-1} feed from yeast and 47.5 mg Cr kg^{-1} feed from aminoniacinate, respectively.

Metabolic effects consisting in the impairment of a number of cytochrome P450-dependent monooxygenases have been observed in laying hens after feeding 50 mg Cr kg^{-1} feed for 28 days as Cr-chloride, -yeast or -aminoniacinate (Guerra et al., 2002). In contrast, Chen et al. (2001) observed metabolic effects, such as an increase of serum triacylglycerol, uric acid and creatinine, and a decrease in glycerol, α- and β-globulin, in turkeys, at much lower levels (3 mg supplemental Cr from Cr nicotinate kg^{-1} feed, 8–22 weeks of age).

Adult black ducks (*Anas rubripes*) fed diets containing 0, 10 or 50 mg Cr(III) kg^{-1} feed, as potassium chromium sulphate ($KCr(SO_4)_2 \bullet 12\ H_2O$), for five months showed a normal pattern of mortality, reproduction and blood chemistry. However, in ducklings from treated groups that were fed the Cr-enriched diets at original parental dosages, growth patterns were altered and survival was decreased (Eisler, 1986)

1.6.5. Farmed fish

To date, there are apparently no studies available in the literature on the toxicity of dietary Cr(III) in farmed fish.

1.6.6. Conclusions

There is no evidence of adverse effects on farm animals arisen from background levels of total Cr in unsupplemented feed.

Many studies on the zootechnical effects of Cr(III) supplemented to animal feeds (see Section 1.5) support the conclusion reached by NRC (2005) on tolerable Cr(III) levels in feed. Most of the recorded inconsistencies and/or species differences in sensitivity may be due to the source of Cr(III), to the (mostly unknown) Cr(III) dietary background, to other dietary factors influencing Cr(III) bioavailability or to analytical uncertainties.

Very few studies investigated potential toxicity biomarkers in target species. Those studies show signs of intolerance or toxicity at considerable lower levels than those established as tolerable by NRC (2005). However, in view of the limited available data, the FEEDAP Panel is not in a position to define maximum tolerable levels of Cr(III) in feed for farm animals.

1.7. Chromium toxicity

Both predominantly occurring Cr oxidation states, i.e. Cr(VI) and Cr(III), should be considered in the assessment of the general toxicity of Cr present in feedingstuffs; several reasons support the concurrent assessment of toxicological information: (i) both forms are currently considered together as ‘chromium’ in national feed monitoring programmes for undesirable substances in feedingstuffs carried out by EU countries; (ii) a separate assessment of Cr(III) in feed needs comparison with the toxic pollutant Cr(VI), and; (iii) the biological effects of the two forms are linked together. Chromium(III) may be the (or one of the) ultimate intracellular toxic form(s) of

Cr(VI) (Stearns, 2000). Moreover, some data suggest that also the oxidation from Cr(III) to Cr(VI) may occur in biological systems under certain circumstances (Nguyen et al., 2008).

1.7.1. Chromium(VI) toxicity

1.7.1.1. General mechanisms

Chromium(VI) has been known for a long time as the most dangerous form of the metal, mostly because of the recognised high ability in entering cell membranes and its powerful oxidising properties. After entering the cells, Cr(VI) is readily reduced by glutathione (GSH), cysteine and other cellular reductants, as well as cytochrome b5 (Plant, 2003). The generation of reactive oxygen species (ROS), including hydroxyl radical ($OH^{\bullet}$), has been proposed as one of the key events in Cr toxicity and is thought to be directly implicated in the mutagenic activity of the metal (Kortenkamp et al., 1996). Chromium(VI) also inhibits the gene transcription regulated by the metal-responsive transcription factor-1 (MTF1), which plays an important role in the defence against oxidative stress by inducing several key antioxidant genes (Laity and Andrews, 2007; Kimura et al., 2008). Thus, in addition to causing the formation of free radicals, Cr(VI) impairs the cell's defence against oxidative stress. Several *in vitro* and *in vivo* experiments have demonstrated the concentration and time-dependent effects of Cr(VI) on ROS production, lipid peroxidation, DNA fragmentation (see below) and apoptotic cell death; in certain instances, the organic moiety (e.g. picolinic acid) seems to enhance those effects (Bagchi et al., 2002). A similar pattern of cellular effects was observed for Cr(III) (Bagchi et al., 2002). Cr(VI) is reduced intracellularly to Cr(V) and subsequently to Cr(III); this process produces the formation of ROS. Moreover, both Cr(V) and Cr(III) react with DNA and other cellular constituents eliciting cytotoxic events (Levina and Lay, 2004; Sugden and Stearns, 2000; Sugiyama, 1992). There is evidence that the products of intracellular reduction of Cr(VI), including Cr(III), are the ultimate responsible for the toxicity of the metal (Stearns, 2000; Costa and Klein, 2006).

1.7.1.2. Genotoxicity and carcinogenicity

Chromium(VI) is genotoxic without exogenous activation in bacteria, in human and in other cultured mammalian cells, by inducing gene mutations in multiple species and strains of bacteria, and gene mutations, DNA-protein crosslinks, DNA strand breaks, chromosomal aberrations, sister chromatid exchanges, unscheduled DNA synthesis and other forms of DNA damage in mammalian cells *in vitro* (ATSDR, 2000). Chromium(VI) has long been recognised as an animal and human carcinogen (Cohen et al., 1993). Whereas Cr(VI) was thought to cause cancer only in the respiratory tract, Costa and Klein (2006) pointed that it must be considered as a human carcinogen also by ingestion. A review concerning the carcinogenicity of Cr(VI) in drinking water stressed the link with stomach and forestomach cancer in humans and female mice, respectively (Sedman et al., 2006). In two chronic toxicity/carcinogenicity studies on F344/N rats and B6C3F1 mice carried out with sodium dichromate dihydrate in drinking water (NTP, 2008), there was a clear evidence of carcinogenic effects in both species; increased incidences of squamous cell neoplasms of the oral cavity and of tumors of the small intestine were observed in rats and mice, respectively (NTP, 2008). Moreover, Cr(VI) exposure has been associated in humans with an increased incidence of cancers in a number of tissues (renal, urinary bladder, prostate, genital tract, Hodgkin's lymphoma, leukaemia) (Cohen et al., 1993): mounting evidence (Costa and Klein, 2006) points to a systemic carcinogenic action beyond the exposure sites in the respiratory or digestive tract. Nevertheless, the mechanisms underlying Cr(VI) carcinogenicity are not fully understood. Three mechanisms are generally accounted for the Cr(VI)-mediated DNA damage:

1) indirect (i.e. through ROS generation) free radical damage (see Section 1.7.1.1);
2) direct oxidative damage (Sugden and Stearns, 2000); the generated free radicals may activate signalling pathways which inhibit apoptosis in neoplastic cells;
3) direct interaction with DNA to form Cr-DNA adducts and DNA-protein crosslinks, (reviewed in Sedman et al., 2006).

