Chromium in Drinking-water

Draft Background document for development of WHO Guidelines for Drinking-water Quality

26 September 2019
Preface

To be updated by WHO Secretariat
Acknowledgements

The first draft of the background document on chromium in drinking-water for the development of the WHO Guidelines for Drinking-water Quality was prepared by Dr Ruth Bevan, Independent Consultant.

To be updated by WHO Secretariat
Abbreviations used in the text

ALT: alkaline phosphatase
ATSDR: Agency for Toxic Substances and Disease Registry (USA)
bw: body weight
Cr: chromium
Cr(III): trivalent chromium
Cr(VI): hexavalent chromium
CrCl: chromium chloride
Cr₂O₃: chromium oxide
CrO₄²⁻: chromate ion
Cr(OH)₃(3-n)⁺: chromium hydroxide Cr(C₆H₄NO₂)₃
Cr(C₆H₄NO₂)₃: chromium picolinate
CrO₃: chromium trioxide
DNA: deoxyribonucleic acid
EFSA: European Food Safety Authority
GI: gastrointestinal
HCrO₄⁻: chromic acid
IOM: Institute of Medicine (USA)
K₂CrO₄: potassium chromate
K₂Cr₂O₇: potassium dichromate
LD₅₀: median lethal dose
L: litre
LOAEL: lowest-observed-adverse-effect level
NADH: nicotinamide adenine dinucleotide
NADPH: nicotinamide adenine dinucleotide phosphate
US NTP: United States National Toxicology Program
NOAEL: no-adverse-effect-level
PBPK: physiologically-based pharmacokinetic model
pH: potential of hydrogen
PPI: proton pump inhibitor
TDI: Tolerable Daily Intake
US EPA: United States Environmental Protection Agency
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1.0 EXECUTIVE SUMMARY

Humans can be exposed to chromium (Cr), primarily as trivalent [Cr(III)] and hexavalent [Cr(VI)] forms, through its wide distribution in air, soil, ground and drinking-water, originating from both natural and anthropogenic sources. Once absorbed, Cr(VI) readily penetrates cell membranes, while Cr(III) does not. General population exposure to Cr compounds through inhalation of ambient air, ingestion of water, or dermal contact, is variable and difficult to quantify. Residents living in close proximity to industrial facilities that use Cr(VI) compounds, or near Cr waste disposal sites, have the greatest potential for exposure. A guideline value (GV) of 50 µg/L is proposed for total Cr based on aspects of achievability by current treatment technologies, measurability by analytical methods, and toxicology. Based on newer, high quality data from chronic drinking water carcinogenicity and mode of action studies for Cr(III) and Cr(VI), the risk assessment of Cr(VI) in drinking water considers both of both cancer (in the case of Cr(VI) and non-cancer (in the case of Cr(III) and Cr(VI)) endpoints and the weight-of-evidence supporting a non-linear mode of action involving hyperplasia in the small intestine as a key precursor event to tumour development. Thus, a GV for Cr(VI) in drinking water considering hyperplasia as the most sensitive endpoint and precursor of tumour formation, is protective of both non-cancer and cancer effects. As Cr is usually found in drinking-water (average of 0.001 mg/L) at concentrations below the guideline value, in general it would only require investigation for monitoring and inclusion in drinking-water regulations and standards if there were indications that a problem might exist. Monitoring can usually be limited to the treatment works.

2.0 GENERAL DESCRIPTION

2.1 Identity

Chromium (Cr) is widely distributed in the earth's crust. It can exist in oxidation states of +2 to +6, with the trivalent (III) and hexavalent (VI) states predominating. Soils and rocks may contain small amounts of Cr, almost always in the trivalent state (ATSDR, 2012).

2.2 Physicochemical properties

<table>
<thead>
<tr>
<th>Property</th>
<th>Chromium (Cr)</th>
<th>Chromium chloride (CrCl₃)</th>
<th>Potassium chromate (K₂CrO₄)</th>
<th>Chromium oxide (Cr₂O₃)</th>
<th>Chromium trioxide (CrO₃)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melting point (°C)</td>
<td>1857</td>
<td>1152</td>
<td>968.3</td>
<td>2266</td>
<td>196</td>
</tr>
<tr>
<td>Boiling point (°C)</td>
<td>2672</td>
<td>-</td>
<td>-</td>
<td>4000</td>
<td></td>
</tr>
<tr>
<td>Solubility</td>
<td>Insoluble</td>
<td>Slightly soluble</td>
<td>790 g/L</td>
<td>Insoluble</td>
<td>624 g/L</td>
</tr>
<tr>
<td>Density (g/cm³)</td>
<td>7.14</td>
<td>2.76</td>
<td>2.73</td>
<td>5.21</td>
<td>2.70</td>
</tr>
</tbody>
</table>

2.3 Organoleptic properties

There is no indication that Cr compounds at the levels normally found in drinking-water cause adverse effects on taste, odour, appearance or colour.
2.4 Major uses and sources

More than 70% of Cr in the environment comes from anthropogenic sources, such as non-ferrous base metal smelters, refineries, leather tanning industries, urban storm water runoff, effluent streams from pulp and paper mills and discharges from thermal generating stations (Health Canada, 2016). Cr and its salts are also used in the manufacture of catalysts, pigments and paints, fungicides, the ceramic and glass industry, in photography, and for chrome alloy and Cr metal production, chrome plating, and corrosion control (ATSDR, 2012; NIOSH, 2013). Cr also occurs naturally in small amounts in rocks and soils, some of which is released into ground water through weathering and erosion processes (Thompson et al., 2007; Health Canada, 2016).

2.5 Environmental fate

The environmental distribution of compounds containing Cr(III) and Cr(VI) depends on redox potential, the pH, the presence of oxidising or reducing compounds, the kinetics of the redox reactions, the formation of Cr(III) complexes or insoluble Cr(III) salts, and the total Cr concentration. In the environment, Cr(VI) occurs mostly as chromate ion (CrO₄²⁻) or chromic acid (HCrO₄), and Cr(III) as chromium hydroxide Cr(OH)₃⁺. In soil, Cr(III) predominates. Cr(VI) can easily be reduced to Cr(III) by organic matter, for example, and its occurrence in soil is often the result of human activities. In water, Cr(III) is a positive ion that forms hydroxides and complexes and is adsorbed at relatively high pH values. In surface waters, the ratio of Cr(III) to Cr(VI) varies widely, and relatively high concentrations of the latter can be found locally. In general, Cr(VI) salts are more soluble than those of Cr(III), making Cr(VI) relatively mobile. In drinking-water treatment, oxidative disinfection techniques such as chlorination may oxidise Cr(III) to Cr(VI) (Health Canada, 2016).

In air, Cr is present in the form of aerosols. It can be removed from the atmosphere by wet and dry deposition. Both trivalent and Cr(VI), from all sources, are released into the air. Because of analytical difficulties, data on Cr speciation in ambient air are rarely available, but the proportion present as Cr(VI) has been estimated as 0.01–30%, based on available studies (Health Canada, 2016).

3.0 ENVIRONMENTAL LEVELS AND HUMAN EXPOSURE

3.1 Water

Background levels of Cr in surface water and groundwater aquifers are a direct function of regional geology, mineral weathering processes, sediment loading rates and precipitation patterns (Health Canada, 2016). High concentrations of Cr may occur naturally in groundwater in areas with mafic or ultramafic volcanic or metamorphic rocks (i.e. rocks that consist mainly of ferromagnesian minerals with no quartz), being particularly prevalent in ophiolite complexes and serpentine-rich units (Thompson et al., 2007). Levels in uncontaminated waters are usually very low (< 1μg/L), although leaching of wastewater from landfill or release through anthropogenic activities may cause contamination of drinking-water (EVM, 2002). Occurrence of Cr(VI) in drinking-water supplies is relatively rare, and studies assessing levels are scarce. Total Cr is regularly monitored in UK drinking-waters and summary results for individual suppliers are published (Environment Agency, 2002). Of over 12,000 drinking-water compliance samples taken in England and Wales in 2016, none exceeded 50 μg/L. The maximum value reported was 15 μg/L and the 95%ile was 1 μg/L (personal communication from P K Marsden to Ruth Bevan). Average Cr(VI) concentrations in Canadian and US drinking-water supplies range from 0.2 to 2 μg/L (Moffat et al., 2018). A survey of 23 sources in the UK over a 12-month period, reported that background Cr(VI) levels were <0.1 μg/l (WRC, 2015). Data from the US collected under the Unregulated Contaminant Monitoring
Rule (UCMR) for the period 2013 – 2015 (UCMR 3¹) showed Cr(VI) to be present in drinking-water across all states at levels between 0.057 and 7.51 µg/L. Recognizing some data anomalies such as paired samplings where the Cr(VI) values were greater than the Total Cr values (Eaton et al., 2018), the majority of states had Cr(VI) levels between 0.1 and 1.0 ppb. In the Netherlands, the total Cr concentration of 76% of the supplies was reported to be below 1 µg/L and of 98% of supplies below 2 µg/L (Fonds et al., 1987). A survey of Canadian drinking-water supplies reported an overall median level of 2 µg of total Cr/L, with maxima of 18.9 µg/L (ground water source) (Health Canada, 2016).

Very little to no data are generally available on the speciation of Cr in drinking-waters (Environment Agency, 2002; WRe, 2015). Recently, a small amount of work has been done in the UK towards resolving this issue. A survey of total Cr, Cr(III) and Cr(VI) has been conducted in finished drinking-water at twenty sites in England and Wales, surveyed, insofar as was possible, four times over a 12-month period. Findings showed that the concentrations of Cr(VI) in drinking-water in England and Wales are very low, generally <1 µg/L, which appears to be consistent with typical background concentrations of Cr(VI) in other countries.

The average concentration of total Cr in rainwater is in the range 0.2 – < 1 µg/L (WRe, 2015). Natural Cr total concentrations in seawater of 0.04–0.5 µg/L have been measured, and in the North Sea, a concentration of 0.7 µg/L was found (WHO, 1988).

