National Centre for Environmental Toxicology

Understanding the Significance of Chromium in Drinking Water

Report Reference: Defra-8930.04
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Glossary

8-oxo-2'-deoxyguanosine	An oxidised form of the DNA base deoxyguanosine that is an indication of oxidative stress in a cell.
Allergic contact dermatitis (ACD)	An immunological reaction that occurs following exposure to a particular allergen.
Apoptosis	The regulated death of a cell.
Ascorbate	Also known as vitamin C, a reducing agent and essential vitamin to humans.
COMET assay	A single cell gel electrophoresis assay that is designed to detect DNA damage.
Cysteine	An amino acid that acts as a reducing agent.
Cytotoxic	A concentration that is toxic to cells.
Erythrocytes	Red blood cells.
Glutathione	A peptide acid that acts as a reducing agent.
Glutathione disulphide (GSSG)	A molecule that is reduced into two molecules of glutathione.
LOAEL	Lowest Observed Adverse Effect Level.
LOEL	Lowest Observed Effect Level.
Lumen	The internal space of a tubular structure, such as the intestine.
Lymphocytes	A type of white blood cell.
Metaphase	A stage of the cell cycle where chromosomes arrange along the metaphase plate in preparation for cell division.
Multinucleated	Contains more than one nucleus per cell.
NADPH	Nicotinamide adenine dinucleotide phosphate, a co-enzyme that can reduce chemicals.

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NOAEL	No Observed Adverse Effect Level.
NOEL	No Observed Effect Level.
NTP	US National Toxicology Program.
Oxidation	The process by which a molecule may lose electrons, it is the opposite of reduction.
Oxidative stress	An imbalance between the manifestation of reactive oxygen species within a cell and the ability of a cell to detoxify these reactive species, which can result in cellular damage.
p53	A protein involved in the arrest of the cell cycle and induction of apoptosis as part of the regulation of the cell cycle.
Peptide	A short chain of amino acids.
Phagocytosis	The process of engulfing a molecule for the purpose of absorption.
Reduction	The process by which a molecule may gain electrons, it is the opposite of oxidation.
Sequestration	The process by which a cell may accumulate a chemical, thus removing its availability to other cells.
Thiol	An organic-sulphur bond.
Transferrin	Iron-binding proteins found in the plasma.

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Summary

i Reasons

Chromium is a naturally occurring element in the environment that primarily exists in one of three oxidative forms; the 0, +3 (chromium (III); trivalent chromium), and +6 (chromium (VI); hexavalent chromium) valency states. Chromium (III) plays an essential role in insulin metabolism in man, however, chromium (VI) is carcinogenic. Carcinogenicity appears to be localised to the point of absorption, as chromium (VI) rapidly penetrates the cell membrane, where it is reduced intracellularly to chromium (III), which in turn binds to macromolecules. The World Health Organization (WHO) has derived a provisional Guideline for Drinking-water Quality (GDWQ) of 50 µg/l for total chromium. WHO states that separate guideline values for chromium (III) and chromium (VI) should be derived, however, there are technical difficulties in analytically measuring chromium at different valencies. Therefore, this study was undertaken to evaluate the current toxicological knowledge on chromium (VI) and undertake a survey in England and Wales to understand the significance of chromium in drinking water.

ii Objectives

1. A review of the toxicokinetics and toxicity of chromium has been undertaken.

2. A review of the fate of chromium in water treatment and supply and previous chromium (VI) monitoring studies was conducted.

3. An analytical method has been developed and tested for the analysis of chromium (VI) and surveys of chromium, chromium (III) and chromium (VI) concentrations in drinking water has been conducted.

iii Benefits

This report provides significant additional understanding on the current state of knowledge of the toxicity and toxicokinetics of chromium. It also highlights several areas where several gaps in knowledge remain and makes suggestions on how these may be addressed. This report also provides the most comprehensive information to date on occurrence of chromium in drinking water supplies in England and Wales.

iv Conclusions

In general, concentrations of chromium (VI) in drinking water in England and Wales are very low, and are consistent with levels reported in other countries (<1 µg/l). One site was the exception to this, where levels of up to 9.94 µg/l were reported. The gaps in the toxicity and

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toxicokinetics data mean that at present it is very difficult to establish a definitive level upon which to propose a new drinking water standard, a range of values have been suggested. For the majority of sites considered in this survey, the concentrations of chromium (VI) were well below even the more conservative of these health-based values, and in the majority of cases were <1 µg/l. However, there would be concern at the one site where concentrations approaching 10 µg chromium (VI)/l were detected if the most conservative lifetime health-based value of 5.4 µg/l is applied. However, it should be noted that this statement is based on the assumption of drinking water only accounting for 20% of total exposure to chromium (VI) (i.e. other sources such food would account for significant exposure). This may be an overly-precautionary assumption, as the available data indicate that food would not provide a significant source of chromium (VI). Therefore, if more realistic assumptions are applied (i.e. drinking water accounts for 80% of total exposure to chromium (VI)), the level detected at this site would be below the derived health-based value (21.6 µg/l).

Overall, in the majority of cases, exposure to chromium (VI) via drinking water in England and Wales is very low and there is no evidence to suggest exposure to the typical concentrations reported in the survey (<1 µg/l) will result in adverse human health effects.

v Recommendations

Further information on the toxicokinetics of chromium (VI) is required to allow the more precise derivation of health-based values. In addition, more information of the effect of drinking water treatment processes on oxidation of chromium (III) to chromium (VI), or the reduction of chromium (VI) to chromium (III) is required.

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1. Introduction

Chromium is a naturally occurring element in the environment that primarily exists in one of three oxidative forms; the 0, +3 (chromium (III); trivalent chromium), and +6 (chromium (VI); hexavalent chromium) valency states.

Chromium has a wide range of industrial uses including in the leather tanning industry, in the manufacture of catalysts, paints, fungicides, ceramics and glass, photography, chrome plating (such as taps), and as a metal in alloys. Chromium (III) plays an essential role in insulin metabolism in man, however, chromium (VI) is carcinogenic. Carcinogenicity appears to be localised to the point of absorption, as chromium (VI) rapidly penetrates the cell membrane, where it is reduced intracellularly to chromium (III), which in turn binds to macromolecules.

In environmental waters, chromium (VI) is readily reduced to chromium (III). Oxidation of chromium (III) to chromium (VI) can also occur, however, this process is much slower, and therefore unlikely to occur at significant levels. However, drinking water treatment can involve a range of treatment processes, which can include different oxidation reactions, and therefore, may promote the formation of chromium (VI).

The use of chromium as in plated fittings in plumbing systems may also represent a potential source of chromium in drinking water supplies, releasing small metallic particles of chromium.

The World Health Organization (WHO) has derived a provisional Guideline for Drinking-water Quality (GDWQ) of 50 µg/l for total chromium. WHO states that separate guideline values for chromium (III) and chromium (VI) should be derived, however, there are technical difficulties in analytically measuring chromium at different valencies.

To understand the significance of chromium in drinking water, the following approach has been adopted.

1. A review of the toxicokinetics of chromium has been undertaken.

2. A critical appraisal of the data on the oral toxicity of chromium has been conducted, with a particular focus on chromium (VI).

3. A review of the fate of chromium in water treatment and supply was conducted.

4. The available data from previous chromium (VI) monitoring studies have been reviewed, evaluated and summarised.

5. An analytical method has been developed and tested for the analysis of chromium (VI).

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6. A survey of total chromium, chromium (III) and chromium (VI) has been conducted in finished drinking water at twenty sites in England and Wales. Each site, insofar as was possible, was surveyed four times over a 12 month period to provide samples for spring, summer, autumn and winter.

7. An additional survey of total chromium, chromium (III) and chromium (VI) has been conducted in raw and finished drinking water sites in England and Wales over the autumn and winter.

2. Review of Chromium Toxicokinetic and Toxicodynamic Data

2.1 Absorption

2.1.1 Chromium (III)

Chromium (III) compounds are poorly absorbed following oral administration. In humans, absorption was estimated to be 0.4% following oral administration of chromium trichloride (Donaldson & Barrera, 1966, cited in US EPA, 1998). Another study in humans has suggested that absorption of chromium (III) is inversely proportional to intake; a dose of 10 µg chromium (III) was 2% absorbed, while a dose of >40 µg was 0.5% absorbed (Anderson *et al.*, 1983, cited in US EPA, 1998). It has been suggested that water-insoluble chromium (III) compounds may be absorbed into cells via phagocytosis (Costa, 1997).

2.1.2 Chromium (VI)

Chromium (VI) is reported to be absorbed more readily than chromium (III) via all routes of exposure. This is due to the chromate ion (CrO_4^{2-}) entering cells via chloride-phosphate anion channels and/or the sulphate channels in the cell membrane, while chromium (III) is transported into cells via passive diffusion or phagocytosis (WHO, 2013; Salnikow and Zhitkovich, 2008).

Chromium (VI) is reported to undergo reduction in saliva and gastric juices to chromium (III). However, Kirman *et al.* (2012) suggest that reduction in saliva is likely to be negligible due to the short transit time within the oral cavity. DeFlora (2000) estimated that an individual can reduce 0.7-2.1 mg chromium (VI)/day in saliva and 80.3-84.5 mg/day in gastric juices. The reduction in gastric juices is reported to be rapid, with at least half of a chromium (VI) dose reduced within 1 minute and complete reduction occurring within 10-20 minutes (DeFlora, 2000).

Intestinal bacteria are also reported to reduce chromium (VI) to chromium (III) and the daily removal of chromium (VI) by faecal bacteria was estimated by DeFlora (2000) to be 15.4-33.4 mg/day. Chromium (VI) is more readily absorbed than chromium (III) (WHO, 2003), and as such reduction by saliva and in the gastrointestinal tract will decrease the absorption of chromium (VI).

Any chromium (VI) that is not reduced will be absorbed by the upper small intestine and released into the blood portal system (ATSDR, 2008; DeFlora, 2000). It has been suggested that red blood cells have a high capacity for sequestering and reducing chromium (VI) to chromium (III). DeFlora (2000) states that the reducing capacity of human whole blood is 234 and 187 mg chromium (VI)/day in males and females, respectively, and the reducing capacity

of human red blood cells is 138 and 100 mg chromium (VI)/day in males and females, respectively.

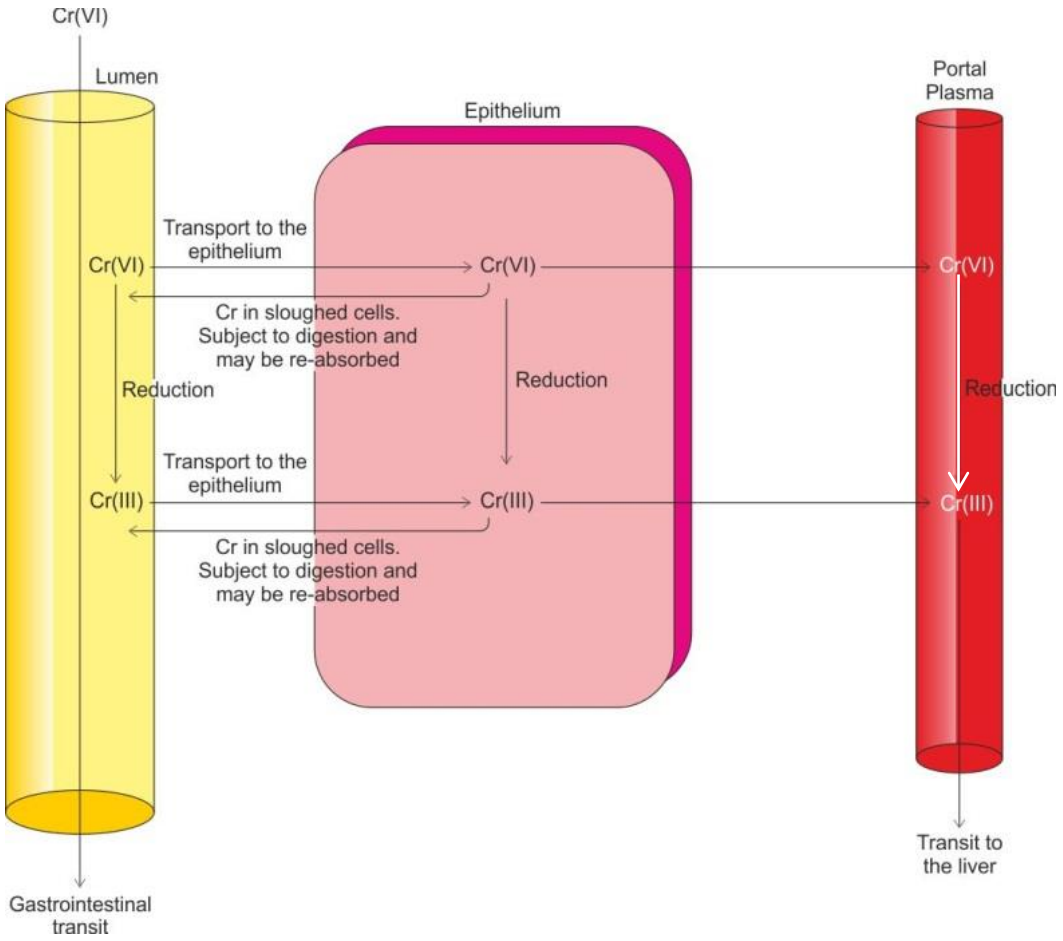
Absorbed chromium (VI) will be in the form of a tetrahydral divalent chromate ion (CrO_4^{2-}), which is similar in structure to physiological sulphate and phosphate ions, and as such can readily enter cells via the sulphate channel (Salnikow and Zhitkovich, 2008). Salnikow and Zhitkovich (2008) have reported that mammalian cells, including human cells, have a high capacity for chromium (VI) accumulation *in vitro*, with cellular levels 10-20 times higher than those outside the cell after three hours. After 24 hours incubation, greater than 100 fold accumulation was observed (Salnikow and Zhitkovich, 2008). Chui *et al.* (2010) have reported that chromate can also enter cells via the chloride-phosphate channels.

The European Union (EU) Risk Assessment Report (RAR) published in 2005 suggested that following administration of highly soluble chromium (VI) compounds via oral gavage or inhalation, approximately 20-30% of a chromium dose is absorbed (EU, 2005). However, absorption via food is much lower due to reduction of chromium (VI) to chromium (III). Administration of chromium (VI) in food to rats and mice resulted in 1-3% absorption, with absorption higher in animals that had not received food for 16-48 hours (EU, 2005). Similarly, in a repeat-dose study in three healthy volunteers, ingestion of chromium (VI) at a dose of 5 mg/day for three days, followed by two days without treatment and a further three days at 10 mg/day resulted in 1.7-3.4% absorption (Kerger *et al.*, 1997, cited in ATSDR, 2008).

In a recent review, Chui *et al.* (2010) examined the differences in bioavailability of a single dose of 5 mg in humans of two chromium compounds, chromium trichloride (a chromium (III) compound) and potassium dichromate (a chromium (VI) compound. The bioavailability of chromium trichloride was 0.13%, while the bioavailability of potassium dichromate was 6.9%, suggesting absorption of the chromium (VI) compound was approximately two orders of magnitude greater than the chromium (III) compound.

Kirman *et al.* (2012) have suggested a conceptual model for the absorption of chromium (VI) across the gastrointestinal tract, consisting of three competing processes; transit to the distal regions of the gastrointestinal lumen; reduction to chromium (III) within the lumen; and absorption and uptake into gastrointestinal tissues. Kirman *et al.* (2012) state that the reduction of chromium (VI) to chromium (III) is pH-dependent, capacity limited and dependent on the concentration of chromium (VI) and reducing agents. Once absorbed by the gastrointestinal tract epithelium, chromium (VI) may be subject to further reduction to chromium (III) prior to absorption into portal plasma. Epithelial cells may also be sloughed off from the oral cavity and stomach, which can release chromium (III) and chromium (VI) back into the lumen, where the cells may be digested in the stomach lumen and the chromium re-absorbed, primarily in the small intestine. However, Kirman *et al*. (2012) suggest that epithelial cells that are sloughed off in the small intestine will transit through the large intestine and be excreted in the faeces. This conceptual model is presented in Figure 2.1.

Figure 2.1 Model for the absorption of chromium (VI) proposed by Kirman *et al.* (2012)



Proctor *et al.* (2012) conducted a study examining the reduction kinetics of chromium (VI) in the stomach contents of rats and mice. In this study, F344/N rats and B6C3F1 mice were administered irradiated NTP-2000 wafers for four days prior to collection of stomach contents (forestomach and glandular stomach). These stomach contents were spiked with sodium dichromate dihydrate at doses, as described in Table 2.1. Proctor *et al.* (2012) determined that the concentrations of chromium (VI) in stomach contents were best described by the following mixed second-order model:

$$\frac{dc_{[Cr(VI)]}}{dt} = k[R][Cr(VI)]$$

Where: [Cr (VI)] is the concentration of chromium (VI), [R] is the concentration of the reducing agent, and k is the second-order rate constant expressed as litre of stomach contents per mg reducing equivalents per hour. The reducing agent was assumed to be consumed at a rate of 1 mg/mg chromium (VI), and was therefore determined to have the units mg chromium (VI) equivalents/litre stomach contents.

Proctor *et al.* (2012) determined that the total reducing capacity of the rat stomach contents was 15.7 mg chromium (VI)-equivalents/l. In the mouse, the reducing capacity of the stomach contents was 16.6 mg chromium (VI)-equivalents/l. Therefore, in both species, at approximately 16 mg chromium (VI)-equivalents/l, all available reducing agents in the stomach contents were consumed, and at higher concentrations the reducing capacity of the stomach would be exceeded.

The results of this study are presented in Table 2.1. These data indicate that in drinking water, the reducing capacity is exceeded at concentrations of 60 mg/l and 21 mg/l in rats and mice, respectively (Proctor *et al.*, 2012). The concentrations at and above the reductive capacity are generally consistent with the those that were reported to result in tumours in rats and mice in the 2-year National Toxicology Program (NTP) studies described in Section 3.4.2. However, the European Food Safety Authority (EFSA) considered that even at low doses, a small percentage of chromium (VI) absorption may occur (EFSA, 2014).

Table 2.1 Stomach chromium (VI)-reduction kinetics study by Proctor *et al.* (2012)

Species	Chromium (VI) drinking water concentration (mg/l)	Chromium (VI) loading (mg Cr (VI)/l stomach contents)	Chromium (VI) intake per dose as a percentage of reducing capacity (%)
Rats	0.1	0.07	0.4
	1.4	0.58	7
	5.0	3.5	22
	21	15	94
	60	42	270
	180	130	810
Mice	0.1	0.1	0.6
	1.4	1.4	8.4
	5.0	5.0	30
	21	21	130
	60	60	360
	180	180	1100

Both chromium (III) and chromium (VI) can undergo some absorption via the skin, and absorption is expected to be higher following application to damaged skin. Following immersion in a warm aqueous bath containing chromium (VI), as potassium dichromate, at a concentration of 22 mg/l, the rate of absorption was estimated to be 3.3×10^{-5} to 4.1×10^{-4} µg chromium/cm²-hour (ATSDR, 2008). In its Risk Assessment Report (RAR), the EU reports that dermal absorption of chromium (VI) compounds in guinea pigs ranges between <1% and 4% of the applied dose (EU, 2005).

2.2 Distribution

2.2.1 Chromium (III)

Following intravenous administration of chromium (III), as chromium chloride, to male rats at a dose of 10 µg/kg bw, rapid clearing from the blood was reported. After 30 minutes, blood chromium concentrations were 94% of the initial dose; after 24 hours, 17% of the initial dose was detectable in the blood; and, after 96 hours, 5% of the initial dose was detected in the blood (Hopkins, 1965, cited in US EPA, 1998).

In a study in humans, following intravenous administration of chromium chloride to six adults, >50% of the dose was distributed to liver, spleen and other organs. After six months, >50% of the dose was still detectable in the liver (Lim *et al.*, 1983, cited in US EPA, 1998).

In rats administered chromium chloride, chromium concentrated in the liver, spleen and bone marrow, with the liver being the only organ to significantly reduce its chromium concentration over 45 days (Visek *et al.*, 1953, cited in US EPA, 1998). In a study in which rats were administered chromium nitrate for 30 or 60 days, the highest concentrations were found in the liver, kidneys, testes and brain (Tandon *et al.*, 1979, cited in US EPA, 1998).

Kirman *et al.* (2012) state that, following administration of chromium (VI) to rodents, essentially all chromium distributed from the hepatic/portal system to systemic plasma will be in the form chromium (III). Distribution of chromium (III) will be dependent upon binding to low molecular weight proteins, and transferrin, an iron transport protein, has a high affinity for chromium (III). Kirman *et al.* (2012) therefore suggest that transferrin plays an important role in the distribution of chromium from the gastrointestinal tract to tissues.

2.2.2 Chromium (VI)

Quinteros *et al.* (2006) have reported that chromium (VI) can be distributed to, and accumulate in, the pituitary gland and the hypothalamus *in vivo*. Chromium (VI) can decrease prolactin levels, but no effect on luteinising hormone was observed (Quinteros *et al.*, 2006).

Burastero *et al.* (2006) investigated the distribution of chromium (VI), as radiolabelled sodium chromate (0.5-5 µM), to either immature or mature human primary dendritic cells *in vitro*. Cellular chromium uptake was reported to follow a near-linear pattern that increased with increasing dose. Forty-eight hours after exposure, chromium was preferentially distributed to the nuclear (44.1-66%) and cytosolic fractions (13.1-31%) (Burastero *et al.,* 2006).

Levina *et al.* (2005) has reported that intracellular chromium (VI) was capable of binding *in vitro* to calf thymus histones under physiological conditions. Histones have a role in the packaging of DNA into nucleosomes, and as such, Levina *et al.* (2005) theorised that bind of chromium (VI) to newly synthesised histones may serve as a mechanism of transporting chromium (VI) into the nucleus of cells.

In a US National Toxicology Program (NTP) distribution study (NTP, 2008), male F344/N rats (40/dose) were administered chromium (VI) as sodium dichromate dihydrate via drinking water at concentrations of 0, 14.3, 57.3, 172 or 516 mg sodium dichromate dihydrate/l (equivalent to 0, 5, 20, 60 and 180 mg chromium/l, respectively) for 53 weeks. Based on water consumption data, doses were reported to be equivalent to 0, 0.6, 2.2, 6 and 17 mg/kg bw/day, respectively. Samples (10 rats/dose) were taken on days 6, 13, 182 and 371. Prior to sampling, urine and faeces samples were collected for 48 hours. The following tissues were then removed for tissue analysis; erythrocytes, plasma, liver, kidneys, glandular stomach and forestomach. The analysis could not differentiate between different oxidation states, therefore, results were reported for total chromium. The tissues at which significantly increased concentrations of chromium were detected at the different time points are presented in Table 2.2.

Table 2.2 Tissues with significantly increased chromium concentrations after 6, 13, 182 and 371 days following administration of sodium dichromate dihydrate (chromium VI) to rats in drinking water (NTP, 2008)

		Concentration (mg/l)			
		14.3	57.3	172	516
Days	6	Plasma Glandular stomach Urine Faeces	Erythrocytes Plasma Liver Glandular stomach Urine Faeces	Erythrocytes Plasma Liver Kidneys Glandular stomach Urine Faeces	Erythrocytes Plasma Liver Kidneys Glandular stomach Urine Faeces
	13	Kidneys Glandular stomach Urine Faeces	Plasma Kidneys Glandular stomach Urine Faeces	Erythrocytes Plasma Liver Kidneys Glandular stomach Forestomach Urine Faeces	Erythrocytes Plasma Liver Kidneys Glandular stomach Forestomach Urine Faeces
	182	Liver Kidneys Glandular stomach Urine Faeces	Plasma Liver Kidneys Glandular stomach Forestomach Urine Faeces	Erythrocytes Plasma Liver Kidneys Glandular stomach Forestomach Urine Faeces	Erythrocytes Plasma Liver Kidneys Glandular stomach Forestomach Urine Faeces
	371	Erythrocytes Liver Kidneys Urine Faeces	Erythrocytes Liver Kidneys Glandular stomach Forestomach Urine Faeces	Erythrocytes Plasma Liver Kidneys Glandular stomach Forestomach Urine Faeces	Erythrocytes Plasma Liver Kidneys Glandular stomach Forestomach Urine Faeces

In another NTP distribution study, female B6C3F1 mice (40/dose) were administered chromium (VI) ,as sodium dichromate dihydrate, via drinking water at concentrations of 0, 14.3, 57.3, 172 or 516 mg sodium dichromate dihydrate/l (equivalent to 0, 5, 20, 60 and 180 mg chromium/l, respectively) for 53 weeks. Based on water consumption data, 0, 14.3, 57.3, 172 or 516 mg sodium dichromate dihydrate/l was reported to be equivalent to 0, 0.6, 2.2, 6 and 17 mg/kg bw/day, respectively. Samples (10 mice/dose) were taken on days 6, 13, 182 and 371. Prior to sampling, urine and faeces samples were collected for 48 hours. The following tissues were then removed for tissue analysis: erythrocytes, plasma, liver, kidneys, glandular stomach and forestomach. The analysis could not differentiate between different oxidation states, therefore, results were reported for total chromium.

