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Chromium in drinking-water

Background document for development of WHO *Guidelines for drinking-water quality*

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Preface

Access to safe drinking-water is essential to health, a basic human right and a component of effective policy for health protection. A major World Health Organization (WHO) function to support access to safe drinking-water is the responsibility "to propose ... regulations, and to make recommendations with respect to international health matters ...", including those related to the safety and management of drinking-water.

The first WHO document dealing specifically with public drinking-water quality was published in 1958 as *International standards for drinking-water*. It was revised in 1963 and 1971 under the same title. In 1984–1985, the first edition of the WHO *Guidelines for drinking-water quality* (GDWQ) was published in three volumes: Volume 1, Recommendations; Volume 2, Health criteria and other supporting information; and Volume 3, Surveillance and control of community supplies. Second editions of these volumes were published in 1993, 1996 and 1997, respectively. Addenda to Volumes 1 and 2 of the second edition were published in 1998, addressing selected chemicals. An addendum on microbiological aspects, reviewing selected microorganisms, was published in 2002. The third edition of the GDWQ was published in 2004, the first addendum to the third edition was published in 2006, and the second addendum to the third edition was published in 2008. The fourth edition was published in 2011, and the first addendum to the fourth edition was published in 2017.

The GDWQ are subject to a rolling revision process. Through this process, microbial, chemical and radiological aspects of drinking-water are subject to periodic review, and documentation relating to aspects of protection and control of drinking-water quality is accordingly prepared and updated.

Since the first edition of the GDWQ, WHO has published information on health criteria and other information to support the GDWQ, describing the approaches used in deriving guideline values, and presenting critical reviews and evaluations of the effects on human health of the substances or contaminants of potential health concern in drinking-water. In the first and second editions, these constituted Volume 2 of the GDWQ. Since publication of the third edition, they comprise a series of free-standing monographs, including this one.

For each chemical contaminant or substance considered, a background document evaluating the risks to human health from exposure to that chemical in drinking-water was prepared. The draft health criteria document was submitted to a number of scientific institutions and selected experts for peer review. The draft document was also released to the public domain for comment. Comments were carefully considered and addressed, as appropriate, taking into consideration the processes outlined in the *Policies and procedures used in updating the WHO guidelines for drinking-water quality* and the WHO *Handbook for guideline development*. The revised draft was submitted for final evaluation at expert consultations.

During preparation of background documents and at expert consultations, careful consideration was given to information available in previous risk assessments carried out by the International Programme on Chemical Safety, in its Environmental Health Criteria monographs and Concise International Chemical Assessment Documents; the International Agency for Research on Cancer; the Joint Food and Agriculture Organization of the United Nations (FAO)/WHO Meeting on Pesticide Residues; and the Joint FAO/WHO Expert Committee on Food Additives (which evaluates contaminants such as lead, cadmium, nitrate and nitrite, in addition to food additives).

Further up-to-date information on the GDWQ and the process of their development is available on the WHO website and in the current edition of the GDWQ.

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The work of the following experts was crucial in the development of this document and others in the second addendum to the fourth edition:

Dr M Asami, National Institute of Public Health, Japan
Dr RJ Bevan, independent consultant, United Kingdom
Mr R Carrier, Health Canada, Canada
Dr J Cotruvo, Joseph Cotruvo & Associates and NSF International WHO Collaborating Centre, United States of America
Dr D Cunliffe, South Australian Department of Health, Australia
Dr L d'Anglada, Environmental Protection Agency, United States of America
Dr A Eckhardt, Umweltbundesamt (Federal Environment Agency), Germany
Professor JK Fawell, Cranfield University, United Kingdom
Dr A Hirose, National Institute of Health Sciences of Japan
Dr A Humpage, University of Adelaide (formerly South Australian Water Corporation), Australia
Dr P Marsden, Drinking Water Inspectorate, United Kingdom
Professor Y Matsui, Hokkaido University, Japan
Dr E Ohanian, Environmental Protection Agency, United States of America
Professor CN Ong, National University of Singapore, Singapore
Dr J Strong, Environmental Protection Agency, United States of America
Dr E Testai, National Institute of Health, Italy

The draft text was discussed at the expert consultations for the second addendum to the fourth edition of the GDWQ, held on 28–30 March 2017 and 13–14 July 2018. The final version of the document takes into consideration comments from both peer reviewers and the public, including P Callan, independent consultant, Australia; D Chad and P Thompson, ToxStrategies, United States of America; S Greene, A Sasso and C Gibbons, Environmental Protection Agency, United States of America; F Lemieux and I Moffat, Health Canada; B Lampe, NSF International, United States of America; S Robjohns, Public Health England, United Kingdom; and M Templeton, Imperial College London, United Kingdom.

The coordinator was Ms J De France, WHO, with support from Dr V Bhat, formerly of NSF International, United States of America. Strategic direction was provided by Mr B Gordon, WHO. Dr A Tritscher, formerly of WHO, and Dr P Verger, WHO, provided liaisons with the Joint FAO/WHO Expert Committee on Food Additives and the Joint FAO/WHO Meeting on Pesticide Residues. Dr R Brown and Ms C Vickers, WHO, provided liaisons with the International Programme on Chemical Safety. Dr M Perez contributed on behalf of the WHO Radiation Programme. Dr Andina Faragher, Biotext, Australia, was responsible for the scientific editing of the document.

Many individuals from various countries contributed to the development of the GDWQ. The efforts of all who contributed to the preparation of this document are greatly appreciated.

Acronyms and abbreviations

bw	body weight
Cr	chromium
Cr(III)	trivalent chromium
Cr(VI)	hexavalent chromium
$Cr(OH)_n^{(3-n)+}$	chromium hydroxide
DNA	deoxyribonucleic acid
EFSA	European Food Safety Authority
GI	gastrointestinal
GV	guideline value
$HCrO_4^-$	chromic acid
HPRT	hypoxanthine-guanine phosphoribosyltransferase
IARC	International Agency for Research on Cancer
LD_{50}	median lethal dose
LOAEL	lowest-observed-adverse-effect level
MOA	mode of action
NADH	nicotinamide adenine dinucleotide
NADPH	nicotinamide adenine dinucleotide phosphate
NTP	United States National Toxicology Program
NOAEL	no-observed-adverse-effect level
PBPK	physiologically based pharmacokinetic (model)
US EPA	United States Environmental Protection Agency
WHO	World Health Organization

Contents

Executive summary

Humans can be exposed to chromium, primarily in its trivalent (III) and hexavalent (VI) forms, through its wide distribution in air, soil, groundwater and drinking-water. Chromium in the environment originates from both natural and anthropogenic sources. Once absorbed, chromium(VI) [Cr(VI)] readily penetrates cell membranes, whereas chromium(III) [Cr(III)] does not.

General population exposure to chromium compounds through inhalation of ambient air, ingestion of water or dermal contact is variable and difficult to quantify. Residents living close to industrial facilities that use Cr(VI) compounds or near chromium waste disposal sites have the greatest potential for exposure.

A guideline value (GV) of 50 µg/L is proposed for total chromium, based on achievability by current treatment technologies, measurability by analytical methods, and toxicology. The risk assessment for chromium in drinking-water is based on recent high-quality data from chronic drinking-water carcinogenicity and mode-of-action studies for Cr(III) and Cr(VI). The risk assessment considers both cancer (in the case of Cr(VI)) and noncancer (in the case of Cr(III) and Cr(VI)) end-points. The weight of evidence supports a nonlinear 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 end-point and precursor of tumour formation is protective of both noncancer and cancer effects.