The natural total Cr content of surface waters is approximately 0.5–2 µg/L and the dissolved Cr content, 0.02–0.3 µg/L (WRe, 2015). Total Cr concentrations in Antarctic lakes increase with depth from <0.6 to 30 µg/L (US EPA, 1987). Most surface waters contain between 1 and 10 µg of total Cr/L. In general, the total Cr content of surface waters reflects the extent of industrial activity. In surface waters in the USA, levels up to 84 µg/L have been found (US EPA, 1987); in central Canada, surface water total concentrations ranged from 0.2 to 44 µg/L [data from the National Water Quality Data Bank (NAQUADAT), Inland Waters Directorate, Environment Canada, 1985]. In the Rhine, total Cr levels are below 10 µg/L, and in 50% of the natural stream waters in India the total concentration is below 2 µg/L (US EPA, 1987).

In general, the total Cr concentration in groundwater is low (<1 µg/L) (Health Canada, 2016). Levels in the UK have been reported to be < 3 µg/L (WRe, 2015). In the Netherlands, a mean total concentration of 0.7 µg/L has been measured, with a maximum of 5 µg/L (WHO, 1988). Most supplies in the USA contain less than 5 µg/L. In 1986, total levels in 17 groundwater supplies and one surface water supply exceeded 50 µg/L, with median levels of 2–10 µg/L (US EPA, 1987; ATSDR, 2012; WRe, 2015). In India, 50% of 1473 water samples from dug wells were reported to contain below 2 µg/L (US EPA, 1987).

### 3.2 Food

The main source of Cr exposure is thought to be from food (with the exception of populations living close to a point source). Food has been found to contain total Cr at concentrations ranging from < 0.0005 to 1.3 µg/g (UK Ministry of Agriculture, Fisheries and Food, 1985; UK Food Standards Agency, 1999; ATSDR, 2012; EFSA, 2014; Health Canada, 2016). Highest concentrations (> 0.1 µg/g) have been found in meat, fish, seafood, cereal products, tea, black pepper, cheese, wheat germ and some fruits and vegetables (UK Ministry of Agriculture, Fisheries and Food, 1985; Copat et al., 2012). However, total Cr levels in fresh foods tend to be extremely low (0.02 – 0.05 µg/kg) (Health Canada, 2016). Beer, wine and spirits contain total Cr concentrations of approximately 450, 300 and 135 µg/L, respectively (US EPA, 1984a). Stainless steel utensils used in food preparation may also contribute to total Cr levels (Health Canada, 2016).

¹ https://www.epa.gov/dwucmr/occurrence-data-unregulated-contaminant-monitoring-rule#3
Based on recent speciation work in food and the recognition of food as a reducing medium, The European Food Safety Authority (EFSA) has stated that there is a lack of Cr(VI) in food and considered that all reported Cr in food could be classed as Cr(III) (EFSA, 2014).

### 3.3 Air

In remote areas including the Arctic region and the Antarctic, Cr concentrations of 0.005 to 2.6 ng/m³ have been measured (Cary, 1982; Barrie & Hoff, 1985; Schroeder et al., 1987; Sheridan & Zoller, 1989). Ambient air at most petrol stations in the USA were found to contain very little Cr; mean levels were generally below 300 ng/m³, and median levels less than 20 ng/m³ (US EPA, 1984b). In non-industrialised areas, concentrations above 10 ng/m³ are uncommon (NAS, 1980). Concentrations in urban areas are 2–4 times higher than regional background concentrations (Nriagu & Nieboer, 1988). Saltzman et al. (1985) compared the levels of atmospheric Cr at 59 sites in US cities during 1968–1971 with data from the US Environmental Protection Agency (US EPA) National Aerometric Data Bank file for 1975–1983. They concluded that atmospheric Cr levels may have declined in the early 1980s from the levels detected in the 1960s and 1970s. The mean concentration of total Cr in air in the Netherlands has been reported to range between 2 to 5 ng/m³ (Sloof et al., 1989). In the UK, Defra² reported average levels of Cr in urban and rural areas during the period 2009 – 2010 to generally be within the range 0.7 to 5 ng/m³; one outlier was identified with an average level of 30.3 ng/m³ in an area with close proximity to steel making industry.

Indoor air concentrations of total Cr can be 10–400 times greater than outdoor concentrations (approximately 1000 ng/m³) as a result of tobacco smoke. An indoor/outdoor air study conducted in the US in 1993 reported Cr(VI) levels of 0.1–0.6 ng/m³ for indoor air (geometric mean 0.2 ng/m³) and 0.10–1.6 ng/m³ for outdoor air (geometric mean 0.55 ng/m³), with the particles being of inhalable size (Bell & Hipfner, 1997). The indoor levels were lower than those of 0.38 to 3,000 ng/m³ (mean of 1.2 ng/m³) reported in an earlier study in the US (Falerios et al., 1992).

### 3.4 Bioaccumulation

Cr is not considered to bioaccumulate along the aquatic food chain (US EPA 1980, 1984a). Cr(VI) is taken up by fish but is transformed to Cr(III) (EU, 2005). Some data indicate that Cr has a low mobility for translocation from roots to above ground parts of plants (Cary, 1982; WHO 1988) but the transfer ratio of Cr from soil to plants and biomagnification in terrestrial food chains is unknown (Health Canada, 2016).

### 3.5 Estimated total exposure and relative contribution of drinking-water

The general population is exposed to Cr by inhaling ambient air and ingesting food and drinking-water containing Cr. The estimated average total intake of total Cr from air, water, and food by the general population in the United Kingdom is approximately 127 μg bw per day. Food contributed around 92% of the total intake and water 8%. The contribution from air was negligible (Defra, 2002). In the Netherlands, the estimated mean daily total Cr intake is 100 μg, with a range of 50–200 μg (Sloof et al., 1989; WHO, 1996). The daily total Cr intake for the US population from consumption of selected diets (diets with 25 and 43% fat) has been estimated to range from 25 to 224 μg, with an average of 76 μg (Kumpulainen et al., 1979). In general, food appears to be the major source of intake. Drinking-water intake can, however, contribute substantially when total Cr levels in drinking-water are above 25 μg/L.

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² https://uk-air.defra.gov.uk/data/metals-data
In Canada, total daily intakes of Cr(VI) from all exposure sources (e.g. drinking-water, food, air, soil, and dust) were estimated for five age groups and the relative source contribution from drinking-water was calculated. Based on the mean total Cr concentration of 2.0 μg/L in unfiltered Ontario drinking-water and assuming that Cr(VI) represents 100% of total Cr, contributions of 99%, 0%, 51%, 51%, 50% and 64% for non-breastfed infants 0–6 months of age, breastfed infants 0–6 months of age, 0.5–4 years, 5–11 years, 12–19 years and 20+ years, respectively were reported (Health Canada, 2016). Food is the second major source of Cr exposure and assuming that 10% of total Cr in food is Cr(VI), exposure through food may represent up to 50% of the total daily intake (Health Canada, 2016). Hence, Health Canada (20016) estimated an allocation factor of 0.5 for drinking-water to indicate the minimum contribution of drinking-water to the total daily intake of Cr(VI) for Canadian adults.

4.0 TOXICOKINETICS AND METABOLISM IN HUMANS AND LABORATORY ANIMALS

4.1 Absorption

The water solubility and oxidation state of the different Cr compounds are important factors influencing their absorption rates via oral, inhalation and dermal routes.

In humans, absorption of Cr following oral administration is relatively low, estimated (through urinary excretion) as < 2% for Cr(III) and around 7% for Cr(VI) (WHO, 1988; ATSDR, 2012).

Oral exposure studies in animals also report low absorption, with < 0.5–6% of Cr compounds being absorbed, depending on solubility. Tissue Cr levels of rats exposed to Cr(VI) (as potassium chromate) in drinking-water were 4–15 times higher than those of rats exposed to Cr(III) (as the trichloride). Cr(VI) appears to be absorbed from the gastrointestinal (GI) tract to a greater extent than Cr(III), due to the involvement of anion transporter (sulfate/phosphate) channels (ATSDR, 2012; WHO, 2013). However, absorption of Cr(VI) is effectively limited in humans, rats and mice because of intragastric reduction to Cr(III) in bodily fluids, including the gastric fluid (ATSDR, 2012). More recent studies reported by De Flora et al. (2016) and Kirman et al. (2016) have confirmed the ability of human gastric fluid to reduce Cr(VI), thereby reducing its biological activity. Cr(VI) reduction was rapid, with 70% of total reduction occurring within 1 min and 98% of reduction within 30 min with post-meal gastric fluid at pH 2.0; decreasing Cr(VI) reducing capacity was observed at higher gastric fluid pH and at higher Cr(VI) concentrations (>0.7 mg/L). Important differences in reduction capabilities were noted between fasted (lower stomach pH) and fed samples (higher stomach pH). This may be of significance for individuals with elevated gastric pH levels, including neonates, users of proton pump inhibitors (PPIs) and those with hypochlorhydria, as Cr(VI) may be reduced to Cr(III) at a lower rate (Kirman et al., 2016).

Moffatt et al. (2018) report that the reduction of Cr(VI) in both rodents and humans can be described mathematically as a three-pool model containing reducing agents of differing capacities and rates. Specifically, a fast acting, low-capacity pool, a slower-acting high capacity pool and a very slow-acting high-capacity pool. At low concentrations of Cr (VI) typical of those found in drinking water, reduction is rapid and efficient, whereas at the high concentrations used in the mice studies, reduction is slower and less efficient.

4.2 Distribution

Once absorbed, the fate of Cr will depend on the oxidation state. Cr(VI) readily penetrates cell membranes, and Cr(III) does not. Cr is therefore found in both erythrocytes and plasma after GI absorption of Cr(VI) but exclusively in the plasma after that of Cr(III). Once transported through the cell membrane, Cr(VI) is rapidly reduced to Cr(III), which subsequently binds to macromolecules.
(predominantly haemoglobin) from which it is slowly released, with a half-life of 30 days (Health Canada, 2016). Transferrin is considered to play a key role in distributing Cr from the GI tract to tissues (Kirman et al., 2012). In humans, the highest concentrations are found in hilar lymph nodes and lungs, followed by spleen, liver, and kidneys (Health Canada, 2016), with tissue Cr levels declining with age.

