

EXPERT GROUP ON VITAMINS AND MINERALS

REVIEW OF CHROMIUM

The attached review of chromium is a slightly revised version of the paper first presented to the Expert Group at the meetings in November 1999, December 2001 and April 2002.

Expert Group on Vitamins and Minerals Secretariat
August 2002

CHROMIUM

Chemistry and Geochemistry

1. The transition element chromium (Cr) belongs to Group VI of the Periodic Table and has an atomic weight of 52.0. Chromium can exist in any oxidation state from -2 to +6; however, oxidation states other than 0, +2, +3 and +6 are uncommon.
2. In biological materials chromium is most stable in the oxidation state +3, as chromium compounds with oxidation states below +3 are reducing, and those above +3, are oxidising. In acidic solutions chromium (III) is soluble, readily forming hexahedral complexes with appropriate ligands, such as oxalate and sulphate ions. At the oxidation state of +6, chromium forms chromates and dichromates, which have strong oxidising potential.

Natural occurrence

3. Chromium is ubiquitous in nature, occurring in air, water, soil and biological materials over a great range of concentrations. Almost all of the sources of chromium in the earth's crust are in the trivalent state, naturally occurring chromium compounds in the hexavalent state are rare. Hexavalent chromium compounds are thus, man-made products (WHO, 1988).

Occurrence in food, food supplements and medicines

Food

4. Most of the chromium ingested with food is in the trivalent form. Early data (pre-1980) on the chromium concentration in foods are flawed, due to the difficulties encountered in contamination control during sampling, sample pre-treatment and analysis (Nordic Council, 1995). Recent data indicate that staple foods are particularly low in chromium. Processed meats, whole grain products, pulses and spices are the best sources of chromium, whilst dairy products and most fruit and vegetables, contain only small amounts.
5. Most of the total chromium in foods derives from food processing in stainless steel containers and processors, which typically contain 18% chromium. Homogenisation of fresh meat in a food processor equipped with standard stainless steel blades almost doubles the chromium concentration of the meat (Kumpulainen *et al.*, 1980). Thus, canned and other processed foods, particularly acidic foods such as fruit juices, are clearly higher in chromium than fresh foods, with the exception of refined sugar which is very low in chromium, compared to brown sugar and molasses (Offenbacher and Pi-Sunyer, 1983). Some brands of beer are very good sources of chromium and a half pint serving may contain as much as 20 µg or approximately two-thirds of the normal dietary chromium intake (Anderson and Bryden, 1983). However, some brands of beer contain much lower concentrations of chromium and

the average chromium intake per half-pint of beer is approximately 7% of the minimum suggested safe and adequate intake (National Research Council, 1989).

Drinking water

6. The concentration of chromium in uncontaminated waters is extremely low, <1 µg/l (<0.02 µmol/l). Industrial activities, such as tanning of leather, the steel industry and sites such as landfills, refuse tips and car scrap yards may cause contamination of the drinking water.

Licensed medicinal products for oral use

7. Six products containing chromium (and other nutrients) may be sold under the supervision of a pharmacist for use in malabsorptive states, conditions leading to hypoproteinaemia and perioperative nutritional support.

8. The maximal daily doses specified in the licences are up to 200 µg elemental chromium.

Intake and exposure

Food

9. Information on dietary intakes of chromium is limited. Most of the chromium ingested with food is in the trivalent form. Chromium is not included in the nutrient databanks for dietary surveys. The most up to date information available is from analysis of samples from the 1997 Total Diet Study (TDS)^{1,2}. This showed that the population average intake of chromium was 0.10 mg/day. This value is lower than that of 0.34 mg/day obtained from the 1994 Total Diet Study¹ but is consistent with intakes from previous TDS prior to 1994. Chromium intake in 1994 was unexpectedly high due to relatively high concentrations in the oils and fats, milk, dairy products and nuts groups. It appears that this is unique to that year and is not part of a trend towards increasing intakes. Table 1 shows the concentration of chromium in each of the TDS food groups in 1997 and the intake from each group.

10. Mean and upper level² (97.5 percentile) chromium intake for adults has been estimated at 0.10 mg/day and 0.17 mg/day respectively using the 1997 TDS concentrations combined with consumption data from the 1986/87 Dietary and Nutritional Survey of British Adults. These figures are lower than those obtained from the 1994 TDS (0.30 mg/day and 0.52 mg/day respectively), which were unexpectedly high.

¹ MAFF (1997) 1994 Total Diet Study: Metals and other elements. Food Surveillance Information Sheet No. 131

² This estimate should be used with caution due to the differences between the Total Diet Study and Adults Survey food groups.

11. The 1997 Total Diet Study shows that the highest concentration of chromium is in the food group meat products followed by oils and fats, bread, nuts, miscellaneous cereals, fish, and sugar and preserves. The main contributors to dietary intake of chromium are beverages, bread, miscellaneous cereals and meat products.

12. Chromium is present in a number of multi-mineral and/or vitamin food supplement products. As a single nutrient product it is available at levels between 200 and 600 µg. Three different chromium salts are available: chromium chloride, chromium picolinate and chromium nicotinate (Kobla and Volpe, 2000). No purity or quality issues have been identified.