As chromium is usually found in drinking-water at an average concentration of 1 µg/L, which is below the GV, in general, monitoring and inclusion in drinking-water regulations and standards would only be necessary if there were indications that a problem might exist. Monitoring can usually be limited to treatment works. Several methods for removing chromium are available, including conventional water treatment with coagulation, flocculation and filtration; adsorption by iron oxides; ion exchange; reverse osmosis; and nanofiltration. Cr(VI) requires reduction to Cr(III) before removal by ferric coagulants as part of conventional water treatment.

1 General description

1.1 Identity

Chromium is widely distributed in Earth's crust. It can exist in oxidation states of –2 to +6; the trivalent (III) and hexavalent (VI) states predominate in the environment. Soils and rocks may contain small amounts of chromium, almost always in the trivalent state (ATSDR, 2012).

1.2 Physicochemical properties

Some physicochemical properties of chromium and chromium compounds are shown in Table 1.1.

Table 1.1. Physicochemical properties of chromium and chromium compounds

Property	Chromium	Chromium chloride ($CrCl_3$)	Potassium chromate (K_2CrO_4)	Chromium oxide (Cr_2O_3)	Chromium trioxide (CrO_3)
Melting point (ºC)	1857	1152	968.3	2266	196
Boiling point (ºC)	2672	NA	NA	4000	NA
Solubility	Insoluble	Slightly soluble	790 g/L	Insoluble	624 g/L
Density (g/cm^3)	7.14	2.76	2.73	5.21	2.70

NA: not applicable

1.3 Organoleptic properties

There is no indication that chromium compounds at the levels normally found in drinking-water cause adverse effects on taste, odour, appearance or colour.

1.4 Major uses and sources

More than 70% of chromium in the environment comes from anthropogenic sources, such as nonferrous base metal smelters, refineries, leather tanning industries, urban stormwater runoff, effluent streams from pulp and paper mills, and discharges from thermal generating stations (Health Canada, 2016). Chromium and its salts are also used in the manufacture of catalysts, pigments, paints and fungicides; in the ceramic and glass industry; in photography; for chrome alloy and chromium metal production; for chrome plating; and for corrosion control (ATSDR, 2012; EFSA, 2014).

Chromium also occurs naturally in small amounts in rocks and soils, from where it can be released into groundwater through weathering and erosion processes (Thompson et al., 2007; Health Canada, 2016).

1.5 Environmental fate

The environmental distribution of compounds containing chromium(III) [Cr(III)] and chromium(IV) [Cr(VI)] depends on redox potential, pH, the presence of oxidizing or reducing compounds, the kinetics of the redox reactions, the formation of Cr(III) complexes or insoluble Cr(III) salts, and the total chromium concentration. In the environment, Cr(VI) occurs mostly

as chromate ion (CrO_4^{2-}) or chromic acid ($HCrO_4^-$), and Cr(III) as chromium hydroxide ($Cr(OH)_n^{(3-n)+}$).

In soil, Cr(III) predominates; for example, Cr(VI) can easily be reduced to Cr(III) by organic matter. 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; relatively high concentrations of Cr(VI) can be found locally, as a result of anthropogenic activities. In general, Cr(VI) salts are more soluble than Cr(III) salts, making Cr(VI) relatively mobile. In drinking-water treatment, oxidative disinfection techniques such as prechlorination and preozonation may oxidize Cr(III) to Cr(VI) (WRc, 2015; Health Canada, 2016); this is discussed further in section 7.2.

In air, chromium is present in the form of aerosols. It can be removed from the atmosphere by wet and dry deposition. Both Cr(III) and Cr(VI), from all sources, are released into the air. Analytical difficulties mean that data on chromium 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).

2 Environmental levels and human exposure

2.1 Water

Background levels of chromium in surface water and groundwater aquifers are determined by regional geology, mineral weathering processes, sediment loading rates and precipitation patterns (Health Canada, 2016). High concentrations of chromium 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), and are particularly prevalent in ophiolite complexes and serpentine-rich units (Thompson et al., 2007). Levels in uncontaminated waters are usually very low (<1 µg/L), although leaching from landfill or release of chromium through anthropogenic activities may cause contamination of drinking-water (WRc, 2015).

Total chromium is regularly monitored in drinking-water in the United Kingdom, and summary results for individual suppliers are published (DEFRA & Environment Agency, 2002). Of more than 12 000 samples taken for drinking-water compliance studies in England and Wales in 2016, none had chromium concentrations above 50 µg/L. The maximum value reported was 15 µg/L, and the 95th percentile was 1 µg/L (PK Marsden, Drinking Water Inspectorate, United Kingdom, personal communication, April 2017). A survey of 23 drinking-water sources in the United Kingdom over a 12-month period reported that background Cr(VI) levels were <0.1 µg/l (WRc, 2015). Average Cr(VI) concentrations in drinking-water supplies in Canada and the United States of America range from 0.2 to 2 µg/L (Moffat et al., 2018). Data from the USA collected under the Unregulated Contaminant Monitoring Rule (UCMR) for 2013–2015 (UCMR 3[1]) 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 in which the Cr(VI) values were greater than the total chromium values (Eaton, Bartrand & Rosen, 2018), the majority of states had Cr(VI) levels between 0.1 and 1.0 µg/L. In the Netherlands, the total chromium concentration was reported to be <1 µg/L for 76% of drinking-water supplies and <2 µg/L for 98% of supplies (Fonds, Van den Eshof & Smit, 1987). A survey of Canadian

[1] https://www.epa.gov/dwucmr/occurrence-data-unregulated-contaminant-monitoring-rule#3

drinking-water supplies reported an overall median level of 2 µg/L for total chromium, with a maximum of 18.9 µg/L (groundwater source) (Health Canada, 2016).

Very little to no data are generally available on the speciation of chromium in drinking-water (DEFRA & Environment Agency, 2002; WRc, 2015). Recently, a small amount of work on this issue has been done in the United Kingdom. Finished drinking-water supplies at 20 sites in England and Wales were surveyed for total chromium, Cr(III) and Cr(VI) four times in 12 months (where possible). The concentrations of Cr(VI) were very low, generally <1 µg/L, which appears to be consistent with typical background concentrations of Cr(VI) in other countries (WRc, 2015)

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

The natural total chromium concentration in surface waters in the UK is approximately 0.5–2 µg/L; the dissolved chromium concentration is 0.02–0.3 µg/L (WRc, 2015). Total chromium concentrations in Antarctic lakes have been reported to increase with depth from <0.6 to 30 µg/L (US EPA, 1987). In general, the total chromium 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 1985, surface water total concentrations in central Canada ranged from 0.2 to 44 µg/L. In the Rhine, total chromium levels were below 10 µg/L. In 50% of the natural stream waters in India, the total chromium concentration was <2 µg/L (US EPA, 1987).

In general, the total chromium concentration in groundwater is low (<1 µg/L) (Health Canada, 2016). Levels in the United Kingdom have been reported as <3 µg/L (WRc, 2015). In the Netherlands, a mean total chromium concentration of 0.7 µg/L has been measured, with a maximum of 5 µg/L (WHO, 1988). Most supplies in the USA contain <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; WRc, 2015). In India, 50% of 1473 water samples from dug wells were reported to contain total chromium at <2 µg/L (US EPA, 1987).

2.2 Food

With the exception of populations living close to a point source of chromium contamination of the environment, the main source of chromium exposure is thought to be from food. Food has been found to contain total chromium at concentrations ranging from <0.0005 to 1.3 µg/g (UK MAFF, 1985; UK Food Standards Agency, 1999; ATSDR, 2012; EFSA, 2014; Health Canada, 2016). The highest concentrations (>0.1 µg/g) have been found in meat, fish, seafood, cereal products, tea, black pepper, cheese, wheatgerm, and some fruits and vegetables (UK MAFF, 1985; Copat et al., 2012). However, total chromium levels in fresh foods tend to be extremely low (0.02–0.05 µg/kg) (Health Canada, 2016). Beer, wine and spirits contain total chromium 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 chromium levels (Health Canada, 2016).