Studies have assessed the distribution of Cr(III) and Cr(VI) following oral exposure via the feed and drinking-water (maximum dose of 182 mg Cr(VI)/L) (NTP, 2008a,b; EFSA, 2014) in male rats and female mice for up to 369 days. Speciation of Cr is not possible in tissues, and therefore studies reported total Cr measurements. The authors reported that in comparison with Cr(III), higher levels of Cr were found in animals in red blood cells, stomach, liver and kidney. Similar levels of Cr were found in plasma, urine and faeces. Species-specific differences were also noted, with a much higher absorption of Cr in mice than rats. Statistically significantly higher levels were apparent in mice in the glandular stomach and liver, and in the kidney in the rat. A time-dependent increase in tissue Cr levels was reported for both species, for both Cr(III) and Cr(VI) over a 6-month period. Longer exposures to Cr(VI) resulted in a decrease in levels in all tissues except red blood cells and plasma.

### 4.3 Metabolism

Reduction of Cr(VI) by GI fluids (gastric juice and saliva) and sequestration by intestinal bacteria result in Cr(VI) being poorly absorbed following oral intake (De Flora et al., 2016). Any Cr(VI) that is absorbed will be reduced in the blood of the portal vein system or the liver. Alternatively, if absorbed into the cell, Cr(VI) undergoes a series of reduction reactions involving direct electron transfer from ascorbate (predominantly) and non-protein thiols, such as glutathione and cysteine, to yield Cr(III) (Health Canada, 2016). Within red blood cells the reduction of Cr(VI) occurs by the action of glutathione, leaving Cr(III) mostly trapped within the erythrocyte for the life-span of the cell (Paustenbach et al., 2003). In addition, in vitro studies have demonstrated reduction of Cr(VI) by microsomal enzymes in an NADPH-dependent process (Gruber & Jennette 1978; Health Canada, 2016). Reduction of Cr(VI) may result in detoxification or activation dependent on the nature of the reducing agents, and the proximity of the intracellular site to DNA. Detoxification will occur when the site of reduction is far away from a DNA source and reactive intermediates can be trapped by components of the intracellular environment (De Flora, 2000). However, activation may occur when the site of reduction is close to a source of DNA, as the production of unstable intermediates may react with intracellular proteins and DNA (De Flora, 2000; Zhitkovich, 2011).

### 4.4 Elimination

Following oral exposure to Cr compounds, especially those of Cr(III), Cr is recovered almost entirely in the faeces due to the poor absorption in the GI tract. Cr is also reported to be excreted in hair and fingernails (WHO, 2013). Urine is the major route of elimination of absorbed Cr. In a 1-year balance study in which two humans received mean daily dietary intakes of 200 and 290 μg of Cr, 60% and 40% of the total amount excreted were recovered in the urine and faeces, respectively (EFSA, 2014). Occupational studies have estimated that 40% of absorbed Cr(VI) is eliminated within 7 days, an additional 50% within 15 – 30 days, with the remaining 10% excreted within 5 years (EFSA, 2014).

### 4.5 PBPK models

Due to the analytical difficulties in speciating Cr(III) and Cr(VI), many studies report total Cr levels in tissues and bodily fluids, which may not be the most appropriate internal dose metric (Kirman et al., 2013). Physiologically-based pharmacokinetic (PBPK) models provide a means to characterise the reduction of Cr(VI) to Cr(III) prior to absorption, under varying conditions, allowing an estimation of internal doses for speciated Cr. Early PBPK models for rats and humans (O’Flaherty et al. 1996; O’Flaherty et al., 2001, respectively) were simplistic in that they did not include compartmentalisation.
and parameterisation of the GI tract and were based on a limited set of data. Kirman et al. (2012) published an improved PBPK model for rats and mice that included compartmentalisation of the target tissues, the small intestine and oral mucosa, and utilised data generated ex vivo to quantify the reduction of Cr(VI) in gastric content of the rat and mice (Proctor et al., 2012). Schlosser and Sasso (2014,) provided modifications of the Kirman et al models, taking into account the pH dependence of reduction and effects due to dilution of gastric juices. The newer models described by Kirman et al. (2012, 2013) and Schlosser and Sasso (2014,) also included consideration of a number of pools of reducing agents associated with the different types present in gastric fluid (e.g. ascorbate, NADH and glutathione). It was proposed that up to three pools were present, reflecting rapid, slower and slow interaction and depletion respectively.

For human risk assessment, Kirman developed a model utilising generated ex vivo data on the rate and Cr(VI) reducing capacity in fasted human gastric fluid. This allowed an estimate of internal doses to the small intestine to be made from available human data for Cr(III) and Cr(VI). In a further update of this model, Kirman et al. (2016) defined data for human gastric fluids under conditions of fasting, feeding, and PPI use, providing improved characterisation of Cr(VI) gastric reduction.

5.0 EFFECTS ON HUMANS

The toxicity of Cr varies with its valence state and the route of exposure. Available data mainly refer to total Cr (i.e. III and VI) and suggests little or no toxicity associated with the trivalent form; data relating to Cr(III) are given below where available. Conversely, a number of studies have indicated toxicity of the hexavalent form, which is soluble in water; available data are summarised below (Health Canada, 2016).

5.1 Nutritional Essentiality

The US Institute of Medicine (IOM) considers Cr(III) to be an essential nutrient required for normal energy metabolism and has determined an adequate intake of 20–45 µg Cr(III) bw per day for adolescents and adults (IOM, 2001). However, this view is equivocal as there is no direct evidence of Cr deficiencies in humans, as there are with other essential minerals and no demonstrated beneficial effects of Cr(III) supplementation (Health Canada, 2016). In animals, although severe Cr deficiency is difficult to induce, when successfully achieved, hyperglycemia, decreased weight gain, elevated serum cholesterol levels, aortic plaques, corneal opacities, impaired fertility, and lethality were observed (ATSDR, 2012). WHO (1996) considered the data to be too limited to recommend a daily allowance or adequate intake.

The database is insufficient to establish a Recommended Dietary Allowance for Cr(III). Adequate intakes (AIs) have been proposed by the US National Academy of Sciences in partnership with Health Canada (IOM, 2001), reflecting current estimates of average Cr intake from well-balanced diets. These AIs range from 0.2 µg Cr per day (for infants) to 45 µg Cr per day (for lactating women). The daily Cr requirement for adults (<50 years) is estimated to be 35 and 25 µg bw per day in males and females. The EFSA Contaminants in the Food Chain (CONTAM) Panel recently derived a Tolerable Daily Intake (TDI) of 300 µg Cr(III)/kg bw per day based on the lowest NOAEL from chronic toxicity studies in rats and mice (EFSA, 2014).

5.2 Acute exposure

Symptoms of acute Cr intoxication in humans include severe GI disorders, respiratory, liver and kidney injury, and cardiovascular collapse due to severe hypovolemia. Death has been reported following Cr(VI) ingestion in several case studies in children and adults at doses ranging from 4.1 to 357 mg/kg bw. Around 1 g of potassium dichromate (K₂Cr₂O₇) is considered a lethal dose (ATSDR, 2012). In human volunteers, exposure to Cr(VI) at a single dose of up to 4mg and to Cr(III) or Cr(VI) at 5 mg in drinking-
water or juice was not associated with any adverse effect (Health Canada, 2016).

5.3 Short-term exposure

No studies relating to short-term human exposure to Cr(III) by any route could be identified. In humans administered Cr(VI) via drinking-water at doses of between 0.03 and 4 mg bw per day for at least 3 days, no apparent clinical changes or health effects were observed (EFSA, 2014; HC, 2016).

5.4 Long-term exposure

5.4.1 Systemic effects
Although not of direct relevance to drinking-water exposure, the respiratory tract is the major target of inhalation exposure to Cr(VI) compounds in humans. Occupational exposure studies and case reports indicate that respiratory effects also occur from inhalational exposure to Cr(III) compounds, however these effects may be due to co-exposure to Cr(VI). Respiratory symptoms following oral exposure to Cr(III) in humans were not identified, however, as discussed in Section 5.2, case studies have reported severe respiratory effects contributing to death following ingestion of high doses of Cr(VI) compounds (ATSDR, 2012).

No information was identified on GI effects in humans due to chronic oral exposure to Cr(III) compounds in isolation. However, chronic oral exposure of the general population in China to Cr(VI) through consumption of well water containing 20 mg Cr(VI)/L (considered to be equivalent to 0.57 mg Cr(VI)/kg bw per day), has been reported to be associated with GI effects including diarrhoea, abdominal pain, indigestion, and vomiting. The reliability of the exposure estimates has been highlighted as being of potential concern due to poor characterisation of the exposure (ATSDR, 2012).

No definitive information was identified on hematological effects in humans following chronic oral exposure to Cr(III) compounds. Haematological changes of leukocytosis and immature neutrophils were reported in the participants of the study carried out in China, described above (ATSDR, 2012).

5.4.2 Neurological effects
Studies to address potential adverse neurological effects in humans following long-term oral exposure to Cr(III) or Cr(VI) could not be identified.

5.4.3 Reproductive and developmental effects
No studies relating to long-term human exposure to Cr(III) by inhalation could be identified. Long-term occupational exposure to Cr(VI) via inhalation has been associated with adverse reproductive effects in males (Health Canada, 2016). However, no studies could be identified regarding the reproductive and developmental toxicity of Cr(III) or Cr(VI) following oral exposure (ATSDR, 2012).

5.4.4 Immunological effects
Exposure to Cr compounds may induce allergic sensitisation in some individuals, through a combination of inhalation, oral, and/or dermal exposure. Only data regarding Cr(VI) could be identified with oral exposure to Cr(VI) being shown to exacerbate dermatitis of sensitive individuals (ATSDR, 2012).