Insufficient mouse urine was collected for analysis at most time points. However, the NTP reported that low urine volumes are normal for mouse metabolism cages. Where sufficient numbers of samples were collected for statistical analysis, the results indicated increased chromium content in the urine at all concentrations and time points. The tissues at which significantly increased concentrations of chromium were detected at the different time points are presented in Table 2.3.

Table 2.3 Tissues with significantly increased chromium concentrations after 6, 13, 182 and 371 days following administration of sodium dichromate dihydrate (chromium VI) to mice in drinking water (NTP, 2008)

		Concentration (mg/l)			
		14.3	57.3	172	516
Days	6	Liver Kidneys Faeces	Erythrocytes Liver Kidneys Glandular stomach Faeces	Erythrocytes Plasma Liver Kidneys Glandular stomach Faeces	Erythrocytes Plasma Liver Kidneys Glandular stomach Faeces
	13	Liver Kidneys Faeces	Plasma Liver Kidneys Glandular stomach Faeces	Erythrocytes Plasma Liver Kidneys Glandular stomach Forestomach Faeces	Erythrocytes Plasma Liver Kidneys Glandular stomach Forestomach Faeces
	182	Liver Kidneys Glandular stomach Faeces	Erythrocytes Plasma Liver Kidneys Glandular stomach Forestomach Faeces	Erythrocytes Plasma Liver Kidneys Glandular stomach Forestomach Faeces	Erythrocytes Plasma Liver Kidneys Glandular stomach Forestomach Faeces
	371	Liver Kidneys Faeces	Plasma Liver Kidneys	Erythrocytes Plasma Liver	Erythrocytes Plasma Liver

		Concentration (mg/l)			
		14.3	57.3	172	516
			Glandular stomach Forestomach Faeces	Kidneys Glandular stomach Forestomach Faeces	Kidneys Glandular stomach Forestomach Faeces

Kirman *et al.* (2012) recently conducted a 90-day toxicokinetic study in female F344 rats and B6C3F1 mice. Rats were administered chromium (VI), as sodium dichromate dihydrate in their drinking water at concentrations of 0, 0.3, 4, 60, 170 or 520 mg/l (reported to be 0, 0.015, 0.21, 2.9, 7.2 and 20.5 mg chromium (VI)/kg bw/day, respectively). Mice were administered concentrations of 0, 0.3, 4, 14, 60, 170 or 520 mg/l (reported to be 0, 0.024, 0.32, 1.1, 4.6, 11.6 and 30.9 mg chromium (VI)/kg bw/day, respectively).

Chromium cannot be separated by speciation in tissues, therefore as with the NTP studies, total chromium measurements were conducted in the oral mucosa, duodenum, jejunum, ileum, stomach, plasma, erthyrocytes and the liver. Iron content was also measured in these tissues. These results are presented in Table 2.4.

A decreasing longitudinal gradient of chromium concentration was noted in the intestines in both species, i.e. the chromium concentration was higher in the duodenum than in the jejunum, which was higher than in the ileum. Kirman *et al.* (2012) also suggest that there is also some evidence to indicate species-specific differences in absorption in the small intestine; the mouse small intestine may absorb chromium to a greater extent than the rat small intestine.

It should be noted that while generally alterations in iron levels in these tissues have resulted in a significant decrease in iron concentrations, the iron levels in the erythrocytes of mice were increased at the top dose. Kirman *et al.* (2012) suggests that this may indicate some adaptation to altered iron levels due to the development of microcytic anaemia (anaemia characterised by small red blood cells).

Table 2.4 Tissues with significant changes in chromium and iron concentrations in rats and mice

		Species			
		Rats		Mice	
Determinand		Chromium	Iron	Chromium	Iron
Tissue	Oral mucosa	Significantly increased at the top three doses	No significant change	Significantly increased at the top three doses	No significant change
	Stomach	Significantly increased at the top two doses	No significant change	Significantly increased at the top three doses	Significantly decreased at the top two doses
	Duodenum	Significantly increased at the top three doses	Significantly decreased at the top dose	Significantly increased at the top four doses	Significantly decreased at the top three doses
	Jejunum	Significantly increased at the top three doses	No significant change	Significantly increased at the top three doses	Significantly decreased at the top four doses
	Ileum	Significantly increased at the top dose	No significant change	Significantly increased at the top three doses	Significantly decreased at the top two doses
	Plasma	Significantly increased at the top three doses	No significant change	Significantly increased at the top two doses	No significant change
	Erthyrocytes	Significantly increased at the top two doses	No significant change	Significantly increased at the top three doses	Significantly increased at the top dose
	Liver	Significantly increased at the top three doses	Significantly decreased at the top three doses	Significantly increased at the top four doses	Significantly decreased at the top three doses

Costa (1997) has reported a distribution study in rats following administration of chromium (VI), as potassium chromate, on the liver, kidneys and femur at concentrations lower than those used in the NTP studies. These data indicate clear dose-related increases in tissue concentrations of all the tissues examined, with a particularly noticeable increase in the liver and kidneys at the highest dose (Table 2.5).

Table 2.5 Distribution of chromium in rat liver, kidneys and femur (Costa, 1997)

Drinking water concentration (mg/l)	Tissue concentration (µg/g)		
	Liver	Kidney	Femur
0.45	0.05	0.26	0.67
2.2	0.12	0.38	1.4
4.5	0.31	0.77	2.3
7.7	0.62	2.8	4.2
11.2	1.4	4.2	5.0
25.0	5.7	12.0	6.4

Another study reported by Costa (1997) examined accumulation of chromium in a wider range of organs in rats and mice following administration of 8 mg potassium chromate/kg bw/day via drinking water to rats and mice for 4 and 8 weeks. These data indicate chromium is distributed to a wide range of organs, but that there is preferential distribution to the liver, kidneys and spleen (Table 2.6).

Table 2.6 Distribution of chromium in rats and mice following exposure for 4 and 8 weeks (Costa, 1997)

	Organ	Concentration (µg/g wet tissue, or µg/ml blood)		
		Control	4-week exposure	8-week exposure
Mice	Liver	0.22 ± 0.14	10.92 ± 5.48	13.83 ± 6.06
	Femur	0.90 ± 0.48	7.43 ± 1.03	12.55 ± 2.99
	Spleen	0.53 ± 0.38	5.04 ± 1.45	10.09 ± 2.50
	Kidney	0.24 ± 0.14	3.77 ± 0.99	4.72 ± 0.68
	Lung	0.24 ± 0.12	0.99 ± 0.10	1.08 ± 0.26
	Heart	0.32 ± 0.15	0.80 ± 0.23	1.02 ± 0.20
	Muscle	0.32 ± 0.23	1.12 ± 0.37	0.60 ± 0.25
	Blood	0.14 ± 0.05	0.71 ± 0.07	0.42 ± 0.04
Rats	Liver	0.0.19 ± 0.14	3.32 ± 0.93	3.59 ± 0.73
	Femur	1.00 ± 0.46	1.85 ± 0.46	1.78 ± 0.99
	Spleen	0.43 ± 0.20	3.65 ± 1.87	4.38 ± 0.84
	Kidney	0.34 ± 0.20	8.62 ± 2.40	9.49 ± 4.38
	Lung	0.39 ± 0.43	1.10 ± 0.38	0.67 ± 0.24
	Heart	0.38 ± 0.22	0.52 ± 0.12	1.05 ± 0.19
	Muscle	0.24 ± 0.14	0.19 ± 0.10	0.17 ± 1.10
	Blood	0.19 ± 0.17	0.73 ± 0.15	0.58 ± 0.13

It has been reported that chromium may be transferred to infants via breast milk, and that the levels of chromium in breast milk may be independent of serum chromium levels, urinary chromium excretion or dietary intake (ATSDR, 2008).

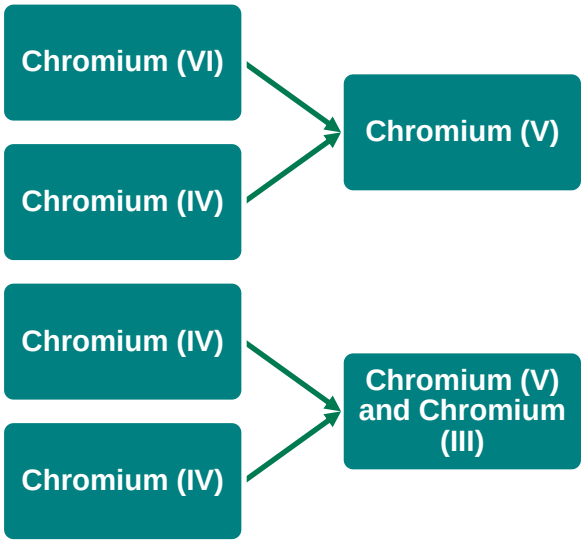
2.3 Metabolism and Mode of Action

Once absorbed into the cell, chromium (VI) undergoes a series of reduction reactions to yield chromium (III). This process does not require enzymes but involves direct electron transfer from ascorbate and non-protein thiols, such as glutathione and cysteine (Salnikow and Zhitkovich, 2008). Salnikow and Zhitkovich (2008) report that ascorbate is the dominant chromium (VI) reducer, accounting for greater than 90% of chromium (VI) metabolism *in vivo*.

Salnikow and Zhitkovich (2008) report that cultured *in vitro* cells tend to contain much lower levels of ascorbate compared to *in vivo* cells. As such, reduction of chromium (VI) by thiols is the dominant process *in vitro*. Salnikow and Zhitkovich (2008) conclude that, therefore, unless cellular ascorbate levels are restored to normal, *in vitro* models provide a non-physiological model of chromium (VI) metabolism, which can underestimate genotoxicity.

The reduction of chromium (VI) to chromium (III) is reported to produce chromium (V), chromium (IV) intermediates and organic radicals at varying amounts that will depend on the reducing agent and the ratio of reactants. Salnikow and Zhitkovich (2008) report that with $\geq$2-fold molar excess of reducing agents, chromium (VI) is first reduced to chromium (IV) by ascorbate prior to reduction to chromium (III). Chromium (V) was only detectable with high concentrations of chromium (VI) and ascorbate present at a 1:1 or lower ratio with chromium (VI). It was suggested that in such cases, the presence of chromium (V) was the result of incomplete reduction to chromium (IV) and the resultant secondary reactions, as shown in Figure 2.2.

Figure 2.2 Proposed formation of chromium (V) from partial reduction of chromium (VI) to chromium (IV) (Salnikow and Zhitkovich, 2008)



Chromium (VI) is also reduced to chromium (III) via reaction with cysteine to chromium (III). This reaction occurs primarily via a one-election transfer, therefore, chromium (V) is more readily detectable through cysteine-driven reactions (Salnikow and Zhitkovich, 2008).

Salnikow and Zhitkovich (2008) also report that chromium (VI) can be reduced to chromium (III) by glutathione-driven reactions. Glutathione reduction is a two-electron transfer process, however, chromium (V) intermediates are also readily detectable via this route.

Suigyama *et al.* (1992) has reported that chromium (VI) can also be reduced to chromium (V) in Chinese hamster V79 cells in the presence of vitamin B2, resulting in an increase in hydroxyl radicals.

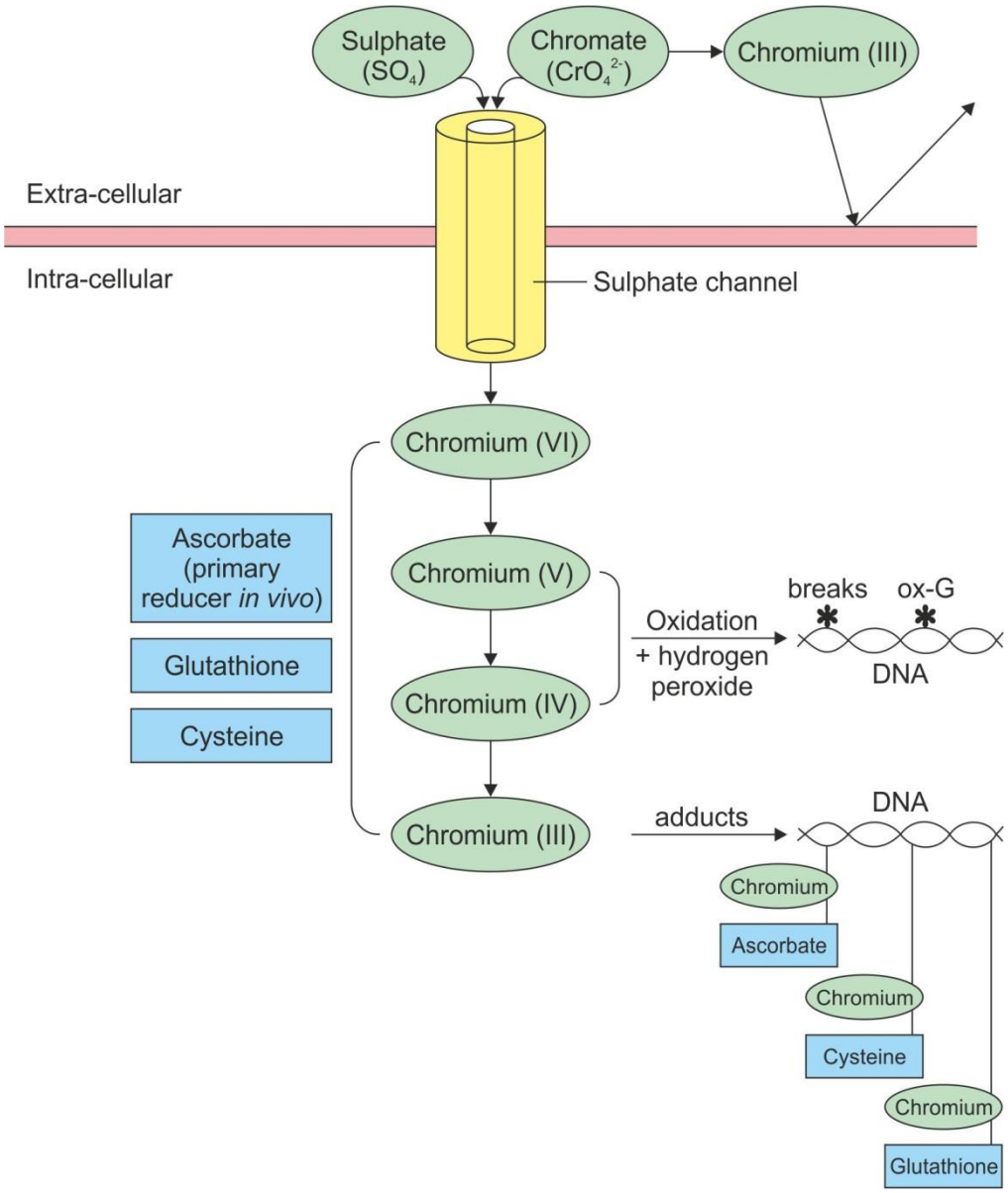
In another review, Zhitkovich (2011) reported that the combined activity of ascorbate, glutathione and cysteine is responsible for >95% reduction of chromium (VI) *in vivo*. Ascorbate reduction is the major metabolism pathway for chromium (VI) *in vivo*. Ascorbate and glutathione are usually present in the cell at similar concentrations, and this faster reaction with ascorbate is due to its high affinity for chromium (VI). At 1 mM concentrations, the half-life for chromium (VI) reduction was 1 minute, 60.7 minutes and 13.3 minutes for ascorbate, glutathione and cysteine, respectively (Zhitkovich, 2011).

The chromium (V) and chromium (VI) intermediates may be capable of inducing DNA breaks by oxidation of DNA by acting as a catalyst for Fenton-like reactions with hydrogen peroxide to produce reactive hydroxyl radicals (Salnikow and Zhitkovich, 2008; Yao *et al.*, 2008). Chromium (VI) is also reported to form DNA-protein cross-links in mammalian cells. This process is reported to be relatively low *in vivo*, with DNA-protein cross-links accounting for 1% of all chromium-DNA adducts (Salnikow and Zhitkovich, 2008).

Once metabolised to chromium (III), additional chromium-DNA adducts can be formed. These adducts are reported to be the predominant form of chromium (VI) induced genetic effect in mammalian cells, with the major adducts being glutathione-chromium-DNA, cysteine-chromium-DNA, histidine-chromium-DNA and ascorbate-chromium-DNA complexes. EFSA has recently reviewed chromium (VI) and also concluded that these two modes of action will occur (EFSA, 2014).

A representation of this process is provided in Figure 2.3.

Figure 2.3 Metabolism of chromium (VI), based on a diagram reported by Salnikow and Zhitkovich (2008)



In a recent review, Chiu *et al.* (2010) hypothesised that chromium (VI) enters cells via chloride channels and will initially be reduced by glutathione, primarily to chromium (IV), but possibly with some reduction to chromium (V) too. Yao *et al*. (2008) have reported that this reaction of chromium (VI) with glutathione yields glutathione-derived thiyl radicals, which may produce direct cellular damage. These thiyl radicals are also capable of reacting with thiol molecules, generating oxygen radicals.

In a review by Yao *et al.* (2008), chromium (VI) stimulated cells were also reported to generate chromium (V)-NADPH complexes and hydroxyl radicals. Addition of NADPH further increased the formation of hydroxyl radicals and reduction of chromium (VI) to chromium (V). It was theorised that chromium (VI) may induce the up-regulation of Reactive Oxygen Species (ROS) generating enzymes such as NADPH oxidase.

Quiervryn *et al.* (2006) have conducted a study which examined the rate of chromium (VI) reduction and the formation of chromium (IV) and (V) in cells under low ascorbate conditions, which could occur in the occupational environment where exposure to high chromate levels may result in transient ascorbate depletion. Using a chromium (VI) concentration of 50 µM and an ascorbate concentration of 0.2 mM, the rate of reduction of chromium was approximately five times slower than at an ascorbate concentration of 1 mM. This difference in reaction rates is consistent with a first-order reaction for the reduction of chromium (VI) by ascorbate. Quiervryn *et al.* (2006) calculated that at a concentration of 0.2 mM ascorbate, ascorbate was responsible for metabolism of 88.5% of the intracellular chromium (VI). At a concentration of 1 mM, ascorbate accounted for 97.5% of the metabolism of chromium (VI). The slower reduction at the lower ascorbate concentration resulted in a 3-5 fold increase in the formation of chromium intermediates (measured by oxidation of a 2,7-dichlorofluoroscin diacetate probe to its fluorescent form, 2,7-dichlorofluorescin).

Quiervryn *et al.* (2006) also investigated the potential formation of DNA breaks and adduct formation during chromium (VI) reduction. DNA breaks were not detectable during chromate reduction. However, significant formation of adducts was observed, and at the highest chromium concentration (200 µM), 9600 chromium adducts per 50 000 DNA base pairs were reported. Quiervryn *et al.* (2006) estimated that the ratio of chromium adducts to DNA breaks was at least 10 000:1.

In a recent review, Yao *et al.* (2008) reported that chromium (VI) can induce the activation of NF-κB and AP-1 (NF-κB and AP-1 are transcription factors that control the transcription of specific genes) in dose and time-dependent manners in RAW264.7 cells (a rodent cell line) and Jurkat cells (a human cell line). The induction of AP-1 following chromium (VI) exposure is associated with the phosphorylation of MAP kinase, p38 and JNK (proteins that regulate various cellular activities, such as gene expression, mitosis, differentiation, proliferation, and cell survival/apoptosis), but not with extracellular signal-regulated protein kinase (ERK). Yao *et al*. (2008) suggest that Reactive Oxygen Species (ROS) may be responsible for the activation of AP-1 and NF-κB signalling pathways.

Chromium (VI) is also reported to be able to induce p53-mediated cell apoptosis. p53 is an important tumour suppressor that can initiate cell cycle arrest and apoptosis. Chromium (VI) metabolites are reported to alter p53 function via binding to DNA, activation of MAP kinases upstream of p53 and by direct oxidation of p53 itself (Yao *et al.* 2008).

Chui *et al.* (2010) has reviewed the biological half-life of a single dose of 5 mg in humans of two chromium compounds, chromium trichloride (a chromium (III) compound) and potassium dichromate (a chromium (VI) compound). The biological half-life of chromium (III) was 10 hours, while the half-life of chromium (VI) was 39 hours.

Thompson *et al*. (2013) investigated the mode of action of chromium (VI) in the small intestine and derived an oral reference dose based on a non-mutagenic mode of action based on intestinal irritation, ultimately leading to intestinal carcinogenesis. However this mechanism of chromium (VI) does not correlate to the genotoxic data presented in section 3.3.

2.4 Excretion

Following oral exposure, chromium compounds are usually excreted via the faeces, due to their poor absorption rate. However, absorbed chromium is excreted via the urine (WHO, 2003). Chromium is also reported to be excreted in hair and fingernails (WHO, 2013).

In rats orally exposed to chromium (VI), as sodium dichromate, at a dose of 0.44 mg/kg bw, peak urinary chromium concentrations were noted after six hours (2947 µg chromium/g creatinine) and had decreased to 339 µg/g creatinine after 72 hours (Gao *et al.*, 1993).

In humans administered chromium (VI), as sodium chromate, or chromium (III), as chromium chloride, at a dose of 20 ng, approximately 89.4 and 99.6% of the chromium (VI) and chromium (III) dose, respectively, were collected in faeces after six days. Twenty-four hours after administration, 2.1 and 0.5% of the chromium (VI) and chromium (III) dose, respectively, were collected in the urine (Donaldson & Barreras, 1966).

Based on studies in occupationally exposed workers, it has been estimated that after 7 days, 40% excretion of an initial chromium (VI) dose occurs, after 15-30 days, an additional 50% excretion has occurred, and after 5 years, the remaining 10% of the initial dose has been excreted (Costa, 1997).

2.5 Summary

2.5.1 Absorption

Chromium (III) compounds are poorly absorbed following oral administration. In humans, absorption was estimated to be 0.4% following oral administration of chromium trichloride and another study in humans has suggested that absorption of chromium (III) is inversely proportional to intake.

Chromium (VI) is more readily absorbed than chromium (III). However, chromium (VI) undergoes reduction in saliva and gastric juices and by intestinal bacteria to chromium (III), and any such reduction will decrease the absorption of chromium (VI). Any chromium (VI) that is not reduced will be absorbed by the upper small intestine and released into the blood portal system and it has been suggested that red blood cells have a high capacity for sequestering and reducing chromium (VI) to chromium (III). The available data indicate the capacity of the rodent stomach to reduce chromium (VI) to chromium (III) can be exceeded, and therefore, substantial chromium (VI) absorption can occur at higher doses. In drinking water, Proctor *et al.* (2012) calculated that the reducing capacity is exceeded in rats and mice at concentrations of 60 mg/l and 21 mg/l, respectively. The concentrations at and above the reductive capacity are generally consistent with the concentration that were reported to result in tumours in rats and mice in the 2-year National Toxicology Program (NTP) studies described in Section 3.4.2. However, the European Food Safety Authority (EFSA) considered that even at low doses, a small percentage of chromium (VI) absorption may occur (EFSA, 2014).

2.5.2 Distribution

In a study in humans, following intravenous administration of chromium (III) (as chromium chloride), distribution primarily occurred in the liver and spleen, with detectable concentrations remaining in the liver after six months. Similar results were reported in rats, with chromium concentrated in the liver, spleen and bone marrow. In a study in rats administered chromium nitrate, the highest concentrations were found in the liver, kidneys, testes and brain. Transferrin, an iron transport protein, has a high affinity for chromium (III) and therefore may play an important role in the distribution of chromium from the gastrointestinal tract to tissues.

Studies by the US National Toxicology Program (NTP) in rats and mice indicate that the distribution of chromium (VI) is dependent upon the concentration of the administered dose and the duration of administration.

In rats at the lowest dose for the shortest duration of exposure, chromium was distributed to the plasma, glandular stomach, urine and faeces, whereas at the highest dose for the longest duration of exposure, chromium was also distributed to the erythrocytes, liver, kidneys and forestomach.