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 chromium in food could be classed as Cr(III) (EFSA, 2014).

2.3 Air

In remote areas, including the Arctic and the Antarctic, chromium concentrations in air of 0.005–2.6 ng/m^3 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 chromium; mean levels were generally <300 ng/m^3, and median levels <20 ng/m^3 (US EPA, 1984b). In non-industrialized areas, concentrations above 10 ng/m^3 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 chromium at 59 sites in cities in the USA during 1968–1971 with data from the United States Environmental Protection Agency (US EPA) National Aerometric Data Bank file for 1975–1983. They concluded that atmospheric chromium levels may have declined in the early 1980s from the levels detected in the 1960s and 1970s. The mean concentration of total chromium in air in the Netherlands has been reported to range between 2 and 5 ng/m^3 (Sloof, 1989). In the United Kingdom, the Department for Environment, Food and Rural Affairs reported average levels of chromium in urban and rural areas during 2009–2010 to be generally within the range 0.7–5 ng/m^3; one outlier of 30.3 ng/m^3 was identified in an area close to steel-making industry (DEFRA, no date).

Indoor air concentrations of total chromium can be around 1000 ng/m^3 – that is, 10–400 times greater than outdoor concentrations – as a result of tobacco smoke. An indoor/outdoor air study conducted in the USA in 1993 reported Cr(VI) levels of 0.1–0.6 ng/m^3 (geometric mean 0.2 ng/m^3) for indoor air and 0.10–1.6 ng/m^3 (geometric mean 0.55 ng/m^3) for outdoor air, with the particles being of inhalable size (Bell & Hipfner, 1997). The indoor levels were lower than the 0.38–3000 ng/m^3 (mean of 1.2 ng/m^3) reported in an earlier study in the USA (Falerios et al., 1992).

2.4 Bioaccumulation

Chromium 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 chromium has a low mobility for translocation from roots to above-ground parts of plants (Cary, 1982; WHO, 1988), but the transfer ratio of chromium from soil to plants and bioaccumulation in terrestrial food chains is unknown (Health Canada, 2016).

2.5 Estimated total exposure and relative contribution of drinking-water

The general population is exposed to chromium by inhaling ambient air, and ingesting food and drinking-water containing chromium. The estimated average total intake of total chromium from air, water and food by the general population in the United Kingdom is approximately 127 µg per day. Food contributes around 92% of the total intake and water 8%. The contribution from air is negligible (DEFRA & Environment Agency, 2002). In the Netherlands, the estimated mean daily total chromium intake is 100 µg, with a range of 50–200 µg (Sloof, 1989; WHO, 1996). The daily total chromium intake for the population of the USA 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). For large segments of the population, food appears to be the major source of intake. Drinking-water intake can, however, contribute substantially, particularly when total chromium levels in drinking-water are above 25 µg/L, although at lower levels may be a significant contributor in certain settings.

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 chromium concentration of 2.0 µg/L in unfiltered Ontario drinking-water and assuming that Cr(VI) represents 100% of total chromium, contributions from drinking-water in the five age groups were as follows: non-breastfed infants 0–6 months of age, 99%; breastfed infants 0–6 months of age, 0%; 0.5–4 years, 51%; 5–11 years, 51%; 12–19 years, 50%; and 20+ years, 64% (Health Canada, 2016). Food is the second major source of chromium exposure. Assuming that 10% of total chromium in food is Cr(VI), exposure through food may represent up to 50% of the total daily intake (Health Canada, 2016). Hence, Health Canada (2016) 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.

3 Toxicokinetics and metabolism in humans and laboratory animals

3.1 Absorption

The water solubility and oxidation state of chromium compounds affect their absorption rates via oral, inhalation and dermal routes.

In humans, absorption of chromium following oral administration is 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 chromium compounds being absorbed, depending on solubility. Tissue chromium levels in rats exposed to Cr(VI) (as potassium chromate) in drinking-water were 4–15 times higher than in 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 body fluids, including gastric fluid (ATSDR, 2012). Recent studies (De Flora et al., 2016; Kirman et al., 2016) have confirmed the ability of human gastric fluid to reduce Cr(VI), thereby reducing its biological activity (see section 3.2). Cr(VI) reduction was rapid: 70% of total reduction occurred within 1 minute and 98% within 30 minutes 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 samples taken after fasting (lower stomach pH) and nonfasting samples (higher stomach pH). This suggests that Cr(VI) may be reduced to Cr(III) at a lower rate in individuals with elevated gastric pH levels, including neonates, users of proton pump inhibitors and people with hypochlorhydria (Kirman et al., 2016).

Moffat et al. (2018) reported that the reduction of Cr(VI) in both rodents and humans can be described mathematically by a three-pool model containing reducing agents of differing capacities and rates: a fast-acting low-capacity pool, a slower-acting high-capacity pool, and a very slow-acting high-capacity pool. At the low concentrations of Cr(VI) that are typically 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.

3.2 Distribution

Once chromium is absorbed, its fate will depend on its oxidation state. Cr(VI) readily penetrates cell membranes, whereas Cr(III) does not. Chromium is therefore found in both erythrocytes

and plasma after GI absorption of Cr(VI), but exclusively in the plasma after GI absorption 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 chromium 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). Tissue chromium levels decline with age.

Studies have assessed the distribution of Cr(III) and Cr(VI) following oral exposure of male rats and female mice to Cr(VI) via feed and drinking-water (maximum dose of 516 mg/L in rats and mice) (NTP, 2008a, b; EFSA, 2014) for up to 369 days. These studies reported only total chromium, as speciation of chromium is not possible in tissues, and therefore the data were only able to suggest the uptake and distribution of Cr(VI). Increases in total chromium relative to controls were seen in multiple tissues, indicating that systemic exposure resulted from both sources of chromium. The authors reported that, for similar external doses, higher levels of chromium were found in red blood cells, stomach, liver and kidney following exposure to Cr(VI), compared with Cr(III). The authors concluded that this showed uptake and distribution of at least a portion of Cr(VI) before reduction. When tissue concentrations were normalised to ingested dose, statistically significantly higher levels of chromium were reported in the glandular stomach and liver in exposed mice, and in the kidney in exposed rats. Similar levels of chromium were found in plasma, urine and faeces of rats and mice. Species-specific differences were also noted, with a much higher absorption of chromium in mice than in rats. A time-dependent increase in tissue chromium levels was reported for rats and mice, for both the studies with Cr(III) and Cr(VI), over a 6-month period. Longer exposures to Cr(VI) resulted in a decrease in levels from that following 6-month exposure in all tissues except red blood cells and plasma.

3.3 Metabolism

Cr(VI) is poorly absorbed following oral intake because it is reduced by GI fluids (gastric juice and saliva) and sequestered by intestinal bacteria (De Flora et al., 2016). Any Cr(VI) that is absorbed is 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 nonprotein thiols, such as glutathione and cysteine, to yield Cr(III) (Health Canada, 2016). Within red blood cells, reduction of Cr(VI) occurs by the action of glutathione, leaving Cr(III) mostly trapped within the erythrocyte for the lifespan of the cell (Paustenbach et al., 1996). In vitro studies have also demonstrated reduction of Cr(VI) by microsomal enzymes in an NADPH-dependent process (Gruber & Jennette, 1978; Health Canada, 2016).