5.4.5 Genotoxicity and carcinogenicity
Studies to assess the potential for genotoxic effects of Cr (III or VI) following oral exposure in humans could not be identified.

In some occupational studies, increased incidences of genotoxic effects including DNA damage have been found in circulating lymphocytes and/or buccal and nasal mucosal cells of workers exposed to Cr(VI) compounds by inhalation. These take the form of DNA strand breaks, DNA protein cross-links, oxidative DNA damage and chromosomal damage (chromosomal aberrations, micronuclei and sister chromatid
exchanges) (ATSDR, 2012; IARC, 2012; EFSA, 2014; Health Canada 2016). It should be noted that, as in all occupational epidemiology studies, a number of limitations are apparent in these studies, particularly concerning actual exposure levels, the small numbers of workers included and the possibility for co-exposures to other mutagenic compounds. In addition, occupational exposure to Cr(VI) is predominately through inhalation and the significance of these findings to exposure through drinking-water is not known.

Findings from occupational exposure studies are supported by results of in vivo studies in animals, in vitro studies in human cell lines, mammalian cells, yeast and bacteria, and studies in cell-free systems (discussed in Section 6.4).

The potential for development of cancer in humans through environmental exposure to Cr has been assessed in several retrospective epidemiological studies (IARC, 2012; HC, 2016). Data from these studies did not show an association between oral exposure to total Cr or Cr(VI) and cancer. It should be noted that these studies did not determine actual exposures of individuals and therefore, exposure misclassification is possible and may bias the reported results (IARC, 2012; Health Canada, 2016).

There is sufficient evidence of respiratory carcinogenicity in humans exposed to Cr(VI) in occupational settings (i.e. high levels of exposure) through inhalation. Data on lung cancer risk in other Cr-associated occupational settings and for cancer at sites other than the lungs, including the GI tract, are considered to be insufficient (IARC, 2012; Health Canada, 2016). The epidemiological data do not allow an evaluation of the relative contributions to carcinogenic risk of metallic Cr, Cr(III), and Cr(VI) or of soluble versus insoluble Cr compounds, but it appears that exposure to a mixture of Cr(VI) compounds of different solubilities results in the highest risk to humans (IARC, 2012; Health Canada, 2016). Cr(VI) compounds were classified as “carcinogenic to humans” (Group 1) by the inhalation route of exposure based on sufficient evidence for carcinogenicity in humans (lung cancer) and sufficient evidence in experimental animals (see also Section 6.3.5.5) (IARC, 2012), recognising that data on human carcinogenicity via the oral route are lacking. The US EPA\(^3\) have also classified Cr(VI) as a group A (known human) carcinogen via the inhalation route.

The conflicting findings associated with different routes of exposure may in part be explained by the reductive capacity of the GI tract that significantly limits and/or prevent Cr(VI) uptake via the oral route. This is supported by findings from a recent study by De Flora et al. (2016) in which patterns of Cr(VI) reduction were evaluated in 16 paired pre- and post-meal gastric fluid samples from 8 volunteers. The mean Cr(VI) reducing capacity of post-meal samples was significantly higher than pre-meal samples, with >70% of total reduction occurring within 1 minute and 98% achieved within 30 minutes in post-meal gastric fluid at pH 2.0. Mutagenicity, as determined in the Ames test, was also attenuated, with reductions being higher in post-meal samples.

6.0 EFFECTS ON EXPERIMENTAL ANIMALS AND IN VITRO TEST SYSTEMS

6.1 Acute exposure

Cr compounds have moderate to high acute oral toxicity in rats and mice, based on oral LD\(_{50}\) values for Cr(III) in rats between 183-422 mg/kg and oral LD\(_{50}\) values for Cr(VI) in rats ranging from 13-811 mg/kg and in mice ranging from 135-175 mg/kg (WHO, 2009; ATSDR, 2012). Variation is again seen with the source of Cr(VI) and females showing greater sensitivity

\(^3\) https://cfpub.epa.gov/ncea/iris2/chemicalLanding.cfm?substance_nmbr=144
6.2 Short-term exposure

A number of short-term (generally ≤ 90 day) repeat-dose toxicity studies have been carried out in rats and mice with Cr(III) administered through the oral route, including via drinking-water. In general, Cr(III) had very little to no toxicity up to the highest dose tested (1368 mg Cr(III)/kg bw/d in rats and 1419 mg Cr(III)/kg bw/d in mice), which may be due to the poor absorption of Cr(III) via this route (NTP, 2010; ATSDR, 2012; EFSA, 2014b).

Several studies addressing the repeat-dose, short-term (generally ≤ 90 day) toxicity of Cr(VI) have been reported for drinking-water in rats and mice at doses up to 60 mg Cr(VI)/kg bw per day in rats, and 80 mg/kg bw per day in mice (NTP, 1996a, b; De Flora et al., 2006; NTP, 2007; Thompson et al., 2011, 2012; Health Canada, 2016). Many of these show a significant decrease in the body weight of animals, however as Cr(VI) decreases the palatability of water this may have impacted on this parameter to some extent.

The 90-day studies conducted by the US National Toxicology Program (NTP, 2007) and Thompson et al. (2011, 2012) were similar in design, with administration of Cr(VI) in drinking-water (as sodium dichromate dihydrate) to F344 rats and BALB/c and C57BL/6 mice. Levels of Cr(VI) utilised in the NTP study corresponded to 0, 22, 44, 88, 175 and 350 mg/L for rats and mice, and in the Thompson et al studies to 0, 21, 60 and 80 mg Cr(VI)/L in rats and mice with additional doses of 0.1, 1.4 and 4.9 mg Cr(VI)/L in mice only. NTP reported that for both species lesions were apparent in the duodenum and jejunum of the small intestine, which showed dose-related increases in incidence and severity. The first (most sensitive) lesions to appear in rats were apoptosis, hyperplasia and histiocytic infiltration in the duodenum. In mice, the first lesions to appear comprised hyperplasia and histiocytic infiltration in the duodenal villi in addition to villous cytoplasmic vacuolization in the duodenum and jejunum. The authors considered the duodenal changes in mice to be secondary to a previous cell injury, which is an important conclusion relating to the MOA of Cr(VI) and is discussed further in Section 7. LOAELs of 2.9 mg Cr(VI)/kg bw per day for rats and 2.6-4.6 Cr(VI)/kg bw per day for mice, relating to the incidence of non-neoplastic lesions were derived (NTP, 2007). Thompson et al. (2011) reported similar lesions in the duodenum of B6C3F1 mice but those in F344 rats were not as reported by NTP (2007). Following publication of the findings of study by Thompson et al. (2011), a re-evaluation of the histopathological findings across the NTP (2007) and Thompson et al. (2011) studies was performed to assess consistency of data (Cullen et al., 2016). The authors concluded that qualitatively similar lesions were present in rats and mice, with the severity being much reduced in rats. The authors postulated that as the severity of the non-neoplastic lesions was milder in rats than mice, a threshold for the progression of carcinogenesis may not have been reached (Cullen et al., 2016).

The NTP have also reported effects on the haematological system in rats and mice following exposure to Cr(VI) in drinking-water for periods between 4 days and 1 year (NTP, 2007, 2008a). In male rats exposed to Cr(VI) (as sodium dichromate dihydrate) in drinking-water at concentrations up to 7.4 mg Cr(VI)/kg bw per day for 4 days, a significant decrease in mean corpuscular haemoglobin (MCH) (by 2.1%) at doses of 2.7 mg Cr(VI)/kg bw per day was seen. Similar effects were noted in male and female rats exposed to Cr(VI) for 5 days, with effects apparent in males at 4.0 mg/Cr(VI)kg bw per day and in females at 4.1 mg/Cr(VI)kg bw per day (NTP, 2007). More severe microcytic, hypochromic anaemia occurred in rats and mice following exposure to sodium dichromate dihydrate in drinking-water for 22 (NTP, 2008a) or 23 days (NTP, 2007). This was evidenced by a dose-dependent decrease in haematocrit, haemoglobin, mean cell volume and MCH at a maximum dose of 0.77 mg/Cr(VI)kg bw per day; no changes were noted at a dose of 0.21 mg/Cr(VI)kg bw per day Male and female rats exposed to 1.7 mg/Cr(VI)kg bw per day for 23 days showed similar changes. The NTP studies also show that longer periods of exposure of between 3 months and 1 year are associated with less severe effects on the haematological system than
those at 22 or 23 days (NTP, 2007, 2008a).

Biochemical and histopathological changes to the liver were also apparent in F344/N rats, but not B6C3F1 mice, following exposure to Cr(VI) via drinking-water for periods of between 5 days and 22 weeks (NTP, 2007). Activity of serum alanine aminotransferase (ALT) was increased in male (30%) and female (15%) rats following 5 days of exposure to Cr(VI) (as disodium dichromate) at levels of 4.0 and 4.1 mg/Cr(VI)kg bw per day respectively (NTP, 2007). The activity of ALT remained raised in both sexes following 14 weeks of exposure at which time serum sorbitol dehydrogenase activity was also significantly raised, with an increase of 77% in males and 359% in females at an exposure level of 1.7 mg/Cr(VI)kg bw per day. After 14 weeks of exposure, histopathological changes were apparent in females only as cellular histiocyte inflammation and chronic focal inflammation (NTP, 2007).

Activity of serum alanine aminotransferase was increased in male (30%) and female (15%) rats following 5 days of exposure to Cr(VI) (as disodium dichromate) at levels of 4.0 and 4.1 mg/Cr(VI)kg bw per day respectively (NTP, 2007). The activity of ALT remained raised in both sexes following 14 weeks of exposure at which time serum sorbitol dehydrogenase activity was also significantly raised, with an increase of 77% in males and 359% in females at an exposure level of 1.7 mg/Cr(VI)kg bw per day. After 14 weeks of exposure, histopathological changes were apparent in females only as cellular histiocyte inflammation and chronic focal inflammation (NTP, 2007).