13. Daily intake of chromium can vary widely, depending on the proportions of various food groups in the diet. Recent reports suggest that many diets in the US supply less than 50 µg of chromium/day. In one study the content of the self-selected diets of 10 men and 22 women, collected by the duplicate portion method, on a daily basis for 7 consecutive days, was determined (Anderson and Kozlovsky, 1985); the daily intake for men was 33 ± 3 µg (mean \pm SEM) with a range of 22 - 48 µg, and for the women 25 ± 1 µg with a range of 13 - 36 µg. Four-day duplicate diets collected 6 times from 80 people aged over 10 years in Maryland, USA in 1995 and 1996 found chromium intakes ranged from 3.3 to 675.9 µg/d with a mean of 43.9 µg/d (Scanlon *et al.*, 1999). The self-selected diets of 23 apparently healthy, well-nourished elderly English volunteers supplied 24.5 µg chromium/day (range 14 - 48 µg) (Bunker *et al.*, 1984). MAFF conducted a duplicate diet study of vegetarian adults to assess exposures to various metals (MAFF, 2000). One hundred and one duplicate diet samples were collected over 7-day periods in 1997 and 1998. The mean dietary exposure to chromium was 0.1mg/day (minimum to maximum range, 0.03 - 0.26 mg/d).

14. Beverages, including milk, account for approximately one fifth of the daily intake of chromium (MAFF, 1999). Normal cows milk is reported to contain 5 to 15 ng chromium/ml and cows colostrum five-fold higher levels. Human breast milk has historically been reported to contain similar levels, but more recent reports indicate that breast milk contains approximately 0.3 - 0.4 ng/ml (Casey and Hambidge, 1984). In contrast an analysis of breast milk samples from 27 Austrian mothers found chromium concentrations ranged widely from <0.8 to 163 ng/ml with a median of 24.3 ng/ml (Krachler *et al.*, 2000). Chromium concentrations were higher in urban smoking mothers.

15. Dietary intake of chromium in Southern Spain was recently measured by sampling duplicate diets (by electrothermal atomisation-atomic absorption spectrometry) for seven consecutive days in different population groups. A total of 161 duplicate diets from 23 subjects were analysed and mean levels of chromium intake ranged from 9.39 to 205.16 µg/d, giving a mean chromium intake of 100 µg/day (Garcia *et al.*, 2001).

Table 1 - Concentrations of Chromium in 1997 Total Diet samples and estimated average intake

Food group (TDS)	Mean Chromium concentrations ³ (mg/kg fresh weight)	Intake of Chromium mg/day ⁴
Bread	0.15	0.016
Miscellaneous cereals	0.14	0.014
Carcase meat	0.09	0.002
Offal	0.08	0.0001
Meat products	0.23	0.011
Poultry	0.09	0.002
Fish	0.13	0.002
Oils & fats	0.17	0.005
Eggs	0.04	0.001
Sugars & preserves	0.13	0.008
Green vegetables	0.02	0.001
Potatoes	0.04	0.005
Other vegetables	0.04	0.003
Canned vegetables	0.06	0.002
Fresh fruit	0.02	0.001
Fruit products	0.03	0.001
Beverages	0.02	0.019
Milk	0.01	0.003
Dairy products	0.09	0.005
Nuts	0.14	0.0003
Total intake (mg/day)		0.10mg/day

Drinking water

16. The concentration of chromium in uncontaminated natural waters is extremely low, <1 µg/l (<0.02 µmol/l), thus its contribution to total dietary intake is negligible. The provisional guideline level, established by the WHO in 1992, for chromium in drinking water is 50 µg/l. This is the limit in most developed countries, including the UK. The current maximum contaminant level for chromium in drinking water, set by the US Environmental Protection Agency (EPA), is also 50 µg/l (Goldhaber and Vogt, 1989). However, the EPA has proposed an increase of the MCL for chromium to 100 µg/l.

³ upper-bound means across the 20 TDS towns, i.e. concentrations below limit of detection taken as the limit of detection

⁴ upper-bound intake, i.e. concentrations below limit of detection taken as the limit of detection

Air/occupational exposure

17. Many dust particles contain high concentrations of chromium; for example, cement particles are particularly high in chromium. No reliable data are available on chromium intake via the lungs in industrial environments. However, the concentration of chromium in stainless steel welding fumes is usually high, ranging from 0.1 – 0.2 $\mu\text{g}/\text{m}^3$ (Gylseth *et al.*, 1977). These fumes contain hexavalent chromium, from 0.05 – 0.16 $\mu\text{g}/\text{m}^3$ (Tola *et al.*, 1977) and have resulted in elevated intakes of chromium by welders.

Recommended amounts

18. The Committee on Medical Aspects of Food Policy (COMA) set no Reference Nutrient Intakes (RNIs) for chromium but suggested that an adequate level of intake is believed to lie above 25 $\mu\text{g}/\text{day}$ for adults and between 0.1 and 1.0 $\mu\text{g}/\text{kg}/\text{day}$ for children and adolescents (COMA, 1991). COMA also noted that no adverse effects had been observed from trivalent chromium at intakes of 1 – 2 g/day. In the US the National Research Council established an Estimated Safe and Adequate Daily Dietary Intake (ESADDI) for chromium of 50 – 200 $\mu\text{g}/\text{day}$ for adults (National Research Council, 1989). The intake of chromium recommended by the National Research Council (1989) for infants aged from 0 to 0.5 years ranges from 10 – 40 $\mu\text{g}/\text{day}$. This recommendation is approximately two orders of magnitude higher than the average intake found in healthy infants (Kumpulainen and Vuori, 1980; Casey *et al.*, 1985). In 2001, the Food and Nutrition Board of the Institute of Medicine released new dietary reference values for Americans and Canadians (Trumbo *et al.*, 2001). Where they could not establish a recommended dietary allowance (the level of intake needed to meet the requirements of 98% of the population) they set adequate intakes (AIs) instead, values based on estimates of nutrient intake by a group of healthy people that are assumed to be adequate. AIs for chromium were set at 0.2 $\mu\text{g}/\text{d}$ for 0-6 months, 5.5 $\mu\text{g}/\text{d}$ for 7-12 months, 11 $\mu\text{g}/\text{d}$ for 1-3y, 15 $\mu\text{g}/\text{d}$ for 4-8y, 25 $\mu\text{g}/\text{d}$ for 9-13y males, 35 $\mu\text{g}/\text{d}$ for 14-50y males, 30 $\mu\text{g}/\text{d}$ for males over 50, 21 $\mu\text{g}/\text{d}$ for 9-13y females, 24 $\mu\text{g}/\text{d}$ for 14-18y females, 25 $\mu\text{g}/\text{d}$ for 19-50y females, 20 $\mu\text{g}/\text{d}$ for females over 50, 30 $\mu\text{g}/\text{d}$ in pregnancy and 45 $\mu\text{g}/\text{d}$ in lactation.