The inter-relationships between the oxidised and the reduced form of the metal (see Section 1.7.1.1) makes it difficult to clearly distinguish the contribution of either Cr form in the mutagenic/carcinogenic process (Costa and Klein, 2006).

1.7.1.3. Other effects

Chromium(VI) compounds produce an allergic contact dermatitis, a delayed-type (class IV) hypersensitivity reaction, characterised by an acute or chronic eczema (Barceloux, 1999). Asthma and related respiratory symptoms in exposed workers have been recorded as the result of inhalation challenge (Olaguibel and Basomba, 1989). Kidney and liver damage have been reported as a feature of acute and prolonged exposure to Cr(VI) (Barceloux, 1999).

Large doses of potassium dichromate (57 mg kg^{-1} bw day^{-1}) in drinking water caused severe fetal toxicity in mice, with increased post-implantation loss, and decreased cranial ossification and fetal weight (Trivedi et al., 1989).

In the chronic toxicity/carcinogenicity studies carried out by the US NTP, a proinflammatory effect of sodium dichromate dihydrate was observed in rats and, to a lower extent, in mice. In rats, histiocytic cellular infiltration in the liver, small intestine and pancreatic and mesenteric lymph nodes was observed at 57.3 mg sodium dichromate dihydrate L^{-1} (equal to approximately 1 mg Cr kg^{-1} bw); the NOAEL was 14.2 mg L^{-1} (equal to approximately 0.25 mg Cr kg^{-1} bw) (NTP, 2008).

Chromium(VI) may act also as endocrine disrupter. When lactating rats received potassium dichromate in drinking water (200 mg L^{-1}, equivalent to 20 mg kg^{-1} bw), the offspring showed delayed puberty, impaired ovarian development and steroidogenesis, as well as reduced synthesis of the pituitary hormones FSH and LH, without any marked signs of general toxicity (Banu et al., 2008).

1.7.1.4. Conclusions

Cr(VI) is an established animal and human carcinogen, also by ingestion. Evidence indicates also a potential for endocrine disruption.

1.7.2. Chromium(III) toxicity

1.7.2.1. General mechanisms

Contrary to Cr(VI), Cr(III) is considered of very low toxic potential, due to its poor intestinal absorption and comparatively low potential to enter the cells (see Section 1.2.1). Nonetheless, Cr(III) is involved in Cr(VI) toxicity mechanism as a consequence of intracellular rapid reduction and trapping in lower valence forms, including Cr(III) (Zhitkovich et al., 1996) (see Section 1.7.1). Evidence of the *in vitro* biological oxidation in extracellular fluids of Cr(III) forms normally included in dietary supplements has been recently provided (Nguyen et al., 2008), resulting in the formation of Cr(VI) species readily taken up by the cell (Levina and Lay, 2008).

In general, Cr(III) has a low order of acute toxicity. As described below, however, excess Cr(III) can induce different adverse effects in a variety of animal species and in humans.

Chromium picolinate has to be considered as special case, since the picolinic acid moiety may exert specific toxic effects and/or exacerbate certain toxic properties of Cr(III) (see Section 1.7.2.2).

1.7.2.2. Adverse effects on metabolism

Interactions with iron and other essential trace elements

Chromium(III) ions are transported by transferrin for tissue distribution and may compete with iron for the same binding site. Thus, Cr(III) may affect iron metabolic pathways, such as heme synthesis, by making this essential metal less available (EVM, 2003). In rats treated with Cr(III) by the intraperitoneal route, signs of iron deficiency and anaemia were elicited (Ani and Moshtaguie, 1992; Lukaski, 1999). In contrast, rats fed diets containing 5 mg Cr kg^{-1} from Cr-nicotinate and Cr-acetate (equivalent to 0.25 mg Cr kg^{-1} bw) showed excess accumulation of iron in the liver compared to other rats fed 0.030 mg background Cr kg^{-1} bw, while in spleen all the Cr(III) sources tested (chromic chloride, chromic acetate, chromic potassium sulphate, chromium dinicotinic acid-diglycine-cysteine-glutamic acid complex, chromium-dinicotinic acid-dihistidine, chromium trihistidine, chromium triglycine, chromium tripicolinic acid, chromium trinicotinate) increased the iron concentration (Anderson et al., 1996). Little is known about the occurrence of similar effects in farm animals. However, an increase of more than 23 % ($P < 0.05$) in iron kidney content over control animals was noticed in barrows fed a basal diet (about 0.26 mg Cr kg^{-1} feed) fortified with a dose as low as 0.30 mg Cr kg^{-1} from Cr-picolinate (Anderson et al., 1997a). Part of this effect, however, might be ascribed to the picolinic moiety, which has been reported to disrupt iron metabolism in cultured rat kidney cells (Fernandez-Pol, 1977).

Chromium(III) may interfere also with selenium. Chromium(III) nitrate at the oral dose of 10 mg Cr kg^{-1} bw counteracts the anti-carcinogenic action of Se on the growth and latency time of virus-induced mammary tumours in mice; the effect was apparently related to reduced Se tissue retention (Schrauzer, 2006).

Effects on lipid metabolism

Chromium(III) can be a significant modulator of lipid metabolism (see also Section 1.3.1). Male rats treated orally with 1, 5 or 10 mg Cr(III) kg^{-1} bw day^{-1} from a nitrate salt of Cr(III) propionate complex for ten weeks showed a number of biochemical changes at study term, without any evident sign of toxicity. Insulin was lowered at ≥5 mg kg^{-1}, triglycerides were reduced at all dose levels and leptin showed a dose-related modulation, increased at 1 mg kg^{-1} and reduced at higher dose levels (Bennett et al., 2006). It is uncertain whether the observed changes might be regarded as potentially adverse, also because no standard indicators of toxicity, such as histopathology, were examined. However, the study indicates that the metabolic parameters relevant to lipid metabolism are affected in rodents by oral exposure to 1 mg Cr(III) kg^{-1} bw.