Histopathological changes to the kidney were also reported in the study by Acharya et al. (2001) and included vacuolization in glomeruli, degeneration of the basement membrane of Bowman’s capsule and renal tubular epithelial degeneration at an exposure level of 1.3 mg Cr(VI)/kg bw per day. However, results of the NTP studies for Cr(VI) administered at levels up to 8.7 mg Cr(VI)/kg bw per day in drinking-water to rats or mice do not show evidence of histopathological changes in the kidney (NTP 2007, 2008a).

Microscopic changes to lymphatic tissues were observed in male and female rats at 1.7 and 20.9 mg Cr(VI)/kg bw per day respectively after 3 months of exposure (NTP, 2007). In mice, microscopic changes to lymphatic tissues were also observed at 3.1 mg Cr(VI)/kg bw per day following exposure for 3 months (ATSDR, 2012; EFSA, 2014; Health Canada, 2016).

A decrease in motor activity and balance was reported in rats given 98 mg Cr(VI)/kg bw per day as sodium chromate in drinking-water for 28 days (Diaz-Mayans et al., 1986).

6.3 Long-term exposure

Long-term (≥ 90 days) oral repeat-dose toxicity studies in animals with Cr(III) support the findings of shorter duration studies, with Cr(III) showing little of no toxicity up to the highest dose tested (1466 mg/kg bw/d in the rat and 783 mg/kg bw per day in mice). Accumulation of Cr in a number of tissues was noted in the longer-term studies (NTP, 2010; Health Canada, 2016).

6.3.1 Systemic effects

In a 2-year study, F344/N rats and B6C3F1 mice were exposed to Cr(VI) in drinking-water at concentrations of 0, 14.3, 57.3, 172, or 516 mg/L (male and female rats and female mice) or 0, 14.3, 28.6, 85.7, or 257.4 mg/L (male mice). The most critical non-neoplastic effects in rats were reported as haematological effects (microcytic, hypochromic anemia NOAEL of 0.21 mg/kg bw per day) and histiocytic cellular infiltration in the liver (NOAEL of 0.21 mg/kg bw per day) mesenteric lymph node (NOAEL of 0.21 mg/kg bw per day) and duodenum (NOAEL of 0.21 mg/kg bw per day). In mice, critical non-neoplastic effects were identified as hyperplasia in duodenum and histiocytic cellular infiltration in the liver (and mesenteric lymph nodes); a LOAEL of 0.38 mg/kg bw per day was determined for each endpoint. In general, effects observed in rats were more severe than those in mice (NTP, 2008b; Cullen et al., 2016; Health Canada, 2016).
6.3.2 Neurological
Histopathological analysis of the brain and nervous system of rats and mice following exposure to Cr(III) (2.040 mg Cr(III)/kg bw per day) or Cr(VI) (8.7 Cr(VI)/kg bw per day) in drinking-water for up to 2 years showed no adverse changes (NTP 2007, 2008b; Health Canada, 2016). Neurological, neurochemical or neurobehavioural tests have not been carried out.

6.3.3 Reproductive and developmental effects
Adverse reproductive effects have been observed in rats and mice orally exposed to Cr(III) through drinking-water and feed, although conflicting results have been reported (; NTP, 1996a,b, 1997; US EPA,1998; NTP, 2010; Health Canada, 2016). Of the drinking-water studies, one found significant alterations in sexual behavior, aggressive behavior toward other males, and significantly lower absolute weight of testes, seminal vesicles, and preputial glands in male Sprague-Dawley rats exposed to 40 mg Cr(III)/kg bw per day (as Cr chloride) in drinking-water for 12 weeks. Although male fertility was not considered to be affected, an increase in the total number of resorptions was seen in unexposed females mated with exposed males. In Swiss mice, fertility was adversely affected in males following exposure to 13 mg Cr(III)/kg bw per day (as Cr chloride) mated to unexposed females, and in females exposed to 5 mg Cr(III)/kg bw per day mated to unexposed males. In addition, increased testes and ovarian weights and decreased preputial gland and uterine weights were also reported at 5 mg Cr(III)/kg bw per day. Decreased spermatogenesis was observed in BALB/c mice treated with 9.1 mg Cr(III)/kg bw per day as Cr sulphate in drinking-water for 7 weeks (Health Canada, 2016).

A limited number of developmental studies relating to oral exposure to Cr(III) provide conflicting findings (EFSA, 2014; Health Canada, 2016). Of the drinking-water studies, no developmental effects were observed in the offspring of rats following exposure to 1.806 mg Cr(III)/kg bw per day (as Cr oxide), 5 days/week for 60 days before mating and throughout gestation. However, in BALB/c mice exposed to 74 mg Cr(III)/kg bw per day (as Cr chloride) in the drinking-water from GD 12 to lactation day 20, significant decreases in the relative weights of reproductive tissues (testes, seminal vesicles, and preputial glands in males; ovaries and uterus in females) and a delay in timing of vaginal opening were observed in offspring (EFSA, 2014; Health Canada, 2016). In the F2 generation mated with unexposed animals, fertility was not affected in males, however a significant decrease in the number of pregnant females (62.5 versus 100% in controls) was observed among the female offspring.

Functional and morphological effects on male reproductive organs have been reported in monkeys, rats and mice exposed via drinking-water to Cr(VI), with the male reproductive system exhibiting the highest sensitivity (ATSDR, 2012). In those relating to drinking-water, decreased sperm count (by 25%) and motility and histopathological changes to the epididymis have been reported in adult monkeys exposed to Cr(VI)/kg bw per day (as potassium dichromate) in drinking-water for 180 days; a LOAEL of 2.1 mg/kg bw per day was derived (ATSDR, 2012). In rats, effects have been observed at concentrations of 1.6 mg Cr(VI)/kg bw per day (ATSDR, 2012). In addition to effects on male reproductive organs (decreased testes seminal vesicles and preputial gland weights), inhibition of sexual behaviour and aggression was reported in male rats exposed to Cr(VI) in drinking-water at levels corresponding to 32 mg Cr(VI)/kg bw per day for 12 weeks (ATSDR, 2012). A 2-year study in which F344 rats were exposed to 5.9 mg Cr(VI)/kg bw per day via drinking-water (as sodium dichromate dihydrate) did not show any morphological changes to male reproductive organs (NTP, 2008b). Similarly, exposure of B6C3F1 mice to 5.9 mg Cr(VI)/kg bw per day did not produce any morphological changes to male reproductive organs (NTP, 2008b). In addition, sperm count or motility were also unaffected in B6C3F1, BALB/c and C57BL/6N mice exposed to 9.1 mg Cr(VI)/kg bw per day via drinking-water for 3 months (NTP, 2007).

Adverse effects on the female reproductive system following Cr(VI) exposure via drinking-water at doses of ≥5 mg Cr(VI)/kg bw per day reported in rats and mice include lengthening of the oestrous cycle, altered reproductive organ weights, reductions in the number of ovarian follicles, and changes to
circulating steroid and pituitary hormone levels (Health Canada, 2016). Cr has been shown to pass the placental barrier and accumulate in foetal tissues (Health Canada, 2016). A number of studies addressing oral exposure to Cr(VI) have shown developmental toxicity following premating and/or in utero or lactational exposure (ATSDR, 2012; Health Canada, 2016). Developmental toxicity has been assessed as embryotoxicity (increases in pre- and post-implantation loss and in resorptions) and/or fetotoxicity (decrease fetal weight, number of fetuses, number of live fetuses, and increased frequency of gross, visceral and skeletal malformations). Effects were observed at doses equivalent to 45 mg Cr(VI)/kg bw per day in rats and 31-52 mg Cr(VI)/kg bw per day in mice. No NOAELs have been determined by the authors of these studies.

A series of studies have reported increased oxidative stress in the offspring of dams exposed to ≥ 6 mg Cr(VI)/kg bw per day in drinking-water during gestation, or the post-natal period, including lactation (Health Canada, 2016). Perinatal exposure to doses ≥2.9 Cr(VI)/kg bw per day as potassium dichromate in the drinking-water caused oxidative stress in the uterus, liver, kidney, and bone from the offspring with associated morphological alterations in the kidney, liver, and bone.

6.3.4 Immunological
Studies in animals suggest that the immune system is a target for ingested Cr(VI) but not Cr(III) compounds (up to maximum dose of 781 mg Cr(III)/kg bw per day as Cr picolinate [Cr(C₆H₄NO₂)₂] in feed for 2 years) (NTP 2007, 2008a; ATSDR, 2012). Functional and structural changes have been reported, including stimulation of the humoral immune system and increased phagocytic activity of macrophages, increased proliferative responses of splenocytes to T- and B-cell mitogens and to the antigen mitomycin C and histopathological alteration (histiocytic cellular infiltration) of pancreatic lymph nodes. Adverse effects in rats were associated with Cr(VI) at levels of 16 mg Cr(VI)/kg bw per day for 3 weeks via drinking-water (ATSDR, 2012). Microscopic changes to lymphatic tissues were observed in male and female rats at 1.7 and 20.9 mg Cr(VI)/kg bw per day respectively after 3 months and at 0.77 mg Cr(VI)/kg bw per day and 2.4 mg Cr(VI)/kg bw per day after 2 years of exposure (NTP, 2008a). In mice, microscopic changes were observed at 3.1 mg Cr(VI)/kg bw per day (NTP, 2007) and 0.38 mg Cr(VI)/kg bw per day in mice (NTP 2008a). following exposure for 3 months and 2 years, respectively. Contact dermatitis has been elicited in guinea pigs and mice (ATSDR, 2012).

6.3.5 In vivo genotoxicity and carcinogenicity
6.3.5.1 In vivo genotoxicity – Cr III
Studies have been conducted in Drosophila melanogaster using Cr(III) compounds. Negative results were obtained for mutagenic and/or recombinogenic events in adults following exposure to Cr chloride in the larval stage (EFSA, 2014). However, positive findings were reported using Cr(III) picolinate in the diet at concentrations equivalent to 260 μg Cr/kg food (EFSA, 2014). No effects on survival, behaviour or fertility of adult Drosophila were reported, however, developmental delays and decreased pupation success were observed in larvae (EFSA, 2014).