Depending on the nature of the reducing agents and the proximity of the intracellular site to DNA, reduction of Cr(VI) may result in detoxification or activation. Detoxification will occur when the site of reduction is far from a DNA source and reactive intermediates can be trapped by components of the intracellular environment (De Flora, 2000). Activation may occur when the site of reduction is close to a source of DNA, so that unstable intermediates that are produced may react with intracellular proteins and DNA (De Flora, 2000; Zhitkovich, 2011).

3.4 Elimination

Following oral exposure to chromium compounds, especially those of Cr(III), a large proportion of the dose is recovered in the faeces, because of the poor absorption in the GI tract. Chromium is also reported to be excreted in hair and fingernails (WHO, 2013). Urine is the

major route of elimination of absorbed chromium. In a 1-year balance study in which two humans received mean daily dietary intakes of 200 and 290 µg of chromium, 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% is excreted within 15–30 days, and the remaining 10% is excreted within 5 years (EFSA, 2014).

3.5 Physiologically based pharmacokinetic modelling

As a result of the analytical difficulties in speciating Cr(III) and Cr(VI), many studies report total chromium levels in tissues and body fluids, which may not be the most appropriate internal dose metric (Kirman et al., 2013). Physiologically based pharmacokinetic (PBPK) models provide a means to characterize the reduction of Cr(VI) to Cr(III) before absorption, under varying conditions, allowing an estimation of internal doses for speciated chromium. Early PBBK models for rats and humans (O'Flaherty, 1996; O'Flaherty et al., 2001, respectively) were simplistic in that they did not include compartmentalization and parameterization 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 compartmentalization of the target tissues, the small intestine and oral mucosa, and used data generated ex vivo to quantify the reduction of Cr(VI) in gastric content of rats and mice (Proctor et al., 2012). Schlosser & Sasso (2014) modified the Kirman et al. (2012, 2013) models, taking into account the pH dependence of reduction and effects due to dilution of gastric juices. The models described by Kirman et al. (2012, 2013) and Schlosser & Sasso (2014) also included consideration of a number of pools of reducing agents present in gastric fluid (e.g. ascorbate, NADH, glutathione). It has been proposed that up to three pools are present, reflecting rapid, slower and slow interaction and depletion, respectively (Moffat et al., 2018).

For human risk assessment, Kirman et al. (2016) developed a model that used data generated ex vivo on the rate and capacity of Cr(VI) reduction in fasted human gastric fluid. This allowed estimation of internal doses to the small intestine 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 proton pump inhibitor use, providing improved characterization of Cr(VI) gastric reduction.

4 Effects on humans

The toxicity of chromium varies with its valence state and the route of exposure. Available data mainly refer to total chromium – that is, Cr(III) and Cr(VI). The data suggest little or no toxicity associated with the trivalent form, but toxicity of the hexavalent form, which is soluble in water. Available data relating to Cr(III) and Cr(VI) are summarized below (Health Canada, 2016).

4.1 Nutritional essentiality

The United States Institute of Medicine considers Cr(III) to be an essential nutrient required for normal energy metabolism and has determined an adequate intake of 20–45 µg/day for adolescents and adults (IOM, 2001). However, this view is equivocal because there is no direct evidence of chromium 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 chromium deficiency is difficult to induce, when successfully achieved, it results in hyperglycemia, decreased weight gain, elevated serum cholesterol levels, aortic plaques, corneal opacities, impaired fertility, and lethality (ATSDR, 2012). The World

Health Organization (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 United States National Academy of Sciences in partnership with Health Canada (IOM, 2001), reflecting current estimates of average chromium intake from well-balanced diets. These AIs range from 0.2 µg/day (for infants) to 45 µg/day (for lactating women). The daily chromium requirement for adults (<50 years of age) is estimated to be 35 and 25 µg/kg bw/day in males and females, respectively.

4.2 Acute exposure

Symptoms of acute chromium 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/day. Around 1 g of potassium dichromate ($K_2Cr_2O_7$) is considered a lethal dose (ATSDR, 2012). In human volunteers, exposure to Cr(VI) at a single dose of up to 4 mg 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).

4.3 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 0.03–4 mg/kg bw/day for at least 3 days, no apparent clinical changes or health effects were observed (EFSA, 2014; Health Canada, 2016).

4.4 Long-term exposure

4.4.1 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 4.2, 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 a rural population in China to Cr(VI) through consumption of well water containing Cr(VI) at up to 20 mg/L (considered to be equivalent to Cr(VI) at 0.57 mg/kg bw/day) was associated with GI effects, including diarrhoea, abdominal pain, indigestion and vomiting. The reliability of the exposure estimates is of potential concern, as a result of poor characterization of the exposure (ATSDR, 2012).

No definitive information was identified on haematological 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 in China, described above (ATSDR, 2012).

4.4.2 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.

4.4.3 Reproductive and developmental effects

No studies could be identified regarding the reproductive and developmental toxicity of Cr(III) or Cr(VI) following long-term oral exposure (ATSDR, 2012), or Cr(III) following long-term inhalation exposure.

Long-term occupational exposure to Cr(VI) via inhalation has been associated with adverse reproductive effects in males (Health Canada, 2016).

4.4.4 Immunological effects

Exposure to chromium compounds may induce allergic sensitization in some individuals, through a combination of inhalation, oral and/or dermal exposure. Oral exposure to Cr(VI) can exacerbate dermatitis in sensitive individuals (ATSDR, 2012). No data on immunological effects of other chromium species could be identified.

4.4.5 Genotoxicity and carcinogenicity

Studies to assess potential genotoxic effects of Cr(III) or Cr(VI) following oral exposure in humans could not be identified.

In some occupational studies, increased incidences of genotoxic effects have been found in circulating lymphocytes and/or buccal and nasal mucosal cells of workers exposed to Cr(VI) compounds by inhalation. These include 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). However, these studies have several limitations, including uncertainty in the quantification of exposure levels, a relatively small numbers of workers included in the studies and potential confounding from co-exposure to other mutagenic compounds. In addition, occupational exposure to Cr(VI) in these studies was predominantly through inhalation, and the relevance of the findings to drinking-water exposure 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 5.4).

The potential for development of cancer in humans through environmental exposure to chromium has been assessed in several retrospective epidemiological studies (IARC, 2012; Health Canada, 2016). These studies did not show an association between oral exposure to total chromium or Cr(VI) and cancer. However, the studies were not considered sufficient for assessing causation (IARC, 2012; Health Canada, 2016) because they did not quantify exposures of individuals; therefore, exposure misclassification is possible and may bias the reported results.

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 chromium-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 chromium, Cr(III) and Cr(VI), or of soluble versus insoluble chromium 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). The International Agency for Research on Cancer (IARC) has classified Cr(VI) compounds as "carcinogenic to humans" (Group 1) by the inhalation route of exposure based on sufficient evidence in both humans (lung cancer) and experimental animals (see also section 5.3.5) (IARC, 2012); data on human carcinogenicity via the oral route are lacking. The US EPA has also classified Cr(VI) as a Group A (known human) carcinogen via the inhalation route (US EPA, no date).

The conflicting findings associated with different routes of exposure may in part be explained by the reductive capacity of the GI tract, which limits or prevents Cr(VI) uptake via the oral route. This is supported by findings from a study (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 eight volunteers. The mean Cr(VI) reducing capacity of post-meal samples was significantly higher than that of pre-meal samples; >70% of total reduction occurred within 1 minute and 98% within 30 minutes in post-meal gastric fluid at pH 2.0. Mutagenicity, as determined in the Ames test, was also attenuated by gastric fluid, with reductions being higher in post-meal samples.