1.7.2.3. Genotoxicity and carcinogenicity

Chromium(III) interacts with DNA *in vitro* (Bridgewater et al., 1994a). Under those conditions, Cr(III) was shown to produce DNA-protein crosslinks, to modify the fidelity and kinetics of DNA replication (Snow, 1994) and to cause guanine-specific polymerase arrest (Bridgewater et al., 1994a, 1994b). Binary and ternary Cr(III)-DNA complexes are mutagenic in human cell

systems (Voitkun et al., 1998; Zhitkovich et al., 2001; Quievryn et al., 2002) and may be involved in the genotoxic action of the metal. The major DNA adducts formed are ternary complexes of Cr and amino acids and glutathione (GSH) (Zhitkovich et al., 1996). Similar to Cr(VI), oxidative stress, and namely the generation of $OH^{\bullet}$ via a Fenton-driven reaction with hydrogen peroxide, has been indicated as one of the mechanisms responsible for Cr(III)-mediated DNA damage (Valavanidis et al., 2005); the reduction in DNA adduct formation (isolated calf thymus DNA) caused by the combined exposure to Cr(III)-H_2O_2 and various free radical scavengers would lend further support to this hypothesis. However, *in vitro* experiments with human keratinocytes performed with various Cr(III) supplements (histidinate, picolinate and chloride) did not disclose oxidative DNA damage but even pointed to a protective (antioxidant) effect brought about by the metal when cells were concomitantly exposed to hydrogen peroxide (Hininger et al., 2007). The role of the oxidative stress in the Cr(VI)-Cr(III)-mediated mutagenicity was also previously questioned by other researchers (Zhitkovich et al., 2001). DNA adduct formation was pointed out by Arakawa et al. (2006): Cr(III)-DNA- and Cr(III)-histidine-DNA adducts were observed at the p53 gene sequence, an event that could initiate lung carcinogenesis. Maximal plasmid DNA modifications occurred at relatively low Cr concentrations, namely 3 μM Cr for $CrCl_3 \bullet 6H_20$ and 0.5 μM Cr for Cr-histidine. Other papers describing similar effects have also been published (O'Brien et al., 2005, 2006).

Contrary to *in vitro* assays, only scant, mostly outdated and controversial *in vivo* genotoxicity studies are available (EC, 2003a), which does not allow any conclusion. Kirpnick-Sobol et al. (2006) reported that Cr(VI) and Cr(III) (Cr-chloride) has a comparable ability in inducing DNA deletions in the offspring of mice exposed during gestation *via* drinking water. The dams were exposed to very high doses of Cr(III) (1875 or 3750 mg L^{-1}, equivalent to 375 or 750 mg kg^{-1} bw); however, the concentrations measured in the embryos (8 to 20 μg kg^{-1}) were of the same order of magnitude as those normally found in tissues (liver, kidney) of food-producing species fed with Cr(III)-supplemented diets. Notwithstanding several controversial points (e.g. very high doses without any toxicity, unusual protocol), those results point to further investigation on the possible transplacental genotoxicity of Cr(III).

Pre-mating i.p. single exposure of male mice to high doses of Cr (III) as Cr-chloride (52 mg kg^{-1} bw) resulted in an increased frequency of various abnormalities in the offspring, including histological lesions and/or neoplasms to thyroid, adrenals, kidney, liver, ovaries and testes (Yu et al., 1999), which has been related to the ability of the metal to disrupt certain endocrine functions such as corticosterone, glucose, insulin-like growth factor-1 (Cheng et al., 2002) and T3 synthesis (Cheng et al., 2004). Those findings may indicate a potential epigenetic action of Cr(III).

Human data are controversial and concern occupational exposure only. In human lymphocytes of chrome alume workers, no induction of chromosomal aberrations could be observed (ATSDR, 2000). In contrast, although the concomitant exposure to other toxic compounds could not be excluded, Medeiros et al. (2003) reported a statistically significant higher incidence (+50 %, $P < 0.001$) of micronuclei formation in circulating lymphocytes from workers exposed to Cr (III) in comparison to those exposed to Cr(VI). Micronuclei assessment is recognised as a significant biomarker of genotoxic risk in humans (Kirsch-Volders et al., 1997, 2000). It is worth mentioning that autopsy studies of workers who were exposed to insoluble Cr (III) (chromite ores) and died of lung cancer, revealed Cr pulmonary levels ranging from 18 to 2060 μg Cr g^{-1} dry tissue, even after 18 years since the last exposure (Mancuso, 1997).

No epidemiological studies are available on possible adverse effects arising from dietary exposure to Cr(III), including supplements. By applying the pharmacokinetic models that have been used to quantify Cr (III) absorption in humans, Stearns et al. (1995) concluded that long-

term ingestion of (Cr III)-dietary supplements can lead to accumulation and retention of Cr(III) to levels at which DNA damage has been observed *in vitro* and *in vivo*.

A recent two-year feeding study on the carcinogenesis of Cr-picolinate monohydrate performed by the National Toxicologic Program (NTP, 2007) in F344/N rats and in B6C3F1 mice concluded that there was equivocal evidence of carcinogenic activity in male F344/N rats at ≥ 52 mg Cr(III) kg^{-1} bw, based on an increase of preputial adenomas, and no evidence of carcinogenic activity in female F344/N rats up to the top dose of 297 mg Cr(III) kg^{-1} bw, and in B6C3F1 mice up to the top dose of 742 and 689 mg Cr(III) kg^{-1} bw in males and females, respectively.

Two recent reviews assess the potential genotoxic effects of Cr(III) with regard to the possible nutritional benefits coming to opposite conclusions. Eastmond et al. (2008) concluded that there is sufficient evidence that the direct exposure of DNA to Cr(III) causes damage that can lead to mutations in a variety of *in vitro* systems; however, the authors considered that the available *in vivo* evidence suggests that genotoxic effects are very unlikely to occur in humans or animals exposed to nutritional or to moderate recommended supplemental levels of Cr(III). The risks would only be restricted to the prolonged intake of high doses of Cr(III), due to the limited bioavailability of this Cr form. According to the authors, who still claim Cr(III) as an essential nutrient, the potential benefits appear to outweigh the theoretical genotoxic risk *in vivo* at 'normal' or 'modestly elevated' intake levels. By contrast, Levina and Lay (2008) have questioned the role of Cr(III) as an essential micronutrient and stressed the importance of recent findings concerning both the generation of highly reactive Cr(VI/V/IV) species arising from the reactions of Cr(III)-complexes with biological oxidants (see Section 1.3.1) and the possible resulting genotoxicity. Therefore, the authors conclude stating that the potential for genotoxic side-effects of Cr(III) complexes may outweigh their possible benefits as insulin enhancers, and that recommendations for their use as either nutritional supplements or antidiabetic drugs need to be reconsidered in the light of those recent findings.

1.7.2.4. Other effects

In a repeated-dose toxicity study (Anderson et al., 1997b) juvenile (four-week old) Harlan Sprague Dawley rats were exposed to diets supplemented with 5, 25, 50 or 100 mg Cr(III) (as Cr-chloride or Cr-picolinate) kg^{-1} (equivalent to 0.25, 1.25, 2.5, 5 mg Cr(III) kg^{-1} bw) for 20 weeks. Fasting blood samples were taken at study weeks 7 and 13. The parameters included also Cr retention in liver and kidney, serum biochemistry (glucose, cholesterol, triglycerides, blood urea nitrogen, lactic acid dehydrogenase, transaminases, total protein and creatinine), histological evaluation of the liver and kidney. Liver and kidney Cr concentrations increased linearly for both the Cr-chloride and Cr-picolinate fed animals. All other parameters, including weight gain and feed consumption, were unaffected by treatment. Under the conditions of this study, the NOAEL was higher than 5 mg Cr(III) kg^{-1} bw.