Analysis of tissue levels and chromium status

19. Tissue chromium stores do not readily equilibrate with blood chromium; thus fasting plasma or serum concentrations may not be good indices of chromium status (Kelsey *et al.*, 1988). The normal concentration of chromium in whole blood is somewhat higher than that in serum, but it is still less than 0.5 ng/ml (WHO, 1988). Plasma or serum levels reflect chromium (III) intake (Moukarzel *et al.*, 1992). However, the chromium concentration in the red blood cells reflects exposure to hexavalent chromium only, as trivalent chromium cannot penetrate the cell membranes (Lewater *et al.*, 1985).

20. Some reports suggest that concentrations of chromium much lower than the normal value of 0.14 – 0.15 ng/ml for serum or 0.26 – 0.28 ng/ml for plasma

(Offenbacher *et al.*, 1986) might indicate the presence of severe chromium deficiency. Elevated serum chromium may be a useful indicator of excessive exposure to chromium. Serum from tannery workers exposed to trivalent chromium had a median chromium concentration of 0.49 ng/ml, whereas that of non-exposed subjects was 0.15 ng/ml (Randall and Gibson, 1987). Urinary excretion of chromium reflects dietary chromium intake in a dose dependant manner (Kumpulainen *et al.*, 1983; Uusitupa *et al.*, 1983; Uusitupa *et al.*, 1992), so high urinary levels may be a good indicator of exposure to excessive amounts.

21. Many reported concentrations of chromium in human tissues are erroneous due to sample contamination and analytical problems; i.e. blood and tissue samples may become highly contaminated by the chromium in needles, knives, blenders and other instruments (WHO, 1988). However, some more recent and reliable data are available on the normal concentrations of chromium in the livers and spleens of accident victims and Sudden Infant Death (SID) infants. These data give the average liver chromium concentration in children as 8 µg/kg dry weight, with a range from 4 to 15 µg/kg dry weight (Vuori and Kumpulainen, 1987). Spleen chromium concentration averaged 15 µg/kg dry weight with a range of 7 to 29 µg/kg dry weight. No statistically significant differences in liver or spleen chromium concentrations were found between sexes, age groups, or cause of death. These levels are much lower than those reported in earlier studies. It is possible that chromium can accumulate in kidneys and the heart, as elevated levels have been reported in these organs in chromium platers (WHO, 1988).

22. Many studies have been conducted in which hair has been suggested as an indicator of chromium status, especially reflecting metabolically incorporated chromium, derived from air contamination. However, a study on washing agents (i.e. shampoo) and times showed a great variability in the levels of chromium in samples from a hair pool (Kumpulainen *et al.*, 1982).

Function

23. Chromium is an essential nutrient that potentiates insulin action and thus, influences carbohydrate, lipid and protein metabolism. However, the nature of the relationship between chromium and insulin function has not been clearly defined. Mertz *et al.* (1974) suggested that the biologically active form of chromium (glucose tolerance factor) is a complex of chromium, nicotinic acid and possibly the amino acids glycine, cysteine and glutamic acid. Many attempts have been made to isolate or synthesise the glucose tolerance factor; none has been successful. Thus, the precise structure of the glucose tolerance factor and whether it is the biologically active form of chromium, remain uncertain.

24. Low-molecular-weight chromuim-binding substance (LMWCr) is a naturally occuring oligopeptide which has recently been proposed as the biologically active form of chromium. Its primary function is proposed to be the activation of insulin receptor tyrosine kinase in response to insulin. Chromium is essential for LMWCr to perform this function. (Vincent, 2000.)

25. Chromium may have a biochemical function that affects the ability of the insulin receptor to interact with insulin. For example, it has been found that *in vitro*, RNA synthesis directed by free DNA is enhanced by the binding of chromium to template (Okada *et al.*, 1981); this suggests that chromium may act similarly to zinc in regulating gene expression, so it may be regulating the synthesis of a molecule that potentiates insulin action. This suggestion is supported by the finding that there is a four-hour lag period between the administration of biologically active chromium and its optimal effect on insulin action *in vivo* (Tuman and Doisy, 1977).

Deficiency

26. Gross chromium deficiency has not been seen in humans, although signs of chromium deficiency have been found in patients receiving long-term parenteral nutrition with infusates low in chromium. Jeejeebhoy *et al.* (1977) reported on a patient receiving long-term parenteral nutrition for three and a half years, exhibiting impaired glucose tolerance and glucose utilisation, weight loss, neuropathy, elevated plasma fatty acids, depressed respiratory quotient and abnormalities in nitrogen metabolism. A woman given total parenteral nutrition low in chromium for five months developed severe glucose intolerance, weight loss and a metabolic encephalopathy-like state (Freund *et al.*, 1979). Both were alleviated by chromium supplementation.