5 Effects on experimental animals and in vitro test systems

5.1 Acute exposure

Chromium compounds have moderate to high acute oral toxicity in rats and mice, based on oral median lethal dose (LD_{50}) values. For Cr(III), the oral LD_{50} is 183–422 mg/kg. For Cr(VI), the oral LD_{50} values are 13–811 mg/kg in rats and 135–175 mg/kg in mice (WHO, 2009; ATSDR, 2012). Variation is again seen with the different chromium compounds containing Cr(VI) and female rats showing greater sensitivity

5.2 Short-term exposure

A number of short-term (generally ≤90 days in duration) repeat-dose toxicity studies have been carried out in rats and mice using Cr(III) administered by the oral route, including via drinking-water. In general, very little toxicity was reported up to the highest dose of Cr(III) tested (1368 mg/kg bw/day in rats and 1419 mg/kg bw/day in mice). This result may be due to the poor absorption of Cr(III) via this route of exposure (NTP, 2010; ATSDR, 2012; EFSA, 2014).

Several repeat-dose, short-term (generally ≤90 days) toxicity studies of Cr(VI) have been conducted in rats and mice exposed via drinking-water at doses up to 60 mg/kg bw/day in rats and 80 mg/kg bw/day in mice (NTP, 1996a,b, 2007; De Flora, Iltcheva & Balansky, 2006; Thompson et al., 2011, 2012; Health Canada, 2016). Many of these studies reported a statistically significant decrease in the body weight of exposed animals compared with controls. However, the authors noted that this is likely to relate to some extent to the effect of Cr(VI) in decreasing the palatability of the drinking-water and therefore reducing drinking-water intake in the experimental groups.

The 90-day studies conducted by the United States 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 to BALB/c, B6C3F1 and

C57BL/6 mice. Drinking-water concentrations of Cr(VI) used in the NTP (2007) study were 0, 22, 44, 88, 175 and 350 mg/L for rats and mice. Drinking-water concentrations of Cr(VI) used in the Thompson et al. (2011, 2012) studies were 0, 0.1, 1.4, 21, 60 and 182 mg/L in rats and mice, with an additional concentration of 4.9 mg/L in mice only. The NTP reported that lesions, which showed dose-related increases in incidence and severity, were apparent in the duodenum and jejunum of the small intestine in both species. The first (most sensitive) lesion to appear in rats was histiocytic infiltration in the duodenum. More severe effects were noted at higher doses in rats, including ulcer and metaplasia of the glandular stomach. In mice, the first lesions to appear were epithelial 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 mode of action of Cr(VI) and is discussed further in section 5.6. Lowest-observed-adverse-effect levels (LOAELs) for Cr(VI) of 2.9 mg/kg bw/day for rats and 2.6–4.6 mg/kg bw/day for mice were identified by the study authors, based on the increased incidence of non-neoplastic lesions (diffuse hyperplasia, which precedes tumour formation) (NTP, 2007). Thompson et al. (2011) reported similar lesions in the duodenum of B6C3F1 mice to those seen in the NTP study, but not to those reported in F344 rats (Thompson et al., 2012). The histopathological findings across the NTP (2007) and Thompson et al. (2011, 2012a) studies were re-evaluated to assess consistency of data (Cullen, Ward & Thompson, 2016). The authors of the re-evaluation concluded that qualitatively similar intestinal lesions were present in rats and mice, as reported by Thompson et al. (2011, 2012a), with the severity being much lower in rats. It was suggested that, because the severity of the non-neoplastic lesions was milder in rats than in mice, a threshold for progression to carcinogenesis may not have been reached (Cullen, Ward & Thompson, 2016).

The NTP has 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 Cr(VI) concentrations up to 7.4 mg/kg bw/day for 4 days, a statistically significant decrease (by 2.1%) was seen in mean corpuscular haemoglobin at a Cr(VI) dose of 2.7 mg/kg bw/day. 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/kg bw/day and in females at 4.1 mg/kg bw/day (NTP, 2007). More severe microcytic, hypochromic anaemia occurred in rats and mice following exposure to sodium dichromate dihydrate in drinking-water for 22 days (NTP, 2008a) or 23 days (NTP, 2007). This was evidenced by a dose-dependent decrease in haematocrit, haemoglobin, mean cell volume and mean corpuscular haemoglobin at a maximum Cr(VI) dose of 0.77 mg/kg bw/day; no changes were noted at a dose of 0.21 mg/kg bw/day. Male and female rats exposed to Cr(VI) at 1.7 mg/kg bw/day for 23 days showed similar changes. The NTP studies showed that longer periods of exposure, ranging from 3 months to 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 in the liver were also apparent in F344/N rats, but not B6C3F1 mice, following exposure to Cr(VI) via drinking-water for periods between 5 days and 22 weeks (NTP, 2007). Activity of serum alanine aminotransferase (ALT) was increased in exposed male rats (by 30%) and female rats (by 15%) compared with controls following 5 days of exposure to Cr(VI) (as disodium dichromate) at Cr(VI) doses of 4.0 and 4.1 mg/kg bw/day, respectively (NTP, 2007). The activity of ALT remained elevated in exposed rats of both sexes following 14 weeks of exposure. At 14 weeks, serum sorbitol dehydrogenase activity was also significantly increased compared with controls, by 77% in exposed males and 359% in exposed females at a Cr(VI) dose of 1.7 mg/kg bw/day. After 14 weeks of exposure,

histopathological changes were apparent in exposed females only as cellular histiocyte inflammation and chronic focal inflammation (NTP, 2007). In a separate study, Acharya et al. (2001) reported increased serum ALT and aspartate aminotransferase in rats following 22 weeks of exposure to Cr(VI) at 1.3 mg/kg bw/day in drinking-water, compared with unexposed rats. In addition, exposed female rats showed increased incidence of morphological liver changes compared with controls, comprising vacuolization, increased sinusoidal space and necrosis.

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

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

A decrease in motor activity and balance was reported in rats given Cr(VI) at 98 mg/kg bw/day (equivalent to 0.7 g/L) as sodium chromate in drinking-water for 28 days (Diaz-Mayans, Laborda & Nuñez, 1986). However, this is a considerably higher dose than that used in the NTP studies.

Shipkowski et al. (2019) reported limited effects on the immune system of female rats and mice exposed to concentrations of 516 and 250 mg/L sodium dichromate dihydrate, respectively, for 28 days in drinking-water. Cr(VI) (as sodium dichromate dihydrate) has also been found to produce adverse immunological effects in rats at doses of Cr(VI) of 16 mg/kg bw/day for 3 weeks via drinking-water (ATSDR, 2012). They included functional and structural changes, 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.

Contact dermatitis has been elicited in guinea pigs and mice following application of a single dose of Cr(VI) but not Cr(III) (ATSDR, 2012).

5.3 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 or no toxicity up to the highest dose tested (1466 mg/kg bw/day in rats and 783 mg/kg bw/day in mice). Accumulation of chromium in several tissues was noted in the longer-term studies (NTP, 2010; Health Canada, 2016).

5.3.1 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). In rats, the most critical non-neoplastic effects

were haematological effects, including microcytic, hypochromic anemia; and histiocytic cellular infiltration in the liver, mesenteric lymph node and duodenum. EFSA reported the no-observed-adverse-effect level (NOAEL) associated with these effects as 0.21 mg/kg bw/day. In mice, critical non-neoplastic effects were hyperplasia in the duodenum, and hystiocytic cellular infiltration in the liver and mesenteric lymph nodes; a LOAEL of 0.38 mg/kg bw/day was determined for each end-point (lowest dose tested) (EFSA, 2014). In general, effects were less severe in rats than in mice (NTP, 2008b; Health Canada, 2016).

5.3.2 Neurological effects

Histopathological analysis of the brain and nervous system of rats and mice following exposure to Cr(III) (2040 mg/kg bw/day) or Cr(VI) (8.7 mg/kg bw/day) in drinking-water for up to 2 years showed no adverse effects (NTP 2007, 2008b; Health Canada, 2016). Neurological, neurochemical or neurobehavioural tests have not been carried out.