An increased incidence in bifurcated cervical arches was noted in the offspring of pregnant mice fed Cr-picolinate but not Cr-chloride (200 mg Cr kg^{-1} feed, equivalent to 20 mg Cr kg^{-1} bw) from GD 6 to GD 17; this points to a significant role of the picolinic moiety in the generation of such skeletal defects (Bailey et al., 2006).

In humans, Cr(III) toxicity was practically unknown until recently, when the claimed beneficial ability of this metal in lowering blood glucose and cholesterol as well as in reducing body weight and increasing body mass and strength have resulted in the widespread availability of Cr over-the-counter supplements, that are mostly taken without medical control. In two out of the three reported cases of toxicity, Cr-picolinate was identified as the causative agent, while the remaining case involved Cr-polynicotinate. Renal failure was the predominant feature in

patients ingesting 0.6–2.4 mg Cr-picolinate day^{-1} (corresponding to 0.01–0.04 mg kg^{-1} bw day^{-1} in a 60 kg individual) for at least six weeks (Wasser et al., 1997; Cerulli et al., 1998). Toxic hepatitis has been reported following the daily ingestion of a much lower (and theoretically non-toxic) amount of Cr-polynicotinate (0.2 mg day^{-1} over five months, corresponding to approximately 0.003 mg kg^{-1} bw day^{-1} in a single 60 kg individual (Lança et al., 2002). However, the concurrent ingestion of botanical products may have contributed to the observed liver toxicity (Kleefstra et al., 2003).

1.7.2.5. Conclusions

The FEEDAP Panel considers that the most recent available literature and the carcinogenicity studies in rats and mice may indicate that Cr(III) is a genotoxic compound under *in vivo* conditions. A similar concern was expressed by the Food Additives and Nutrient Sources added to Food (ANS) Panel who suggested a re-evaluation based on the available information (EFSA, 2008).

1.7.3. Consumer safety assessment

Chromium(III) was regarded as an essential trace element for human metabolism, and the Estimated Safe and Adequate Daily Dietary Intake of Cr for adults was 50–200 µg day^{-1}, the latter figure identifying the safety limit for this element (Food and Nutrition Board, 1989; Hathcock, 1996). However, according to the Food and Nutrition Board (2001), the Estimated Average Requirements for Cr could not be set due to insufficient evidence. Therefore, the above-mentioned body set an Adequate Intake (AI) based on estimated mean intakes of 35 µg day^{-1} and 25 µg day^{-1} for men and women, respectively. Consequently, the Tolerable Upper Intake Level (UL) for Chromium(III) was not established. The Expert Group on Vitamins and Minerals (EVM, 2003) set as guidance level (GL) 10 mg day^{-1} for Cr(III); the EVM excluded the picolinate form from its recommendation.

The SCF assessed a possible UL for Cr(III) in 2003 (EC, 2003a). With the specific reference to the possible carcinogenicity of Cr(III), in reference to the International Agency for Research on Cancer (IARC, 1990), the SCF stated that '*there is limited evidence in experimental animals for the carcinogenicity of Cr trioxide (chromic acid) and sodium dichromate*" and "*there is inadequate evidence in experimental animals for the carcinogenicity of metallic Cr, barium chromate and Cr(III) compounds*.' The SCF also highlighted in its opinion the rather conflicting outcomes from the WHO (1996) and the UK Expert Group on Vitamins and Minerals (EVM, 2002); the former considered that supplementation of Cr should not exceed 0.25 mg day^{-1}, whilst the latter concluded that, notwithstanding the limitations of available data, a dose of 0.15 mg kg^{-1} bw (9 mg day^{-1} in a 60 kg adult) is not expected to pose a health risk based on results from laboratory animal studies. Overall, the SCF was unable to derive a UL for Cr(III), concluding that '*the limited data from studies on subchronic, chronic, and reproductive toxicity on soluble trivalent Cr salts and the available human data do not give clear information on the dose-response relationship. Therefore, a tolerable upper intake level can not be derived*.'

The SCF reported that, according to the available data, the background dietary intake of Cr(III) is not expected to exceed 0.3 mg day^{-1} and is likely to be no more than 0.1 mg day^{-1}. In most foodstuffs, natural Cr concentrations are well below 0.1 mg kg^{-1}. However, it should be kept in mind that the metal or its compounds are also used in electroplating and in surface treatment of food cans, and that stainless steel may contain Cr at relatively high percentages. Therefore, Cr migration from cookware and cans has been postulated, even though only small quantities have

been generally observed in foodstuff as a result of leaching (Berg et al., 2000; Flint and Packirisamy, 1997; Jorhem and Slorach, 1987; Smart and Sherlock, 1985).

The mean contribution of foodstuffs of animal origin from unsupplemented Cr(III) animals to the background dietary intake of Cr for the adult population has been estimated in the range of 16–26 % (Ysart et al., 2000; Leblanc et al., 2005). Therefore, a 25 % contribution can be regarded as a conservative, yet still realistic estimate.

No reliable data have been found concerning the additional consumer's exposure from the use of feed additives in animal nutrition in the countries where such additives are authorised. In this respect, it should be noted that Cr(III)-based additives are organic forms (e.g. picolinate); the organic complexes with aminoacids or other endogenous compounds are expected to have a significantly higher bioavailability than the Cr present as background content in feedingstuffs (Stearns, 2000). Such higher bioavailability would lead to higher retention in edible tissues and products from exposed food-producing animals. The lack of reliable figures, however, prevents an estimation of the additional contribution of Cr(III)-based feed additives to the total consumer exposure.

The FEEDAP Panel highlights the following issues:

a) The Panel is not in a position to fully exclude that oral Cr(III) is a genotoxic compound under *in vivo* conditions and to conclude on the potential carcinogenicity of Cr(III).
b) Chromium(III) interferes with trace elements (e.g. Fe, Se) as well as with hormones (e.g. insulin, leptin, cortisol).
c) Indications of transgenerational effects represent an additional point of concern for Cr(III), warranting further investigations.
d) Chromium picolinate may induce teratogenic effects.

The above concerns prevent proposing a maximum upper safe level for consumers for Cr(III).

Given the concerns expressed above on consumer safety for Cr(III), the FEEDAP Panel considers it prudent to avoid any additional exposure of the consumers resulting from the use of supplementary Cr in animal nutrition.

1.8. Safety for the user

The exposure to Cr(III) compounds may elicit an allergic contact dermatitis similar to that described for hexavalent compounds and is mainly associated with the exposure to tanned leather (Hansen et al., 2006).

A critical point for user safety is the observation that occupational exposure to Cr(III) may be associated with increased genotoxic risk (Medeiros et al., 2003; see Section 1.7.2.3).

The FEEDAP Panel concludes that, due to concerns for allergenicity and potential genotoxicity, any occupational exposure to Cr(III) in feeds should be kept to a minimum.

1.9. Safety for the environment

The environmental fate and behaviour of chromium (III) and (VI) has been extensively assessed in the EU risk assessment report on chromium trioxide, sodium chromate, sodium dichromate, ammonium dichromate and potassium dichromate (EC, 2005).