In mice at the lowest dose for the shortest duration of exposure, chromium was distributed to the liver, kidneys and faeces. At the highest dose for the longest duration of exposure, chromium was also distributed to the erythrocytes, plasma, glandular stomach and forestomach.

2.5.3 Metabolism

Once absorbed into the cell, chromium (VI) undergoes a series of reduction reactions to yield chromium (III). This process does not require enzymes but involves direct electron transfer

from ascorbate and non-protein thiols, such as glutathione and cysteine. The reduction of chromium (VI) is reported to produce chromium (V), chromium (IV) intermediates and organic radicals at varying amounts that will depend on the reducing agent and the ratio of reactants.

Zhitkovich (2011) reported that the combined activity of ascorbate, glutathione and cysteine is responsible for >95% reduction of chromium (VI) *in vivo*, with ascorbate reduction being the major metabolism pathway (Figure 2.3).

The chromium (V) and chromium (VI) intermediates may be capable of inducing DNA breaks by oxidation of DNA by acting as a catalyst for Fenton-like reactions with hydrogen peroxide to produce reactive hydroxyl radicals.

Chromium (VI) is also reported to form DNA-protein cross-links in mammalian cells. This process is reported to be relatively low *in vivo*, with DNA-protein cross-links accounting for 1% of all chromium-DNA adducts.

Once metabolised to chromium (III), additional chromium-DNA adducts can be formed. These adducts are reported to be the predominant form of chromium (VI) induced genetic effect in mammalian cells, with the major adducts being glutathione-chromium-DNA, cysteine-chromium-DNA, histidine-chromium-DNA and ascorbate-chromium-DNA complexes.

2.5.4 Excretion

Following oral exposure, chromium compounds are usually excreted via the faeces, due to their poor absorption rate. However, absorbed chromium is excreted via the urine.

3. Toxicological Review of Chromium

This section of the report provides an overview of the available toxicological data identified in the publically available literature. Where applicable, these studies have been assessed and classified according to the Klimisch classification system.

3.1 Acute Toxicity

3.1.1 Chromium (III)

Data on the acute toxicity of chromium (III) compounds are presented in Table 3.1. These data indicate that chromium (III) compounds are of low acute oral toxicity to experimental animals.

3.1.2 Chromium (VI)

Data on the acute toxicity of chromium (VI) compounds are presented in Table 3.2. These data are highly variable, depending upon the chromium compound, however they generally indicate that chromium (VI) compounds are of high to moderate acute oral toxicity, of moderate to low acute dermal toxicity, and of high acute inhalation toxicity.

Table 3.1 Acute Toxicity of Chromium (III) Compounds

Compound	Species	Route	Endpoint	Concentration/Dose	Reference
Chromium sulphate	Rats	Oral	LD50	3530 mg/kg bw	Bayer (1978), cited in WHO (2009)
Chromium oxide	Rats	Oral	LD50	>15 000 mg/kg bw	Bayer (1972), cited in WHO (2009)
Chromium nitrate nonahydrate	Rats	Oral	LD50	1270-3010 mg/kg bw	Smyth *et al.* (1969), cited in WHO (2009)
Triaqua-μ3-oxohexakis-μ-propionatorichromium(1^+) ($Cr_3O(O_2CCH_2CH_3)_6(H_2O)_3^+$ cation)	Wistar rats (male and female)	Oral	LD50	>2000 mg/kg bw	Staniek *et al.* (2010).
Unspecified chromium (III) compound	Rats	Oral	LD50	185-615 mg/kg bw	Janus *et al.* (1990), cited in WHO (2003)

Table 3.2 Acute Toxicity of Chromium (VI) Compounds

Compound	Species	Route	Endpoint	Concentration/Dose	Reference
Sodium chromate, potassium dichromate, ammonium dichromate	Rats (male)	Oral	LD50	21-28 mg/kg bw	Gad *et al.* (1986), cited in IPCS, (2011)
Sodium chromate, potassium dichromate, ammonium dichromate	Rats (female)	Oral	LD50	13-19 mg/kg bw	Gad *et al.* (1986), cited in IPCS, (2011)
Potassium dichromate	Rats (male)	Oral	LD50	74 mg/kg bw	EU (2005)
Potassium dichromate	Rats (female)	Oral	LD50	48 mg/kg bw	EU (2005)
Sodium dichromate	Rats (male)	Oral	LD50	59 mg/kg bw	EU (2005)
Sodium dichromate	Rats (female)	Oral	LD50	46 mg/kg bw	EU (2005)

Compound	Species	Route	Endpoint	Concentration/Dose	Reference
Ammonium dichromate	Rats (male)	Oral	LD50	55 mg/kg bw	EU (2005)
Ammonium dichromate	Rats (female)	Oral	LD50	48 mg/kg bw	EU (2005)
Sodium chromate	Rats (male)	Oral	LD50	87 mg/kg bw	EU (2005)
Sodium chromate	Rats (female)	Oral	LD50	40 mg/kg bw	EU (2005)
Chromium trioxide	Rats (male)	Oral	LD50	29 mg/kg bw	American Chrome and Chemicals (1989) cited in IPCS, (2011)
Chromium trioxide	Rats (female)	Oral	LD50	25 mg/kg bw	American Chrome and Chemicals (1989) cited in IPCS, (2011)
Chromium trioxide	Rats	Oral	LD50	52-113 mg/kg bw	EU (2005)
Chromium trioxide	Mice	Oral	LD50	135-175 mg/kg bw	EU (2005)
Strontium chromate	Rats (male)	Oral	LD50	811 mg/kg bw	Shubochkin and Pokhodzie (1980) cited in IPCS, (2011)
Unspecified chromium (VI) compound	Rats	Oral	LD50	20-250 mg/kg bw	Janus *et al.* (1990), cited in WHO (2003)
Sodium chromate, potassium dichromate, ammonium dichromate	Rabbits (male)	Dermal	LD50	339-763 mg/kg bw	Gad *et al.* (1986), cited in IPCS, (2011)
Sodium chromate, potassium dichromate, ammonium dichromate	Rabbits (female)	Dermal	LD50	361-553 mg/kg bw	Gad *et al.* (1986), cited in IPCS, (2011)
Sodium dichromate	Rabbits	Dermal	LD50	960 mg/kg bw	EU (2005)
Potassium dichromate	Rabbits	Dermal	LD50	1150 mg/kg bw	EU (2005)
Ammonium dichromate	Rabbits	Dermal	LD50	1860 mg/kg bw	EU (2005)
Sodium chromate	Rabbits	Dermal	LD50	1330 mg/kg bw	EU (2005)
Chromium trioxide	Rats (male and female)	Dermal	LD50	30 mg/kg bw	American Chrome and Chemicals (1989) cited in IPCS, (2011)
Chromium trioxide	Rabbits	Dermal	LD50	57 mg/kg bw	EU (2005)

Compound	Species	Route	Endpoint	Concentration/Dose	Reference
Sodium chromate, potassium dichromate, ammonium dichromate	Rats (male)	Inhalation	LC50	33-82 mg/m³	Gad *et al.* (1986), cited in IPCS, (2011)
Sodium chromate, potassium dichromate, ammonium dichromate	Rats (female)	Inhalation	LC50	29-45 mg/m³	Gad *et al.* (1986), cited in IPCS, (2011)
Sodium dichromate, potassium dichromate, ammonium dichromate	Rats	Inhalation	LC50	200 mg/m³	EU (2005)
Potassium dichromate	Rats	Inhalation	LC50	99 mg/m³	EU (2005)
Chromium trioxide	Rats (male)	Inhalation	LC50	137 mg/m³	American Chrome and Chemicals (1989) cited in IPCS, (2011)
Chromium trioxide	Rats (female)	Inhalation	LC50	87 mg/m³	American Chrome and Chemicals (1989) cited in IPCS, (2011)

3.2 Irritation and Sensitisation

Chromium (VI) compounds are regarded as skin sensitisers. Shelnutt *et al.* (2007) have conducted a review of the available data relating to chromium (VI) induced allergic contact dermatitis (ACD) and skin ulcers. ACD is an immunological reaction that occurs following exposure to a particular allergen. A number of studies have been conducted assessing the prevalence of chromium-induced ACD, and Shellnut *et al.* (2007) note that these estimates vary by 100-fold, ranging from 0.08 to 7%. This range is a result of the different populations that have been studied in the literature and prevalence rates will be higher among populations with the greatest exposure to chromium. In a study of the general population, 567 individuals in Denmark were assessed for chromium (VI) sensitisation in 1992. The overall prevalence was found to be 0.53%, with males exhibiting slightly higher sensitivity (0.7%) (Nielsen and Menne, 1992, cited in Shelnutt *et al.,* 2007). Shelnutt *et al.* (2007) report that induction of ACD by chromium (VI) requires years of chronic, low-level exposure.

Chromium (VI) can be found in cement at concentrations of 10-20 mg/kg, and as a result, cement workers may be chronically exposed to chromium, although the concentration will be diluted in water. One study reported 4-5% of cement workers displayed chromium induced ACD, compared to 0.5% in typical European populations (Stern *et al.,* 1993, cited in Shelnutt *et al.,* 2007). Other studies have reported chromium sensitisation rates in cement workers of 40% in Singapore (Goh *et al.,* 1986, cited in Shelnutt *et al.,* 2007), 13% in Taiwan (Guo *et al.*, 1999, cited in Shelnutt *et al.,* 2007) and 23% in Poland (Kiec-Swierczynska, 1990, cited in Shelnutt *et al.,* 2007). Although no measured data were available in the USA, Shelnutt *et al.* (2007) suggested the prevalence of chromium induced ACD in cement workers may be higher than that in the EU due to the use of ferrous sulphate in cement in the EU, which reduces the concentration of chromium (VI). Estimates of chromium (VI) ACD have been made in the USA, based on the number of patients with dermatitis reported by the US North American Contact Dermatitis Group, which suggest prevalence rates between 1972 and 2004 ranged from 4.3 to 7.6%.

Chromium (VI) induced ACD has also been associated with exposure via consumer detergents. In a study in Spain, detergents were found to contain chromium (VI) concentrations of 2.1-6.2 mg/kg, with an average concentration of 4.3 mg/kg, and a 90.5% correlation was determined between detergent dermatitis and allergic reactions to chromium (VI) (Quinones & Garcia-Munoz, 1965, cited in Shelnutt *et al.,* 2007). A study in Israel reported a 92% correlation between hand dermatitis and chromium (VI) (Feuerman, 1971, cited in Shelnutt *et al.,* 2007).

Chromium (VI) can also illicit the formation of necrotising ulcers known as ‘chrome ulcers’ or ‘chrome holes’. These lesions generally occur in individuals that do not display chromium induced ACD and can occur as a result of exposure to high concentrations, or exposure to low concentrations under certain conditions, such as abraded skin or high temperatures or humidity (Shelnutt *et al.,* 2007). Shelnutt *et al.* (2007) stated that the concentration required to produce these ulcers is unknown, but note that application of 0.005% sodium dichromate to a

patch of superficially scratched skin for 8 hours a day for three days produced chrome ulcers that took two weeks to heal.

Patch tests have demonstrated that the threshold for irritation from hexavalent chromium is dependent upon pH. In a patch tests, 3 of 33 test subjects provided a positive response to 10 mg sodium dichromate/l at pH 11.7, however none of these subjects responded to application of 10 mg potassium dichromate/l at pH 1.5 (Zelger, 1964, cited in Shelnutt *et al.,* 2007).

3.3 Genotoxicity

3.3.1 In vitro

Chromium (III)

Andersson *et al.* (2007) evaluated the genotoxic potential of chromium (III) as chromium picolinate on human lymphocytes and L5178Y mouse lymphoma cells *in vitro* under different conditions and micronucleated polychromatic erythrocytes in peripheral blood and DNA damage in lymphocytes and hepatocytes *in vivo* in mice.

A significant increase in the incidence of DNA damage was noted in human lymphocytes exposed to chromium picolinate without serum. No increase was observed in human lymphocytes or mouse lymphoma cells under biologically relevant conditions. No increase in the incidence of micronucleated polychromatic erythrocytes or DNA damage was noted *in vivo*.

This study has been classified as Klimisch Code 2 (reliable with restrictions), on the basis that, although it does not follow a standard regulatory protocol, it is a well-documented and scientifically acceptable study.

Gudi *et al.* (2005) assessed the genotoxicity of chromium (III), as chromium picolinate, in Chinese Hamster ovary K1 cells at concentrations of 96.25, 192.5, 385 or 770 µg/ml for 4 hours (with and without metabolic activation) and 20 hours (without metabolic activation). Visible precipitation of chromium picolinate was noted at the top dose. However, no increase in structural or numerical chromosomal aberrations was noted at any dose.

This study has been classified as Klimisch Code 1 (reliable without restrictions) on the basis that it is a well-documented and scientifically acceptable study.

The genotoxicity of chromium (III), as chromium picolinate was evaluated *in vitro* in a Chinese hamster ovary cell hypoxanthine phosphoribosyltransferase (Hprt) gene locus assay by Slesinski *et al*. (2005). Concentrations of 15.6-500 µg/ml, with and without metabolic activation for 5 hours and 31.3-500 µg/ml, without metabolic activation for 48 hours were used

in this assay. Slight cytotoxicity was noted at the limit of solubility of the medium, but no evidence of increased mutations was noted at any concentration.

This study has been classified as Klimisch Code 1 (reliable without restrictions) on the basis that it is a well-documented and scientifically acceptable study.

El-Yamani *et al.* (2011) compared the genotoxicity of chromium (III), as chromium chloride, and chromium (VI), as sodium chromate, in human lymphoblastoid (TK6) cells exposed to chromium concentrations of 0, 0.2, 0.4, 0.6, 0.8 or 1 mM by the COMET assay. The authors also assessed the type of DNA occurring in these cells by treatment with enzymes that recognise oxidised bases, formamidopyrimidine (FPG) and endonuclease III (endoIII) at concentrations of 0.8 or 1 mM, and conducted a kinetic repair study at a concentration of 1 mM.

In the COMET assay, the percentage of DNA in the tail was significantly increased in with both chromium compounds at the top four doses. Treatment with FPG and endoIII revealed a higher degree of DNA damage with both chromium compounds at both doses indicating the induction of oxidised bases. The kinetic repair study demonstrated that DNA damage was removed after 8 hours, with the damage more rapidly repaired or removed following exposure of the cells to chromium (III).

This study has been classified as Klimisch Code 2 (reliable with restrictions) as the abstract to the report states that sodium chromate was significantly more genotoxic in the COMET assay than chromium chloride. However, this is not reported in the main text of the paper, and therefore a full evaluation cannot be conducted.

In 2008, Eastmond *et al.* (2008) conducted a review of studies on the genotoxicity of chromium (III) compounds. A summary of the *in vitro* genotoxicity results from this review is presented in Table 3.3. Details on the evaluation of chromium (III) compounds *in vivo* are presented in Table 3.5. Although a weight-of-evidence view of these results would suggest that the data for genotoxicity are equivocal, or possibly weakly positive, Eastmond *et al.* (2008) considered that for the majority of the bacterial and mammalian cell assays, results were negative, and where positive results are recorded, these assays were only weakly positive. Eastmond *et al.* (2008) did note that many positive results have been reported in more recent years, but were unable to determine if this is a result of using more bioavailable forms of chromium (III) or new bioassays or dilutants.

Table 3.3 Summary of *in vitro* genotoxicity of chromium (III) compounds reported by Eastmond *et al.* (2008)

Assay	Result	Chromium compound(s)
Bacterial assays		
Reverse mutation assays in *Salmonella sp.*	Positive	Chromium chloride Cis-dichlorobis(2,2'-bipyridyl)chromium Chromium chloride phenanthroline complex ($[Cr(phen)_2Cl_2]^{1+}$) Chromium hydroxide phenanthroline complex ($[Cr(phen)_2OH_2]^{1+}$) Chromium(2,2'- bipyridine)chloride Chromium(2,2'- bipyridine)hydroxide Tris(2,2'-bipyridine)chromium^{3+}
Reverse mutation assays in *Salmonella sp.*	Negative	Potassium chromic cyanide Tris(ethylenediamine)chromium Tris(ethylenediamine)chromium chloride Chromium picolinate Chromium picolinate anhydrous Chromium picolinate monohydrate Chromium chloride Nicin-bound chromium
Reverse mutation assay in *Escherichia coli*	Negative	Chromium picolinate monohydrate
Mutation induction by replicated chromium treated DNA in *Escherichia coli*	Positive	Chromium chloride
Promotor induction of DNA damage responding genes in *Escherichia coli*	Positive	Chromium chloride Chromium nitrate
Promotor induction of DNA damage responding genes in *Escherichia coli*	Negative	Chromium oxalate
Yeast assays		
Deletion mutation assay in *Saccharomyces cerevisiae*	Positive	Chromium nitrate
Mammalian cell assays		
Mutation induction by chromium-treated shuttle vectors in human fibroblasts	Positive	Chromium chloride with and without cysteine, histidine and glutathione
DNA fragmentation in J774A.1 macrophage cells	Positive	Chromium picolinate Chromium nicotinate
DNA damage (COMET assay) in mouse lymphoma cells	Negative	Chromium picolinate
DNA damage (COMET assay) in human lymphocytes	Positive	Chromium chloride

Assay	Result	Chromium compound(s)
DNA damage (COMET assay) in human lymphocytes	Negative (weakly positive with DMSO and serum)	Chromium picolinate
Micronucleus assay in Chinese hamster lung V79 cells	Negative	Tris(ethylenediamine)chromium Chromium glycyl complex ($[Cr(glycly)_2]^-$)
Micronucleus assay in Chinese hamster lung V79 cells	Positive	Chromium hydroxide phenanthroline complex ($[Cr(phen)_2(OH_2)s]^{3+}$) Chromium hydroxide salen complex ($[Cr(salen)(OH_2)_2]^{3+}$)
Chromosomal aberration assays conducted in Chinese hamster ovary cells	Negative	Chromium nicotinate Chromium chloride Chromium picolinate Chromium nitrate nonahydrate
Chromosomal aberration assay conducted in Chinese hamster ovary cells	Positive	Chromium picolinate
Cen+ and – micronuclei in human fibroblasts	Positive	Chromium chloride
Sister chromatid exchange assays conducted in Chinese hamster ovary cells	Positive	Chromium nitrate nonahydrate
Anchorage independence assay in human fibroblasts	Positive	Chromium oxide Chromium chloride Chromium sulphide Chromium chloride hexahydrate
Hprt mutation assay in human fibroblasts	Positive	Chromium oxide Chromium chloride
Hprt mutation assay in human fibroblasts	Equivocal	Chromium chloride hexahydrate
Hprt mutation assay in Chinese hamster ovary cells	Negative	Chromium picolinate
Hprt mutation assay in Chinese hamster ovary cells	Positive	Chromium picolinate Chromium chloride
Tk mutation in mouse lymphoma L5178Y cells	Equivocal	Chromium chloride
Tk mutation in mouse lymphoma L5178Y cells	Positive	Chromium picolinate
Tk mutation in mouse lymphoma L5178Y cells	Negative	Niacin-bound chromium

Chromium (VI)

In an *in vitro* study, Wise *et al.* (2010) compared the cytotoxicity and genotoxicity of three insoluble, particulate chromium (VI) compounds – zinc chromate, barium chromate and lead chromate – and one soluble chromium (VI) compound – sodium chromate – in primary human bronchial fibroblasts (WTHBF-6 clonal line).

All chromium (VI) compounds induced a concentration-dependent increase in cytotoxicity. Of the particulate compounds, zinc chromate and barium chromate were significantly more cytotoxic than lead chromate. The authors stated that sodium chromate concentrations could not be directly compared with the other compounds due to its water solubility.

However, based on intracellular chromium concentrations, Wise *et al.* (2010) suggested that sodium chromate is of similar cytotoxicity to primary human bronchial fibroblasts as lead chromate. The authors also noted that, based on intracellular concentrations, the difference in cytotoxicity between lead, barium and zinc chromate is reduced, although the trend remains the same, i.e. zinc chromate was the most cytotoxic of the three particulate compounds and lead chromate was the least cytotoxic.

DNA double-strand breaks were induced by all four chromium compounds in a concentration-related manner, with no statistically significant difference in the incidence of double strand breaks between any of the compounds.

Evaluation of chromosomal damage also indicated concentration-dependent increases in the incidence of percentage of cells in metaphase with damage and the total number of aberrations per 100 metaphases. These data indicated that zinc chromate was the most clastogenic compound and lead chromate was the least clastogenic. The authors suggest that the greater potency of zinc chromate may be due not only to the presence of chromate, but also that the zinc may act as a co-carcinogen due to its effects on DNA repair.

This study has been classified as Klimisch Code 2 (reliable with restrictions), on the basis that, although it does not follow a standard regulatory protocol, it is a well-documented and scientifically acceptable study.

Patlolla *et al.* (2009) have evaluated the potential for chromium (VI), as potassium dichromate, to induced cytotoxicity, genotoxicity and oxidative stress in human liver carcinoma (HepG2) cells using concentrations of 0, 3.12, 6.25, 12.5 and 25 µM. The cytotoxicity assay revealed dose- and time-related effects, and 24 and 48-hour LD50s were determined to be 8.83 ± 3.5 and 6.76 ± 0.7 µg/ml, respectively. The formation of reactive oxygen species was increased in a concentration-related manner and was statistically significant at the top two doses. Genotoxicity was evaluated by the COMET assay, and indicated DNA damage at all concentrations.

This study has been classified as Klimisch Code 2 (reliable with restrictions), on the basis that, although it does not follow a standard regulatory protocol, it is a well-documented and scientifically acceptable study.

In 2008, the US Agency for Toxicity Substances and Disease Registry (ATSDR) (ATSDR, 2008) conducted a review of the toxicity of chromium compounds. As part of this review, the data were gathered on the genotoxicity of various chromium (III) and chromium (VI) compounds. A summary of the chromium (VI) *in vitro* genotoxicity data collated by ATSDR are presented in Table 3.4. Overall, these data indicate that chromium (VI) compounds are genotoxic *in vitro*.

To assess the risks of wear and tear from metal-on-metal implants, the genotoxicity of chromium (III) and chromium (VI), with and without cobalt, were examined in cultured human primary fibroblasts. Chromium (III) (as chromium chloride hexahydrate) and chromium (VI) were used at concentrations of 2, 20 or 40 µg/l, with or without cobalt at concentrations of 1.3, 25 and 50 µg/l, respectively. Cobalt was also tested at these concentrations without chromium. The lowest concentrations were reported to be typical concentrations found in the blood of patients with well-functioning metal-on-metal implants, while the higher concentrations are similar to those found with worn metal-on-metal implants. Cells were exposed to the metals for 24 hours prior to examination.

The incidence of total chromosomal aberrations was significantly increased in all treatments at all doses. This effect was least apparent in cells treated only with cobalt, and was most apparent in cells treated with chromium (VI), especially in combination with cobalt. The incidence of simple aneuploidy (46±3 or fewer chromosomes) was significantly increased chromium (III) plus cobalt and cobalt-only treated cells at the mid and top concentrations, and in chromium (III) treated cells, chromium (VI) treated cells and chromium (VI) plus cobalt treated cells at all doses. Chromium (VI), with and without cobalt, also produced complex aneuploidy (>49 chromosomes) at all doses in a dose-related manner. Chromium (III) with and without cobalt, and cobalt-only treated cells also produced complex aneuploidy, but only at the top dose.

Chromosomal fragments were seen in chromium (III) and chromium (VI) administered cells, with and without cobalt, at the top dose, and persisted in chromium (VI) treated cells for 10 days after exposure, implying persistent breakage events (Figgitt *et al.,* 2010).

This study has been classified as Klimisch Code 2 (reliable with restrictions), on the basis that, although it does not follow a standard regulatory protocol, it is a well-documented and scientifically acceptable study.