5.3.3 Reproductive and developmental effects

Conflicting results have been reported on the occurrence of adverse reproductive effects in rats and mice orally exposed to Cr(III) through drinking-water and feed (NTP, 1996a,b, 1997, 2010; US EPA, 1998; Health Canada, 2016). Of the drinking-water studies, one found significant alterations in sexual behaviour and aggressive behaviour towards other males, and significantly lower absolute weight of testes, seminal vesicles and preputial glands in male Sprague–Dawley rats exposed to Cr(III) (as chromium chloride) at 40 mg/kg bw/day in drinking-water for 12 weeks. Although male fertility was not considered to be affected, an increase in the total number of fetal resorptions was seen in unexposed females mated with exposed males. In Swiss mice, fertility was adversely affected in males following exposure to Cr(III) (as chromium chloride) at 13 mg/kg bw/day when they were mated with unexposed females, and in females exposed to Cr(III) at 5 mg/kg bw/day when they were mated with unexposed males. In addition, increased testes and ovarian weights, and decreased preputial gland and uterine weights were reported at a Cr(III) dose of 5 mg/kg bw/day. Decreased spermatogenesis was observed in BALB/c mice treated with Cr(III) (as chromium sulfate) at 9.1 mg/kg bw/day 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 Cr(III) (as chromium oxide) at 1806 mg/kg bw/day, 5 days/week for 60 days before mating and throughout gestation. However, in BALB/c mice exposed to Cr(III) (as chromium chloride) at 74 mg/kg bw/day in drinking-water from gestation day 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 to Cr(VI) via drinking-water, with the male reproductive system showing the highest sensitivity (ATSDR, 2012). A statistically significant (p value not stated) decrease in sperm count (by 13%) and motility (by 12%), as well as increased incidence of histopathological changes to the epididymis, have been reported in adult monkeys exposed

to Cr(VI) (as potassium dichromate) at ≥ 2.1 mg/kg bw/day in drinking-water for 180 days; a LOAEL of 2.1 mg/kg bw/day was derived (lowest dose tested) (ATSDR, 2012). In rats, effects have been observed at Cr(VI) concentrations of 1.6 mg/kg bw/day (ATSDR, 2012). In addition to effects on male reproductive organs (decreased weights of testes, seminal vesicles and preputial glands), inhibition of sexual behaviour and aggression was reported in male rats exposed to Cr(VI) in drinking-water at 32 mg/kg bw/day for 12 weeks (ATSDR, 2012). A 2-year study in which F344 rats were exposed to sodium dichromate dihydrate to give a dose of Cr(VI) of 5.9 mg/kg bw/day via drinking-water did not show any morphological changes to male reproductive organs (NTP, 2008b). Similarly, exposure of B6C3F1 mice to Cr(VI) at 5.9 mg/kg bw/day did not produce any morphological changes to male reproductive organs (NTP, 2008b). In addition, sperm count and motility were also unaffected in B6C3F1, BALB/c and C57BL/6N mice exposed to Cr(VI) at 9.1 mg/kg bw/day via drinking-water for 3 months (NTP, 2007).

Adverse effects reported in the female reproductive system of rats and mice following Cr(VI) exposure via drinking-water at doses of ≥5 mg/kg bw/day include lengthening of the estrus cycle, altered weights of reproductive organs, reductions in the number of ovarian follicles, and changes in circulating steroid and pituitary hormone levels (Health Canada, 2016). Chromium has been shown to pass the placental barrier and accumulate in fetal tissues (Health Canada, 2016).

A number of studies addressing oral exposure to Cr(VI) have shown developmental toxicity following premating exposure and/or in utero or lactational exposure (ATSDR, 2012; Health Canada, 2016). Effects included embryotoxicity (increases in pre- and post-implantation loss, and in resorptions) and fetotoxicity (decreased fetal weight, number of fetuses and number of live fetuses; and increased frequency of gross, visceral and skeletal malformations), and were observed at Cr(VI) doses of 45 mg/kg bw/day in rats and 31–52 mg/kg bw/day in mice. No NOAELs were identified by the authors of these studies.

A series of studies in rats have reported increased oxidative stress in the offspring of dams exposed to Cr(VI) at ≥6 mg/kg bw/day in drinking-water during the gestational or postnatal periods, including lactation (Health Canada, 2016). Perinatal exposure to Cr(VI) (as potassium dichromate) at ≥2.9 mg/kg bw/day in drinking-water was associated with oxidative stress in the uterus, liver, kidney and bone of the offspring, with associated morphological alterations in the kidney, liver and bone.

5.3.4 Immunological effects

Two-year feeding studies in rodents , using Cr(III) (as chromium picolinate [$Cr(C_6H_4NO_2)_3$]) up to a maximum dose of 781 mg/kg bw/day, suggest that the immune system is not a target for ingested Cr(III) (NTP, 2007; ATSDR, 2012; Shipkowski et al., 2017). However, Cr(VI) produced adverse immunological effects in rats in short-term studies (see section 5.2). Microscopic changes to lymphatic tissues were observed in male and female rats at doses of Cr(VI) of 1.7 and 20.9 mg/kg bw/day, respectively, after 3 months, and at 0.77 and 2.4 mg Cr(VI)/kg bw/day in male and female rats, respectively, after 2 years of exposure (NTP, 2008a). In mice, microscopic changes were observed at Cr(VI) doses of 3.1 mg/kg bw/day (NTP, 2007) for 3 months and 0.38 mg/kg bw/day for 2 years (NTP 2008a).

5.3.5 In vivo genotoxicity and carcinogenicity

5.3.5.1 In vivo genotoxicity – Cr(III)

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

Komorowski, Greenberg & Juturu (2008) reported no induction of chromosomal aberrations in the bone marrow cells of rats 18 or 42 hours after exposures to single oral doses of Cr(III) of 4.1, 30.8 or 246 mg/kg bw/day.

Studies conducted on Cr(III) compounds in animal models using the oral route have yielded negative results. In an NTP study (NTP, 2010), male F344/N rats treated with Cr(III) picolinate (anhydrous) (156–2500 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 (80–50 000 mg/kg diet, corresponding to Cr(III) doses of 2–1419 and 1.7–1090 mg/kg bw/day for males and females, respectively) in feed for 3 months, no micronuclei were found in peripheral blood erythrocytes of males. Weak increases in the micronuclei frequency in erythrocytes of female mice were considered equivocal, because the anhydrous form of Cr(III) picolinate was inactive (NTP, 2010). De Flora, Iltcheva & Balansky (2006) analysed the frequency of micronuclei in bone marrow and peripheral blood cells of male and female BDF1 mice administered Cr(III), as chromium potassium chromate ($CrK(SO_4)_2 \cdot 12H_2O$), in drinking-water, equivalent to a Cr(III) dose of 165 and 140 mg/kg bw/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 reversion assay in C57BL/6 mice following administration of Cr(III) chloride to dams in the drinking-water at an average dose of Cr(III) of 375 or 750 mg/kg bw/day. Significant increases in the frequency of DNA deletions in embryos harvested at 17.5 days postcoitum were reported. The authors confirmed absorption of Cr(III) by measuring tissue levels (Health Canada, 2016).

5.3.5.2 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/6J mice to Cr(VI) via drinking-water at a concentration of 62.5 mg/L, induction of mutations was reported (Health Canada, 2016). A significant increase in micronuclei formation was reported in peripheral erythrocytes of am3-C57BL/6 mice following exposure to Cr(VI) at 43.6 mg/L; in B6C3F1 strain BALB/c mice, a positive, but nonsignificant, trend was observed (NTP, 2007). Other studies have reported negative results in bone marrow, peripheral blood cells or tissues following oral exposure to Cr(VI) compounds (De Flora, Iltcheva & Balansky, 2006; ATSDR, 2012; De Flora et al., 2016; Thompson et al., 2017a; Aoki et al., 2019).