Chromium exists in the environment in a number of valency states. Chromium(VI) and chromium(III) are the major forms found (Bartlett, 1991). However, in the environment, kinetic and other non-equilibrium factors result in that chromium(III) species dominate in nature, with

high levels of chromium(VI) species generally only found as a result of anthropogenic pollution.

The predominant forms of chromium(III) present in solutions are Cr^{3+} at very low pH, then $Cr(OH)^{2+}$ and $Cr(OH)^{3+}$ with increasing pH, and finally $Cr(OH)^{4-}$ at very high pH. Chromium(VI) is a strong oxidising agent and as such only exists as oxygenated species in the environment. It is likely that Cr(VI) will be reduced to Cr(III) in soil, and that such conversion would have taken place in many of the toxicity tests.

Chromium(III) is expected to be rapidly and strongly adsorbed onto soil, particularly by iron and manganese oxides, clay minerals and sand. About 90 % of the added chromium has been found to be adsorbed onto clay minerals and iron oxides in 24 hours. The adsorption of Cr(III) onto soil follows the pattern typical of cationic metals: it increases with pH and the organic matter content of the soil and decreases when other competing (metal) cations are present.

The Cr concentration of soils varies greatly from traces up to 250 mg kg^{-1} or more. In most soils, Cr occurs at concentrations between 2 and 60 mg kg^{-1}. The concentrations of chromium in rivers and freshwaters are usually between 1 and 10 µg L^{-1} (although levels in lakes in Scandinavia tend to be lower than this).

Chromium(III) has generally been shown to be less toxic than Cr(VI) to soil organisms, partly because Cr(III) adsorbs more strongly onto soil than chromium(VI). Based on an extensive data set, the Predicted No Effect Concentration in soil ($PNEC_{soil}$) for Cr(VI) and Cr(III) is taken to be 0.031 and 2.8 mg kg^{-1} wet weight, respectively.

For the risk assessment of Cr intended for use as feed additive, the PNEC for Cr(III) is considered to be the most relevant. From the available data, Cr(III) appears to be less toxic than chromium(VI) in waters of medium hardness (> 50 mg $CaCO_3$). There are indications that the toxicity of Cr is negatively correlated with water hardness. The PNEC values for the surface water compartment are taken to be 3.4 µg L^{-1} for Cr(VI) and 4.7 µg L^{-1} for Cr(III). There is insufficient data available to derive a PNEC from studies on sediment dwelling organisms. Using the equilibrium partitioning approach, the $PNEC_{sediment}$ for Cr(III) was estimated to be 31 mg kg^{-1} wet weight. Given that the vast majority of Cr(VI) entering into sediment will be converted to Cr(III), this PNEC is considered to be the most relevant for the risk assessment.

CONCLUSIONS

Dietary Cr is poorly absorbed upon ingestion. There is limited evidence that organic forms are better absorbed than inorganic forms and may have a higher bioavailability. Chromium(III) is found in animal tissues (including liver, kidney and muscle). Urine is by far the main excretion route for absorbed Cr.

Chromium(III) potentiates insulin-dependent glucose entry into the cells. Other biological effects are less well established (e.g. on immune response and lipid metabolism).

Since Cr(III) deficiency in farm animals has not been recognised under experimental or field conditions, the FEEDAP Panel considers that there is no evidence of the essentiality of Cr(III) as a trace element in animal nutrition.

The assessment of data on Cr concentration in feeds, foods and biological samples need to be interpreted with caution due to the uncertainties associated with the analytical procedures.

The main sources of Cr for animals are minerals. Concerning food of animal origin, the highest levels of Cr are found in offals, followed by muscle tissue, fish flesh and eggs.

A consistent pattern of tissue deposition could not be identified in farm animals given feed supplemented with Cr, regardless of source.

Conditions adversely affecting health/welfare and/or metabolic challenges generally appear to favour the occurrence of beneficial zootechnical effects of supplemental Cr(III). However, the responses are highly inconsistent, possibly due to the wide range of natural Cr background in feed, the sources and levels of supplementary Cr and the presence of other dietary factors (e.g. phytate, ascorbic acid, organic acids, other trace elements).

There is no evidence of adverse effects on farm animals arisen from the background levels of total Cr in unsupplemented feed. In view of the limited available data on supplementary Cr, the FEEDAP Panel is not in a position to define maximum tolerable levels of Cr(III) in feed for farm animals.

Available data clearly indicate that Cr(III) is much less toxic than Cr(VI), an established animal and human carcinogen. However, Cr(III) is the likely ultimate intracellular toxic form of Cr(VI). The FEEDAP Panel notes that the most recent available literature and the carcinogenicity studies in rats and mice indicate that Cr(III) may be a genotoxic compound under *in vivo* conditions. Given the concerns on consumer safety for Cr(III), the FEEDAP Panel considers it prudent to avoid any additional exposure of the consumers resulting from the use of supplementary Cr in animal nutrition.

The consumer background dietary intake of Cr(III) is not expected to exceed 0.3 mg day^{-1} and is likely to be no more than 0.1 mg day^{-1}. The mean contribution of foodstuffs of animal origin from unsupplemented Cr(III) animals to the background dietary intake of Cr for the adult population has been estimated at approximately 25 %. No reliable data have been found concerning the additional consumer's exposure resulting from the use of supplementary Cr in animal nutrition.

The FEEDAP Panel concludes that, due to concerns for allergenicity and potential genotoxicity, any occupational exposure to Cr(III) in the feed industry should be kept to a minimum.

Chromium, as a natural element, is ubiquitous in the environment, occurring in a number of oxidation states. Chromium(III) is the predominant naturally occurring form. PNECs for Cr(III) for soil, water and sediment are established.

DOCUMENTATION PROVIDED TO EFSA

1. Dossier Availa®Cr as nutritional additive for all species. April 2006. Submitted by Zinpro Animal Nutrition Inc.
2. Availa®Cr Supplementary information. October 2006. Submitted by Zinpro Animal Nutrition Inc.
3. Availa®Cr Supplementary information. May 2007. Submitted by Zinpro Animal Nutrition Inc.
4. Availa®Cr Supplementary information. January 2009. Submitted by Zinpro Animal Nutrition Inc.
5. Evaluation report of the Community Reference Laboratory feed additives authorisation on the analytical methods of Availa®Cr. (February 2007)
6. Comments from Member States received through the EFSAnet.