Table 3.4 Summary of *in vitro* genotoxicity of chromium (VI) compounds reported by ATSDR (2008) and WHO (2013)

Assay	Result	Chromium compound(s)
Bacterial assays		
DNA protein cross-links in *Escherichia coli*	Negative	Potassium chromate
Forward mutations in double-stranded M13mp2 bacteriophage DNA transferred to *Escherichia coli*	Positive	Potassium chromate
Recombination assay conducted in *Bacillus subtilis*	Positive	Potassium chromate Potassium dichromate
Induction of SOS response in *Escherichia coli* strains PQ37, PQ35, AB1157, GC2375, UA4202 and PQ30	Positive	Potassium chromate Potassium dichromate
Induction of SOS response in *Escherichia coli* strains AB1157, GC2375, UA4202 and PQ30	Positive	Chromium trioxide
Base-pair substitutions in *Salmonella typhimurium* strains TA92, TA100 and TA102	Positive	Sodium dichromate
Frame-shift mutations in *Salmonella typhimurium* strain TA97	Positive	Sodium dichromate
Frame-shift mutations in *Salmonella typhimurium* strains TA1535, TA1537 and TA1538	Negative	Sodium dichromate
Frame-shift mutations in *Salmonella typhimurium* strain TA1978	Equivocal	Sodium dichromate
Base-pair substitutions in *Salmonella typhimurium* strain TA100	Positive	Potassium dichromate
Base-pair substitutions in *Salmonella typhimurium* strain TA1535	Equivocal	Potassium dichromate
Yeast assays		
Mitotic gene conversions in *Saccharomyces cerevisiae* D7	Positive	Chromium trioxide
Reverse mutations in *Saccharomyces cerevisiae* D7	Positive	Potassium dichromate
DNA deletions in *Saccharomyces cerevisiae*	Positive	Potassium dichromate
Mitotic gene conversions and forward mutations in *Schizosacharomyces pombe*	Positive	Potassium dichromate

Assay	Result	Chromium compound(s)
Mammalian cell assays		
DNA-protein crosslinks and DNA fragmentation in human embryonic lung fibroblasts and mouse L1210 leukaemia cells	Positive	Potassium chromate
DNA fragmentation in human bronchial epithelial cells	Positive	Potassium chromate
Single strand breaks in human lymphocytes	Positive	Potassium chromate
DNA damage in human lymphocytes	Positive	Potassium dichromate
Sister chromatid exchange and chromosomal aberrations in Chinese hamster lung DON cells	Positive	Chromium trioxide Calcium chromate Potassium chromate
Chromosomal aberrations and DNA fragmentation in Chinese hamster ovary cells	Positive	Sodium chromate Lead chromate
Chromosomal aberrations in mouse embryo fibroblasts	Positive	Calcium chromate
DNA damage (DNA interstrand crosslinks, DNA strand breaks and DNA-protein crosslinks) in chick embryo hepatocytes	Positive	Sodium chromate
Unscheduled DNA synthesis in mouse A18BcR cells	Positive	Potassium chromate
Transformations and chromosomal aberrations in mouse primary foetal cells	Positive	Potassium chromate
DNA damage in human gastric mucosa and peripheral blood lymphocytes	Positive	Potassium dichromate

3.3.2 *In vivo*

Chromium (III)

In an *in vivo* study, Sprague-Dawley rats (5/sex/dose) were orally administered a single dose of chromium (III) as chromium picolinate at doses of 0, 33, 250 or 2000 mg/kg bw. Bone marrow cells in metaphase were examined for interstitial deletions, chromatid and chromosome gaps, breaks or other anomalies. No evidence of genotoxic effects were noted at any dose (Komorowski *et al.*, 2008).

This study has been classified as Klimisch Code 1 (reliable without restrictions), on the basis that it is a well-documented and scientifically acceptable study.

In 2008, Eastmond *et al.* (2008) conducted a review of studies on the genotoxicity of chromium (III) compounds. A summary of the *in vivo* genotoxicity results from this review is presented in Table 3.5. Details on the evaluation of chromium (III) compounds *in vitro* are presented in Table 3.3. The weight of evidence of these data indicate that chromium (III) compounds are not genotoxic *in vivo*.

Table 3.5 Summary of *in vivo* genotoxicity of chromium (III) compounds reported by Eastmond *et al.* (2008)

Assay	Result	Chromium compound(s)
Chromosomal aberration assays conducted in male and female Sprauge Dawley rats	Negative	Chromium picolinate
Bone marrow micronuclei assay conducted in male Slc:ddY mice	Negative	Chromium chloride
Bone marrow micronuclei assay conducted in male F344/N rats	Negative	Chromium picolinate
Bone marrow micronucleus assay conducted in male and female Cr1:CD1(ICR) mice	Negative	Niacin-bound chromium
Peripheral blood micronuclei assay in male and female B6C3F1 mice	Negative (females), equivocal (males)	Chromium picolinate monohydrate
Bone marrow and peripheral blood micronuclei assays conducted in male and female BDF1 mice	Negative	Chromium potassium sulphate dodecahydrate
DNA single strand breaks in female Sprague Dawley rats	Weakly positive	Chromium chloride

Assay	Result	Chromium compound(s)
DNA damage (COMET assay) in lymphocytes and hepatocytes of male CBA/Ca mice	Negative	Chromium picolinate
Deletion mutation in male and female C57BL/6Jp^{un}/p^{un} mice	Weakly positive	Chromium chloride
K-ras mutation in tumours of female NIH Swiss mice	Negative	Chromium chloride
Hypomethylation in sperm of male Cr:NIH Swiss mice	Positive	Chromium chloride
8-Hydroxydeoxyguanosine (8-OHdG) formation in Sprague Dawley rats	Positive	Chromium picolinate

Chromium (VI)

In a 3-month study, B6C3F1 mice (10/sex/dose) were administered chromium (VI) as sodium dichromate dihydrate via their drinking water at doses of 0, 62.5, 125, 250, 500 or 1000 mg/l (reported to be 0, 9, 15, 26, 45 and 80 mg/kg bw/day, respectively). In additional studies three strains of male mice (B6C3F1, BALB/c and am3-C57BL/6; 5/strain/dose) were administered sodium dichromate dihydrate via their drinking water at doses of 0, 62.5, 125 or 250 mg/l (reported to be 0, 62.5, 125 or 250 mg/l was reported to be 0, 8, 15 and 26 mg/kg bw/day in B6C3F1 mice, 0, 9, 14 and 24 mg/kg bw/day in BALB/c mice and 0, 8, 15 and 25 mg/kg bw/day in am3-C57BL/6, respectively). Micronucleus frequencies were determined from peripheral blood samples and the percentage of polychromatic erythrocytes was determined from bone marrow assay.

No significant increase in micronucelated erythrocytes in the first study in B6C3F1 mice. The percentage of polychromatic erythrocytes was slightly decreased, indicating possible bone marrow toxicity, but the effect was small and did not appear to be concentration related.

In the second study, equivocal results were noted in the incidence of micronucelated erythrocytes in male B6C3F1 mice, no increase was noted in BALB/c mice, and a significant increase was noted in am3-C57BL/6 mice. Overall, the evidence for micronucleus formation is equivocal. No significant effects on the incidence of polychromatic erythrocytes were noted in any of the assays (National Toxicology Program, 2007).

This study has been classified as Klimisch Code 1 (reliable without restrictions), on the basis that it is a well-documented and scientifically acceptable study.

The genotoxicity of chromium (VI), as potassium chromate, was investigated by Itoh and Shimada (1997) in male lacZ transgenic mice administered intraperitoneal doses of

40 mg/kg bw for two consecutive days. A significant increase in the incidence of micronucleated peripheral blood reticulocytes was noted. Examinations seven days after termination of treatment revealed a significant increase in the frequency of mutations in the liver, but not in the bone marrow.

This study has been classified as Klimisch Code 2 (reliable with restrictions) on the basis that it is an acceptable study, but the seven day rest period prior to examination of mutation frequency means that possible genotoxic effects may have been 'masked' by repair mechanisms.

In a combined *in vitro*/*in vivo* study, female SKH-1 hairless mice (10/sex/dose) were administered chromium (VI) as sodium dichromate dihydrate via their drinking water at doses of 0, 5 or 20 mg/l (reported to be 0, 1.2 and 4.7 mg/kg bw/day, respectively) for 9 months. Portions of the forestomach, glandular stomach and duodenum were scrapped and DNA-protein crosslinked (DPXL) and 8-oxo-2'-deoxyguanosine (8-oxo-dG) levels were determined. Potions of the forestomach, glandular stomach and duodenum from control animals were also scrapped and collected for *in vitro* treatment with sodium dichromate dihydrate at a concentration of 1.6 mM chromium (VI) for 1 hour at 37°C and DPXL and 8-oxo-dG levels were determined.

All mice survived until the end of the study and no effects on bodyweight gain at any dose. No treatment-related neoplastic lesions were detected in the forestomach, glandular stomach, duodenum or lungs at any dose. No statistically significant effects DPXK or 8-oxo-dG levels in the forestomach, glandular stomach or duodenum were noted at any dose. In the *in vitro* study, chromium (VI) induced significant increases in both DPXL and 8-oxo-dG levels in the forestomach, glandular stomach and the duodenum. The authors concluded that chromium (VI) induced clastogenic effects *in vitro* that were not apparent *in vivo* (DeFlora *et al.*, 2008).

This study has been classified as Klimisch Code 2 (reliable with restrictions), on the basis that it is a well-documented and scientifically acceptable study. However, the authors also make conclusions with regards to no evidence of clastogenicity in the foetuses of these mice, but these data are not presented in the report.

DeFlora *et al.* (2006) have conducted a series of micronucleus assays in mice following administration via several routes. In the first study, male BDF1 mice were administered chromium (VI), as potassium dichromate, at doses of 0, 10 or 20 mg/l via drinking water for 20 days or at a dose of 50 mg/kg bw as a single oral gavage dose or a single intraperitoneal injection. In the second study, male and female BDF1 mice were administered sodium dichromate dihydrate at doses of 0, 5, 50 or 500 mg chromium (VI)/l via drinking water for 210 days or chrome alum at a dose of 500 mg chromium (III)/l for 210 days. In the third study, pregnant Swiss albino mice were administered sodium dichromate at doses of 5 or 10 mg chromium (VI)/l or potassium dichromate at a dose of 10 mg chromium (VI)/ for 18 days, or a

single intraperitoneal injection of sodium dichromate dihydrate or potassium dichromate at a dose of 50 mg/kg bw.

In the first study, no effects on the frequency of micronucleated polychromatic erythrocytes (PCEs) or the ratio of PCE/normochromatic erythrocytes (NCEs) of the bone marrow or the frequency of PCE in peripheral blood samples were observed at any dose following administration via drinking water and oral gavage. Administration via the intraperitoneal route did not increase the PCE/NCE ratio, but significantly increased the frequency of PCE in bone marrow. The second study also reported no effect on the frequency of micronucleated PCEs or the ratio of PCE/NCEs of the bone marrow or the frequency of PCE in peripheral blood samples at any dose following administration of either sodium dichromate dihydrate or chrome alum. In the third study, no effects on the frequency of micronucleated PCEs or the ratio of PCE/NCEs of the bone marrow or the frequency of PCE in peripheral blood samples were noted following administration of test material via drinking water, however, via the intraperitoneal route chromium increased the frequency of PCE, but did not increase the PCE/NCE ratio in foetal livers (DeFlora *et al.,* 2006).

These results indicate that chromium (VI) does not alter the frequency of micronuclei via the oral route.

This study has been classified as Klimisch Code 1 (reliable without restrictions), on the basis that it is a well-documented and scientifically acceptable study.

The European Food Safety Authority (EFSA) has also reviewed the available data on *in vivo* genotoxicity of chromium (VI) (EFSA, 2014). In addition to the studies detailed above, this review also includes some additional oral studies. These studies, alongside those detailed above are summarised in Table 3.6. These results are mixed, with the majority of oral micronucleus studies indicating negative results, but positive results in Comet assays, although this assay has not been validated for all endpoints (only liver and stomach). These negative results may be a reflection of the reductive capacity of the gastrointestinal tract, which will serve to limit chromium (VI) absorption. However, as several positive results are reported, the possibility of *in vivo* genotoxicity via the oral route cannot be discounted.

Table 3.6 Summary of *in vivo* genotoxicity of chromium (VI)

Assay	Result	Chromium compound(s)
Micronucleus assay in B6C3F1 mice (oral administration)	Negative	Sodium dichromate dihydrate
Micronucleus assay in B6C3F1 mice (oral administration)	Equivocal	Sodium dichromate dihydrate
Micronucleus assay in male lacZ transgenic mice (intrapertioneal administration)	Positive in blood reticulocytes and liver, negative in bone marrow	Potassium chromate
DNA-protein crosslinked (DPXL) and 8-oxo-2'-deoxyguanosine (8-oxo-dG) in SKH-1 hairless mice (oral administration)	Negative	Sodium dichromate dihydrate
Micronucleus assay in male BDF1 mice (oral administration)	Negative	Potassium dichromate
DNA deletions in 20-day-old offspring of Female C57BL/ 6Jpun/pun mice (oral administration)	Positive	Potassium dichromate
Micronucleus assay in Swiss albino mice (oral administration)	Negative	Sodium dichromate dihydrate
Micronucleus assay in male MS/Ae and CD-1 mice (oral administration)	Negative	Potassium chromate
DNA damage Comet assay in peripheral lymphocytes of Swiss albino mice (oral administration)	Positive	Potassium dichromate
DNA damage Comet assay in leukocytes of Swiss albino mice (oral administration)	Positive	Potassium dichromate
DNA damage Comet assay in stomach, colon, liver, kidney, bladder, lung, brain and bone marrow of ddY mice (oral administration)	Positive	Potassium dichromate

3.4 Repeat Dose and Carcinogenicity

3.4.1 Chromium (III)

In a 6-week study, bile duct ligation (BDL) treated male Sprague-Dawley (6/group) rats were administered chromium (III) as a complex consisting of chromium chloride hexahydrate, lactoferrin, whey protein concentrate and milk powder at a concentration of 0 or 80 µg chromium/kg bw/day via oral gavage. Three BDL groups were used, with a treatment regimen as follows:

- A control group was administered placebo milk for the entire six weeks and underwent a BDL operation after three weeks.

- A pre-BDL chromium treated group were administered the chromium complex for 6 weeks, starting three weeks prior to the BDL operation.

- A post-BDL chromium treated group received placebo milk powder for three weeks prior to the BDL operation and were administered the chromium complex for three weeks after the operation.

An additional control group (3 rats) received placebo milk for the entire six weeks and underwent a sham operation after three weeks.

No effect on food intake was noted in any group. Bodyweight gain was slightly decreased in the BDL rats, however, no significant effects were noted due to chromium treatment. The BDL controls displayed severe bile duct hyperplasia and biochemical alterations, which were consistent with hepatocellular injury. In chromium-treated rats, these effects were also significantly compared to the sham-operated controls, and the incidence of histopathological alterations was unaffected when compared to the BDL controls. However, the severity of these changes was reduced and the levels of biochemical changes were moderately decreased. There was no significant difference between pre- and post-BDL treated groups.

These data indicate that chromium (III) treatment may have attenuated the effects of liver injury caused by the BDL operation. The authors suggested that chromium (III) may act as a hepatoprotective agent against cholestasis-related injury (Chen *et al.*, 2009).

This study has been classified as Klimisch Code 2 (reliable with restrictions) due to the small number of animals used for each treatment.

In a 14-week study, F344/N rats (10/sex/dose) were administered chromium (III) as chromium picolinate via the diet at doses of 0, 80, 240, 2000, 10 000 or 50 000 mg/kg diet (reported to be 0, 7, 20, 160, 800 and 4240 mg/kg bw/day in males and 0, 6, 20, 160, 780 and 4250 mg/kg bw/day in females, respectively). Chromium (III) plays an essential role in insulin metabolism, and as such chromium picolinate is available as a nutritional supplement to treat or prevent chromium deficiency.

No effect on final bodyweight, bodyweight gain or food consumption was noted and no clinical signs of toxicity were noted at any dose. No consistent or significant haematology or clinical chemistry alterations were noted at any dose. Absolute and relative kidney weights and relative liver weight were significantly increased in females at all doses. However, the toxicological significance of these effects is unclear given the lack of histological or clinical chemistry effects. No significant effects on the weight of reproductive organs, sperm parameters or oestrous cycle were noted at any dose (National Toxicology Program, 2010).

Based on the lack of clear treatment-related effects at any dose, a No Observed Adverse Effect Level (NOAEL) of 50 000 mg/kg diet (reported to be 4240 and 4250 mg/kg bw/day in males and females, respectively) can be identified from this study.

This study has been classified as Klimisch Code 1 (reliable without restrictions) on the basis that it is a well-documented and scientifically acceptable study.

The National Toxicology Program (2010) also conducted a similar study in mice. In a 14-week study, B6C3F1 mice (10/sex/dose) were administered chromium (III) as chromium picolinate via the diet at doses of 0, 80, 240, 2000, 10 000 or 50 000 mg/kg diet (reported to be 0, 17, 50, 450, 2300 and 11 900 mg/kg bw/day in males and 0, 14, 40, 370, 1775 and 9140 mg/kg bw/day in females, respectively.

No effects on bodyweight, bodyweight gain or food consumption were noted and no clinical signs of toxicity were noted at any dose. There were also no significant haematological effects, organ weights changes or effects on reproductive organs at any dose (National Toxicology Program (2010)).

Therefore, NOAELs of 50 000 mg/kg diet (reported to be 11 900 and 9140 mg/kg bw/day in males and females, respectively) can be identified.

This study has been classified as Klimisch Code 1 (reliable without restrictions) on the basis that it is a well-documented and scientifically acceptable study.

In a 26-week study, 6-week old male obese Zucker rats (10/dose) were administered chromium (III) as chromium picolinate via their diet at doses of 0, 5 or 10 mg chromium/kg diet for 20 weeks. An additional group of 10 6-week old lean Zucker rats served as an additional control. Dietary doses of 0, 5 or 10 mg chromium/kg diet were calculated to be equivalent to bodyweight of 0, 0.19 ± 0.02 and 0.41 ± 0.02 mg chromium/kg bw/day, or 0, 1.58 ± 0.16 and 3.29 ± 0.12 mg chromium picolinate/kg bw/day, respectively. As chromium has an essential role in insulin metabolism, it has been suggested that chromium (III) supplementation may prevent obesity.

One control obese rat and one rat administered 5 mg chromium/kg diet died before the end of the study. Bodyweight gain was increased in all rats, including the controls, but was significantly greater in obese rats compared to the lean rats. No effect on bodyweight was observed due to chromium picolinate administration.

A number of effects were reported to occur in obese rats compared to lean rats, including increased kidney weight, fasting plasma glucose, plasma insulin concentration, Homeostatic Model Assessment (HOMA) insulin resistance index, albumin and 8-oxo-2'-deoxyguanosine (8-OHdG; a measure of oxidative stress, which may indicate damage to DNA) excretion, and

histopathological changes to the pancreas and kidneys. However, none of these effects were attributable to administration of chromium picolinate treatment.

Obese rats displayed higher water intake and excretion than lean rats, with this effect being significant in chromium picolinate-treated rats. However, the ratio of urine output to water intake and urine osmolality was similar among all groups. Urinary monocyte chemotactic protein-1 (MCP-1) concentration was significantly increased in untreated obese rats compared to lean rats. However, MCP-1 excretion was attenuated in chromium picolinate-treated rats.

These data appear to indicate that dietary administration of chromium picolinate to Zucker rats did not result in any significant changes to the glycaemic status of the rats or increase the risk of oxidative damage (Mozaffari *et al.*, 2009).

This study has been classified as Klimisch Code 2 (reliable with restrictions), on the basis that although it appears to be a well conducted and reported study, it does not follow any internationally recognised guideline.

In a chronic study, male and female Sprague-Dawley rats were administered chromium (III), as niacin bound chromium (NBC), in their diet for several phases over 1.2 years (Perricone *et al.*, 2010). During phase 1A, male and female rats were administered NBC in their diet at doses of 0, 2.8, 8.4 or 28 mg/kg diet during days 0-150 of the study. During phase 1B (days 151-312 of the study), half of the rats administered 8.4 and 28 mg/kg diet were administered control diet (satellite groups), while all rats at 2.8 mg/kg diet and remaining rats at 8.4 and 28 mg/kg diet were continued with their respective concentrations. Only male rats were continued to phase 2 (days 313-460 of the study). During phase two, rats in the main study and in the satellite groups were administered 8.4 mg/kg diet.

Phase 1A and 1B

Systolic blood pressure was significantly decreased in both sexes at the mid and top doses and was also significantly decreased at the low dose for most of phase 1A and 1B, but began to increase from 3 months of the study, and was not significantly different from the control by the end of phase 1B. Average glucose level was significantly decreased in males at the top dose by the end of phase 1A. Glycosylated haemoglobin levels were significantly decreased in males at the mid and top doses and in females at the top dose. Glucose tolerance tests (GTT) indicated significant decreased glucose levels in males at the top dose and females at the mid and top doses. However, no significant effects on insulin concentrations were noted.

Following administration of control diets to the satellite rats, systolic blood pressure returned to baseline levels after 2-3 months. Following administration of a nitric oxide synthase (NOS) inhibitor towards the end of phase 1B, systolic blood pressure was significantly increased in

males of the main study at the mid and top doses, and females of the main study at the top dose. No significant effect on systolic blood pressure was noted on satellite rats.

Phase 2

During phase two, male rats in the main study (of 0, 2.8, 8.4 or 28 mg/kg diet) and in the satellite groups (8.4 and 28 mg/kg diet) were administered 8.4 mg/kg diet. However, for the ease of reporting, these rats will continue to be referred to as low, mid and top dose and satellite rats. Bodyweight was significantly decreased in all rats at all doses. Glucose challenge revealed significantly decreased glucose levels at all doses. However, no significant differences in insulin levels were noted. Circulating glucose levels were significantly decreased and circulating creatinine levels were significantly increased at the mid dose. The authors suggested the effects on creatinine levels were likely secondary to muscle build-up, rather than renal failure (Perricone *et al.*, 2010).

The authors concluded that these data indicate that long-term administration of NBC at sufficient doses may have beneficial effects and there was no evidence of adverse effects to rats in this study.

This study has been classified as Klimisch Code 2 (reliable with restrictions).

In a 2-year carcinogenicity study F344/N rats (50/sex/dose) were administered chromium (III) as chromium picolinate via their diet at concentrations of 0, 2000, 10 000 or 50 000 mg/kg diet.

A decreased survival was noted in males. However, this effect was not considered to be treatment related. No effect on survival was noted in females at any dose. No effects on bodyweight or food consumption or incidence of non-neoplastic lesions were noted in either sex at any dose. In males, the incidence of preputial gland adenoma was significantly increased at the mid dose. No increase in the incidence of preputial gland hyperplasia or preputial gland carcinoma was noted at any dose. In females, there was no increase in the incidence of clitoral gland hyperplasia, adenoma or carcinoma at any dose.

The authors considered that the evidence for carcinogenicity in male rats was equivocal, but there was no evidence for carcinogenicity in female rats (Stout *et al.*, 2009).

Based on the lack of treatment related effects at any dose, a NOAEL of 50 000 mg/kg diet (reported to be 286.2 and 313.7 mg chromium (III)/kg bw/day in males and females, respectively) can be identified.

This study has been classified as Klimisch Code 1 (reliable without restrictions), on the basis that is a well reported and conducted study.

In a similar study, B6C3F1 mice (50/sex/dose) were administered chromium (III) as chromium picolinate via their diet at concentrations of 0, 2000, 10 000 or 50 000 mg/kg diet for 105 weeks from 5-6 weeks of age.

No effects on survival or food consumption were noted in either sex at any dose. Bodyweight was transiently decreased by 10% in treated females during the study, but was comparable to the control at the study termination. No effects on bodyweight were noted in males at any dose. No clinical signs of toxicity and treatment-related neoplastic or non-neoplastic lesions were noted in either sex at any dose (Stout *et al.,* 2009).

Based on the lack of treatment related effects at any dose, a NOAEL of 50 000 mg/kg diet (reported to be 783 and 727.5 mg chromium (III)/kg bw/day in males and females, respectively) can be identified.

This study has been classified as Klimisch Code 1 (reliable without restrictions), on the basis that is a well reported and conducted study.

3.4.2 Chromium (VI)

In a limited study, Wang *et al.* (2006) administered chromium (VI) as potassium dichromate to Swiss mice at doses of 0, 25, 50 or 100 mg/kg bw/day for 1 or 5 days. Examination of effects was limited to oxidative stress in the liver and kidneys and DNA damage in peripheral blood lymphocytes.

In the liver, levels of reactive oxygen species were increased and doses dependent decreases in superoxide dismutase and catalase activities were noted at all doses after 1 and 5 days administration of potassium dichromate. No effect was noted on malondialdehyde levels. No evidence of oxidative stress was noted in the kidneys. Dose and time dependent DNA damage was observed in the COMET assay (Wang *et al.*, 2006).

This study has been classified as Klimisch Code 2 (reliable with restrictions), on the basis that the study is reasonably well conducted, but only considers a few effects in two organs and no examination of pathological effects to the target tissues was conducted.