The route-dependent genotoxicity of Cr(VI) has also been demonstrated in MS/Ae and CD-1 mice. When administered by intraperitoneal injection, potassium chromate (equivalent to Cr(VI) at 17.7 mg/kg) induced micronuclei in a dose-dependent manner in both strains. However, when administered orally via drinking-water, potassium chromate (equivalent to Cr(VI) at 113.1 mg/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 equivalent to a Cr(VI) concentration of 500 mg/L for up to 210 consecutive days, no increase in micronucleus frequency was observed in either bone marrow or peripheral blood erythrocytes. However, following intraperitoneal injection (equivalent to Cr(VI) at 50 mg/kg), the compounds induced clastogenic damage. In the same study, pregnant mice were treated with Cr(VI) up to 10 mg/L drinking-water. No genotoxic effects were observed either in bone marrow of pregnant mice or in liver and peripheral blood of their fetuses. EFSA concluded that the determinant for the genotoxic effects of Cr(VI) in vivo is the reductive capacity of the GI tract, which limits or completely prevents uptake in the blood and/or systemic distribution (although, even at low levels, not all Cr(VI) may be converted to Cr(III) in the human GI tract) (EFSA, 2014). However, as noted in section 3.3, it is considered that any Cr(VI) that is absorbed from the GI tract will be reduced to Cr(III) in the blood of the portal vein system or the liver, and any absorbed into the cells will be reduced by intracellular mechanisms (Health Canada, 2016). DNA damage as measured by the comet assay has been observed in several tissues in mice and rats, including stomach, colon, liver, kidney, bladder, brain and peripheral leukocytes (ATSDR, 2012).

5.3.5.3 Carcinogenicity – Cr(III)

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

A long-term study has been carried out by the NTP (2010) to assess the carcinogenicity of Cr(III) (as chromium 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 Cr(III) doses of 286.2 and 313.7 mg/kg bw/day for male and female rats, respectively, and to 783.0 and 727.5 mg/kg bw/day for male and female mice, respectively. No significant changes were seen 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/kg bw/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.

5.3.5.4 Carcinogenicity – Cr(VI)

A long-term study was conducted by the 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 Cr(VI) doses of 5.9, 7.0 and 8.7 mg/kg bw/day for male mice and rats, female rats and female mice, respectively (NTP, 2007, 2008b). Significant increases in the incidence of squamous cell carcinoma of the oral mucosa, and 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 exposures in each sex for adenomas and carcinomas combined ($P < 0.001$), and at the highest concentration for carcinomas in the duodenum, jejunum and ileum combined ($P < 0.05$), both for males and females (EFSA, 2014).

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

5.3.5.5 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 the IARC as Group 3 – that is, they are *not classifiable as to their carcinogenicity in humans* (IARC, 2012).

Data on evaluation of Cr(VI) compounds for carcinogenicity via the oral route are also limited. However, there is sufficient evidence from animal studies that 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 IARC has classified Cr(VI) compounds as Group 1 – that is, there is *sufficient evidence in humans for their carcinogenicity* (IARC, 2012).

5.4 In vitro genotoxicity studies

5.4.1 Bacteria and yeast – Cr(III)

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 number of *Salmonella* Typhimurium strains and concentrations of Cr(III) picolinate up to 10 000 µg/plate, in the presence or absence of metabolic activation (EFSA, 2014). Cr(III) chloride and chromium 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. *Salmonella* Typhimurium strains TA102 and TA2638) (ATSDR, 2012).

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

5.4.2 Bacteria and yeast – Cr(VI)

Cr(VI) compounds have generally tested positive for gene mutations in bacterial cells. As described in EFSA (2014), reverse mutations were observed after exposure to Cr(VI) compounds in multiple species and strains of *Salmonella* Typhimurium and *Escherichia coli*, using assays that can detect a wide spectrum of DNA lesions, including oxidative damage and DNA cross-links, and 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).

5.4.3 Mammalian cells – Cr(III)

Cr(III) compounds, particularly chromium picolinate, have been tested in numerous bioassays using cultured mammalian cells. Results have been mixed, but often positive (EFSA, 2014).

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

Cr(III) chloride induced chromosomal aberrations in phytohaemagglutinin-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, micronuclei were found when Cr(III) imine complexes, which can be oxidized to Cr(V) complexes, were tested (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 with 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 using the comet assay with and without hydrogen peroxide–induced stress in human HaCaT keratinocytes. Whereas Cr(III) picolinate did not induce any DNA damage at a concentration of 120 mM, significant induction of DNA breaks was reported after exposure to Cr(III) chloride at 6 mM (ATSDR, 2012).

5.4.4 Mammalian cells – Cr(VI)

Cr(VI) compounds are also mutagenic in mammalian cell lines. Clastogenic activity (micronuclei, chromosomal aberrations and sister chromatid exchanges) of several Cr(VI) compounds has 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 showed a significant increase in mutation frequency at the TK locus (ATSDR, 2012).

5.4.5 Summary of genotoxic and carcinogenic effects

Cr(VI) compounds cause mutations and related 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 are active 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 Cr(VI) and Cr(III) in mutagenic action 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.

5.5 Mode of action

The toxicity potential of chromium depends on its oxidation state: Cr(VI) has greater toxic potential than Cr(III). Evidence from health effects following oral exposure to Cr(VI), as the most potent species, indicates that the small intestine is the target for both neoplastic and non-neoplastic effects. In the animal studies identified, small intestinal tumours in mice were the most sensitive chronic carcinogenic end-point (observed at Cr(VI) doses as low as 1.4 mg/kg bw/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 Cr(VI) doses of 0.8 and 0.2 mg/kg bw/day, respectively (NTP, 2008b). Intestinal tumour development is thought to be related to these early 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 mode of action (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 as follows:

- Absorption of Cr(VI) from the intestinal lumen. Extracellular reduction of Cr(VI) to Cr(III) is a vital process to limit toxicity. It is dependent on pH, the levels of reducing agents present, and whether fed or fasting conditions prevail. The reduction capacity is best represented by several sources of reducing compounds present in gastric fluids that become depleted at different rates (Kirman et al., 2016). Some Cr(VI) may escape reduction; in humans, total reduction (98%) has been estimated to occur by 30 minutes following absorption (De Flora et al., 2016). If still present, nonreduced Cr(VI) is taken up by intestinal cells through anion transporters or excreted unchanged.
- Toxicity to intestinal villi. Data suggest that the nonproliferating, nonpluripotent cells of the intestinal villi are the primary target of Cr(VI) toxicity. That is, toxicity occurs at the point of contact, triggering 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 to Cr(VI) leads to blunted villi and elongated crypts in the duodenum (Thompson et al., 2015b); however, DNA damage is confined to villi (O'Brien et al., 2013; Thompson et al., 2015b).
- Sustained compensatory crypt hyperplasia. This occurs as a result of 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, 2015a,b).