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APPENDICES

APPENDIX A

Total chromium in selected feed materials, compound feeds and food

Table A.1. **Total chromium in feed materials on an *as is* basis (mg kg^{-1})**

Item	Mean	Range	n	Reference
Wheat grain, durum	0.027	0.020-0.032	3	Cubadda et al., 2005
Wheat grain	0.020-0.058	0.013–0.071	12	Plessi and Monzani, 1990
Wheat bran	0.124–0.141	0.078–0.177	5	Plessi and Monzani, 1990
Corn grain	0.006–0.144	0.004–0.186	7	Plessi and Monzani, 1990
Barley grain	0.018	0.013–0.021	4	Plessi and Monzani, 1990
Barley bran	0.058	0.029–0.095	4	Plessi and Monzani, 1990
Soybeans	0.497	0.383–0.639	5	Plessi and Monzani, 1990
Feed grade phosphates	49	21–72	4	Lima et al., 1999
Mineral mixture	45.9[a]	1.5–220	21	Li et al., 2005

[a] Median

Table A.2. **Total chromium in compound feed on an *as is* basis (mg kg^{-1})**

Item	Mean	Range	n	Reference
Poultry feed	0.82–0.85		2	Kroliczewska et al., 2004
	1.27		1	Yildiz et al., 2004
Swine feed [a]	0.75–1.50			NRC, 1998
Rabbit feed	0.5		1	Sahin et al., 2001
Ruminant feed [a]	0.3–1.6			Bunting, 1999
Various feeds	1.93	0.20-6.87	70	Soares et al., 1994

[a] Review of published data

Table A.3. **Total chromium in food on an *as is* basis (mg kg^{-1})**

Item	Mean	Range	n	Reference
Cereal-based products				
Pasta	0.006[a]	0.029–0.094	*n.a.*	Santos et al., 2004
	0.013		2	Cubadda et al., 2005
	0.04		*n.a.*	Leblanc et al., 2005
Rice	0.026[a]	0.006–0.300	*n.a.*	Santos et al., 2004
	0.008	< 0.003–0.033	49	Jorhem et al., 2008
Rice and semolina	0.06		*n.a.*	Leblanc et al., 2005
Various products (inc. bread)	0.09	0.01–0.16	*n.a.*	Leblanc et al., 2005
Wheat flour	0.022[a]	0.005–0.048	*n.a.*	Santos et al., 2004
Corn flour	0.018[a]	0.006–0.117	*n.a.*	Santos et al., 2004
Fruit, vegetables and other plant food				

Beans, various types	0.006–0.063	0.005–0.083	14	Plessi and Monzani, 1990
Chick peas	0.154	0.129–0.189	3	Plessi and Monzani, 1990
Peas, frozen	0.006	0.005–0.007	4	Plessi and Monzani, 1990
Red lentils	0.006	0.005–0.007	2	Plessi and Monzani, 1990
Pulses	0.08		*n.a.*	Leblanc et al., 2005
Peas	0.003	0.0005–0.009	171	Gundersen et al., 2000
Onion	0.010	0.002–0.047	192	Gundersen et al., 2000
Vegetables (no potatoes)	0.02	0.002.0.120	*n.a.*	Santos et al., 2004
	0.05		*n.a.*	Leblanc et al., 2005
Potatoes	0.01		*n.a.*	Santos et al., 2004
Starchy vegetables	0.05		*n.a.*	Leblanc et al., 2005
Fruits	0.01		*n.a.*	Leblanc et al., 2005
Sugars, confectionery	0.12		*n.a.*	Leblanc et al., 2005
Sugar	0.005	0.004–0.007	*n.a.*	Santos et al., 2004
Fats and oils				
Olive oil	-	0.001–0.030	*n.a.*	Karadjova et al., 1998
Oils	0.04		*n.a.*	Leblanc et al., 2005
Margarine	0.06		*n.a.*	Leblanc et al., 2005
Milk and dairy products				
Milk	0.001[a]		*n.a.*	Santos et al., 2004
	0.001		*n.a.*	Rivero Martino et al., 2001
	0.02		*n.a.*	Leblanc et al., 2005
Ultra-fresh dairy products	0.03		*n.a.*	Leblanc et al., 2005
Cheeses	0.14		*n.a.*	Leblanc et al., 2005
Butter	0.07		*n.a.*	Leblanc et al., 2005
Ice creams	0.10		*n.a.*	Leblanc et al., 2005
Meat and offal				
Beef meat	0.004	< 0.004–0.020	36	Jorhem et al., 1996
Pork meat	0.007	< 0.004–0.074	22	Jorhem et al., 1996
Meat	0.052[a]	0.032–0.154	*n.a.*	Santos et al., 2004
	0.05		*n.a.*	Leblanc et al., 2005
Poultry and game birds	0.03		*n.a.*	Leblanc et al., 2005
Offals	0.10		*n.a.*	Leblanc et al., 2005
Fish and seafood				
Fish 13 species	0.004–0.013	< 0.003–0.020	40	Engman and Jorhem, 1998
Plaice	0.064	0.010-0.13	4	Engman and Jorhem, 1998
6 species	0.014	< 0.001–0.061	*n.a.*	Sepe et al., 2003
	0.025[a]	0.018–0.032	*n.a.*	Santos et al., 2004
Mussel	0.09–0.20	0.04–0.25	30	Cubadda et al., 2006
Other				
Eggs and egg products	0.05		*n.a.*	Leblanc et al., 2005
Honey	0.003		3	Caroli et al., 1999

[a] Geometric mean

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APPENDIX B

Chromium concentrations in tissues and animal products fed diets supplemented with various sources of trivalent chromium

Table B.1. **Chromium concentrations in tissues (in µg kg^{-1}) of cattle for fattening and in milk (µg L^{-1}) from dairy cows fed diets supplemented with various sources of trivalent chromium**

Animal species	Cr content of complete control diet (mg kg^{-1})	Cr(III) dose supplemented to control complete feed (mg kg^{-1})	Source of Cr(III) supplemented	Duration of study	Muscle	Liver	Kidney	Heart	Rib fat	Milk	Method of Cr analysis	Reference
Cattle[1]	ND	Control		146 d		25	13				GF-AAS	Spears et al., 2004
		0.4	$CrCl_3$			21	12					
		0.8	Cr nicotinate			20	11					
Cattle[1]	ND	Control		125 d	116[a]	41	108				GF-AAS	Spears et al., 2004
		0.8	Cr-methionine		71[b]	38	127					
Dairy cows[3]	ND	Control		28 d before calving and 28 d during lactation						55	ICP-AES	Hayirli et al., 2001
		0.03	Cr-methionine							55		
		0.06	Cr-methionine							57		
		0.12	Cr-methionine							57		
Steers[1,4]	1.65[6]	Control	Cr-yeast	138 d	510	140	200		430		GF-AAS	Chang et al., 1992
		0.2			560	200	270		200			
Steers[1,5]	1.65[6]	Control		138 d	730	230	380		330		GF-AAS	Chang et al., 1992
		0.2	Cr-yeast		600	290	220		170			

[1] chromium tissue concentrations on dry matter basis
[2] chromium tissue concentrations on wet weight basis
[3] supplemented dose in mg kg^{-1} body weight$^{0.75}$ which corresponded in three Cr(III) supplemented groups to Cr contents of 0.22, 0.39 and 0.83 mg kg^{-1} complete feed, respectively
[4] 70-d growing period on urea-corn feed
[5] 70-d growing period on soybean meal
[6] Cr contents of complete diet to finishing steers
ND – not detected
GF-AAS – graphite furnace atomic absorption spectrophotometry
ICP-AES – inductively coupled plasma atomic emission spectroscopy
[a,b] Means in a column with different letters in superscripts are statistically different ($P < 0.01$)