Komorowski et al. (2008) reported no induction of chromosomal aberrations in the bone marrow cells of rats, 18 or 42 h following exposure to a single oral dose corresponding to 4.1, 30.8 and 246 mg Cr(III)/kg bw per day.

Studies conducted on Cr(III) compounds in animal models for the oral route have yielded negative results. In an NTP study (NTP, 2010) male F344/N rats treated with Cr(III) picolinate (anhydrous) (156 to 2500 mg/kg bw) by oral gavage three times at 24-hour intervals showed no presence of micronuclei in bone marrow. Similarly, in male and female B6C3F1 mice administered Cr(III) picolinate monohydrate (80 to 50000 mg/kg diet corresponding to 2-1419 and to 1.7-1090 mg Cr(III)/kg bw per day for male and female respectively) in feed for 3 months, no micronuclei were found in peripheral blood erythrocytes of males. Weak increases in the micronuclei frequency observed in erythrocytes of female mice were considered
equivocal findings as the anhydrous form was inactive (NTP, 2010). De Flora et al. (2006) analysed the frequency of micronuclei in bone marrow and peripheral blood cells of BDF1 mice, both males and females, administered Cr(III), as CrK(SO₄)₂·12H₂O, in the drinking-water, equivalent to 165 and 140 mg Cr(III)/kg bw per day (for males and females, respectively), for 7 months. Cr(III) did not affect the micronuclei frequency at any dose tested.

Frequencies of DNA deletions have been measured using the in vivo p(un) reversion assay in C57BL/6J p(un)/p(un) mice following administered to dams of Cr(III) chloride in the drinking-water at an average dose of 375 or 750 mg of Cr/kg bw per day. Significant increases in the frequency of DNA deletions in embryos harvested at 17.5 days post-coitum were reported. The authors confirmed absorption of Cr(III) through the measurement of tissue levels (Health Canada, 2016).

6.3.5.2 In vivo genotoxicity – Cr(VI)
Cr(VI) compounds have tested positive for mutations in Drosophila melanogaster in several studies following exposure of larvae via feed at concentrations of 0.1 mM (ATSDR, 2012).

Genotoxicity has been shown in rats and mice following exposure to Cr(VI) via the parenteral, intratracheal or inhalation routes. Following exposure of C57BL/6Jpun/pun mice to Cr(VI) via drinking-water at a dose of 62.5 mg/L, induction of mutations were reported (Health Canada, 2016). A significant increase in micronuclei formation was reported in peripheral erythrocytes of am3-C57BL/6 mice following exposure to 43.6 mg Cr(VI)/L, whereas in B6C3F1 strain BALB/c mice a positive, but non-significant, trend was observed (NTP, 2007). Other studies have reported negative results in bone marrow or peripheral blood cells following oral exposure to Cr(VI) compounds (De Flora et al., 2006; ATSDR, 2012).

The route-dependent genotoxicity of Cr(VI) has also been demonstrated in MS/Ae and CD-1 mice. When administered by i.p injection, potassium chromate (17.7 mg Cr(VI)/kg) induced micronuclei in a dose-dependent manner in both strains. However, when administered orally via drinking-water, potassium chromate (113.1 mg Cr(VI)/kg) failed to induce micronuclei (ATSDR, 2012). Similarly, sodium dichromate dihydrate and potassium dichromate were administered to BDF1 and Swiss mice through the drinking-water or as a single intragastric dose (De Flora et al. 2006). Following oral administration (500 mg Cr(VI)/L for up to 210 consecutive days), no increase of the micronucleus frequency was observed in either bone marrow or peripheral blood erythrocytes. However, following i.p. injection (50 mg Cr(VI)/kg), the compounds induced a clastogenic damage. In the same study pregnant mice were also treated up to a concentration of 10 mg Cr(VI)/L drinking-water. No genotoxic effects were observed either in bone marrow of pregnant mice or in liver and peripheral blood of their foetuses. EFSA concluded that the determinant for the genotoxic effects of C(VI) in vivo is the reductive capacity of the GI tract which plays a significant part in limiting or preventing completely the uptake in blood and/or systemic distribution and recognized that there was some uncertainty that even at low levels not all Cr(VI) may be converted to Cr(III) in the human GI tract (EFSA, 2014). However, as stated previously (section 4.3) it is considered that any Cr(VI) that is absorbed from the GI tract will be reduced in the blood of the portal vein system or the liver, and any absorbed into the cells will be reduced by intracellular mechanisms to Cr(III) (Health Canada, 2016). DNA damage as measured by the Comet assay has been observed in mice and rats in several tissues including stomach, colon, liver, kidney, bladder, brain and peripheral leukocytes (ATSDR, 2012).

6.3.5.3 Carcinogenicity – Cr(III)
In a lifetime carcinogenicity study in which 3-month-old inbred male and female BD rats (60 per dose) were exposed, 5 days per week for 2 years, to 2040 mg Cr(III)/kg bw per day of insoluble, nonhydrated Cr(III) oxide pigment in feed, tumour incidence was not affected (ATSDR, 2012). Rats and mice exposed to 0.46 and 0.48 mg Cr(III)/kg bw per day respectively in drinking-water for 2 years did not show any
adverse changes (ATSDR, 2012). In addition, ddY mice were unaffected following exposure to between 25 and 100 mg Cr(III)/L in drinking-water for 1 year (ATSDR, 2012).

A long-term study has been carried out by US NTP (2010) to assess the carcinogenicity of Cr(III) (as Cr picolinate monohydrate). Male and female F344/N rats and B6C3F1 mice were exposed in feed to concentrations from 2000 to 50,000 mg/kg for 2 years, corresponding to average daily doses of 286.2 and 313.7 mg Cr(III)/kg bw per day for male and female rats, respectively, and of 783.0 and 727.5 mg Cr(III)/kg bw per day for male and female mice, respectively. No significant changes in mortality, body weight, feed consumption or the occurrence of non-neoplastic lesions in rats or mice. In male rats, a statistically significant increase in the incidence of preputial gland adenomas was reported at a dose of 54.9 mg Cr(III)/kg bw per day, although this was not associated with increased incidence of preputial gland hyperplasia (at any dose) or preputial gland carcinoma (at any dose). Examination of the clitoral gland in exposed females (female counterpart of the preputial gland) showed no evidence of hyperplasia or adenomas.

6.3.5.4 Carcinogenicity – Cr(VI)

A long-term study has been carried out by US NTP to assess the carcinogenicity of Cr(VI) (as sodium dichromate dehydrate). Male and female F344/N rats and B6C3F1 mice were exposed in drinking-water at maximum concentrations of 5.9, 7.0 and 8.7 mg Cr(VI)/kg for male mice and rats, female rats and male and female mice, respectively (NTP, 2007, 2008). Significant increases in the incidence of squamous cell carcinoma in the oral mucosa and for squamous cell papilloma or carcinoma (combined) of the oral mucosa or tongue were reported in male and female rats at the highest doses used. In mice only, a dose-dependent increase in the incidence of adenomas as well as carcinomas in duodenum and jejunum was reported in males and females, with higher incidences in the duodenum. The increases were statistically significant (poly-3 test) at the two highest exposure concentrations in each sex for the adenoma and carcinoma combined (p < 0.001), and at the highest concentration for the carcinoma in the duodenum, jejunum or ileum combined (p < 0.05), both for males and females (EFSA, 2014).

Exposure of NMRI mice in a 29-month three-generation study to 135 mg of Cr(VI)/L (as potassium chromate) in drinking-water did not result in carcinogenic activity in the stomach ATSDR, 2012).

6.3.5.5 Summary of carcinogenicity studies

There is no definitive evidence relating to the carcinogenicity of Cr(III) following short-term or chronic oral exposure. Cr(III) compounds have been classified by IARC as Group 3, i.e. they are not classifiable as to their carcinogenicity in humans (IARC, 2012).

Data on the evaluations for Cr(VI) compounds for carcinogenicity via the oral route are also limited, however there is sufficient evidence from animal studies that the development of hyperplasia in the small intestine is indicative of non-genotoxic carcinogenicity, There is a stronger association between the inhalation of Cr(VI) and the development of lung cancer in humans, and sufficient evidence from animal studies. The International Agency for Research on Cancer (IARC) has classified Cr(VI) compounds as Group 1, i.e. there is sufficient evidence in humans for the carcinogenicity (IARC, 2012).

6.4 In vitro genotoxicity studies

6.4.1 Cr(III) - Bacteria and yeast

Cr(III) compounds have been reported to be generally inactive in bacterial mutagenicity assays. No genotoxic effects have been reported for Cr(III) picolinate in Ames assays using a variety of Salmonella typhimurium strains and concentrations up to 10 000 µg Cr(III) picolinate/plate in the presence or absence of metabolic activation (EFSA, 2014). Cr(III) chloride and Cr picolinate monohydrate have also shown negative results in assays with Escherichia coli strain WP2uvr/pKM101, when tested with or without exogenous metabolic activation (S9) (NTP, 2010). Some evidence has been reported for Cr(III)
compounds showing mutagenicity in bacterial strains that are sensitive to oxidative stress (e.g. *S. typhimurium* strains TA102 and TA2638) (ATSDR, 2012).

A significant increase in the frequency of DNA deletions in *Saccharomyces cerevisiae* with Cr(III) chloride has also been reported (ATSDR, 2012).

### 6.4.2 Chromium VI – Bacteria and yeast
Cr(VI) compounds have generally tested positive for gene mutations in bacterial cells. As described in the Scientific Opinion by EFSA reverse mutations were observed after exposure to Cr(VI) compounds in multiple species and strains of *Salmonella typhimurium* and *Escherichia coli* able to detect a wide spectrum of DNA lesions, including oxidative damage and DNA crosslinks, and of mutations such as base pair substitutions and frame-shift mutations (EFSA, 2014).

Positive results were also found for forward mutations and mitotic gene conversion in yeast (*Saccharomyces cerevisiae*) (EFSA, 2014).