27. Brown *et al.* (1986) reported that chromium supplementation reversed the development of unexplained hyperglycaemia and glycosuria, during administration of a total parenteral nutrition regime of several months duration. All subjects in these studies exhibited impaired glucose tolerance or hyperglycaemia, with glycosuria and a refractiveness to insulin. These symptoms, it is suggested, should therefore be considered as signs of chromium deficiency.

Overview of reported beneficial effects

28. Extravagant claims about the benefits of chromium have been made. The suggested beneficial effects associated with increased chromium intake appear to be a result of its effect on insulin sensitivity and include muscle and strength enhancing properties, aiding weight and fat loss, delaying ageing and treating diabetes (Nielsen, 1996). Clinical studies in diabetics have shown that supplementing the diet with chromium can decrease fasting blood glucose levels, improve glucose tolerance, lower insulin levels and decrease total cholesterol and triglyceride levels while increasing HDL-cholesterol levels (Mooradian *et al.*, 1994). In a double-blind, placebo-controlled, randomised trial in China, Anderson *et al.* (1997a) supplemented adults with type II diabetes with either placebo, 200 µg chromium or 1000 µg chromium. Over 4 months there were pronounced and significant decreases in fasting blood glucose and insulin and 2-hour blood glucose and insulin in the 1000 µg group. Evidence relating to the other claims is equivocal (Nielsen, 1996) but it has been suggested that initial chromium status of study participants may be an important mediating factor and benefits will only be seen in those who have marginal or poor chromium status (Vincent, 2000).

29. In one double-blind crossover study of eight female patients, 200 µg of chromium (as chromium chloride), given twice daily for three months, alleviated hypoglycaemic symptoms and improved the results of glucose tolerance tests (Anderson *et al.*, 1987).

30. The effect of chromium on lipid metabolism has led to speculation that it could decrease the risk of cardiovascular disease. A number, but not all, studies have observed beneficial effects of chromium supplements on blood cholesterol and triglyceride levels (discussed by Kobla and Volpe, 2000).

31. Due to its actions on increasing the body's sensitivity to insulin, it has been suggested that chromium may have a role to play in promoting weight loss. It has been demonstrated that chromium can lower body weight and yet increase lean body mass as a result of greater insulin sensitivity (Evans and Pouchnik, 1993; Evans, 1993).

32. McCleod and colleagues (1999) have observed striking effects of chromium picolinate supplements on the symptoms of dysthymic disorder. They report 5 case studies of adults suffering from this mood disorder who experienced dramatic improvements in their symptoms within days of taking the supplements, and rapid return of symptoms on ceasing supplementation. Whilst the authors attempted some simple single-blind experiments with these patients, they conclude that a controlled prospective trial is needed to clarify these observations.

Bioavailability

33. Absorption of hexavalent chromium species is much higher than that of trivalent compounds. For example, oral administration of sodium chromate in trace doses in humans resulted in absorption of 10% of the administered dose from the gastrointestinal tract. However, orally administered hexavalent chromium compounds are rapidly reduced to trivalent compounds, thus reducing the gastrointestinal absorption (Donaldson and Barreras, 1966).

Interactions

34. Chromium interacts with iron in binding to transferrin. Consequently, chromium has been shown to impair iron metabolism and storage. Significant reductions in serum iron, total iron-binding capacity, ferritin and haemoglobin have been reported (Ani and Moshtague, 1992). Haemochromatosis, is a pathological condition characterised by an overly high gastrointestinal absorption of dietary iron, leading to saturation of transferrin with iron and iron accumulation in the liver. In this condition, transferrin cannot bind absorbed chromium (III) (Lim *et al.*, 1983). However, there is no evidence of haemochromatosis causing any ill health effects due to low circulating chromium levels.

35. In humans, Lukaski *et al.* (1996) reported a tendency for decreased transferrin saturation in men supplemented with chromium (III) picolinate, but Campbell *et al.* (1997) found no effects of much higher chromium (III) picolinate supplements on indices of iron status.

Absorption

36. Intestinal absorption of trivalent chromium is low in both humans and animals, varying between approximately 0.5 and 2.0% depending on dietary intake (Kumpulainen *et al.*, 1983; Anderson and Kozlovsky, 1985; Offenbacher *et al.*, 1986; Felter and Dourson, 1997). Some data indicates chromium absorption to be inversely related to its dietary intake. If the dietary intake in humans is excessively low, 10 µg/day, absorption is approximately 2% and with increasing chromium intake, to an approximate level of 40 µg/day, chromium absorption decreases to about 0.5% (Kumpulainen *et al.*, 1983; Anderson and Kozlovsky, 1985).

37. Preliminary findings led to the suggestion that biologically active chromium (glucose tolerance factor – see para 33) is more readily absorbed than trivalent chromium. Later studies however, indicate that this may not be the case (Offenbacher and Pi-Sunyer, 1988). Some evidence suggests that while organic chromium may be readily absorbed, it passes quickly through the body, without being utilised (Anderson *et al.*, 1980/WHO, 1996).

38. The mechanism of absorption of chromium (III) from the intestine has not been clearly identified, but it apparently involves processes other than passive diffusion.

Distribution

39. Transport of chromium in the blood is governed by two processes depending on chromium valency. Hexavalent chromium species enter red blood cells, where reduction to trivalent chromium and subsequent binding to haemoglobin, take place. Assimilation of hexavalent chromium in excess of the amount that can be reduced and sequestered, results in longer residence times of hexavalent chromium in the blood and hence, greater exposure of body tissues. In contrast, absorbed trivalent chromium does not enter blood cells. A portion of it is incorporated into glucose tolerance factor, while the remainder is bound to serum transferrin. Transferrin-bound chromium is rapidly cleared from the blood and appears mainly in the liver (Outridge and Scheuhammer, 1993). In contrast, the organ distribution of hexavalent chromium is more widespread, with the kidneys, spleen, liver, lungs and bone accumulating significant concentrations of the metal.