In a 30-day study, Nudler *et al.* (2009) examined the effect of chromium (VI) on the hypothalamus and anterior pituitary gland of male Wistar rats. Rats were administered chromium (VI), as potassium dichromate, via their drinking water at doses of 0 or 30 mg/l. No effects on water consumption, food consumption or bodyweight were noted at any dose. Accumulation of chromium varied between tissues, with chromium increases of 100, 10 and

4-fold in the liver, anterior pituitary and hypothalamus, respectively. The effects of chromium administration on lipid peroxidation and antioxidant enzyme activities are reported in Table 3.7.

Table 3.7 Effects of chromium administration on lipid peroxidation and antioxidant enzyme activities in the liver, hypothalamus and anterior pituitary (Nudler *et al.*, 2009)

Assessment	Organ	Effect
Lipid peroxidation	Hypothalamus and anterior pituitary	Increased
	Liver	No effect
Superoxide desmutase (SOD) activity	Anterior pituitary	Increased
	Hypothalamus and liver	No effect
Catalase activity	Liver	Increased
	Hypothalamus and anterior pituitary	No effect
Glutathione reductase activity	Hypothalamus	Increased
	Anterior pituitary and liver	No effect
Glutathione peroxidase (GPx) activity	Hypothalamus, anterior pituitary and liver	No effect

Nudler *et al*. (2009) also examined the expression of mRNA of haem oxygenase-1 (HO-1), an adaptive mechanism that serves to protect cells from damage due to oxidative stress, and two metallothioneins, MT-1 and MT-3, proteins that have a role in controlling the detoxification of heavy metals. HO-1 mRNA was significantly increased in the hypothalamus and the anterior pituitary, but was not increased in the liver. MT-3 mRNA expression was significantly increased in the hypothalamus, but was not expressed in the anterior pituitary. MT-1 mRNA expression was increased in the anterior pituitary, but was not increased in the hypothalamus.

This study has been classified as Klimisch Code 2 (reliable with restrictions), on the basis that although well conducted and reported, only a single dose was used, and it is not a regulatory study.

In a 3-month study, F344/N rats (10/sex/dose) were administered chromium (VI) as sodium dichromate dihydrate via their drinking water at doses of 0, 62.5, 125, 250, 500 or 1000 mg/l. Based on water consumption data, these doses were reported to be equivalent to 0, 5, 9, 17, 32 and 60 mg/kg bw/day in males, and 0, 5, 10, 18, 33 and 61 mg/kg bw/day in females, respectively. No effect on survival was noted in either sex at any dose. Bodyweight gain was slightly, but significantly, reduced in both sexes at the top dose. Water consumption was decreased in both sexes at the top three doses. No clinical signs of toxicity were noted at any dose.

Bile acid concentration was significantly increased in males at the top two doses and in females at all doses except 250 mg/l with clinical chemistry data suggesting the cause was being hepatocellular effects rather than cholestasis.

Absolute and relative liver weights were significantly decreased in males at the top two doses. Absolute spleen weight was significantly decreased in males at the top two doses. Relative spleen and kidney weights were increased in females at the top two doses.

Increased incidences of non-neoplastic lesions of the glandular stomach were noted in both sexes at the top dose. Minimal to mild histiocytic cell infiltration of the pancreatic lymph node was noted in females at the top dose and males at all doses except 125 mg/l. Minimal to mild histiocytic cell infiltration was noted in both sexes at the top three doses (National Toxicology Program, 2007).

Based on alterations in haematological parameters, a Lowest Observed Adverse Effect Level (LOAEL) of 62.5 mg/l (reported to be 5 mg/kg bw/day in males and females) can be identified.

This study has been classified as Klimisch Code 1 (reliable without restrictions), on the basis that is a well reported and conducted study.

In another 3-month study, B6C3F1 mice (10/sex/dose) were administered chromium (VI) as sodium dichromate dihydrate via their drinking water at doses of 0, 62.5, 125, 250, 500 or 1000 mg/l (reported to be 0, 9, 15, 26, 45 and 80 mg/kg bw/day, respectively).

Bodyweight gain was significantly increased in both sexes at the top four doses, while bodyweight gain was decreased in males at the low dose. Water consumption was decreased in males at the top three doses and females at the top four doses. No clinical signs of toxicity were noted at any dose. Haematology revealed decreased mean cell volume in both sexes at all doses.

Absolute liver weight was significantly decreased in males at the top three doses and in females at the top dose. Relative liver weight was significantly increased in males at the top dose.

A significantly increased incidence of minimal to mild epithelial hyperplasia was noted in the duodenum of both sexes at all doses, with the severity increasing with increasing chromium concentration. Increased incidence of minimal to mild histiocytic cellular infiltration was noted in males at 125, 250 and 1000 mg/l and in females at the top four doses doses (National Toxicology Program, 2007).

Based on alterations in haematological parameters, a LOAEL of 62.5 mg/l (reported to be 9 mg/kg bw/day) can be identified.

This study has been classified as Klimisch Code 1 (reliable without restrictions), on the basis that is a well reported and conducted study.

The US National Toxicology Program (NTP) has also conducted comparative 3-month toxicity studies in three strains of male mice. B6C3F1, BALB/c and am3-C57BL/6 (10/strain/dose). were administered chromium (VI) as sodium dichromate dihydrate via their drinking water at doses of 0, 62.5, 125 or 250 mg/l (reported to be 0, 8, 15 and 26 mg/kg bw/day in B6C3F1 mice, 0, 9, 14 and 24 mg/kg bw/day in BALB/c mice and 0, 8, 15 and 25 mg/kg bw/day in am3-C57BL/6, respectively).

Comparison of these three strains of mice produced broadly similar toxicological responses. There was evidence of glycogen depletion in B6C3F1 and am3-C57BL/6 mice. However, this may have been due to decreased food consumption. There was also some evidence of alterations in serum alanine aminotransferase levels in BALB/c and am3-C57BL/6 which were not apparent in B6C3F1 mice. However, overall the authors concluded that any differences between strains were likely to be due to nutritional inequalities rather than a toxic effect (National Toxicology Program, 2007).

This study has been classified as Klimisch Code 2 (reliable with restrictions), on the basis that is a well reported and conducted study. However, only partial histopathological examinations were conducted.

Thompson *et al.* (2011) administered sodium dichromate dihydrate to female B6C3F1 mice (10/dose) in their drinking water for 3 months at concentrations of 0, 0.3, 4, 14, 60, 170 and 520 mg/l (equivalent to 0, 0.1, 1.4, 4.9, 20.9, 59.3 and 181 mg chromium (VI)/l, respectively), with an interim kill at 8 days.

At the 8-day interim sacrifice, microscopic examinations revealed villious atrophy and crypt cell hyperplasia in the duodenum at the top dose and cytoplasmic vacuolisation of the villious epithelium at the top two doses. Cytoplasmic vacuolisation of the villious epithelium was also noted in the jejunum at the top two doses, but the effect was milder and less prevalent than in the duodenum.

Glutathione disulphide (GSSG) was increased in the oral epithelium at the top dose and glutathione (GSH)/GSSG ratio was significantly decreased at the top two doses. In the duodenum, GSSG was increased, GSH/GSSG ratio was significantly decreased at the top three doses and redox potential was decreased at the top two doses. In the jejunum, GSH levels were decreased and redox potential was increased at the top two doses.

In the plasma, GSH and GSSG levels were increased at the top three doses, but there was no alteration in redox potential.

At the end of the study, bodyweight was decreased at the top dose and water consumption was decreased at the top two doses.

Cytoplasmic vacuolisation of the villious epithelium of the duodenum was noted at the top three doses. Villious atrophy was noted in one mouse and apoptosis were noted at the top two doses. Crypt cell hyperplasia was noted at the top dose. Histocytic cellular infiltration of the villous lamina propria was noted in all mice at the top two doses and fused cells in the villous lamina propria were noted at the top dose.

In the jejunum, cytoplasmic vacuolisation was noted in at the top three doses, crypt cell hyperplasia was at the top two doses, and villous atrophy was noted at the top dose. Histocytic cellular infiltration of the villous lamina propria was noted at the mid and top doses.

In the duodenum, GSH and GSSG levels and GSH/GSSG ratios were significantly altered at the top four doses. In the jejunum, GSH/GSSG ratio was significantly decreased at the top four doses. In the plasma, GSH and GSSG levels were significantly increased at 14 and 60 mg/l, and GSH/GSSG ratio was significantly decreased at the top two doses.

No significant increase in 8-OHdG was noted at any dose in the duodenum or oral mucosa, indicating that treatment did not induce oxidative DNA damage.

Significant alterations in cytokine levels were noted in the duodenum at the top three doses, however, with the exception of tumor necrosis factor-α (TNFα) and interleukin-1β (IL-1β), these effects were not dose-related decrease. Both TNFα and IL-1β are regulated by NF-κB, and the authors suggest that chromium (VI) may disrupt this inflammatory signal, or a decrease in protein expression levels due to toxicity.

Measurement of total chromium content in tissues indicated that total chromium content was significantly increased in the oral cavity, glandular stomach, jejunum and ileum at the top three doses, and in the duodenum at the top four doses.

A NOAEL of 4 mg/l (reported to be 1.4 mg chromium (VI)/l; approximately 0.35 mg/kg bw/day) can be identified from this study based on alterations of GSH and GSSG levels.

This study has been classified as Klimisch Code 2 (reliable with restrictions), on the basis that is a well reported and conducted study. It is not stated if 8-OHdG was measured in the jejunum. However, as the results from the study seem to indicate that the duodenum is the most sensitive tissue, and no effect was noted in the duodenum, it is presumed that no effect was observed in the jejunum.

In a 6-month study, Davidson *et al.* (2004) investigated the potential for chromium (VI) exposure to increase susceptibility to UV-induced skin tumours. Female CRL:SK1-hrBR mice were administered chromium (VI), as potassium chromate, in their drinking water at doses of

0, 2.5 or 5 mg/l without UV exposure or 0, 0.5, 2.5 or 5 mg/l with UV exposure. No visible signs of skin tumours were noted in mice administered potassium without UV exposure. However, potassium chromate increased the incidence of skin tumours in UV exposed mice in a dose-dependent manner. In mice that were not administered UV treatment, a significant increase in the levels of chromium in the skin was noted at the top dose. In mice administered UV and chromium (VI), levels of chromium in the skin were significantly increased at the top dose, and levels in the area of the skin exposed to UV were significantly higher than in unexposed areas of the skin.

The authors suggest that chromium (VI) may enhance the UV-induction of skin cancers, possibly by UV increasing the ability of chromium (VI) to penetrate cells. However, without detailed pathology of the nature of these tumours and data from a second test species under similar condition, caution should be taken in interpreting these data.

This study has been classified as Klimisch Code 2 (reliable with restrictions), on the basis that is a well reported and conducted study. However, limited information on these tumours was reported.

In a 2-year study, F344/N rats (50/sex/dose) were administered chromium (VI) as sodium dichromate dihydrate via drinking water at concentrations of 0, 14.3, 57.3, 172 or 516 mg sodium dichromate dihydrate/l (equivalent to 0, 5, 20, 60 and 180 mg chromium/l, respectively) for two years. Based on water consumption data, 0, 14.3, 57.3, 172 or 516 mg/l was reported to be equivalent to 0, 0.6, 2.2, 6 and 17 mg/kg bw/day in males, and 0, 0.7, 2.7, 7 and 20 mg/kg bw/day in females, respectively.

No significant effects on survival were noted at any dose. Bodyweight was decreased in both sexes at the top dose at the end of the study. However, this affect was attributed to poor palatability of the water rather than a toxic effect. Water consumption was decreased at the top two doses throughout the study.

Haematological examinations revealed dose-related evidence of anaemia that started on day 4 and persisted throughout the study at the top two doses. The anaemia was most severe during examinations on day 22 or month 3, but became more tolerable over time. Decreased packed cell volume, haemoglobin and erythrocyte counts were noted at the top three doses, and was most severe on day 22.

Incidences of squamous cell carcinomas of the oral mucosa were significantly increased in both sexes at the top dose. Squamous cell carcinomas of the oral mucosa were also noted in two females administered 171 mg/l, which exceeded the historical control range. The incidence of squamous cell papilloma or squamous cell carcinomas (combined) of the oral mucosa or the tongue was significantly increased in both sexes at the top dose.

In the liver, incidences of minimal to moderate histiocytic cellular infiltration was significantly increased in males at the top dose and in females at the top three doses, and the incidence of chronic inflammation (generally minimal to mild) was significantly increased in males at 172 mg/l and in females at all doses.

In the small intestine, the incidences of minimal to mild histiocytic cellular infiltration of the duodenum was significantly increased in males at the top three doses and in females at the top two doses. The incidences of histiocytic cellular infiltration were also increased in the mesenteric lymph node in males at the top three doses and in females at the top two doses, and in the pancreatic lymph node in males at the top dose and in females at the top three doses.

The incidence of minimal lymph node haemorrhage in the mesenteric lymph node was significantly increased in males at the top three doses and in females at the top dose.

The US National Toxicology Program (NTP) concluded that there was clear evidence of carcinogenicity in the oral cavity of male and female rats in this study (National Toxicology Program, 2008b). It is worth noting that these tumours occurred at concentrations at, and above, the reductive capacity identified by Proctor et al. (2012) for rats of 60 mg chromium (VI)/l.

Based on the incidence of chronic inflammation of the liver occurring in females at all doses, a LOAEL for non-carcinogenic endpoints of 14.3 mg/l (17 mg/kg bw/day and 20 mg/kg bw/day in males and females, respectively) can be identified from this study.

This study has been classified as Klimisch Code 1 (reliable without restrictions), on the basis that is a well reported and conducted study.

In a 2-year study, male B6C3F1 mice (50/dose) were administered chromium (VI) as sodium dichromate dihydrate via drinking water at concentrations of 0, 14.3, 28.6, 85.7 or 257.4 mg/l and female were administered sodium dichromate dihydrate via drinking water at concentrations of 0, 14.3, 57.3, 172 or 516 mg/l. Based on water consumption data, doses were reported to be equivalent to 0, 1.1, 2.6, 7 and 17 mg/kg bw/day in males, and 0, 1.1, 3.9, 9 and 25 mg/kg bw/day in females, respectively.

No significant effects on survival were noted at any dose. Bodyweight was decreased in females at the top two doses at the end of the study. However, this affect was attributed to poor palatability of the water rather than a toxic effect. Water consumption was decreased in both sexes at the top two doses throughout the study. No clinical signs of toxicity were noted at any dose. Haematological examinations in female mice revealed minimal, but statistically significant, evidence of anaemia at the top two doses. Erythrocyte counts were slightly increased at all doses, with a consistent, statistically significant increase at the top dose.

Histopathological examinations of the small intestine revealed a concentration-related increase in the incidence of adenoma of the duodenum in both sexes, with statistically significant increases occurring in males at the top dose and in females at the top two doses. The incidences of carcinoma of the duodenum were increased in both sexes at the top two doses (statistically significant in females at the top dose) and exceeded the historical control range for in both sexes at the top dose. In the jejunum of the small intestine, the incidence of adenomas was significantly increased in females at the top dose. Incidence of carcinoma in the jejunum exceeded the historical control range in females at the top two doses. The incidences of adenoma or carcinoma (combined) for the entire small intestine (duodenum, jejunum and ileum) were significantly increased in both sexes at the top two doses.

In the liver, decreased incidences of neoplastic lesions were noted. However, NTP state that the toxicological significance of this effect is unclear.

The incidence of histiocytic cellular infiltration of the liver was significantly increased in females at all doses, and the incidence of chronic inflammation of the liver was significantly increased in females at the top dose. Minimal to moderate histiocytic cellular infiltration was noted in the mesenteric lymph node in both sexes at all doses and in the pancreatic lymph node of both sexes at the top two doses. Incidences of cytoplasmic alteration of the pancreatic acini were increased in males at the top two doses and in females at all doses.

The US National Toxicology Program (NTP) concluded that there was clear evidence of carcinogenicity in the duodenum and jejunum of male and female mice in this study (National Toxicology Program, 2008b). These tumours occurred at concentrations that are broadly similar to, although in some cases, slightly below, the reductive capacity for chromium (VI) reported in mice by Proctor *et al.* (2012) of 21 mg/l.

Based on the incidence of histiocytic cellular infiltration, a LOAEL for non-carcinogenic endpoints of 14.3 mg/l (1.1 mg/kg bw/day in males and females) can be identified from this study.

This study has been classified as Klimisch Code 1 (reliable without restrictions), on the basis that is a well reported and conducted study.

3.5 Reproductive and Developmental Toxicity

3.5.1 Chromium (III)

Bailey *et al.* (2008) have reported on the potential for developmental toxicity following administration of two chromium (III) compounds; chromium picolinate or triaqua-µ3-oxohexakis-µ-propionatorichromium(1+) in the diet of CD-1 mice. Chromium picolinate was administered at 200 mg/kg diet (reported to be 25 mg chromium/kg bw/day) and triaqua-µ3-oxohexakis-µ-propionatorichromium(1+) was administered at 15 or 120 mg/kg diet (reported

to be 3.3 and 26 mg/kg bw/day, respectively). No effects on food consumption and no clinical signs of toxicity were noted in any of the dams at any dose. No effects on foetal weight, number of live or dead foetuses or incidence of gross or skeletal malformations were noted at any dose. However, this study reports a high incidence of cervical arch defects in the control offspring, which may have masked the significance of this effect in chromium-treated foetuses.

This study has been classified as Klimisch Code 3 (not reliable), as there are several details lacking from the paper (e.g. number of mice treated at each dose and haematology, clinical chemistry and gross pathology of parental mice).

Deshmukh *et al.* (2009a) has conducted a 2-generation study in rats administered chromium (III) as a novel oxygen-coordinated niacin-bound chromium(III) complex (NBC). Thirty rats per sex per dose were administered NBC in their diet at concentrations of 0, 4, 15 or 60 mg/kg diet for 10 weeks prior to mating and throughout mating until termination at weaning. At weaning, one male and one female pup from each litter were selected for the F1 generation and underwent the same treatment from 10 weeks prior to mating to produce the F2 generation.

No effects on mortality, food consumption, clinical signs of toxicity, bodyweight, reproductive performance, oestrous cycle length, sperm parameters or live birth indices were noted at any dose in either generation. Necropsy of parental animals revealed no effects on absolute or relative organ weights and histopathological examinations did not reveal any treatment-related effects. No effect on offspring bodyweight, organ weights, survival, clinical signs of toxicity or time to sexual maturation at any dose in either generation (Deshmukh *et al.,* 2009a).

Based on the lack of effects at any dose, parental, reproductive and offspring NOAELs of 60 mg/kg diet (reported to be 5.88, 8.24, 9.71 and 9.83 mg/kg bw/day in F0 males, F0 females, F1 males and F1 females, respectively) can be identified from this study.

This study has been classified as Klimisch Code 1 (reliable without restrictions), on the basis that is a well reported and conducted study.

In an extension to the 2-generation reproductive study described above, male and female Sprague-Dawley rats (25/sex/dose) from the F2b generation were mated at 10-12 weeks of age and pregnant females were administered an oxygen co-ordinated niacin-bound chromium (III) complex (NBC) in their diet at concentrations of 0, 4, 15 or 60 mg/kg diet (approximately 0, 0.2, 0.75 and 3 mg/kg bw/day, respectively) until day 20 of gestation. In dams, no effects on bodyweight, clinical signs of toxicity, mating behaviour, maternal deaths during pregnancy, number of pregnant/non-pregnant females, pregnancy rate or resorption rate were noted at any dose. Necropsy revealed no treatment-related effects. No treatment-related effects on

absolute and relative uterus weight, number of corpora lutea, number of implantations, number of live or dead implants, number of early and late resorptions or pre- and post-implantation loss at any dose. No significant effects were noted on litter size, number of foetuses, sex ratio or foetal weight at any dose. No treatment-related effects on external examinations, the incidence of soft tissue or skeletal alterations were noted at any dose (Deshmukh *et al.*, 2009b).

Based on the lack of treatment-related effects at any dose, maternal and developmental NOAELs of 60 mg/kg diet (approximately 3 mg/kg bw/day) can be identified.

This study has been classified as Klimisch Code 1 (reliable without restrictions), on the basis that is a well reported and conducted study.

3.5.2 Chromium (VI)

In a limited developmental study, Soudani *et al.* (2011) examined the effects of chromium (VI) (as potassium dichromate; $K_2Cr_2O_7$) to the kidneys of pregnant Wistar rats and their offspring. Rats were administered potassium dichromate via their drinking water at concentrations of 0 or 700 mg/l (approximately 0 and 67 mg/kg bw/day, respectively) from the 14th day of pregnancy until 14 days after delivery. Alterations in a number of kidney enzymes and evidence of oxidative stress were noted in treated dams and their pups. Histopathological examinations revealed infiltration of mononuclear cells, necrosis and vascular congestion in the kidneys of treated dams and their offspring. These results suggest that administration of potassium dichromate via drinking water to pregnant rats produced kidney damage in both the parental animals and their offspring.

This study has been classified as Klimisch Code 3 (not reliable), as although it is a well reported study, only a single dose was used, and endpoints other than those relevant to the kidney were not considered. Therefore, the developmental effects cannot be fully evaluated.

Banu *et al.* (2008) considered the effects of exposure of chromium (VI) via lactation on the development of sexual maturity in rats. Lactating female rats were administered chromium (VI) as potassium dichromate via drinking water at a concentration of 0 or 200 mg/l with or without vitamin C at a concentration of 500 mg/l during postnatal days 1-21. During this time, pups were fed on their mother's milk and continued on a regular diet and water from day 21.

In vitro studies were also conducted in a spontaneously immortalised rat granulosa cell line (SIGC). These cells were treated with chromium (VI) at concentrations of 0 or 12.5 µM chromium (VI), with and without 1 mM vitamin C for 12 or 24 hours. Ribonucleic acid was extracted and mRNA expression of a range of proteins associated with steriodogenesis, follicular development and expression of oestrogens.

In the pups of rats administered chromium, concentrations were significantly increased in the plasma and ovarian tissues of pups, but gradually decreased with age. In the pups of rats administered chromium and vitamin C, plasma and ovarian chromium levels were decreased compared to chromium-only exposed pups, but were still higher than control pups. The onset of puberty was significantly delayed in chromium treated rats. However, in the pups of rats where chromium treatment was supplemented with vitamin C no significant alteration in the onset of puberty was observed. In the pups of chromium treated rats, the duration of the oestrous cycle was significantly extended during the dioestrous phase. No alterations in the length of the proestrous, oestrous or metoestrous phases were observed. Supplementation with vitamin C mitigated the effects of chromium on the dioestrous phase. The effects of chromium (VI) exposure, with and without vitamin C supplementation in the pups on ovarian follicle number and hormone levels are reported in Table 3.8.

Table 3.8 Effect of lactational exposure to chromium (VI) on ovarian follicle number and hormone levels

Postnatal day	Effect of lactational exposure to chromium (VI)	Effect of lactational exposure to chromium (VI) and vitamin C
21	Significantly decreased primordial, primary and secondary follicle numbers, and no antral follicle development was detected	Vitamin C mitigated the effects on pup antral follicle development
	Decreased plasma oestradial and progesterone levels	Vitamin C mitigated these effects
	Decreased plasma luteinising hormone (LH) and follicle stimulating hormone (FSH)	Vitamin C mitigated these effects
	Decreased growth hormone level	Vitamin C reduce this effect, but did not return growth hormone levels to control level
	Decreased prolactin levels	Vitamin C mitigated this effect
45	Significantly decreased the number of primordial, primary, secondary and antral follicles	Vitamin C mitigated these effects
	Decreased plasma LH and FSH	Vitamin C mitigated these effects
	Decreased plasma oestradial and progesterone levels	Vitamin C mitigated these effects
	Decreased growth hormone level	Vitamin C reduce this effect, but did not return growth hormone levels to control level
	Decreased prolactin levels	Vitamin C mitigated this effect
65	Decreased primordial and primary follicle	Vitamin C mitigated these effects
	Decreased plasma oestradial and progesterone levels	Vitamin C mitigated these effects
	Decreased growth hormone level	Vitamin C mitigated this effect
	Decreased prolactin levels	Vitamin C mitigated this effect

In the *in vitro* studies in SIGC cells, an IC50 of 12.5 µM was calculated for potassium chromate. When the cells were pre-treated with 1 mM vitamin C, the IC50 was 200 µM.

Chromium (VI) treatment for 12 and 24 hours decreased the expression of steroidogenic acute regulatory protein (StAR), steroidogenic factor 1 (SF-1) and 17β-hydroxysteroid dehydrogenase 1 and 2 (17β-HSD-1 and -2), FSH, LH, oestrogen receptor-α (ERα) and oestrogen receptor-β (ERβ) mRNA. Pre-treatment with vitamin C completely mitigated the effects of chromium on the expression of all these mRNAs, with the exception of ERβ expression, where vitamin C only partially mitigated this effect.