### 6.4.3 Cr(III) - Mammalian cells
Cr(III) compounds, particularly Cr picolinate, have been tested in numerous bioassays using cultured mammalian cells with mixed, often positive, results (EFSA, 2014).

Cr(III) chloride was shown to induce micronuclei originated from chromosome breakage and loss of entire chromosomes, in human fibroblasts (ATSDR, 2012).

Cr(III) chloride induced chromosomal aberrations in phytohemagglutinin(PHA)-stimulated human lymphocytes, considered to be mediated through production of oxygen free radicals (Health Canada, 2016). No induction of micronuclei was observed following exposure of V79 Chinese hamster lung cells to a variety of Cr(III) complexes. However, when Cr(III) imine complexes, which could be oxidized to Cr(V) complexes, were used, positive findings of micronuclei were reported (Health Canada, 2016).

In Chinese Hamster Ovary (CHO) cells, Cr(III) picolinate up to 1 mM was found to induce HPRT mutations by up to 40-fold compared to controls. Picolinic acid at concentrations up to 3 mM did not induce mutations (ATSDR, 2012). Negative results were reported for the HPRT assay in CHO cells exposed to Cr(III) picolinate at concentrations up to 1.43 mM for 5- and 48-hour periods (ATSDR, 2012). Chromosomal aberration assays with CHO cells exposed to Cr(III) picolinate at concentrations up to 770 μg/mL (for 4 hours in the presence of metabolic activation and 20 hours in the absence of metabolic activation) were also negative (ATSDR, 2012).

The induction of DNA damage by Cr(III) compounds has been analysed by the Comet assay with and without hydrogen peroxide-induced stress in human HaCaT keratinocytes. Whilst Cr(III) picolinate did not induce any DNA damage at concentrations of 120 mM, significant induction of DNA breaks was reported after exposure to Cr(III) chloride at 6 mM (ATSDR, 2012).

### 6.4.4 Cr(VI) – Mammalian cells
Cr(VI) compounds are also mutagenic in mammalian cell lines. Clastogenic activity (micronuclei, chromosomal aberrations and sister chromatid exchanges) of a number of Cr(VI) compounds have been reported in CHO cells, mouse mammary FM3A carcinoma cells, human fibroblasts, human epithelial cells and human lymphocytes (ATSDR, 2012). Clastogenic and mutagenic effects were observed in the absence of metabolic activation, indicating Cr VI to be a direct-acting mutagen. However, nucleotide excision repair has been shown to effectively repair Cr VI-induced mutagenicity (Health Canada, 2016).

Chinese hamster cells AT3-2 and V79 exposed to potassium dichromate showed a significant increase in
mutation frequency at the HPRT locus and mouse lymphoma cells L5178Y exposed to calcium chromate at the TK locus (ATSDR, 2012).

6.4.5 Summary of genotoxic and carcinogenic effects
Cr(VI) compounds cause mutations and allied effects such as chromosomal aberrations in a wide range of prokaryotic and eukaryotic test systems, both in vitro and in vivo. Cr(III) compounds are not active in similar systems, or only at high, cytotoxic concentrations. It has therefore been concluded that Cr(VI) is mutagenic, whereas Cr(III) is not.

The mutagenic activity of Cr(VI) is decreased or abolished by reducing agents such as human gastric juice and rat liver microsomal fraction. Inactive Cr(III) compounds are not converted to mutagens by biological systems, but only by treatment with strong oxidizing agents. The difference between the mutagenic action of Cr(VI) and Cr(III) can be explained by differences in physicochemical properties. Although Cr(VI), which readily penetrates cell membranes, is the causative agent, there are strong indications that Cr(III) or intermediates such as Cr(V) formed during the intracellular reduction of Cr(VI) are the genetically active agents that form ligands with macromolecules such as DNA.

6.5 Mode of Action

The toxicity potential of Cr is dependent on the oxidation state, with Cr(VI) having greater toxic potential than Cr(III). Evidence of the health effects following oral exposure to Cr(VI), as the most potent species, indicates the small intestine to be the target for both neoplastic and non-neoplastic effects. Of the animal studies identified, small intestinal tumours in mice were the most sensitive chronic carcinogenic endpoint (observed at doses as low as 1.4 mg Cr(VI)/kg bw per day in mice; NTP, 2008b). The most sensitive non-neoplastic chronic effects were also in the small intestine, with evidence of histiocytic cellular infiltration in the rat and diffuse epithelial hyperplasia in the mouse, at doses of 0.8 and 0.2 mg Cr(VI)/kg bw per day, respectively (NTP, 2008b). Intestinal tumour development is thought to be related to these earlier changes in the small intestine (Health Canada, 2016).

The mechanisms of Cr(VI) toxicity and carcinogenicity are very complex and still under debate. A considerable body of literature on target tissue-specific mechanisms of Cr(VI) toxicity has been published since the last WHO (2003) background document that strengthens the proposed MOA. Thompson et al. (2013) performed a weight of evidence analysis of numerous studies from their group (Thompson et al., 2011b, 2012a,b,c; Kirman et al., 2012; Kopec et al., 2012a,b; Proctor et al., 2012) which supported a cytotoxic, threshold MOA for Cr(VI). The authors proposed the key events to be:

(a) Absorption of Cr(VI) from the intestinal lumen: extracellular reduction of Cr(VI) to Cr(III) is a vital process to limit toxicity and is dependent on prevailing conditions of pH, the levels of reducing agents present and whether fed or fasting conditions prevail. If still present, non-reduced Cr(VI) is taken up by intestinal cells through anion transporters or excreted unchanged. Reduction capacity is considered best represented by several pools of reducing compounds which become depleted at different rates (Kirman et al., 2016). Some Cr(VI) may escape reduction as, in humans, total reduction by 30 min has been estimated as 98% (De Flora et al., 2016).

(b) Toxicity to intestinal villi: data supports the non-proliferating, non-pluripotent cells of the intestinal villi to be the primary target of Cr(VI) toxicity, i.e. toxicity occurs at the point of contact, causing subsequent triggering of compensatory cell proliferation of crypt enterocytes. Oxidative stress is considered to contribute to intestinal villi cytotoxicity, even at low doses of Cr(VI) in the absence of oxidative DNA damage. Continued exposure leads to blunted villi and elongated crypts in the duodenum (Thompson et al., 2015b) however, DNA damage is confined to villi (Thompson et al., 2015c).
(c) **Sustained compensatory crypt hyperplasia**: occurs as a result of the repair or replacement of damaged intestinal mucosa. Continued exposure to Cr(VI) is associated with diffuse hyperplasia in crypt cells but not with focal hyperplasia, indicating that proliferation is secondary to mucosal injury (O’Brien et al., 2013; Thompson et al., 2013, 2015b,c).

(d) **Clonal expansion of mutations within the crypt stem cells, resulting in late onset tumorigenesis**: Thompson et al. (2013) concluded that the weight of evidence does not support a mutagenic MOA for Cr(VI), particularly as an early key event (Thompson et al., 2013, 2017). The authors proposed that the late onset tumours seen at high doses in some studies may have resulted from spontaneous mutations due to sustained cell proliferation (Thompson et al., 2013; O’Brien et al., 2013; Health Canada, 2016).

The incidence of small intestine cancers in mice following oral exposure to Cr(VI) has been used in a number of quantitative risk assessments (Haney, 2015). As such, it is important to assess whether the excess cancer risk observed at high doses (approx. 3 orders of magnitude higher than drinking-water exposure levels) in mice are applicable to much lower oral doses in drinking-water in humans. Use of the MOA specific to small intestine cancers will most reliably inform the basis for such extrapolations (Thompson et al., 2013). It is considered that all of the key events outlined above are of relevance to humans (Thompson et al., 2013; Health Canada, 2016).

Additional MOAs for Cr(VI) toxicity following exposure through the oral route have been proposed, the most prominent of which is the direct-acting mutagen MOA proposed by McCarroll et al. (2010). It should be noted that this predates the large body of evidence relating to the MOA for cytotoxicity, as described above. The authors propose three key steps as follows:

a) **Intracellular reduction of Cr(VI) to Cr(III)**: following intracellular uptake, Cr(VI) is reduced to Cr(III) which is associated with the formation of reactive intermediates and resultant oxidative stress. Some evidence of DNA damage, Cr(III)-DNA adducts and DNA-protein cross-links has been observed in a limited number of in vivo and in vitro studies.

b) **Mutagenesis**: there is evidence of mutagenesis following exposure to Cr(VI) via i.p. injection and oral gavage, however, there is no evidence following exposure through drinking-water.

c) **Cell proliferation**: duodenal hyperplasia was observed in 90-day and 2-year mouse studies, but not in the rat.

EFSA (2014) also notes that in addition to the reduction of Cr(VI) to Cr(III) (step ‘a’ above), reactive oxygen species can be produced, which may also contribute to generation of DNA adducts. Due to the uncertainties in the available data, the MOA cannot currently be definitively confirmed (EFSA, 2014; HC, 2016; COM, 2015). However, the overall weight-of-evidence supports a threshold MOA. This is based on the following points of evidence: the absence of mutagenicity in target tissues; lack of concordance of mutagenicity and tumour development; absence of mutagenesis from drinking-water studies; lack of evidence of mutagenesis in highly proliferative intestinal tissue following drinking-water exposure; no evidence of tumours in other tissues in which Cr is present; and early onset of crypt proliferation (following 7 days of exposure to Cr(VI) is unlikely to result from a fixed mutation (Health Canada, 2016, Moffat et al., 2018).

### 7.0 OVERALL DATABASE AND QUALITY OF EVIDENCE

#### 7.1 Summary of Health Effects

Following oral exposure, Cr(VI) compounds are generally more toxic than Cr(III) compounds. Health effects can vary with route of exposure and some adverse effects are at the point of contact, e.g.
respiratory effects are associated with inhalation of Cr compounds, but not with oral or dermal exposures, and GI effects are primarily associated with oral exposure.