40. Mice given hexavalent chromium (potassium chromate) in drinking water for up to one year accumulated chromium in all major body organs, but particularly spleen and liver. In contrast, those given trivalent chromium (chromium chloride) accumulated chromium, only in the liver. Liver concentrations were 40 – 90 times higher in the hexavalent treated group, than in the trivalent treated group (ATSDR, 1993).

Metabolism

41. In plasma, absorbed chromium (III) is bound mostly to transferrin and to other plasma proteins, which are responsible for its transport in the body. Long-term storage occurs particularly in the liver, spleen, bones and other organs (Lim *et al.*, 1983).

42. Chromates can penetrate red blood cells after which they are reduced to chromium (III). Reduction of chromium (VI) during transport in the blood is consistent with the finding that chromium is present in the urine only as chromium (III) (WHO, 1988).

Excretion

43. Most dietary chromium (> 98%) is not absorbed and is excreted via the faeces. Urine is the main excretory route, in both animals and humans for assimilated chromium, with only small amounts being lost in perspiration and bile. Urinary chromium excretion reflects the dietary chromium intake in a dose dependent manner (Kumpulainen *et al.*, 1983; Uusitupa *et al.*, 1983; Aitio *et al.*, 1988). The normal chromium excretion via urine is from 0.05 – 0.5 µg/day, representing a dietary intake range of 10 – 200 µg/day (Anderson and Kozlovsky, 1985; Uusitupa *et al.*, 1983; Uusitupa *et al.*, 1992). Urinary chromium increases after physical exercise and the amount excreted may be related to the fitness of the individual, with fitter people having lower basal urinary chromium but greater losses in response to exercise (Haymes, 1998).

Toxicity

44. The toxicity of chromium compounds has been reviewed by COT (1995), the US Agency for Toxic Substances and Disease Registry (ATSDR, 1993), IARC (1990), WHO (1988) and HSE (1988). The 1995 COT review of chromium is attached at Annex 1.

45. The oxidation state of chromium is a critical factor, determining not only the route dependent bioavailability, but also its toxicity. Compounds of both hexavalent and trivalent chromium can cause contact dermatitis and hexavalent chromium is locally irritating and corrosive due to its acidity and oxidising potential.

46. Acute exposure of experimental animals to hexavalent chromium leads to acute nephrotoxicity, which can be sufficiently severe to cause renal failure and death. Humans can also suffer renal tubular damage following massive exposure to hexavalent chromium. The main clinical features of acute toxicity of chromium administered orally are vomiting, diarrhoea, haemorrhagic diathesis and blood loss into the gastrointestinal tract causing cardiovascular shock. Other effects that have been noted under extreme conditions include liver necrosis, tubular necrosis of the kidneys and oedema of the brain. Systemic effects of low-level hexavalent chromium have been difficult to document in any species. Systemic effects of trivalent chromium are not known.

*Human toxicity**Acute toxicity*

47. These studies have mainly been in persons, occupationally exposed via the inhalation or dermal routes. Studies investigating the effects of oral exposure are mainly on accidental or intentional ingestion. Deaths have been reported following ingestion of 7.5 mg of chromium (VI)/kg as dichromate and from ingesting 4.1 mg chromium (VI)/kg as chromic acid (ATSDR, 1993). Deaths were preceded by gastrointestinal haemorrhage, and severe kidney and liver damage. Haematological effects have also been reported in individuals after consuming lethal or sublethal doses.

48. Van Heerden *et al.* (1994) report on a case of acute toxicity from ingestion of trivalent chromium. A woman is reported to have ingested 400 ml of a leather tanning solution containing 48 g of basic chromium sulphate (CrOH_2SO_4). The patient died of cardiogenic shock, complicated acute renal shock, pancreatitis, haemorrhage and gut mucosal necrosis.

Chronic and subchronic toxicity

49. The reported acute and chronic effects of chromium are associated with the hexavalent form. Hexavalent chromium is an unstable oxidising compound, in contrast to trivalent chromium, that is thought to be stable and essentially non-toxic on oral administration. A review of the literature (pre-1997) failed to reveal any reports in which trivalent chromium administered orally, to humans, induced renal toxicity or any lesion, other than transient gastrointestinal irritation associated with massive bolus doses (McCarty, 1997). Subsequent to this, the following two case reports describe suspected toxic effects of trivalent chromium (see also para 46).

50. A patient, who developed renal failure after using over the counter oral chromium picolinate, 600 μg daily for six weeks (12 -14 times the normal chromium intake and 3 times the manufacturers recommended dose for dietary supplementation), to aid weight reduction, presented for evaluation of renal insufficiency (Wasser and Feldman, 1997). Tests had shown normal renal function two years previously, but now showed a blood nitrogen urea level of 74 mg/dl and creatinine of 5.9 mg/dl. Urinalysis showed a protein level of 30 mg/dl and trace amounts of blood. Findings from a renal biopsy supported a diagnosis of nephrotoxicity, which the authors attribute to ingestion of chromium.