These data indicate that chromium (VI) can delay the development of puberty in rats by altering expression of mRNA that is involved in the regulation of hormone secretion and that vitamin C can largely mitigate these effects.

This study has been classified as Klimisch Code 2 (reliable with restrictions), on the basis that is a well reported and conducted study. However, the study was not conducted using standard reproductive toxicity study protocols.

In an embryotoxicity/foetoxicity study, Marouani *et al.* (2011) administered chromium (VI) as potassium dichromate in 9% sodium chloride solution via intraperitoneal administration of 0, 1 or 2 mg/kg bw/day to pregnant Wistar rats on days 6-15 of gestation.

No clinical signs of toxicity were noted in dams at any dose. Bodyweight gain, uterine weight and placental weight were significantly reduced in dams treated with chromium, with the effects most severe at the top dose. Examinations of the placenta revealed pathological changes. The number of foetuses per litter, foetal weight, crown rump and number of implantations were significantly decreased in chromium treated groups. Increased incidences of dead foetuses, resorptions and post-implantation losses were noted in chromium treated groups. Examinations of the foetuses revealed oedema and subdermal haemorrhagic patches in the thoracic and abdominal regions of chromium-treated foetuses. Examinations also revealed facial defect, lack of tail and hypertrophy at the top dose. Skeletal examinations revealed incomplete ossification of the nasal, cranium, abdominal and/or caudal bones in foetuses at the low dose and the absence of ossification in the sacral vertebrae at the top dose (Marouani *et al.,* 2011).

Based on reported effects at all doses, maternal, embryo and foetal LOAELs of 1 mg/kg bw/day can be identified.

This study has been classified as Klimisch Code 2 (reliable with restrictions) on the basis that is a well reported and conducted study. However, only two doses were used.

In a 6-month study, wild-caught adult male bonnet monkeys were administered chromium (VI), as potassium dichromate, via their drinking water at concentrations of 0, 50, 100, 200 or 400 mg/l. Additional groups of monkeys were administered 400 mg chromium (VI)/l supplemented with vitamin C at doses of 500, 1000 or 2000 mg/l for 6 months. A further group of monkeys was administered 400 mg chromium (VI)/l for six months and then observed for a further 6 months without treatment.

Sperm count was decreased at the top three doses in a time and dose-dependent manner. Sperm count was significantly decreased after 4, 3 and 2 months at 100, 200 and 400 mg/l, respectively. After cessation of treatment with 400 mg/l, sperm count was restored to normal levels after 3 months. Supplementation with vitamin C mitigated the effects on sperm count. Sperm motility was decreased at the top three doses in a time and dose-dependent manner. Significant decreases in motility were noted after 3, 3 and 2 months at 100, 200 and 400 mg/l, respectively. Following the withdrawal of chromium treatment, sperm motility returned to control levels after 3 months. Supplementation with vitamin C mitigated the effects on sperm motility.

Analysis of the seminal plasma and sperm revealed decreased superoxide dismutase (SOD) activity at the top three doses, which was restored to control levels 4 months after the cessation of treatment. Catalase activity was decreased at the top three doses, and following withdrawal of treatment, was similar to the control in the seminal plasma after 1 month, and in the sperm after 4 months. Glutathione concentrations were decreased at the top two doses, and following withdrawal of treatment, were restored after 5 and 4 months in the seminal plasma and sperm, respectively. Hydrogen peroxide formation was increased at the top three doses. After the withdrawal of treatment, hydrogen peroxide concentrations returned to normal in the seminal plasma and sperm after 4 and 3 months, respectively. Supplementation of treatment with vitamin C mitigated all of these effects (Subramanian *et al.,* 2006).

Based on the lack of effects at the low dose, a NOAEL of 400 mg/l can be identified from this study.

This study has been classified as Klimisch Code 3 (not reliable) as the monkeys were collected from the wild, and it is therefore unclear if they would have been exposed to any external environmental factors prior to this study that would have affected these results.

Bataineh (1997) conducted a limited fertility study in adult male rats. Rats were administered chromium (III), as chromium chloride, or chromium (VI), as potassium dichromate, via drinking water at a concentration of 1000 mg/l for 12 weeks prior to assessment for sexual behaviour and fertility. The number of mounts and number of ejaculating rats were significantly decreased and the post-ejaculatory interval was significantly increased following administration of either chromium chloride or potassium dichromate. Potassium dichromate also increased the time to ejaculation. No effect on fertility was noted with either chromium

chloride or potassium dichromate. However, the total number of resorptions and dead foetuses were increased.

This study has been classified as Klimisch Code 3 (not reliable) as only a single, large dose was used for each chemical, this study is only of limited value for the evaluation the reproductive toxicity.

In a similar study, Elbetieha & Al-Hamood (1997) evaluated the effects of chromium (III), as chromium chloride, and chromium (VI), as potassium dichromate, to fertility in mice via drinking water in a series of experiments. In the first study, male mice were administered chromium chloride at a dose of 0, 1000 or 5000 mg/l or potassium dichromate at a dose of 0, 1000, 2000, 4000 or 5000 mg/l for 12 weeks prior to mating with two untreated females for 10 days. In the second study, female mice were administered either chromium chloride or potassium dichromate at a dose of 0, 2000 or 5000 mg/l prior to mating with untreated males for 10 days. In the third study, body and sexual organ weights were assessed in males administered chromium chloride or potassium dichromate at doses of 2000 or 5000 mg/l and females at a dose of 5000 mg/l.

In the first study, the number of pregnant females was significantly reduced in males administered chromium chloride at the top dose. The number of implantation sites and viable foetuses were significantly decreased in males administered potassium chromate at the top two doses. The number of resorptions or dead foetuses was increased at all doses with both chromium compounds. In the second study, the number of implantations and the number of viable foetuses was significantly decreased in females administered either chromium compound at all doses and number of females with resorptions was also significantly increased in mice administered potassium dichromate.

In the third study, bodyweight was significantly decreased in males at all doses with both chromium chloride and potassium dichromate. Testes weight was significantly increased, and preputial gland weight was significantly increased at both doses of chromium chloride and 5000 mg potassium dichromate/l. Seminal vesicle weights were significantly reduced at 5000 mg chromium chloride/l and both doses of potassium dichromate. No significant effect on female bodyweight. Ovarian weight was significantly increased following administration of either chromium compound, and uterine weight was significantly decreased following administration of chromium chloride.

This study has been classified as Klimisch Code 3 (not reliable) as the limited dose range in the third study means that no evaluation can be made on whether the effects reported in the first two studies were at levels below those that produce parental toxicity, or if the effects on reproductive parameters could be secondary to parental toxicity.

In a teratogenicity study, female Swiss albino mice were administered chromium (VI), as potassium dichromate, via drinking water at doses of 0, 250, 500 or 750 mg/l for 20 days prior to mating with untreated males overnight. Based on water intake, 0, 250, 500 or 750 mg/l was reported to be equivalent to 0, 6.44, 12.2 and 15.28 mg/kg bw/day, respectively. No clinical signs of toxicity and no alterations in behaviour or mortalities were noted at any dose. Decreased maternal bodyweight gain, and increased chromium blood levels were noted at the mid and top doses. Oestrous cycle length was significantly increased at the top dose. The number of corpora lutea, implantations and foetuses per litter were significantly decreased at the mid and top doses and the number of resorptions was increased at all doses. Examinations of foetuses revealed a significant increase in the incidence of sub dermal haemorrhagic patches on the thoracic and abdominal areas and kinky tails at the top dose and incidences of reduced ossification of bones at that the mid and top doses (Kanojia *et al.*, 1996).

Based on the increased incidence of resorptions, a reproductive LOAEL of 250 mg/l (reported to be 6.44 mg/kg bw/day) can be identified from this study, and based on the incidence of reduced bone ossification, a teratrogenic NOAEL of 250 mg/l (reported to be 6.44 mg/kg bw/day) can be identified.

This study has been classified as Klimisch Code 2 (reliable with restrictions).

3.6 Immunotoxicity

In an *in vitro* immunotoxicity assay, Akbar *et al.* (2011) exposed resting and active (anti-CD3 ± anti-CD28 antibodies) primary human lymphocytes were exposed to chromium (VI) in vitro at concentrations of 0.1-100 µM. Concentrations of 10-100 µM chromium (VI) significantly decreased cell viability and increased apoptosis in rested and activated lymphocytes. Cell proliferation and cytokine release were significantly reduced in activated lymphocytes. These data indicate that chromium (VI) may alter immune function by increasing apoptosis and inhibiting cell proliferation and cytokine release.

This study has been classified as Klimisch Code 2 (reliable with restrictions).

Mignini *et al.* (2009) have conducted an investigation of the effects of chromium (VI) on immunological parameters on eighty-four healthy human volunteers (40 exposed, 44 controls) from the shoe, hide and leather industry. Total chromium concentrations in the blood were not significantly different between the exposed and unexposed groups, however, urinary chromium concentrations were significantly higher in exposed volunteers. Particularly high concentrations were noted among 14 exposed individuals, all of whom worked in tanning, therefore the exposed group was sub-divided into lower (Group A) and higher (Group B) exposure. Analysis of neutrophils, macrophages, lymphocytes, lymphocyte phenotypes and cytotoxic natural killer cell-mediated activity revealed no significant differences between any of

the groups. GR density was significantly decreased and lymphocyte proliferation activity was increased in Group B in the presence of the mitogens ConA and PHA. Interleukin-6 (IL-6) and IL-2 levels were significantly increased and IL-12 levels were significantly decreased in Group B. Mignini *et al.* (2009) also conducted an *in vitro* assay using chromium (VI) concentrations of 0.1 and 100 µg/l. At the lower concentration, ConA and PHA increased lymphocyte proliferation activity and LPS inhibited lymphocyte proliferation activity. At the higher concentration, no effect on proliferation activity was noted following exposure to ConA, PHA or LPS.

These data suggest that chromium (VI) may be an immunological environmental stressor, and have biphasic activity on lymphocyte stimulation. At low doses, it may stimulate lymphocyte activity, while at higher doses, it may inhibit lymphocyte proliferation.

This study has been classified as Klimisch Code 2 (reliable with restrictions).

3.7 Human Toxicity

Goullé *et al.* (2011) have reported the case of a 58-year-old male who was admitted to hospital after the accidental ingestion of approximately 30 g potassium dichromate/l, which the authors reported to be equivalent to 3000 mg chromium (VI) (approximately 50 mg/kg bw, based on a 60 kg bodyweight). Although Goullé *et al.* (2011) state that 2000-3000 mg chromium (VI) typically produces gastrointestinal injury and hepatic and renal failure, resulting in rapid death, in this case, the patient was discharged from hospital after 7 days with no evidence of hepatic or renal failure. Over 49 days, chromium concentrations decreased from 2088 µg/l to 5 µg/l, 631 µg/l to 129 µg/l and 3512 µg/g to 10 µg/g creatinine in plasma, red blood cells and urine, respectively. Chromium was more quickly cleared from plasma than from red blood cells, suggesting a cellular trapping of the metal. Chromium elimination in the plasma followed a two-phase model; a first half-life of 5.6 hours and a second half-life of 191 hours were reported. The half-life for the red blood cells was calculated to be 440 hours.

In another case of accidental poisoning, for which only limited details are available, a 1-year-old girl ingested an unreported quantity of chromium (VI), as ammonium dichromate. Prior to death, the child became severely dehydrated and experienced caustic burns to the mouth and pharynx, blood in her vomit, diarrhoea, irregular respiration and laboured breathing. The cause of death was identified as shock and haemorrhage into the small intestine (Reichelderfer, 1968, cited in ATSDR, 2008).

Death has been reported in a 17-year old male following ingestion of chromium (VI), as potassium dichromate, at a dose of 29 mg chromium (VI)/kg bw. Death occurred after 14 hours as a result of respiratory distress and severe haemorrhage. Examinations of the body also revealed caustic burns to the stomach and duodenum and gastric haemorrhage

(Clochesy, 1984, cited in ATSDR, 2008). Death, due to gastrointestinal ulcerations and severe liver and kidney damage, has been reported in a 14-year old boy eight days after ingestion of potassium dichromate at a dose of 7.5 mg chromium (VI)/kg bw (Kaufman *et al.,* 1970, cited in ATSDR, 2008).

Zhang *et al.* (2011) recently conducted a study examining DNA damage in humans as a result of occupational (inhalation) exposure to chromium (assumed to be chromium (VI), although this is not specified in the study) from electroplating in China. 157 electroplating workers and 93 control subjects from 20 electroplating factories in Hangzhou, China, were examined between 2009 and 2010. Considerations were made for age, smoking habits, alcohol consumption and years of exposure.

Blood and urine samples were collected for 8-oxo-2'-deoxyguanosine (8-OHdG) determination, a measure of oxidative stress which can indicate damage to DNA. A modified COMET assay was also conducted, with Olive tail moment, tail length and % tail DNA measurements providing an indication of DNA damage.

Erythrocyte chromium concentrations were significantly higher in occupationally exposed workers than in the controls. The overall concentrations in exposed workers were approximately twice the controls, and erythrocyte chromium concentrations were also significantly increased in smokers when compared to non-smokers. Urinary 8-OHdG was significantly higher in exposed workers than in controls. The COMET assay revealed that Olive tail moment, tail length and % tail DNA were significantly higher in exposed workers than in the controls. These data indicate that there was evidence of oxidative DNA damage following occupational exposure to chromium.

In an epidemiological study conducted in Greece, Linos *et al.* (2011) examined the incidence of death due to cancer in the Oinofita municipality, an area with historic contamination of the water supply from industrial waste containing chromium (VI).

Between November 2007 and February 2008, chromium (VI) was detected in 35 of 87 well water samples in the area at concentrations of 10-156 µg/l. Concentrations of chromium (VI) in public water supplies between September 2008 and December 2008 were 41-53 µg/l.

Measurements of chromium (VI) in drinking water supplies between June 2007 and June 2008 revealed concentrations ranging from 10 µg/l to 51 µg/l. In 2009, the water supply from the district was diverted to receive water from Mornos lake, and concentrations of chromium (VI) decreased to <0.01-1.53 µg/l.

5842 individuals were identified for this study who were legally registered citizens permanently living within Oinofita between 1st January 1999 and 31st December 2009. From this group, a total of 474 deaths were noted, with 118 of these deaths due to cancer.

The number of deaths due to primary liver cancer was statistically significantly increased in both sexes, and was eleven-fold higher than the number of expected deaths. The number of deaths due to kidney and genitourinary cancers were significantly increased in females, and were three-fold higher than the number of expected deaths. The number of deaths due to lung, trachea and bronchial tumours was significantly increased in males, and both sexes when the data were combined, but was not statistically significant in females.

Although this study has a relatively small cohort, the actual intake of chromium has not been measured, and a number of confounding factors, such as the number of smokers have not been considered, it does indicate a significant association between consumption of chromium (VI) via drinking water and incidence of cancer in humans.

Beaumont *et al.* (2008) conducted an evaluation on the incidence of mortality from cancer in China following chromium exposure (presumed by the authors to be chromium (VI) in nine regions near a ferrochromium factory. The factory began small-scale operations in 1959 and was operating at full scale in 1965. From mid-1964, residents from a nearby village had noted that water from drinking wells had turned yellow. In 1965, chromium (VI) was detected in drinking water wells of villages 5.5 km from the factory, and in 1974, chromium (VI) was detected in wells over an 11.25 km² area. Pollution prevention measures were initiated from 1965, and chromium concentrations began to rapidly decrease from 1967. The nine study regions were divided into two groups; five regions where chromium exposure via drinking water was known to have occurred, and four regions that were considered 'unexposed'. The data were too limited to perform assessment of the relative risks based on exposure dose.

The rate of mortality from all cancers was slightly increased in the regions with chromium exposure compared to the unexposed region and compared with the whole province. The incidence of mortality from stomach cancer was significantly increased in the chromium exposed regions compared to the unexposed regions and the whole province. The risk ratio for mortality due to lung cancer was only very slightly increased in the chromium exposed regions compared to the unexposed regions, but was significantly increased compared to the whole province. Risk ratios from mortality from cancers at sites other than the lungs or stomach were not increased in the chromium exposed regions compared to the unexposed regions or the whole province.

The authors conclude that these data are consistent with the theory that chromium (VI) exposure via drinking water may increase the risk of the development of stomach cancers.

3.8 Other Toxicity Studies

3.8.1 Chromium (III)

In a recent study, three chromium (III) complexes, $[Cr(salen)(H_2O)_2]^+$, chromium picolinate and $[Cr(ttpy)_2]^{3+}$, were assessed in their ability to inhibit the formation of transcription factor-DNA (TF-DNA) complexes using two different oligonucleotide sequences (Sp1 and TFIID)

and to inhibit transcription of the cytomegalovirus (CMV) promoter. Chromium picolinate did not inhibit the formation of TF-DNA or the transcription of the CMV promoter. $[Cr(salen)(H_2O)_2]^+$ and $[Cr(ttpy)_2]^{3+}$ inhibited TF-DNA formation in a dose-dependent manner, with $[Cr(salen)(H_2O)_2]^+$ partially blocking TF-DNA formation at doses lower than $[Cr(ttpy)_2]^{3+}$. $[Cr(ttpy)_2]^{3+}$ did not inhibit transcription of the CMV promoter at any dose, but $[Cr(salen)(H_2O)_2]^+$ inhibit transcription in a dose-dependent manner. The authors conclude that some chromium (III) complexes may be able to interact with biological molecules and thus interfere with processes such as transcription, which may result in toxic effects. However, it is unclear whether chromium (III) complexes can cross cell membranes in sufficient concentrations to allow this interaction to occur (Raja *et al.*, 2008).

3.8.2 Chromium (VI)

Asatiani *et al.* (2010) recently investigated the potential for chromium (VI), as potassium chromate, to induce cytotoxic effects in foetal human lung fibroblast (HLF) cells. HLF cells were exposed to chromium (VI) concentrations of 0, 2, 5, 10, 15, 20, 25 or 30 µM for 48 hours and then allowed to continue to grow for either 24 or 48 hours.

Additional assays were conducted measuring DNA content for cell cycle analysis, the formation of Reactive Oxygen Species (ROS) and gluathione reductase (GR) and glutathione peroxidase (GPx).

The results of the cell viability assay are presented in Table 3.9.

Table 3.9 Foetal human lung fibroblast cell viability following exposure to various concentrations of chromium (VI)

Chromium concentration (µM)	Decrease cell viability (%)		Effect of replacement of media with fresh media for 24 hours
	24 hours	48 hours	
2	-	10	Not reported
5	10	15	Decreased viability was completely reversed
10 and 15	20-35	60-70	Decreased cell viability of 15-20%
≥20	50	80	Progressive cell death indicating irreversible chromium toxicity

Asatiani *et al.* (2010) classified the chromium concentrations into three groups; non-toxic (2-5 µM), sub-toxic (10-15 µM) and toxic (>20 µM). For the subsequent assays, concentrations of 5, 15 and 30 µM chromium (VI) were used in 24 hour exposure studies.

In the cell cycle assay, Asatiani *et al.* (2010) concluded that the non-toxic concentration induced cell cycle arrest, the sub-toxic concentration induced growth arrest and the toxic concentration induced apoptosis. In the ROS analysis, 5 and 15 µM significantly increased ROS levels by greater than 2-fold after 2 hours. After 24 hours, the ROS levels at 5 µM had decreased to control levels. ROS levels also decreased at 15 and 30 µM, and were considered to be the result of loss of cell viability and apoptosis. SOD levels were increased at all doses after 2 hours, and was significantly more pronounced at 30 µM. SOD levels were similar to controls after 24 hours following exposure to 5 and 15 µM chromium (VI).

In the GR and GPx assays, 5 µM chromium (VI) produced an intensification of oxidative stress and the successful functioning of reductive enzymes which was apparent after 24 and 48 hours. Treatment with 15 µM produced similar alterations after 2 and 4 hours. However, after 24 hours, GPx activity continued to increase, but GR activity significantly decreased indicating disruption of the glutathione cycle and decreased cell viability. Treatment with 30 µM produced similar results to 15 µM after 2 and 4 hours, but by 24 hours both GR and GPx activities were significantly decreased indicating disruption of the glutathione dependent antioxidant defence system (Asatiani *et al.,* 2010).

In an *in vitro* study, rat hepatocytes were exposed to chromium (VI) ions at concentrations of 0, 50, 100 or 250 µM for three hours. These concentrations were considered by the authors to be typical of those found in the livers of cadavers with worn metal-on-metal hip implants. The effects of exposure on phase I metabolism was measured by measured by the hydroxylation of testosterone and the effects on phase II metabolism was measured by the glucuronidation and sulphonation of 7-hydroxycoumarin (7-HC) and 1-naphthol. Chromium (VI) had no effect on the formation of testosterone metabolites. Glucuronidation and sulphonation of 7-HC and 1-naphthol were inhibited and ATP levels were also reduced. The authors suggested that chromium (VI) may inhibit the formation of 3'-phosphoadenosine-5'-phosphosulphate (PAPS), a co-factor of sulphonation by reducing ATP and competing with sulphate for ATP-sulfurylase (Afolaranmi *et al.,* 2011).

Chang *et al.* (2011) have conducted a combined *in vitro* and *in vivo* study examining the effects of chromium (VI) on cell function of cardiovascular tissue via the inhibition of raf kinase inhibitor protein (RKIP). Raf kinases are protein kinases that form part of the mitogen-activated protein kinase (MAPK) cell-signalling cascade, which regulate a range of transcription factors that alter the expression of proteins related to the cell cycle.

In the *in vivo* study, male Wistar rats (5/dose) were administered chromium (VI) as sodium dichromate via their diet at concentrations of 0, 250, 500, 750, 1000 or 1250 mg/kg diet (reported to be 0, 5, 10, 15, 20 and 25 mg/kg bw/day, respectively) for 60 days. In the *in vitro* study, cultured myocardial cells were exposed to chromium (VI) as sodium dichromate at concentrations of 0, 0.25, 0.5, 1.5, 3 or 4.5 mg/l for 24 hours.

In the *in vivo* study, no significant effect was noted on bodyweight at any dose. Chromium concentrations were significantly increased in heart tissue at all doses in a dose-dependent

manner and RKIP was significantly decreased in at the top three doses. In the *in vitro* study, RKIP expression was significantly decreased in cells at the top four doses. However, a caspase-3 colourimetric assay indicated that chromium (VI) did not induce cell apoptosis. The authors suggest that these results may indicate chromium (VI) exposure may induce decreased heart function.

Wakeman *et al.* (2004) investigated the effects of chromium (VI) on S-phase in HeLa cells (a human cell line derived from cervical cancer cells). HeLa cells were either mock treated or treated with 10 µM chromium (VI) or 6Gy ionising radiation. Chromium (VI) induced Ataxia telangiectasia mutated kinase (ATM)-dependent phosphorylation of Structural Maintenance of Chromosomes 1 (SMC1) protein, activating the S-phase checkpoint, and thereby inducing inhibition of DNA synthesis.

Chiu *et al.* (2010) have reported that chromium (VI) can also arrest the G2 cell cycle checkpoint. The function of this checkpoint is to prevent cells with DNA damage proceed through the cell cycle to the M phase and undergo mitosis. In a study in in human lung epithelial A549 cells treated with chromium (VI) for 24 hours. The percentages in G2/M phase were 10.88, 6.53, 10.02, 18.10, 26.16% at chromium (VI) concentrations of 0, 1, 5, 10 and 25 µM, respectively. Evidence of chromium-induced cell apoptosis was also noted at 25 µM.

Raghunathan *et al.* (2009a) have investigated the chronic effects of chromium (VI), as chromium oxide, on immortalised osteoblasts from a rat neonatal calvaria and human leukemic monocytes. Cells were exposed to chromium (VI) at concentrations of 0.05, 0.1 or 0.5 µM for 4 weeks. Chromium (VI) was more cytotoxic to human monocytes than rat osteoblasts. In osteoblasts, glutathione levels were significantly increased at all doses during the first week of the study and remained significantly increased at the top two doses by week three of the study. Glutathione levels were decreased at the low dose during the third week, and were significantly lower than the control at the end of the study. In monocytes, glutathione levels were significantly increased at the top dose during the second week of the study, but were significantly decreased at the end of the study. At the mid and low doses, glutathione levels were significantly increased in monocytes at the end of the study. Expression of glutathione reductase activity was increased in osteoblasts during the first two weeks of the study. No similar increase in expression was noted in monocytes. Raghunathan *et al.* (2009a) concluded that these data indicate that both osteoblasts and monocytes mounted an adaptive response to chromium (VI) exposure, however, the increase in glutathione reductase expression in osteoblasts suggests that these cells were able to mount a more potent response than monocytes.