Table B.2. **Chromium concentrations in tissues (in µg kg^{-1}) of pigs for fattening and sows fed diets supplemented with various sources of trivalent chromium**

Animal species	Cr content of complete control diet (mg kg^{-1})	Cr(III) dose supplemented to control complete feed (mg kg^{-1})	Source of Cr(III) supplemented	Duration of study	Muscle	Liver	Kidney	Heart	Pancreas	Bone	Ovary	Adrenals	Method of Cr analysis	Reference
Pigs[1]	ND	Control		From 18 to	2	9^{d}	10^{d}	8^{e}	3^{de}				GF-AAS	Ward, 1995
		0.2	$CrCl_3$	100 kg b.w.	6	17^{cd}	15^{cd}	19^{bcd}	30^{b}					
		0.2	Cr-acetate		5	35^{b}	78^{b}	15^{bcde}	2^{e}					
		0.2	Cr-oxalate		4	35^{b}	50^{bc}	22^{b}	4^{cde}					
		0.2	Cr-nicotinate		3	13^{cd}	35^{cd}	9^{de}	12^{cd}					
		0.2	Cr-picolinate		5	20^{c}	38^{cd}	16^{bcde}	8^{cde}					
		0.2	CrNGCG[3]		7	9^{cd}	10^{d}	20^{bc}	4^{cde}					
Pigs[1]	2.7	Control		50 d	< 1.5	21^{a}	27^{a}	4					GF-AAS	Anderson et al., 1997a
		0.3	Cr-picolinate		< 1.5	29^{b}	57^{b}	11						
Pigs[1]	2.1	Control		75 d	96	12^{a}	42^{a}			27^{a}	4^{a}		ICP-MS	Lindemann et al., 2008
		5.0	Cr-picolinate		151	91^{c}	185^{d}			95^{d}	51^{c}			
		5.0	Cr-propionate		219	21^{a}	61^{ab}			45^{ab}	4^{a}			
		5.0	Cr-methionine		82	41^{b}	106^{c}			81^{cd}	24^{b}			
		5.0	Cr-yeast		205	14^{a}	77^{bc}			59^{bc}	12^{ab}			
Pigs[2]	ND		Control	35 d	33^{a}	24^{a}	48^{a}	49^{a}					GF-AAS	Wang and Xu, 2004
		0.2	Cr-nanopartic.		93^{b}	59^{b}	90^{b}	75^{b}						
Sows[1]	ND	Control		3 parities		23	36				11	16	ICP-MS	Lindemann et al., 2004
		0.2	Cr-picolinate			37^{L}	56^{L}				31	20^{L}		
		0.6	Cr-picolinate			88^{L}	133^{L}				49	34^{L}		
		1.0	Cr-picolinate			92^{L}	176^{L}				32	48^{L}		

[1] chromium tissue concentrations on dry matter basis
[2] chromium tissue concentrations on wet weight basis
[3]Cr-nicotinate-glycine-cysteine- glutamate complex
ND – not detected
GF-AAS – graphite furnace atomic absorption spectrophotometry
ICP-MS – inductively coupled plasma mass spectrometry
[a,b,c] Means in a column with different letters in superscripts are statistically different ($P < 0.05$)
[L] Means in column show significant linear response ($P < 0.005$)

Table B.3. **Chromium concentrations in tissues and animal products (in µg kg^{-1}) from poultry fed diets supplemented with various sources of trivalent chromium**

Animal species	Cr content of complete control diet (mg kg^{-1})	Cr(III) dose supplemented to control complete feed (mg kg^{-1})	Source of Cr(III) supplemented	Duration of study	Muscle		Liver	Kidney	Heart	Lungs	Spleen	Gizzard	Egg white	Egg yolk	Method of Cr analysis	Reference
Broilers[2]	4,6			21 d	Breast	Thigh									AAS	Amatya et al., 2004
		Control			60[a]	230[a]	360[b]		1760[a]	880[a]	140[a]					
		0.2	$CrCl_3$		400[b]	1030[b]	250[a]		1850[a]	6000[c]	1970[b]					
		0.2	Cr-yeast		390[b]	1420[b]	210[a]		2870[b]	4900[bc]	3760[b]					
Broilers[2]	4,6 in starter			35 d	Breast	Thigh									AAS	Amatya et al., 2004
		Control			200[a]	1860[a]	420[b]		350	460	1440[a]					
	4.9 in grower	0.2	$CrCl_3$		460[b]	4170[b]	300[a]		440	550	2200[b]					
		0.2	Cr-yeast		660[b]	4540[b]	310[a]		440	790	2840[b]					
Broilers[1]	1.1			35 d	Carcase										AAS	Samanta et al., 2008
		Control			270[a]											
		0.5	$CrCl_3$		510[b]											
Broilers[1]	1.7			35 d	Breast	Thigh									AAS	Ahmed et al., 2005
		Control			400	450	590									
		0.2	$CrCl_3$		600	750	370									
Broilers	ND			56 d	Breast										AAS	Debski et al., 2004
		Control			40											
		0.2	Cr-yeast		52											
Laying hens[1]	0.22	Control		28 d										160[a]	GF-AAS	Dębski et al., 2001
		0.5	Cr-yeast											380[b]		
Laying hens[1]	3.0	Control		35 d										390	ICP-AES	Piva et al., 2003
		21.2[3]	$CrCl_3$											400		
		31.9[3]	Cr-yeast											380		
		41.8[3]	Cr-AN[4]											430		
Turkeys[2]	0.5			35 d	Breast	Thigh									AAS	Anderson et al., 1989
		Control			0.8	0.8	1.9	3.3	0.9	12	3.7	1.0	15	1.8		
		25	$CrCl_3$		1.1[L]	1.0	36[L]	88[L]	1.8[L]	12[L]	13[L]	1.9[L]	14	1.2		
		100	$CrCl_3$		2.8[L]	2.0	168[L]	224[L]	8.0[L]	41[L]	50[L]	8.0[L]	29	2.0		
		200	$CrCl_3$		3.5[L]	3.7	326[L]	541[L]	12.0[L]	59[L]	67[L]	12.0[L]				
Japanese quails[1]	4.0	Control		38 d	1.3[b]		2.6[b]	2.4[c]							GF-AAS	Uyanik et al., 2005
		20	$CrCl_3$		1.4[b]		2.6[b]	2.4[c]								
		40	$CrCl_3$		2.0[ab]		3.7[ab]	3.1[bc]								
		80	$CrCl_3$		2.6[a]		4.5[a]	4.2[b]								