51. Cerulli *et al.* (1998) describe a case of toxicity due to ingestion of 6 – 12 times the daily recommended allowance of over the counter chromium (III) picolinate (1200 – 2400 $\mu\text{g}/\text{day}$ for 4 – 5 months. The 33 year old woman presented with weight loss, anaemia, haemolysis, liver dysfunction (aspartate aminotransferases 15- 20 times normal, total bilirubin 3 times normal) and renal failure (blood urea nitrogen 152 mg/dl, serum creatinine 5.3 mg/dl). The patient had plasma chromium concentrations 2 – 3 times normal. The patient received blood product transfusions and haemodialysis, haemolysis and liver function improved over six days and renal function began to

return on day 12. One year later, all measured parameters were within normal limits (Cerulli *et al.*, 1998).

Genotoxicity

52. Few reports exist dealing with chromium-induced mutagenicity in human subjects, and only hexavalent chromium causes any effects. Studying cultured lymphocytes, obtained from workers occupationally exposed to chromic acid, Sarto *et al.* (1982) found an increased frequency of sister chromatid exchanges compared with controls, which was correlated with urinary-chromium levels and enhanced by smoking.

Carcinogenicity

53. Hexavalent chromium is carcinogenic via the inhalation route; epidemiological studies have found an association between exposure to chromium (VI) and lung cancer (WHO, 1988). The potential for such carcinogenicity appears to be associated with the inhalation of the less soluble or insoluble hexavalent chromium compounds, possibly due to the fact that these compounds stay in contact with the tissues for long periods of time (depot effect). A few cases of cancer in the upper respiratory tract have been reported, but cancer has not been convincingly demonstrated in other body tissues.

54. Carcinogenicity via the oral route, in humans has not been adequately studied. A limited retrospective mortality study, conducted on a population which resided in a polluted area near an alloy plant that smelted chromium, and where contamination of water supplies had occurred (20 mg Cr VI/l), found increased incidences of lung and stomach cancer (Zhang and Li, 1987). However, as this was not a controlled study, the population would have been exposed to chromium by all routes, i.e. air, water, food and soil and all other contaminants that were in the environment.

55. Trivalent chromium is not considered to be carcinogenic (IARC, 1990) and there is no evidence of excess cancer in studies of two industries, where only trivalent compounds were present. This is further supported by the chemical and biological characteristics of the trivalent state, i.e. non-oxidising, non-irritating and probably unable to penetrate cell membranes.

56. IARC (1990) has classified hexavalent chromium, on the basis of combined results of epidemiological studies (exposure to chromium by inhalation) and carcinogenicity studies in experimental animals, into Group 1 (i.e. it is a known lung carcinogen) and trivalent chromium into Group 3 (i.e. it is not classifiable as to its carcinogenicity in humans).

Human supplementation studies

57. In 19 randomised controlled trials, in which individuals took between 175 and 1000 µg/day chromium for durations between 6 and 64 weeks, no evidence of toxic effects was reported (Reviewed by Jeejeebhoy, 1999; all original sources checked). Chromium was generally in the form of chromium picolinate, but also as chromium

chloride and chromium nicotinate. In addition to those described below, some reports considered in Jeejeebhoy's review are described elsewhere in this review (see Overview of reported beneficial effects and Interactions sections).

58. In one study, antibody titres of an oxidised DNA base, 5-hydroxymethyl-2'-deoxyuridine, were measured, following an eight-week course of 400 µg chromium picolinate/day and indicated no detectable DNA damage (Kato *et al.*, 1998). Anderson *et al.* (1985) reported increased serum chromium levels following three months supplementation with 200 µg Cr/day (as chromic chloride). Mean basal serum chromium levels were 0.13 ng/ml and increased significantly to 0.38 ng/ml following supplementation, but no adverse effects were noted in this study.

59. Abraham *et al.* (1992) reported a significant increase in mean serum chromium levels from 2.69 nmol/l to 12.12 nmol/l in a randomised clinical trial in which seventy-six patients with atherosclerotic disease were given 150 µg/day chromium chloride or placebo for a period of 7 to 16 months. Clinical chemistry indicated no adverse effects on liver or renal function and haematology indicated no haematological abnormalities. The authors reported that both placebo and chromium supplement were well tolerated and that no adverse effects were reported by patients.

60. A low number of side effects were reported, including mild gastrointestinal symptoms and decreased appetite in a randomised, double-blind placebo-controlled study in which 63 male patients, prescribed beta-blockers for the treatment of hypertension, were given 600 µg/day trivalent chromium or placebo for 8 weeks. However, these were of similar low frequency in both treated and control groups (Roeback *et al.*, 1991).

61. A randomised, double-blind study was conducted in which 23 men aged 50-75 yrs were given 924 µg/day chromium (as picolinate) or placebo for a period of 12 weeks, during which time the subjects participated in a resistance training programme. Five subjects did not complete the study due to reasons unrelated to chromium or placebo treatment (injury, aggravation of injuries, family commitments). No adverse effects were discussed (Campbell *et al.*, 1999).

Animal toxicity

62. In general hexavalent chromium compounds, in experimental animals, are more toxic than trivalent and it is evident that the toxicity of hexavalent chromium, in animals, varies with the route of entry into the body. Water soluble trivalent chromium compounds were moderately toxic when given orally in single doses to rats and mice, with LD₅₀ values varying from 140 - 422 mg/kg, whereas values for hexavalent chromium ranged from 40 - 795 mg/kg, with a positive correlation between toxicity and water solubility (HSE, 1989). Oral exposures to chromium (III and VI) compounds have resulted in gastrointestinal, hepatic, renal, immunological, neurological, developmental and reproductive effects.