Another study on human monocytes by Raghunathan *et al.* (2009b) exposed cells to continuous concentrations of chromium (VI), as chromium oxide, at concentrations of 0.05, 0.1 or 0.5 µM for 4 weeks. Superoxide dismutase (SOD) activity, catalase (CAT) activity and total glutathione and expression of SOD1, SOD2, CAT, GPx1, GSTπ expression were determined. Protein levels were significantly decreased in the low and mid doses at the end of the first week, recovered by the third week and decreased again at the end of the fourth week.

Protein levels were decreased at the top dose throughout the study and continued to decrease over the course of the study. Total SOD activity was significantly increased at the top dose throughout the study and at the mid dose at the end of the 1st week. CAT activity was significantly increased at the top dose during weeks 2 to 4 of the study. Glutathione activity was significantly increased at the top dose throughout the study. SOD1 expression was significantly increased at the mid and top doses during the 2nd week of the study, but was significantly decreased at the top dose by the end of the study. SOD2 expression was significantly increased at the mid and top doses at the end of the 3rd week and significantly decreased at the top dose at the end of the study. CAT expression was significantly increased at the top dose from the 2nd week and at the mid dose at the 4th week. GPx1 expression was significantly increased at the top dose during weeks 2 and 3 and at the mid dose during week 2. GSTπ expression was significantly increased at the top dose during the 1st week, at the mid dose throughout the study, and at the low dose during the first 3 weeks of the study.

The up-regulation of expression of these proteins indicates that chromium (VI) induced an adaptive response for the production of reactive oxygen species savaging enzymes. With the expression of SOD, CAT and GPx at the top dose generally reaching a maximum before the end of the study prior to declining to the end of the study, it would appear these cells were under significant oxidative stress as a result of chronic exposure to chromium.

Xia *et al.* (2011) exposed human bronchial epithelial cells to chromium (VI), as potassium chromate, at doses of 0, 1.56, 3.13, 6.25 or 12.5 µM for 24 hours to investigate the effects of chromium (VI) exposure on biotinidase (BTD) and holocarboxylase synthetase (HCS), two proteins involved in biotin (vitamin B7) homeostasis. BTD levels were significantly decreased at the top dose, and BTD mRNA expression was significantly reduced at the top three doses. HCS levels were significantly increased at the top two doses, while HCS mRNA expression was significantly increased at the bottom two doses and significantly decreased at the top dose.

In the second part of this study, investigations were conducted on cells administered 0, 12.5 or 25 µM potassium chromate. Administration of 5-aza-2'-deoxycytidine (Aza), an inhibitor of DNA methlytransferase activity at doses of 0, 12.5 or 25 µM, had no effect on BTD levels, but significantly increased HCS levels in the controls and at the low dose. Administration of trichostatin A (TSA), an inhibitor of mammalian histone deacetylase, significantly increased the up-regulation of BDT in control cells and at the low dose, and significantly inhibited HCS in at both doses, but not in the control.

These results suggest that chromium (VI) exposure may result in alterations in biotin (vitamin B7) homeostasis by the down-regulation of BTD, and TSA may be able to reverse this inhibition of BTD following chromium exposure, but only at low doses.

In a similar study, J774.1 murine macrophages were exposed to chromium (VI), as chromium oxide at concentrations of 0, 0.1, 0.5, 1, 2.5, 5, 10, 25, 50 or 100 µM for 24 hours. Concentrations of lactate dehydrogenase (LDH), glutathione and oxidized glutathione (GSSG)

were determined. In addition, superoxide desmutase (SOD), glutathione reductase (GR) and catalase activities were measured following exposure of J774.1 murine macrophages to 10 µM chromium oxide. Toxicity was observed in macrophages at concentrations of >1 µM, as measured by the MTT and neutral red assays. Leakage of LDH out of the macrophages was noted at >5 µM. Glutathione concentration was significantly reduced at >25 µM, which was associated with a rise of GSSG excretion out of the cell. It was calculated that oxidation of glutathione to GSSG accounted for 86% of the glutathione loss. No effect was noted on SOD or catalase activity, however GR activity was completely inhibited. The authors suggested that the loss of glutathione at concentrations above the observed level of toxicity indicate that glutathione loss is a consequence, rather than a cause of loss of cell viability (Lalaouni *et al.*, 2007).

In a study of the effects of acute exposure of chromium (VI) on the concentrations of proteins involved in apoptosis, male Swiss albino mice were administered potassium chromate as a single oral gavage dose of 0, 25, 50 or 100 mg/kg bw. The liver was extracted and analysed for protein content. A significantly increase in p53 and a significant decrease in Bcl-2 were noted at the top dose. Bax concentration was increased in a dose-dependent manner, but this effect was not statistically significant. Cytochrome c levels in the cytoplasm were significantly increased at the mid and top doses. These data indicate that chromium (VI) may induce dose-dependent apoptosis in the liver of mice (Wang *et al.*, 2010).

Bagchi *et al*. (2001) have conducted a series of *in vitro* and *in vivo* studies investigating chromium (VI) induced oxidative stress. In the *in vitro* studies, human chronic myelogenous leukaemic K562 cells and normal human donor peripheral blood mononuclear cells (HPBM) were exposed to chromium (VI), as sodium dichromate, at concentrations of 0, 12.5 or 25 µM for 24 or 48 hours. Murine macrophage J774A.1 cells were exposed to concentrations of 0, 0.2, 0.4 or 0.6 µM for 24 or 48 hours. In the *in vivo* studies, female C57BL/6Ntac and p53-deficient C57BL/6TSG p53 mice were administered sodium dichromate as a single oral dose of 95 mg/kg bw and sacrificed after 24 hours for collection of brain and hepatic tissue.

In the *in vitro studies*, extensive cell necrosis was noted in myelogenous leukaemic K562 cells, which prevent analysis of these cells after 48 hours. Apoptotic cell death was not observed in HPBM cells, however, in human chronic myelogenous leukaemic K562 cells, 40% apoptotic cell death and a significant decrease in the proportion of cells in the G2/M phase were noted at 12.5 µM after 24 hours. A summary of the effects measured in these two cell types is provided in Table 3.10. These data indicate that chromium (VI) enhanced reactive oxygen species formation, DNA fragmentation and apoptotic cell pathways in myelogenous leukaemic K562 cells to a greater extent than HPBM cells.

Table 3.10 Effects of chromium (VI) to HPBM and human chronic myelogenous leukaemic K562 cells

Effect	Study duration (hours)	Chromium (VI) concentration (µM)	HPBM cells	Human chronic myelogenous leukaemic K562 cells
Cytochrome c reduction	24	12.5	1.4-fold reduction in cytochrome c	2.7-fold reduction in cytochrome c
		25	1.55-fold reduction in cytochrome c	4.8-fold reduction in cytochrome c
	48	12.5	1.4-fold reduction in cytochrome c	-
		25	1.7-fold reduction in cytochrome c	-
Hydroxyl radical production	24	12.5	2.4-fold increase in hydroxyl radical production	10.7-fold increase in hydroxyl radical production
		25	2.5-fold increase in hydroxyl radical production	14.1-fold increase in hydroxyl radical production
	48	12.5	2.7-fold increase in hydroxyl radical production	-
		25	3.2-fold increase in hydroxyl radical production	-
DNA fragmentation	24	12.5	1.0-fold increase in DNA fragmentation	2.2-fold increase in DNA fragmentation
		25	1.3-fold increase in DNA fragmentation	3.0-fold increase in DNA fragmentation
	48	12.5	1.1-fold increase in DNA fragmentation	-
		25	1.4-fold increase in DNA fragmentation	-

In murine macrophage J774A.1 cells, no effect on succinate dehydrogenase was noted at the low and mid dose (as measured by the reduction of tetrazolium MTT dye) after 24 hours, but a slight, non-significant decrease was noted at the top dose. After 48 hours, a dose-dependent decrease in succinate dehydrogenase activity was noted at all doses. DNA fragmentation after 24 hours was increased by 1.8, 2.8 and 2.9-fold at the low, mid and top dose, respectively. A dose-related increase in apoptotic cell death was noted and cultured cells lost their adhesion to plates and became rounded in appearance.

The results of the *in vivo* studies are presented in Table 3.11. The greater enhancement of cytochrome c reduction, DNA fragmentation and lipid peroxidation in p53 deficient mice suggest that the p53 regulatory protein may have a role in chromium (VI) induced oxidative stress and toxicity.

Table 3.11 Effects of chromium (VI) to C57BL/6Ntac mice and p53-deficient C57BL/6TSG p53 mice

Effect	Organ	C57BL/6Ntac mice	p53-deficient C57BL/6TSG p53 mice
Cytochrome c reduction	Liver	6.0-fold reduction in cytochrome c	11.1-fold reduction in cytochrome c
	Brain	4.1-fold reduction in cytochrome c	7.7-fold reduction in cytochrome c
DNA fragmentation	Liver	2.2-fold increase in DNA fragmentation	4.1-fold increase in DNA fragmentation
	Brain	2.1-fold increase in DNA fragmentation	4.6-fold increase in DNA fragmentation
Lipid peroxidation	Liver	3.3- fold increase in lipid peroxidation	10.8-fold increase in lipid peroxidation
	Brain	3.5-fold increase in lipid peroxidation	7.3-fold increase in lipid peroxidation

3.9 Summary

The toxicology of chromium is highly complex and dependent upon the form of chromium. In general, the data indicate that chromium (III) compounds are far less toxic than chromium (VI) compounds.

Both chromium (III) and chromium (VI) compounds display some evidence of genotoxicity *in vitro*. However, the evidence of *in vitro* genotoxicity is much stronger for chromium (VI) compounds than for chromium (III). The weight of evidence would also suggest that chromium (III) compounds are not genotoxic *in vivo*. *In vivo* studies with chromium (VI) are mixed, with the majority of oral micronucleus studies indicating negative results, but positive results in Comet assays, although this assay has not been validated for all endpoints. These negative results may be a reflection of the reductive capacity of the gastrointestinal tract, which will serve to limit chromium (VI) absorption. However, as several positive results are reported, the possibility of in vivo genotoxicity via the oral route cannot be discounted.

The available literature indicates that there is evidence of chromium (VI)-induced carcinogenicity via the oral route. In a 6-month study, mice administered chromium (VI) in drinking water whilst simultaneously undergoing UV-treatment displayed a dose-related

increase in skin tumours. The significance of this effect is unclear, as this test has only been conducted in a single species, and the nature of these tumours was not reported, however, it may indicate that chromium (VI) has the potential to enhance tumour formation under certain circumstances.

Carcinogenicity has also been reported in two studies conducted by the US National Toxicology Program (NTP). In these studies, rats and mice were administered chromium (VI) via drinking water (concentrations of 0, 14.3, 57.3, 172 or 516 mg sodium dichromate dihydrate/l, 0, 14.3, 28.6, 85.7 or 257.4 mg/l and 0, 14.3, 57.3, 172 or 516 mg/l in rats and male mice and female mice, respectively). Rats displayed significant increases in the incidence of tumours in the oral mucosa, while mice displayed significant increases in the duodenum and jejunum. It is interesting to note that in both these species, these tumours were only observed at the higher doses and were restricted to the early parts of the gastro-intestinal system. This restriction of tumours to the early parts of the gastro-intestinal system is consistent with the reported data on toxicokinetics of chromium, as chromium (VI) undergoes significant reduction to chromium (III) as it passes through the gastro-intestinal system, effectively reducing its ability to be absorbed by tissues in the latter part of the gastro-intestinal system. However, it is somewhat unexpected that tumours formed in the oral cavity of male and female rats, as the transit time through the oral cavity is expected to be very brief, and therefore, significant absorption of material would not normally be anticipated.

A number of *in vitro*, and some *in vivo* studies, have examined the effects of chromium exposure on the expression of a number of proteins, such as gluathione reductase (GR) and glutathione peroxidase (GPx), which respond to oxidative stress, and p53 and other proteins involved in the regulation of the cell cycle and apoptosis. These data suggest that following exposure to low concentrations of chromium (VI), cells adapt by increasing the production of proteins that respond to oxidative stress and arresting the cycle, or induce apoptosis. However, at higher concentrations, chromium (VI) either overwhelms the proteins that deal with oxidative stress, or inhibit their production, and affect some of the proteins that regulate the cell cycle and induce apoptosis. Co-administration of chromium (VI) with ascorbate (vitamin C) appears to mitigate many of the effects of chromium toxicity. Ascorbate is a reducing agent and thus would likely reduce chromium (VI) to chromium (III).

Chromium (VI) may also induce effects on reproductive systems. Banu *et al.* (2008) have reported effects on a range of proteins associated with steroidogenesis, follicular development and expression of oestrogens. Other studies have reported increased resorptions, decreased implantations and alterations of oestrous cycle length.

These data indicate that understanding the toxicity of chromium (VI), particularly in consideration of its induction of genotoxic or carcinogenic effects, is not a simple process. Significant reduction of chromium (VI) to chromium (III) occurs in gastric juices, acting to limit the absorption of chromium. If this process completely reduces chromium (VI) to chromium (III), it would effectively prevent chromium (VI) toxicity. However, there is significant uncertainty in the threshold for this reductive capacity. Furthermore, once absorbed, there is

some evidence that cells appear to be able to adapt to chromium (VI) to mitigate against any toxic effects, assuming the concentrations of chromium (VI) are not high enough to overwhelm cellular function. This would indicate a threshold for toxicity, and as such, when considering an 'acceptable' health-based level of chromium (VI), it would not be appropriate to apply a linear extrapolation (i.e. use of uncertainty factors) from a high dose to derive this acceptable level.

However, as stated above, the data with regards to *in vivo* genotoxicity are incomplete, and overall, mixed results have been reported from *in vivo* assays. The majority of oral micronucleus studies indicating negative results, but positive results in Comet assays, although this assay has not been validated for many endpoints. These negative results may be a reflection of the reductive capacity of the gastrointestinal tract, which will serve to limit chromium (VI) absorption. However, as several positive results are reported, the possibility of *in vivo* genotoxicity via the oral route cannot be discounted. Therefore, although a range of approaches have been considered by various authoritative bodies using extrapolation approaches, such as benchmark dosing to derive health-based values (detailed in Section 4), the outstanding issues with regards to these data mean that uncertainty remains within these values. A more appropriate method of deriving an acceptable health-based level would be through a physiologically based toxicokinetic (PBTK) model. However, before such a model can be applied, several gaps in the existing data must be addressed:

- No studies were located on the measurement of absorption of chromium (VI) from water in humans. Differences in absorption have been reported between administration via oral gavage and via the diet in humans; therefore, there may also be differences in absorption via drinking water.
- The data indicate that significant reduction of chromium (VI) to chromium (III) occurs in gastric juices, acting to limit the absorption of chromium. If this process completely reduces chromium (VI) to chromium (III), it would effectively act to detoxify chromium (VI) prior to absorption. However, there is significant uncertainty in the threshold for this reductive capacity.
- There were differences in chromium concentrations in the small intestines of mice and rats that suggested a greater tendency to absorb chromium in mice. However, there are also substaintial differences between the rodent and human gastrointestinal system. The rodent forestomach has two defined areas, a non-glandular forestomach and a glandular stomach. This forestomach helps maintain a consistent volume of GI lumen contents and reduces the occurrence of significant peaks in gastric acid production. In contrast, humans have no forestomach and therefore gastic acid production is subject to far greater variation, with post-prandial peaks (increases after consumption of a meal) and more acidic conditions during times of fasting. Therefore, there are likely to be significant differences in the capacity of the human stomach to reduce chromium (VI) to chromium (III) than in rodents. This is particularly important when considering that many of the toxicokinetic studies indicate that the duodenum, the part of the small intestine into which the gastric contents are emptied, represents the most significant region for absorption of chromium into the portal system.

4. Authoritative Evaluations

4.1 Chromium (III)

In 1990, the International Agency for Research on Cancer (IARC) evaluated chromium. Metallic chromium and chromium (III) compounds were classified as Group 3, i.e. they are not classifiable as to their carcinogenicity in humans (WHO, 2009).

In 1998, the United States Environmental Protection Agency (US EPA) derived a chromium (III) oral Reference Dose (RfD) of 1.5 mg/kg bw/day. The oral RfD was based on a NOEL of 1800 g chromium oxide/kg bw (reported to be 1468 mg chromium (III)/kg bw/day) identified in a 840-day rat study. An uncertainty factor of 100 (to account for inter- and intra-species variation) and an additional modifying factor of 10 (to account for limitations within the database, which include the lack of a non-rodent mammalian studies and the lack of unequivocal data on the reproductive toxicity of chromium (III)) were applied to the NOEL to derive the oral RfD (US EPA, 2012).

In 2014, the European Food Safety Authority (EFSA) derived a Tolerable Daily Intake (TDI) for chromium (III) of 300 µg/kg bw/day (rounded). This TDI was based on a NOAEL of 286 mg/kg bw/day identified in a chronic study in rats with application of an uncertainty factor of 100 (to account for inter- and intra-species variation) (EFSA, 2014).

4.2 Chromium (VI)

Most of the evaluations on chromium (VI) to date have used hyperplasia as the point of departure (POD) for chromium (VI)-induced toxicity. Hyperplasia can indicate a mechanism of non-genotoxic carcinogenicity, where tumours are formed due to increased DNA synthesis, resulting in an increased probability of errors in cellular replication. Where a chemical also causes mutation, hyperplasia can also increase the risk of cancer developing.

In 1990 IARC evaluated chromium (VI) compounds and classified them as Group 1, i.e. sufficient evidence in humans for the carcinogenicity (WHO, 2013). In 2012, IARC re-evaluated chromium (VI) compounds and confirmed the Group 1 classification. The classification was based on sufficient evidence in humans for chromium (VI)-induced lung cancers following occupational exposure and positive associations between chromium (VI) exposure and cancer of the nose and nasal sinuses. Additionally, there is also sufficient evidence of chromium (VI) carcinogenicity in experimental animals (IARC, 2012).

In 2008, and confirmed in the 2012 update, the US Agency for Toxic Substances and Disease Registry (ATSDR) derived an intermediate (14-365 days) Minimal Risk Level (MRL) of 0.005 mg/kg bw/day for chromium (VI), based on a 2-year study conducted in rats, exposed to sodium dichromate dihydrate in drinking water. This intermediate MRL was derived using a

Benchmark Dosing (BMD) approach, for which a BMD of 0.52 mg/kg bw/day was identified, based on microcytic hypochromic anaemia. An uncertainty factor of 100 (to account for inter- and intra-species variation) was applied to the BMD to derive the intermediate MRL (ATSDR, 2008; ATSDR, 2012).

In 2012, the US ATSDR derived a chronic Minimal Risk Level (MRL) of 0.0009 mg/kg bw/day for chromium (VI), based on a 2-year mice study exposed to sodium dichromate dihydrate in drinking water. The MRL was derived using a BMD approach. The $BMDL_{10}$ of 0.094 mg/kg bw/day was identified, based on increased incidence of diffuse epithelial hyperplasia of the duodenum in female mice. An uncertainty factor of 100 (to account for inter- and intra-species variation) was applied to the $BMDL_{10}$ to derive the chronic MRL (ATSDR, 2012).

In 2010, the US EPA re-evaluated chromium (VI) and drafted a revised oral RfD of 0.0009 mg/kg bw/day (0.9 µg/kg bw/day) from the previous oral RfD of 0.003 mg/kg bw/day. The revised oral RfD is based on the lowest 95% lower confidence limit of the benchmark dose ($BMDL_{10}$) of 0.094 mg chromium (VI)/kg bw/day. The $BMDL_{10}$ was identified from a 2-year mice study based on an increased incidence of diffuse epithelial hyperplasia of the duodenum in female mice. An uncertainty factor of 100 (to account for inter- and intra-species variation) was applied to the $BMDL_{10}$ to derive the oral RfD (US EPA, 2010b).

In 2013, in their Concise International Chemical Assessment Documents (CICAD) document WHO derived an oral Tolerable Daily Intake (TDI) of 0.9 µg/kg bw/day based on $BMDL_{10}$ of 0.094 mg chromium (VI)/kg bw/day. The $BMDL_{10}$ was identified from a 2-year mice study based on the increased incidence in diffuse epithelial hyperplasia of the duodenum in female mice. An uncertainty factor of 100 (to account for inter- and intra-species variation) was applied to the $BMDL_{10}$ to derive the oral TDI (WHO, 2013). WHO did not conduct a quantitative risk assessment for the carcinogenic risk to humans due to uncertainties within the data (WHO, 2013).

In 2014, the European Food Safety Authority (EFSA) derived two approaches for identifying a point of departure for chromium (VI) (EFSA, 2014):

- The first approach, for non-neoplastic effects, was based on diffuse epithelial hyperplasia identified in a 2-year mice study exposed to sodium dichromate dihydrate in drinking water. Using this endpoint, and a benchmark dosing approach, a $BMDL_{10}$ of 0.11 mg chromium (VI)/kg bw/day was identified.

- The second approach, for neoplastic effects, was based on the same study, and a BMDL10 of 1.0 mg chromium (VI)/kg bw/day was derived based on increased incidence of adenoma and carcinoma (combined) in the mouse small intestine.

It is notable that these health-values are significantly higher than those proposed by other organisations.

4.3 Total Chromium

In 2003 and confirmed in the current 4th edition of the Guidelines for Drinking-Water Quality (GDWQ) in 2011, the World Health Organization (WHO) derived a provisional GDWQ of 0.05 mg/l for total chromium. The provisional GDWQ was based on the gentoxicity and carcinogenicity of chromium (VI) via inhalation exposure. The WHO acknowledged that there were uncertainties with the relevant toxicological data, but concluded that the current data did not support the derivation of a new guideline and that the current GDWQ of 0.05 mg/l is unlikely to cause significant health effects (WHO, 2003; WHO, 2011).

The US EPA has established a maximum contaminant level (MCL) of 0.1 mg/l based on total chromium in drinking water (ATSDR, 2012).

In the EU and the UK, water that is abstracted for drinking water should contain less than 0.05 mg/l of total chromium (EU, 2005).

4.4 Other Evaluations/Toxicological Peer Review

Thompson *et al*. (2013) reported a chronic oral RfD of 6 µg/kg bw/day for chromium (VI) designed to be protective against intestinal cancer via a non-mutagenic mechanism of action. A pharmacokinetic model which was based on mice exposed to chromium (VI) was used to estimate the amount of chromium (VI) at the intestine (duodenum, jejunum and ileum). Using the results from the model, a point of departure (POD) for diffuse hyperplasia was derived from benchmark dose and constrained non-linear regression modelling. The derived POD was converted to a human equivalent dose. An uncertainty factor of 10 was applied to the human equivalent dose to derive an oral RfD for diffuse hyperplasia. This derived oral RfD is based on a non-genotoxic mechanism of chromium (VI). However, the view that chromium (VI) acts via a non-genotoxic mechanism is in disagreement with the current opinions of both WHO and Public Health England (PHE) (HPA, 2007; WHO, 2013).

4.4.1 WRc opinion of this assessment

The evaluation of the genotoxicity of chromium (VI) within this report (Section 3.3) also supports the view that chromium (VI) is genotoxic, as the weight of evidence indicates chromium (VI)-induced genotoxicity and clastogenicity *in vivo*, although some negative results have been reported in micronuclei assays. These negative results may be due to substantial reduction of chromium (VI) to chromium (III) in the gastrointestinal tract prior to absorption (see Section 2.1.2). While there may not be a biological threshold for chromium (VI), the reduction of chromium (VI) to chromium (III) in the saliva and gastric juices prior to absorption may act as a possible chemical threshold. Therefore, the oral RfD derived by Thompson *et al*. (2013) may still be appropriate, even if the justification for that oral RfD is not supported by the views of WHO or PHE.

4.5 Application of Evaluations in the Derivation of Potential Drinking Water Standards or Guidelines

The current EU and English and Welsh drinking water standard for total chromium is 0.05 mg/l (50 µg/l). This standard is derived from the WHO guideline value which was initially based on chromium (VI) health concerns however the guideline was changed to total chromium due to difficulty in analysing chromium (VI).

A significant body of data on the toxicological properties of chromium (VI) has become available subsequent to the setting of this standard. However there remain gaps in the data and, in particular, uncertainty whether there is a threshold for the effects observed., Nonetheless a number of groups have derived TDIs and equivalent values (eg RfDs). Caution must be used in interpreting these values given the uncertainties and data gaps.