		100	$CrCl_3$		2.3[a]	4.1[ab]	5.9[a]			
Japanese quails[2], laying hens	1.1	Control		93 d	260	130	230	GF-AAS	Sahin et al., 2002	
		0.2	Cr-picolinate		300[L]	200[L]	290[L]			
		0.4	Cr-picolinate		310[L]	270[L]	310[L]			
		0.8	Cr-picolinate		340[L]	350[L]	390[L]			
		1.2	Cr-picolinate		370[L]	380[L]	440[L]			

[1] chromium tissue concentrations on dry matter basis; [2] chromium tissue concentrations on wet weight basis; [3] analysed Cr contents in supplemented complete feed; [4] chromium aminoniacinate
ND – not detected; AAS - atomic absorption spectrophotometry; ICP-AES - inductively coupled plasma atomic emission spectroscopy; GF-AAS – graphite furnace atomic absorption spectrophotometry; [a,b,c] Means in a column with different letters in superscripts are statistically different ($P < 0.05$);
[L] Means in column show significant linear response ($P < 0.05$)

Table B.4. **Chromium concentrations in tissues (in µg kg^{-1}) of rabbits and rainbow trouts fed diets supplemented with various sources of trivalent chromium**

Animal species	Cr content of complete control diet (mg kg^{-1})	Cr(III) dose supplemented to control complete feed (mg kg^{-1})	Source of Cr(III) supplemented	Duration of study	Muscle	Liver	Kidney	Heart	Lungs	Spleen	Method of Cr analysis	Reference
Rabbits, pregnant[1]	0.54	Control		One gravidity plus lactation period	200	180	400	90	180	100	GF-AAS	Sahin et al., 2001
		0.2	$CrCl_3$		220	260	420	120	220	140		
		0.4	$CrCl_3$		240	310	480	180	300	150		
Newborns from rabbits above[1]	-	Control			320	240	440	110	140	110	GF-AAS	Sahin et al., 2001
		0.2	$CrCl_3$		340	280	430	140	180	130		
		0.4	$CrCl_3$		370	350	470	170	210	140		
Rabbits, weaned growing[1]	0.54	Control		4 months	330	260	450	120	180	120	GF-AAS	Sahin et al., 2001
		0.2	$CrCl_3$		350	380	480	150	190	160		
		0.4	$CrCl_3$		390	400	470	170	240	170		
Rainbow trouts[2]	ND	Control		58 d		300					GF-AAS	Kucukbay et al., 2006
		0.4	Cr-picolinate			350^{L}						
		0.8	Cr-picolinate			380^{L}						
		1.6	Cr-picolinate			420^{L}						

[1] chromium tissue concentrations on dry matter basis
[2] chromium tissue concentrations on wet weight basis
ND – not detected
GF-AAS – graphite furnace atomic absorption spectrophotometry
[L] Means in column show significant linear response ($P < 0.001$)

Table B.5. **Chromium concentrations in tissues (in µg kg^{-1}) of rats and mice fed diets supplemented with various sources of trivalent chromium**

Animal species	Cr content of complete control diet (mg kg^{-1})	Cr(III) dose supplemented to control complete feed (mg kg^{-1})	Source of Cr(III) supplemented	Duration of study	Muscle	Liver	Kidney	Heart	Spleen	Testicle	Bone	Method of Cr analysis	Reference
Rats[1]	ND	Control		20 wk		6^{a}	8^{a}					GF-AAS	Anderson et al., 1997b
		100	$CrCl_3$			91^{b}	700^{b}						
		100	Cr-picolinate			550^{c}	2200^{c}						
Rats[2]	ND	Control		42 d	11.3^{a}	6.1^{a}	9.1^{a}	1.1	1.2			GF-AAS	Zha et al., 2007a
		0.3	$CrCl_3$		13.0^{a}	9.4^{a}	25.1^{b}	1.8	1.3				
		0.3	Cr-picolinate		11.4^{a}	27.6^{b}	59.9^{c}	1.8	1.8				
		0.3	Cr-nanopartic.		20.6^{b}	31.6^{b}	75.5^{d}	1.9	1.8				
Rats[2]	0.2	Control		42 d	11.3^{c}	16.1^{b}	49.1^{b}	1.1	1.2	0.8		GF-AAS	Zha et al., 2007b
		0.075	Cr-nanopartic.		11.6^{c}	24.0^{ab}	59.0^{ab}	1.7	1.5	1.3			
		0.150	Cr-nanopartic.		15.1^{c}	29.9^{a}	69.6^{a}	1.9	1.0	0.8			
		0.300	Cr-nanopartic.		20.6^{ab}	31.6^{a}	75.5^{a}	1.9	1.8	0.9			
		0.450	Cr-nanopartic.		23.6^{a}	32.1^{a}	76.8^{a}	2.3	1.2	1.0			
		0.600	Cr-nanopartic.		22.6^{a}	29.8^{a}	76.7^{a}	2.1	1.3	0.9			
		1.200	Cr-nanopartic.		15.6^{bc}	32.9^{a}	85.9^{a}	2.5	1.7	0.2			
Rats[1,5]	0.03	Control		42 d	16^{abc}	10^{f}	23^{d}	12^{bc}				GF-AAS	Anderson et al., 1996
		5.0	$CrCl_3$		14^{bc}	10^{def}	74^{cd}	5^{d}					
		5.0	Cr-acetate		27^{a}	40^{abc}	397^{b}	17^{b}					
		5.0	$CrK(SO_4)_2$		13^{abc}	5^{bcd}	407^{b}	8^{bc}					
		5.0	Cr-histidinate		17^{abc}	7^{ef}	49^{d}	9^{cd}					
		5.0	Cr-glycinate		21^{abc}	40^{cde}	343^{b}	20^{b}					
		5.0	Cr-nicotinate		12^{c}	15^{def}	133^{c}	12^{bc}					
		5.0	Cr-NAHIS[3]		16^{abc}	19^{def}	394^{b}	13^{bc}					
		5.0	Cr-picolinate		24^{ab}	48^{a}	368^{b}	28^{a}					
		5.0	Cr-NGCG[4]		27^{a}	49^{ab}	850^{a}	27^{a}					
Mice[1]	0.07	Control		26 d		44	63^{a}		98	62	25^{a}	GF-AAS	Seaborn and Stoecker, 1989
		1.0	$CrCl_3$			37	89^{b}		116	66	42^{b}		
Mice[2]	0.12	Control		7 wk		156^{a}		1200^{a}				AAS	Schrauzer et al., 1986
		5.0	Cr-yeast			270^{b}		1800^{b}				X-ray fluorescence	

[1] chromium tissue concentrations on dry matter basis; [2] chromium tissue concentrations on wet weight basis; [3]Cr-dinicotinic acid dihistidine
[4]Cr-nicotinate-glycine-cysteine- glutamate complex; [5] Cr levels in muscle, liver and heart are given in values approximated from figures only
ND – not detected; GF-AAS – graphite furnace atomic absorption spectrophotometry; AAS – atomic absorption spectrophotometry;
[a,b,c] Means in a column with different letters in superscripts are statistically different ($P < 0.05$);

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