Chronic toxicity

63. Only a limited number of studies exist that have investigated the chronic effects of chromium administered via the oral route; these studies are also very old. Chromium (III) compounds are generally observed to be of very low toxicity following repeated oral administration. No signs of toxicity were observed in rats and mice when levels of up to 100 ppm were administered in diet (HSE, 1989). No adverse effects were observed at any dose level in rats fed chromic dioxide in the diet at levels of 0, 1, 2, or 5 % (estimated equivalent to 150, 300 or 750 mg/kg bw/day), for five days a week for a total of 840 days (600 feeds) (Ivankovic and Preussman, 1975). These data were used by the EPA to calculate an oral reference dose (RfD) for insoluble salts of trivalent chromium. The EPA calculated the high dose to be equivalent to 1468 mg/kg/day.

64. There were no statistically significant differences in body weight, organ weights or blood variables (glucose, cholesterol, triglycerides, blood urea nitrogen, lactic acid dehydrogenase, transaminases, total protein and creatinine) in rats fed diet supplemented with 0, 5, 25, 50 or 100 mg trivalent chromium/kg diet (estimated equivalent to 0.75, 3.75, 7.5, or 15 mg/kg bw/day) (Anderson *et al.*, 1997b). Trivalent chromium was supplemented as both chromium chloride and in the more bioavailable form of chromium picolinate. Histological evaluation of the liver and kidney of control animals and animals fed 100 mg/kg of diet, also did not show any detectable differences. Liver and kidney chromium concentrations increased linearly for both chromium chloride and picolinate.

65. Limited studies of repeat oral doses of hexavalent chromium in experimental animals have also produced little evidence of toxicity. Reduced enzyme activity was reported in the small intestine of rats receiving oral doses of 10 mg/kg/day for 14 days (HSE, 1989). Decreased body weight gain, increased lipid content of the liver and kidneys and reduced activity of several liver and kidney enzymes were also reported in rats given 14 mg/kg/day by oral gavage for 20 days (HSE, 1989). No effects were seen after administration of chromium (VI) in drinking water up to 200 ppm for 33 days in rats, at 100 ppm for up to one year in mice, or at 11 ppm for four years in dogs (HSE, 1989). The EPA used the results of a study by Mackenzie *et al.* (1958) to calculate an RfD for the soluble salts of hexavalent chromium. No significant adverse effects were reported in rats receiving drinking water containing potassium chromate at concentrations up to 25 mg/l (equivalent to 2.4 mg/kg/day) for one year.

Reproductive toxicity

66. Elbetieha and Al-Hamood (1997) exposed adult male and female mice to drinking water containing 2,000 or 5,000 mg hexavalent chromium/l for 12 weeks (in the form of potassium dichromate) and 2,000 or 5000 mg trivalent chromium/l for 12 weeks (in the form of chromium chloride). The dose can be estimated to be equivalent to 500 or 1250 mg/kg bw/day. Fertility was significantly decreased in males exposed to the trivalent chromium compound and the number of implantation sites and viable foetuses was significantly reduced in females impregnated by males exposed to either trivalent or hexavalent chromium. The exposure of female mice to trivalent or hexavalent chromium compounds significantly reduced the number of implantation sites and the number of viable foetuses. The number of resorptions was increased in females exposed to either trivalent or hexavalent chromium, but this was only

significant ($p < 0.001$) in the hexavalent chromium exposed females (Elbetieha and Al-Hamood, 1997). However, it should be noted that although no mortality, or clinical signs of toxicity, were noted in any of the groups, both levels of chromium supplementation were high enough to produce a significant decline in body weight.

67. In a study by Junaid *et al.* (1995), female mice received 250, 500 or 750 ppm of hexavalent chromium, as potassium dichromate, in drinking water on days 14 to 19 of pregnancy. The dose can be estimated to be equivalent to 62.5, 125 or 187.5 mg/kg bw. Gestational weight gain of the mothers, foetal weight and crown length decreased in the 500 and 750 ppm treatment groups. The high dose group (750 ppm) also had significantly higher incidences of post-implantation loss. Chromium levels were reported to increase in a dose-dependent manner in maternal blood and placenta, and in the foetuses.

Carcinogenicity

68. Evidence exists from studies in experimental animals demonstrating the carcinogenicity of inhaled chromium. However, limited data are available concerning ingested doses. Administration of low levels of trivalent chromium acetate (5 mg/l in drinking water) to rats and mice for life did not result in increased incidence of tumours compared with controls (WHO, 1988). Trivalent chromium oxide, incorporated in diet at concentrations of 1, 2 or 5 % fed to rats did not increase tumour incidence compared with controls (WHO, 1988). Hexavalent chromium compounds have not been tested by oral administration.

Genotoxicity

69. Hexavalent chromium compounds are historically reported to produce a variety of genotoxic effects including DNA damage, mutations and chromosomal aberrations, in a number of *in vivo* and *in vitro* test systems. It is widely accepted that hexavalent chromium is genetically active because of its ability to cross cell membranes and enter cells. If reduction of hexavalent chromium takes place outside the cell (or even outside the cell nucleus, e.g. in mitochondria or endoplasmic reticulum) its genotoxic activity is suppressed. If the reduction takes place in the nucleus, alterations in the DNA may still occur, depending on the oxidation power of hexavalent chromium or the formation of trivalent chromium complexes with nucleophilic sites of the DNA.

In vitro

70. In contrast, negative genotoxicity results were obtained when trivalent chromium compounds were tested *in vitro* for point mutations or DNA damage in bacteria, and for gene mutations, unscheduled DNA synthesis or cell transformation in mammalian cells (HSE, 1989). These results are consistent with very poor uptake of trivalent chromium in cells.