- 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, 2017b). 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 (O'Brien et al., 2013; Thompson 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; Health Canada, 2016; TCEQ, 2016; FSCJ, 2019). It is therefore important to assess whether the excess cancer risk observed at high doses (approximately three orders of magnitude higher than drinking-water exposure levels) in mice is 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 via the oral route have been proposed, the most prominent of which is the direct-acting mutagenic MOA proposed by McCarroll et al. (2010). It should be noted that this pre-dates the large body of evidence relating to the MOA for cytotoxicity, as described above. The authors propose three key steps:

- Intracellular reduction of Cr(VI) to Cr(III). Following intracellular uptake, Cr(VI) is reduced to Cr(III). This is associated with 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 (EFSA, 2014).
- Mutagenesis. There is evidence of mutagenesis following exposure to Cr(VI) via intraperitoneal injection and oral gavage. However, there is no evidence following exposure through drinking-water.
- Cell proliferation. Duodenal hyperplasia was observed in 90-day and 2-year studies in mice, but not in rats.

Because of the uncertainties in the available data, the MOA cannot currently be definitively confirmed (EFSA, 2014; UK Committees, 2015; Health Canada, 2016). However, the overall weight of evidence supports a threshold MOA. This is based on the following points of evidence (Health Canada, 2016; Moffat et al., 2018):

- absence of mutagenicity in target tissues
- lack of concordance of mutagenicity and tumour development
- absence of mutagenesis in drinking-water studies
- lack of evidence of mutagenesis in highly proliferative intestinal tissue following drinking-water exposure
- lack of evidence of tumours in other tissues in which chromium is present
- early onset of crypt proliferation (following 7 days of exposure to Cr(VI)), which is unlikely to result from a fixed mutation.

6 Overall database and quality of evidence

6.1 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; for example, respiratory effects are associated with inhalation of chromium 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 subchronic or long-term oral toxicity studies of Cr(III) in mice or rats. NOAELs for Cr(III) of 506 and 286 mg/kg bw/day can be derived from the subchronic and long-term studies in rats (NTP, 2010). Cr(III) did not show reproductive toxicity in male rats or female mice following subchronic oral exposure via drinking-water, with NOAELs of 506 and 1090 mg/kg bw/day, 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), although some published risk assessments consider that a proportion of Cr(VI) may remain available for absorption. If so, this is likely to also be converted to Cr(III) in the liver intracellularly. It is considered here that the key carcinogenicity study for derivation of a guideline value is the 2-year NTP (2008a) study investigating oral intake of Cr(VI) (as sodium dichromate dihydrate) via drinking-water in rats and mice. Doses of Cr(VI) were 0–5.9 and 0–7.0 mg/kg bw/day in male and female rats, respectively, and 0–5.9 and 0–8.7 mg/kg bw/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 rats and mice (both male and female), respectively, with identified LOAELs for Cr(VI) of 0.38 and 1.79 mg/kg bw/day, respectively (NTP, 2008a).

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 those for neoplastic changes (NTP, 2008a).

6.2 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 of ecologic design (which preclude the ability to determine causation between exposure and effect), and do not encompass the oral route of exposure. The database of information for laboratory animals is more complete than that for humans. It includes chronic exposure to Cr(III) or Cr(VI) though drinking-water in well-conducted studies that conform to current testing guidelines. Substantial new data and an increasing weight of evidence support a threshold MOA, recognizing some remaining uncertainty regarding clear negative results for genotoxicity at low doses (Suh et al., 2019). Gaps in the database also relate to sensitive tests of immune function after oral exposure, and reproductive or developmental effects of Cr(III) and Cr(VI) after oral exposure, including potential neurobehavioural end-points across life stages.

7 Practical considerations

7.1 Analytical methods and achievability

Methods for the determination of chromium in biological and environmental samples are developing rapidly, and early results (especially for the lower chromium levels) should be interpreted with caution. The International Organization for Standardization (ISO, 1998) specifies two methods for the determination of total chromium 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 wastewater when the concentration range for chromium is 0.5–20 mg/L. ISO (1998) notes that the use of evaporation will increase the effect of interfering substances; therefore, the method in clause 4 is given for concentrations below 0.1 mg/L.

Methods for the analysis of total chromium approved by the US EPA (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 limits of detection between 0.08 and 7 μg/L (US EPA, 1994a,b,c, 2003a; APHA et al., 2017).

The current method for the analysis of low-level Cr(VI) in drinking-water recommended by the US EPA (EPA method 218.7) uses ion chromatography with post-column derivatization and UV–visible spectroscopy. This has a detection limit in the range 0.0044–0.015 μg/L (US EPA, 2011). More recently, the United Kingdom Drinking Water Inspectorate reported development and use of ion chromatography followed by ICP-MS to measure Cr(VI) and Cr(III) concentrations in drinking-water samples, with a limit of quantitation of 0.5 μg/L; total chromium was measured separately using ICP-MS (WRc, 2015). However, the determination of chromium species remains a very sophisticated procedure. Reliable and validated methods to separate analysis of Cr(III) and Cr(VI) in collected samples are still required.

7.2 Treatment methods and performance

Chromium normally exists in two redox states in aqueous solutions: Cr(III) and Cr(VI). 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_3^+ and chromium hydroxide ($CrOH_2^+$, $Cr(OH)_2^+$, $Cr(OH)_3(aq)$ and $Cr(OH)_4^-$). Cr(VI) forms chromate (CrO_4^{2-}), which can be protonated to form chromic acid ($HCrO_4^-$) and, under very acidic conditions (pH <2), H_2CrO_4. At high Cr(VI) concentrations and low pH, the dichromate ion ($Cr_2O_7^{2-}$) also forms. It should be noted that some disinfectant procedures, such as pre-chlorination and pre-ozonation, can oxidize Cr(III) to Cr(VI), thereby increasing the soluble chromium content for removal. However, as the levels of reducing agents used during treatment are likely to be in excess of the low concentrations of Cr(VI) in source water, this should mitigate any potential toxicological effects in humans from an increased level of Cr(VI) resulting from oxidation of Cr(III) during water treatment (WRc, 2015). Technologies for chromium removal can be categorized into five general groups (Sharma, Petrusevski & Amy, 2008):

- Coagulation–precipitation–filtration. Cr(VI) requires reduction to Cr(III) before removal by ferric coagulants.
- Adsorption by iron oxides (ferrihydrite and goethite) and iron oxide–coated sand. Removal of both Cr(III) and Cr(VI) requires pH changes and the removal to be carried out in stages.

- Ion exchange. This is effective in removing both Cr(III) and Cr(VI), with 80–96% of the ions removed (US EPA, 2003b).
- Membrane technologies. Together with reverse osmosis, these are considered one of the best technologies available for chromium removal; reverse osmosis has an efficiency of 82–97%. Nanofiltration has also been used for chromium removal and shows similar efficiency for both Cr(III) and Cr(VI) (e.g. Hafiane, Lemordant & Dhahbi, 2000; Taleb-Ahmed et al., 2002).
- Microbiological removal. Bacteria have been to shown to be effective in reducing Cr(VI) to Cr(III), which is precipitated within biomass. However, this method may not be suitable for drinking-water treatment because optimum removal requires anaerobic conditions (e.g. Chen & Hao, 1998; Komori et al., 2004; Chen & Gu, 2005).

8 Conclusions

8.1 Derivation of the guideline value

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

As levels of total chromium in drinking-water (average of 1 µg/L) are generally below the previously derived provisional GV of 50 µg/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 GV, 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, b), 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 a risk assessment of Cr(VI) in drinking-water that considers both cancer and noncancer effects, and provides evidence to support an 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 end-point and precursor of tumour formation is protective of both cancer and noncancer effects. The current GV of 50 µg/L (total chromium) is therefore considered to be adequately protective of health and is retained, with the previously allocated ‘provisional’ status removed.

8.2 Considerations in applying the guideline value

As chromium is usually found in drinking-water at concentrations below the GV, monitoring and inclusion in drinking-water regulations and standards would usually only be necessary if there were indications that a problem might exist. Monitoring can normally be limited to the treatment works; however, specific pollution events may need to be considered on a case-by-case basis.

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