Cr(III) compounds present low oral toxicity because they are poorly absorbed. No carcinogenic or other adverse effects have been observed in the sub-chronic or long-term oral toxicity studies of Cr(III) in mice or rats. NOAELs of 506 and 286 mg Cr(III)/kg bw per day can be derived from the sub-chronic and long-term studies in rats (NTP, 2010). Cr(III) did not show reproductive toxicity in male or female animals following sub-chronic oral exposure via drinking-water, with NOAELs of 506 and 1090 mg/kg bw per day, being identified respectively (NTP, 2010).

Oral exposure to Cr(VI) was carcinogenic in rats and mice and genotoxic in some in vivo studies. Cr(VI) is rapidly and efficiently reduced in the GI tract to Cr(III) in the liver of intracellularly. It is considered here that the key carcinogenicity study for derivation of a guideline value is a 2-year NTP study investigating oral intake of Cr(VI) (as sodium dichromate dihydrate) via drinking-water in rats and mice. Doses were between 0 and 5.9/7.0 mg Cr(VI)/kg bw per day in male and female rats, respectively, and between 0 and 5.9/8.7 mg Cr(VI)/kg bw per day in male and female mice, respectively. An increased incidence of tumours of the oral cavity squamous epithelium and of the small intestinal epithelium were reported in male and female rats and mice, respectively, with identified LOAELs of 0.38 and 1.79 mg Cr(VI)/kg bw per day (NTP, 2008b).

Non-neoplastic effects following oral exposure to Cr(VI) in the 2-year NTP study included lesions in liver, duodenum, mesenteric lymph nodes and pancreas and haematological effects in rats and mice at NOAELs higher than that for neoplastic changes (NTP, 2008b).

7.2 Quality of Evidence

The database of information regarding adverse health effects in humans following exposure to Cr(III) and/or Cr(VI) though drinking-water is limited to case reports of acute accidental or intentional ingestion, or epidemiological studies that are weak in design and do not encompass the oral route of exposure. The database of information in laboratory animals is more complete than that for humans and includes chronic exposure to Cr(III) or Cr(VI) though drinking-water in well-conducted studies that conform to current testing guidelines. There are substantial new data and increasing weight-of-evidence to support a threshold MOA, recognizing some remaining uncertainty regarding clear negative results for genotoxicity at low doses. Gaps in the database also relate to sensitive tests of immune function after oral exposure and reproductive or developmental effects of Cr(III) and/or Cr(VI) after oral exposure, including potential neuro/behavioural end points across life stages.

8.0 PRACTICAL CONSIDERATIONS

8.1 Analytical methods and achievability

Methods for the determination of Cr in biological and environmental samples are developing rapidly, and early results (especially for the lower Cr levels) should be interpreted with caution. The International Organization for Standardization (ISO, 1998) specifies two methods for the determination of total Cr in water: Determination of chromium by flame atomic absorption spectrometry (Clause 3) and Determination of chromium by electrothermal atomization atomic absorption spectrometry (Clause 4). Clause 3 is applicable to the analysis of water and waste water when the concentration range is between 0.5 mg/L and 20 mg/L Cr. When the concentration is below 0.5 mg/L, the determination can be carried out after carefully evaporating an acidified sample to small volume, taking care to avoid the formation of a precipitate. ISO (1998) notes that the use of evaporation will increase the effect of interfering
substances and therefore for concentrations below 0.1 mg/L the method in Clause 4 is given. Clause 4 is applicable to the analysis of water and waste water when the concentration range is between 5 µg/L and 100 µg/L Cr by injecting a sample volume of 20 µL. It is applicable to the determination of higher concentrations by using a smaller sample volume.

Methods for the analysis of total Cr approved by the US Environmental Protection Agency (US EPA, 2014) include inductively-coupled plasma atomic emission spectroscopy (ICP-AES), inductively-coupled plasma mass spectrometry (ICP-MS), atomic emission spectroscopy and graphite furnace atomic emission spectroscopy (GFAA), with detection limits between 0.08 and 7 µg/L (WRc, 2015).

The current method for the analysis of low-level Cr(VI) in drinking-water recommended US EPA (EPA Method 218.7) uses ion chromatography with post-column derivatisation and UV–visible spectroscopy. This has a detection limit in the range 0.0044 to 0.015 µg/L. Methods using high performance liquid chromatography (HPLC) with a direct current plasma emission spectrometer have also been used for the determination of Cr(III) and Cr(VI) in water samples (Krull et al. 1983) with detection limits of 0.011 µg/L. More recently, the UK DWI reported development and use of ion chromatography followed by ICP-MS method to measure both Cr(VI) and Cr(III) concentrations in drinking-water samples, with the limit of quantitation being 0.5 µg/L, total Cr was measured separately using ICP-MS. However, the determination of Cr species remains a very sophisticated procedure for which reliable and validated methods to separate Cr(III) analysis from Cr(VI) analysis in collected samples are still required.

### 8.3 Treatment methods and performance

Cr normally exists in two redox states in aqueous solutions, Cr(III) and Cr(VI), where Cr(VI) salts are generally more soluble than Cr(III) salts (WHO, 2003). Depending on pH, Cr(III) can be hydrolysed to varying degrees forming Cr(OH)₃, CrO₂⁻, Cr(OH)₂⁺, Cr(OH)₃(aq) and Cr(OH)₆⁺. Cr(VI) forms chromate (CrO₄²⁻) that can be protonated to form HCrO₄⁻ and, under very acidic conditions (pH<2), H₂CrO₄. At high Cr(VI) concentrations and low pH, the dichromate ion (Cr₂O₇²⁻) also forms. It should be noted that some disinfectants can oxidise Cr(III) to Cr(VI) and treatments such as pre-chlorination and pre-ozonation can make the Cr more mobile and difficult to remove (WRc, 2015). Removal technologies for Cr removal can be categorised into five general groups (Sharma et al., 2008):

- **Coagulation-precipitation-filtration - Cr(VI) requires reduction to Cr(III) prior to removal by ferric coagulants.**
- **Adsorption - iron oxides (ferrihydrite and goethite) and iron oxide coated sand (IOCS); removal of both Cr(III) and Cr(VI) requires pH changes and the removal carried out in stages.**
- **Ion exchange - effective in removing both Cr(III) and Cr(VI), with between 80-96% of the ions removed (US EPA, 2003).**
- **Membrane technologies – together with reverse osmosis (RO) these are considered one of the best technologies available for Cr removal, with efficiency at 82-97% for RO. Nanofiltration has also been used for Cr removal and shows similar efficiency for both Cr(III) and Cr(VI) (e.g. Hafiane et al., 2000; Taleb-Ahmed, 2002).**
- **Microbiological removal - bacteria has been to shown to be effective with Cr(VI) being reduced to Cr(III) and precipitated within biomass. However, may not be suitable for drinking-water treatment as anaerobic conditions are employed for optimum removal (e.g. Chen & Hao, 1997; Komori et al., 2004; Chen & Gu, 2005).**
9.0 CONCLUSIONS

9.1 Derivation of the guideline value

In principle, as the health effects of Cr are determined largely by the oxidation state, different guideline values for Cr(III) and Cr(VI) should be derived. However, current analytical methods and the variable speciation of Cr in water still favour a guideline value for total Cr. A guideline value is therefore proposed for total Cr based on aspects of achievability by current treatment technologies, measurability by analytical methods, and toxicology, which is intended to be protective of both cancer (in the case of Cr(VI)) and non-cancer (in the case of Cr(III) and Cr(VI)) endpoints.

As occurrence levels of total Cr in drinking-water (average of 0.001 mg/L) are generally below the previously derived provisional Guideline Value (pGV) of 0.05 mg/L (WHO, 2003), it is unlikely that this level will be exceeded. Toxicological data at that time did not support the derivation of a new value and this level was considered unlikely to give rise to significant risks to health.

Using the newer, high quality data from chronic drinking water carcinogenicity studies for Cr(III) and Cr(VI) (NTP 2008a, 2008b) and weight-of-evidence analyses supporting a threshold MOA (Health Canada, 2016) a GV of 50 µg/L remains valid (Moffat et al., 2018). The NTP (2008b) study allows for the risk assessment of Cr(VI) in drinking water considering both cancer and non-cancer effects and provides evidence to support a MOA involving hyperplasia in the small intestine as a key precursor event to tumour development. Thus, a GV for Cr(VI) in drinking water considering hyperplasia as the most sensitive endpoint and precursor of tumour formation, is protective of both non-cancer and cancer effects. The current guideline value of 0.05 mg/L is therefore considered to be adequately protective of health and is retained, with the previously allocated ‘provisional’ status removed.

9.2 Considerations in applying the guideline value

As Cr is usually found in drinking-water at concentrations below the guideline value, in general it would only require investigation for monitoring and inclusion in drinking-water regulations and standards if there were indications that a problem might exist. Monitoring can usually be limited to the treatment works.

10.0 APPENDICES

10.1 References


EFSA. 2014. Scientific Opinion on the risks to public health related to the presence of chromium in food and drinking water. EFSA Panel on Contaminants in the Food Chain (CONTAM), European Food Safety Authority (EFSA), Parma, Italy. EFSA Journal 2014;12(3):3595


NTP. 2007. NTP technical report on the toxicity studies of sodium dichromate dihydrate (CAS No. 7789-12-0) administered in drinking water to male and female F344/N rats and B6C3F1 mice and male BALB/c and am3-C57BL/6 mice. US National Institute of Environmental Health Sciences, National Toxicology Program. NTP TR 72.

NTP. 2008a. NTP technical report on the toxicology and carcinogenesis studies of sodium dichromate dihydrate (CAS No. 7789-12-0) in F344/N rats and B6C3F1 mice (drinking water studies). US National Institute of Environmental Health Sciences, National Toxicology Program. NTP TR 546.

NTP. 2008b. NTP technical report on the toxicology and carcinogenesis studies of chromium picolinate monohydrate (CAS No. 27882-76-4) in F344/N rats and B6C3F1 mice (feed studies). US National Institute of Environmental Health Sciences, National Toxicology Program. NTP TR 556.