71. More recently, Gao *et al.* (1993) reported that chromium (VI) and not chromium (III) stimulated minimal unscheduled DNA synthesis (UDS) at sub-toxic concentrations in cultured hepatocytes. A positive UDS response was only observed at cytotoxic concentrations of hexavalent chromium. Cytotoxicity (measured using

tetrazolium salt (MTT) reduction assay) was reported at a much higher dose of chromium (III), (> 50 μM) compared to that of chromium (VI), (> 2.5 μM).

72. Studies of chromium (III) complexes for their ability to cause chromosomal aberrations in Chinese hamster ovary cells, found chromium picolinate able to produce damage 3 to 18-fold above control levels, at doses of 0.05, 0.10, 0.50 or 1.0 mM for 24 hours (Stearns *et al.*, 1995). Chromium nicotinate and chromium hexahydrate did not produce chromosomal damage at equivalent doses. The authors suggested that the damage seen with chromium picolinate was due to the picolinate moiety, because picolinic acid in the absence of chromium was found to be clastogenic (Stearns *et al.*, 1995).

73. In a further study, chromium picolinate, in the presence of 5 M ascorbic acid, was shown to cause DNA cleavage when added to pUC19 plasmid DNA at concentrations of 20 nmol/l and higher for periods of 20 minutes or more (Speetjens *et al.*, 1999). The authors suggested that chromium picolinate is reduced by ascorbic acid and other reductants to chromium II-containing species, which are susceptible to oxidation, producing hydroxyl radicals.

74. Dartsch *et al.* (1998) reported that chromium (VI), but not chromium (III), has an acute cytotoxic effect and causes a dose-dependent loss in cell viability, in human kidney and liver derived cell lines *in vitro*. The effective dose that caused 50% cell death was 5 $\mu\text{mol/l}$ for kidney epithelial cells and 50 $\mu\text{mol/l}$ for liver epithelial cells. The observation that the kidney epithelial cells are ten times more sensitive towards chromium (VI) than the liver epithelial cells may explain the known nephrotoxicity *in vivo*.

In vivo

75. Chronic low dose administration of sodium (VI) dichromate (2.5 mg/kg/day for 75 days) induces an oxidative stress, as determined by increased hepatic lipid peroxidation, hepatic glutathione depletion hepatic nuclear DNA damage and excretion of urinary lipid metabolites. The resulting tissue damaging effects, may contribute to the toxicity and carcinogenicity associated with chromium VI (Bagchi *et al.*, 1997).

Regulatory Considerations

76. There are no specific regulations on maximum levels of chromium in foods.

Existing recommendations on maximum intake levels

77. As noted previously, COMA (1991) have recommended a safe dietary intake of >25 $\mu\text{g/day}$. In 1991, the joint MAFF/Department of Health Working Group identified 1 g/day as an undesirable dose and recommended that intake should not exceed 1/10th of that (MAFF/DH, 1991).

Existing recommendations on maximum supplementation levels

78. The UK trade association, the Council for Responsible Nutrition recommend an upper safe limit of 300 µg/day trivalent chromium for short term supplementation and 200 µg/day for long term supplementation (CRN, 1999).

Summary

79. Chromium is ubiquitous in nature, occurring in air, water, soil and biological materials, over a range of concentrations. Most chromium in foods is in the trivalent form and derives from food processing with stainless steel instruments and containers. The average intake of chromium from diet is estimated at 0.1 mg/day.

80. Chromium is an essential element that potentiates insulin action and thus, influences carbohydrate, lipid and protein metabolism. The nature of this relationship however, has not been clearly defined. Chromium deficiency has only been observed in patients on long-term parenteral nutrition with infusates low in chromium and has been induced in experimental animals.

81. Intestinal absorption of chromium is low (0.5 – 2.0 %) and some data indicates that it is inversely related to its dietary intake. In plasma absorbed chromium is bound mainly to transferrin and other plasma proteins, which are responsible for its transport in the body. Long-term storage of chromium occurs in the liver, spleen and bones. Most dietary chromium is not absorbed (98 %) and is excreted in the faeces. Urine is the main excretory route for assimilated chromium in both humans and animals.

82. The oxidation state of chromium is a critical factor in determining its toxicity. In humans acute toxicity (of trivalent or hexavalent chromium) is characterised by gastrointestinal haemorrhage, and severe liver and kidney damage and may lead to death. Chronic exposure to trivalent chromium is reported to induce renal failure, anaemia, haemolysis and liver failure. Where follow-up was carried out symptoms were reversible and returned to normal parameters in one year.

83. The effects of chromium in animals are summarised in Tables 1 & 2. In general hexavalent chromium compounds, in experimental animals, are reported to be more toxic than trivalent. Acute oral exposure to both trivalent and hexavalent chromium has resulted in gastrointestinal, hepatic, renal, immunological, neurological, developmental and reproductive effects.

84. Chronic administration of trivalent chromium to rats and mice resulted in increased kidney and liver chromium content, but no significant adverse effects at levels up to 750 mg/kg/day. In contrast to available data regarding the carcinogenicity of inhaled chromium, administration of levels of chromium in drinking water to rats and mice for life, did not result in any increase in tumour incidence.

85. Positive results are obtained with hexavalent chromium in genotoxicity studies *in vitro* and *in vivo*. In contrast, trivalent chromium gave negative results when tested *in vitro* for point mutations or DNA damage in bacteria, and for unscheduled DNA

synthesis or cell transformation in mammalian cells. An increase in chromosomal aberrations has been reported in Chinese hamster ovary cells, caused by chromium (III) picolinate, but not by other trivalent chromium compounds and this was considered to be due to the picolinate moiety.

86. A reduction in fertility in male mice exposed to either trivalent or hexavalent chromium has been reported, and a decrease in implantation sites and viable fetuses in female mice. Hexavalent chromium also increased the number of resorptions.

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