In 2013, in their CICAD, WHO derived an oral TDI of 0.9 µg/kg bw/day for chromium (VI) (WHO, 2013). The CICAD is limited in that it does not consider all the latest data available on reduction of chromium (VI) in the gastrointestinal tract and mode of action. Though it does acknowledge that processes that determine absorption and metabolism of chromium(VI) following ingestion are not fully understood. By assuming the same principles that WHO would adopt in the derivation of their lifetime Guidelines for Drinking-water Quality (GDWQ), i.e. a 60 kg adult drinking 2 litres of water per day, with an allocation of 20% of the TDI to water, a lifetime health-based value of 5.4 µg/l can be derived.

The European Food Safety Authority (EFSA) states that there are a lack of data on the presence of chromium (VI) in food. However, it continues that all reported chromium data in food can be considered as chromium (III). This assumption was based on recent speciation work and the "*fact that by-and-large food is a reducing medium, and that oxidation of Cr(III) to Cr(VI) would not be favoured in such a medium*" (EFSA, 2014). Therefore, it can be considered that an allocation of 20% of the TDI to drinking water for lifetime exposure may be overly precautionary, and a higher allocation can be applied. Assuming an 80% allocation, as some exposure via other sources, such as industrial emissions cannot be excluded, a lifetime health-based value of 22 µg/l is obtained.

Similarly, by using the oral RfD of 6 µg/kg bw/day for chromium (VI) derived by Thompson *et al*. (2013) a lifetime health-based value of 36 µg/l is derived based on a 20% allocation or 144 µg/l based on an allocation of 80%. Use of the EFSA values would result in even higher lifetime health-based values for drinking water; applying a 20 and 80% allocation of the BMDL10 derived for non-neoplastic endpoints (110 µg/kg bw/day) (EFSA, 2014) would result values of 660 and 2640 µg/l, respectively.

Some of these values are lower than the current drinking water standard of 50 µg/l for total chromium. Therefore, it may be appropriate to review or reconsider the current drinking water standard but the uncertainties described above make this difficult at present.

Using each of these values for chromium (VI), and assuming a 60 kg adult drinking 2 litres of water per day, with an allocation of either 20 or 80% of the health-value to drinking water, a comparison against a selected concentration in water can be made to determine a margin of safety (MOS) for life-time exposure to that selected concentration. A MOS of greater than 1 would indicate no health concern following life-time exposure to a chosen concentration. However, if the MOS is less than 1, the possibility of adverse health effects cannot be disregarded.

For the purposes of this example, concentrations of 1 µg/l and 10 µg/l have been used. The concentration of 1 µg/l represents an 'upper limit' concentration for most of the sites surveyed within this project (see Sections 9 and 10). However, one site had levels of chromium (VI) that were significantly higher, at just below 10 µg/l. Therefore, a concentration of 10 µg/l would represent a 'worst-case' for this one site. The results of this comparison are provided in Table 4.1 (20% allocation) and Table 4.2 (80% allocation). These data indicate that regardless of the health value selected, the MOS for a concentration of chromium (VI) in drinking water of 1 µg/l is greater than 1, even if the precautionary assumption of only using a 20% allocation of the health value to drinking water is applied. At a concentration of 10 µg/l, if the health values of 0.9 µg/kg bw/day were used to derive drinking water standards, the MOS would be less than 1 if a 20% allocation was applied, but slightly above 1 (2.16) is an 80% allocation to water was applied. However, it should be emphasised that a concentration of 10 µg chromium (VI)/l would not be typical in UK drinking water, and as discussed above, exposure to chromium (VI) via other sources is likely to be minimal, and therefore, this example would represent a very extreme situation. At the most realistic allocation of 80%, the MOS for both concentrations is above 1.

Table 4.1 Margins of safety for each proposed health-based value for chromium (VI) assuming concentrations of 1 or 10 µg chromium (VI)/l in drinking water, and assuming an allocation of 20% of the health-value to drinking water

Evaluating Organisation	Proposed health value (µg/kg bw/day)	Lifetime health-based value in drinking water, assuming a 20% allocation to water (µg/l)	Assumed concentration in drinking water (µg/l)	Margin of safety
ATSDR intermediate MRL	5	30	1	30
			10	3
ATSDR chronic MRL	0.9	5.4	1	5.4
			10	0.54
US EPA RfD	0.9	5.4	1	5.4
			10	0.54
WHO CICAD	0.9	5.4	1	5.4
			10	0.54

Evaluating Organisation	Proposed health value (µg/kg bw/day)	Lifetime health-based value in drinking water, assuming a 20% allocation to water (µg/l)	Assumed concentration in drinking water (µg/l)	Margin of safety
EFSA BDML10 (non-neoplastic effects)	110	660	1	660
			10	66
EFSA BDML10 (neoplastic effects)	1000	6000	1	6000
			10	600
Thompson *et al*., 2013	6	36	1	36
			10	3.6
Current drinking water standard	50 µg/l	-	1	50
			10	5

Table 4.2 Margins of safety for each proposed health-based value for chromium (VI) assuming concentrations of 1 or 10 µg chromium (VI)/l in drinking water, and assuming an allocation of 80% of the health-value to drinking water

Evaluating Organisation	Proposed health value (µg/kg bw/day)	Lifetime health-based value in drinking water, assuming a 80% allocation to water (µg/l)	Assumed concentration in drinking water (µg/l)	Margin of safety
ATSDR intermediate MRL	5	120	1	120
			10	12
ATSDR chronic MRL	0.9	21.6	1	21.6
			10	2.16
US EPA RfD	0.9	21.6	1	21.6
			10	2.16
WHO CICAD	0.9	21.6	1	21.6
			10	2.16
EFSA BDML10 (non-neoplastic effects)	110	2640	1	2640
			10	264
EFSA BDML10 (neoplastic effects)	1000	24000	1	24000
			10	2400
Thompson *et al*., 2013	6	144	1	144
			10	14.4
Current drinking water standard	50 µg/l	-	1	50
			10	5

5. Fate of Chromium During Water Treatment

5.1 Aqueous chromium chemistry

Chromium normally exists in two redox states in aqueous solutions, chromium (III) and chromium (VI), where chromium (VI) salts are generally more soluble than chromium (III) salt (WHO, 2003). Depending on pH, chromium (III) can be hydrolysed to varying degrees forming Cr^{3+}, $CrOH^{2+}$, $Cr(OH)_2^{+}$, $Cr(OH)_3$(aq) and $Cr(OH)_4^{-}$. Chromium (VI) forms chromate (CrO_4^{2-}) that can be protonated to form $HCrO_4^{-}$ and, under very acidic conditions (pH<2), H_2CrO_4. At high chromium (VI) concentrations and low pH, the dichromate ion ($Cr_2O_7^{2-}$) also forms.

5.2 Removal of chromium

Removal technologies for chromium removal can be categorised into five general groups (Sharma *et al.*, 2008):

- Coagulation-precipitation-filtration
- Adsorption
- Ion exchange
- Membrane technologies
- Biological removal

The techniques and their main advantages and disadvantages are summarised below.

5.2.1 Coagulation-precipitation-filtration

Coagulation using aluminium sulphate (alum) and ferric (Fe(III)) is widespread practice in the water industry and these coagulants have been used for chromium (III) removal. The removal is due to the formation and precipitation of $Cr(OH)_3$(s) and co-precipitation with aluminium and ferric hydroxides ($Al(OH)_3$ and $Fe(OH)_3$, respectively). For a relatively high chromium (III) concentration of 460 µg/l, Fatoki and Ogunfowokan (2002) reported >85% removal using alum and ferric sulphate at near neutral pH and coagulant doses of 10 mg $Al_2(SO_4)_3$/l or 9 mg $Fe_2(SO_4)_3$/l.

Chromium (VI) is not effectively removed by alum and ferric coagulants and needs to be reduced to chromium (III) for the removal to take place. Reducing agents used for chromium (VI) reduction are ferrous (Fe(II)) sulphate, zero valent iron, sulphur dioxide (SO_2), and sodium bisulphite ($NaHSO_3$). Stannous chloride ($SnCl_2$) is also being investigated as a

suitable reducing agent (US EPA, 2003). The use of ferrous sulphate has been shown to remove nearly 100% of the chromium (VI) present through reduction to chromium (III), precipitation to $Cr(OH)_3$ and co-precipitation with ferric hydroxides (Faust and Aly, 1998; Lee and Hering, 2003; Quin *et al.*, 2005). The reduction of chromium (VI) to chromium (III) is fast (minutes to hours), more effective at lower pH and requires a 3-5 times excess of Fe(II) to chromium (VI) (Beukes *et al.*, 1999; Lee and Hering, 2003, Sharma *et al.*, 2008). Alternatively, the Fe(II) can be added electrochemically through the oxidation of iron electrodes using an electrocoagulation process, although this is not common in water treatment.

As the reduction of chromium (VI) is more effective at lower pH, chromium (VI) removal is usually carried out in two steps. The chromium (VI) is first reduced at low pH and then the pH is raised to precipitate $Cr(OH)_3$. If chromium is only present as chromium (III), raising the pH using NaOH or lime can be enough to precipitate $Cr(OH)_3$. US EPA (2003) estimates that lime softening has a 72-99% efficiency for chromium (III) removal.

The disadvantages with coagulation-precipitation are that:

- precipitation is less effective if the metal ions are complexed or in the form of anions;
- the concentration achieved depends on the solubility product, although higher concentrations can be expected if the kinetics are slow;
- the precipitate may form small particles that do not readily settle and might need micro- or ultrafiltration as a polishing step;
- chromium-rich sludge may form. A hazard assessment will have to be carried out as high concentrations of chromium (and other compounds) in the sludge could lead to the sludge being regarded as hazardous waste that would need to be disposed of accordingly.

It should be noted that some disinfectants can oxidise chromium (III) to chromium (VI) and treatments such as pre-chlorination and pre-ozonation can make the chromium more mobile and difficult to remove. The addition of ferrous sulphate can under certain conditions increase the leaching of chromium (VI) from chromium-rich ore but these conditions are unlikely to be met during drinking water treatment (Geelhoed *et al*., 2003).

5.2.2 Adsorption

Iron oxides, such as ferrihydrite and goethite, and iron oxide coated sand (IOCS) are effective in removing both anions and cations but the removal is highly pH-dependent; cations are generally more readily removed at high pH and anions more readily at low pH. Removal of both chromium (III) and chromium (VI) is therefore likely to require pH changes and the removal carried out in stages. These types of adsorbents can achieve a considerably lower aqueous chromium solution concentration than precipitation processes, e.g. Bailey *et al*.

(1992) reported >99% removal of a 20 mg chromium (VI)/l. They also generate less sludge than the coagulation procedure and the adsorbents can be regenerated. They need, however, to be disposed of when exhausted and they have a relatively limited capacity. A hazard assessment will have to be carried out as high concentrations of chromium (and other compounds) on the adsorbed could lead to the adsorbed being regarded as hazardous waste that would need to be disposed of accordingly. This is also valid for when the adsorbed is regenerated.

Other ions can also form soluble complexes with chromium and reduce the efficiency of the adsorbents and, for example, carbonate is known to compete for available surface sites and reduce the adsorption of chromium (VI) (Villalobos *et al.*, 2001).

Other possible adsorbents are activated carbon, coal, bone charcoal, pyrite fines, calcined Mg-AlCO_3 hydrotalcite, and manganese oxide coated sand (MnOCS) but these have either been found not to be effective in chromium removal (activated carbon) or they have only been used in laboratory trials (Sharma *et al*., 2008). Huang (1977) found that chromium (VI) was more readily removed by activated carbon than chromium (III). Bailey *et al.* (1999) reviewed potential low-cost sorbents for heavy metal removal and chitosan, rice hulls, bentonite, orange peel, wool, senna leaves and peat showed reasonably high adsorption capacities (50-330 mg chromium/g) for either chromium (III) or chromium (VI).

5.2.3 Ion exchange

Ion exchange is effective in removing both chromium (III) and chromium (VI), between 80-96% of the ions can be expected to be removed (US EPA, 2003). As chromium (III) generally forms cations and chromium (VI) forms anions, a cation exchanger is effective for chromium (III) removal and an anion exchanger is effective for chromium (VI). Chromate is the preferentially most removed anion of the common anions present in water (Galan *et al.*, 2005) and ion exchange is therefore, at least theoretically, seen as an ideal technique for chromate removal (Sharma *et al.*, 2008). At low pH, a weak-base anion exchange resin is generally used for chromate removal whereas at neutral pH, a strong-base anion exchanger must be used (Sharma *et al.*, 2008).

5.2.4 Membrane technologies

Membrane technologies and particularly reverse osmosis (RO) are considered one of the best technologies available for chromium removal, US EPA (2003) estimating the efficiency at 82-97% for RO. Nanofiltration has also been used for chromium removal and shows similar efficiency for both chromium (III) and chromium (VI) (e.g. Hafiane *et al.*, 2000; Taleb-Ahmed, 2002). Chromium (VI) removal is enhanced at alkaline pH (Hafiane *et al.*, 2000; Mortazavi *et al.*, 2010) and chromium (III) removal is enhanced under acidic conditions (Taleb-Ahmed, 2002).

Coagulation followed by micro- or ultrafiltration are processes that are likely to show good removal for chromium (III), but also chromium (VI) if a ferrous coagulant is used to reduce chromium (VI) to chromium (III). No good references have been found but e.g. Zouboulisa *et al.* (2010) reported that the method was “very effective” in removing copper.

There are a number of membrane products available and these have different properties that will affect the chromium removal. The composition of the water will also affect the performance of membrane technologies.

The main drawbacks with the membrane technologies are that they are working at relatively high pressures that require energy to maintain and the fouling and subsequent cleaning of the membranes can also be costly.

5.2.5 Biological removal

Biological removal of chromium (VI) by bacteria has been to shown to be effective by a number of researchers. The chromium (VI) is reduced to chromium (III) and precipitated within the biomass. However, all studies have shown that anaerobic conditions work best and this is not suitable for drinking water treatment (e.g. Chen and Hao, 1997; Komori *et al*., 2004; Chen and Gu, 2005).

5.3 Formation of chromium (VI) during drinking water treatment

In 2012, the US Water Research Foundation (WRF) published a review on the current knowledge of chromium in drinking water. Within this review, WRF examined drinking water treatment processes that may result in the conversion of chromium (III) to chromium (VI) and *vis versa*. These data were summarised as a table of fast and slow reaction process, which are detailed in Table 5.1 (McNeill *et al.*, 2011).

Table 5.1 Summary of treatment processes that may lead to the conversion of chromium (III) to chromium (VI) and chromium (VI) to chromium (III)

Treatment	Rate	Reaction
Oxidation of chromium (III) to chromium (VI)		
Manganese dioxide (MnO_2)	Minutes to hours	$2Cr^{3+} + 3MnO_2 + 2H_2O = 2CrO_4^{2-} + 3Mn^{2+} + 4H^+$
Chlorine (Cl_2)	Minutes to hours	$2Cr^{3+} + 3HOCl + 5H_2O = 2CrO_4^{2-} + 3Cl^- + 13H^+$
Hydrogen peroxide (H_2O_2)	Minutes to hours	$2Cr^{3+} + 3H_2O_2 + 2H_2O = 2CrO_4^{2-} + 10H^+$
Potassium permanganate ($KMnO_4$)	Minutes to hours	$5Cr^{3+} + 3MnO_4^- + 8H_2O = 5CrO_4^{2-} + 3Mn^{2+} + 16H^+$
Chloramine (NH_2Cl)	Hours to days	$2Cr^{3+} + 3NH_2Cl + 8H_2O = 2CrO_4^{2-} + 3NH_3 + 3Cl^- + 13H^+$
Dissolved Oxygen (O_2)	Days to years	$4Cr^{3+} + 3O_2 + 10H_2O = 4CrO_4^{2-} + 20H^+$
Reduction of chromium (VI) to chromium (III)		
Iron (Fe^{2+})	Minutes to hours	$CrO_4^{2-} + 3Fe^{2+} + 8H^+ = Cr^{3+} + 3Fe^{3+} + 4H_2O$
Stannous chloride ($SnCl_2$)	Minutes to hours	$2CrO_4^{2-} + 3Sn^{2+} + 16H^+ = 2Cr^{3+} + 3Sn^{4+} + 8H_2O$
Sulphites (SO_3^{2-})	Minutes to hours	$2CrO_4^{2-} + 3SO_3^{2-} + 10H^+ = 2Cr^{3+} + 3SO_4^{2-} + 5H_2O$
Absence of dissolved oxygen, sulphides, numerous bacteria	Days to years	$2CrO_4^{2-} + 3S^{2-} + 16H^+ = 2Cr^{3+} + 3S^o + 8H_2O$

6. Summary of Previous Monitoring Studies and Occurrence Data

6.1 Introduction

Gathering data on the occurrence of chromium (VI) in drinking water supplies is difficult. The importance of chromium (VI) as a possible risk to human health through ingestion is a relatively new discovery and previously chromium (VI) has not been seen as a potential problem. Occurrence of chromium (VI) in drinking water supplies is rare, but more frequent is the analysis of chromium (VI) in the environment where specific industries have caused pollution incidents or have polluted surface or ground waters over a number of years. More general studies into the occurrence of chromium (VI) in potential drinking water sources have become more common in recent years but the data are still relatively scarce. It has been possible to gather some data on the occurrence of chromium (VI) in places like California and China where specific pollution incidents of chromium (VI) have been linked to human health issues and the risk of people being exposed in their drinking water. A small amount of work has been done in the UK and Europe but this is mostly linked with specific pollution incidents and very little has been found on the general exposure of populations to chromium (VI).

6.2 United Kingdom

Even though total chromium is regularly monitored in UK drinking waters the results are rarely published (Environment Agency, 2002). There are very little to no data available on the speciation of chromium in drinking waters and so levels of chromium (VI) in UK drinking waters are unknown (Rowbotham *et al.*, 2010; Environment Agency, 2002).

Rowbotham *et al.* (2010) reported levels of total chromium in rainwater and surface water and levels of total chromium in UK soils (Table 6.1) the speciation of total chromium in the UK environment is not known. For the purposes of their study into the exposure of the UK population to chromium, the Environment Agency (EA) assumed that UK drinking waters contained no more than 5 µg/l of total chromium (Environment Agency, 2002). Rowbotham *et al.* (2010) assumed a concentration of 2 µg/l in their similar study.

Table 6.1 Environmental concentrations of total chromium in UK waters and soils (Rowbotham *et al.*, 2010)

Source	Total chromium range
Water	
Rainwater, rural 1982-1991	<0.4-<0.9 µg/l
Estuaries, 1991-1994	1.1-17.7 µg/l
Glasgow, landfill water course	3 920 µg/l
Glasgow background water course	0.02 µg/l
Soils	
General UK	39 mg/kg
Background UK	5-1 500 mg/kg
Serpentine soil, Lizard	2 221 mg/kg
Serpentine soil, Scotland	10 347 mg/kg
British Chrome and Chemicals, 6cm depth	22-1 236 mg/kg
British Chrome and Chemicals, 10-25 cm depth	5-1 680 mg/kg
British Chrome and Chemicals background soils	8-14 mg/kg
Glasgow landfill areas	9 400-26 150 mg/kg

As part of this project, WRc contacted all water companies within the United Kingdom to request any data they may have on the occurrence of chromium within their respective water catchments that may not be in the public domain. Four companies reported at least one occurrence of detectable concentrations of chromium in raw water, but no occurrences of chromium in finished water. These data are presented in Table 6.2.

Table 6.2 Chromium concentrations reported in raw water in the UK (WRc data)

Water Company	Site Details	Concentration
1	Groundwater	Maximum concentration of <3 µg/l
1	Surface water	Maximum concentration of <1 µg/l
2	Occasional detections in raw water from annual sampling	<1-10 µg/l
3	One borehole	~10 µg/l
4	Groundwater	>LOD for all samples (13 samples over 4 years) Maximum concentration of 2.4 µg/l
4	Surface water	25% of samples >LOD (32 samples over 4 years)

6.3 Europe

There are various studies on the presence on chromium (VI) in groundwaters but these do not directly translate to groundwaters used for drinking water purposes. Generally most investigations into concentrations of chromium (VI) in groundwaters, surface waters or drinking waters have come directly from concerns about pollution incidents or prolonged historical industrial pollution into surface or groundwater supplies. This includes a study into the surface water pollution from tanneries in Albania which have grown in number over the last decade or so with many facilities running illegally out of private farmsteads. This has caused concerns for water pollution in Albania. The study found concentrations of total chromium that exceed the limits within domestic and EU standards (Floqi *et al.*, 2007). Turkey has also reported similar pollution incidences, and one creek which, discharges into the Izmit Gulf, was found to be heavily polluted with chromium (VI) from a factory dyeing waste water to the extent that it was affecting the ecosystem and entering the human food chain (Oktor *et al.*, 2008). Slovenia however reports that only one of their groundwater wells appears to be polluted with chromium (VI) and incidents of toxic metal pollution are rare in Slovenia (Petresin, 1996).

Chromium (VI) contamination of drinking water has been reported in the Meulan District of Paris France, where it is believed chromium (VI) contaminated groundwaters used for supplying drinking water in 1974. This contamination originated from a metal works upstream of the extraction wells which discharged untreated effluent, had no storage for parts to be plated with chromium and emissions from the stacks also contaminated local water supplies (Philipot *et al.*, 1985). An investigation into the source of the contamination concluded that level of chromium (VI) were higher in the groundwater than in the river. They reported levels of total chromium between 300 and 400 µg/l in the raw water samples and investigated various chromium removal methods. Unfortunately this study did not indicate the level of chromium (VI) in the drinking water supplies, although did state that the addition of ferric sulphate and filtration was the most effective method investigated.

In the 1990's within the Oinofita municipality of Greece a community of villages raised concerns of discoloration and turbidity in their drinking water. In 2007, 20 industries in the area were fined for disposing of wastes containing high levels of chromium (VI) into the local Aspos River, which supplies drinking water to these villages. The concentrations of chromium (VI) in the area were investigated from 2007 by three different bodies.

1. Institute of Geology and Mineral Exploration surveyed 87 groundwater wells in the area and found 35 wells had chromium (VI) concentrations $\geq$10 µg/l with a maximum of 156 µg/l.

2. The Geology and Geography Department of the University of Athens took three drinking water samples between September and December 2008 and found concentrations ranging from 41-53 µg/l.

3. The Oinofita Municipality took 16 samples of drinking water between 2007 and 2008. The concentrations of chromium (VI) ranged from 8.3-51 µg/l. Of the 16 samples taken, 13 were above 10 µg/l. A sample taken in 1996 from the drinking water supply in the municipality contained a concentration of 54 µg/l.

This study looked at the possibility that these high chromium (VI) concentrations were contributing to the cancer mortality rates in the Oinofita region. It concluded that in this case there was a slight relationship between the mortality rates in the area and the concentration of chromium (VI) in the drinking water.

6.4 USA

McNeill *et al.* (2011) observed that deducing the full extent of chromium (VI) occurrence in drinking water in the USA is a difficult task. They found that reported levels of total chromium were usually very low and the extremely low levels of chromium (VI) were difficult to detect. In the USA there is a national Maximum Concentration Limit (MCL) of 100 µg/l for total chromium in drinking water (US EPA, 2010a). Each state can however set their own lower limits or goals for contaminants in drinking water. California established a Public Health Goal (PHG) for chromium (VI) in 1999 of 0.2 µg/l, it was been suggested by the Office of Environmental Health Hazard Assessment within the California EPA that the PHG should be lowered to 0.06 µg/l, but since the publication of the Toxicological Review of Hexavalent Chromium by the EPA (US EPA, 2010b) they have lowered this proposal to 0.02 µg/l. Thomas *et al*. (2002) examined the determination methods for chromium (VI) in drinking water and concluded that the method proposed by the California unit of the Environmental Protection Agency is not sufficient to detect chromium (VI) in drinking water at levels proposed by the PHG.

The most prominent case of chromium (VI) in drinking water supplies in the USA is that of the case of Pacific Gas and Electric (PG&E) and the residents of Hinkley, California. PG&E used chromium (VI) as an anti-corrosion agent in its compressor plant located in Hinkley. The chromium (VI) leachate from the plant contaminated the local groundwater that fed private water groundwater wells. This contamination was then blamed for the apparent health problems such as skin irritation, cancer and birth defects in the local residents. The famous legal battle ended in PG&E settling out of court to the sum of $333 million (Sharma, 2003). Ground water concentrations at the time were reported as high as 580 µg/l. The concentration of chromium (VI) in the local distribution systems was however not investigated. It is worth noting that an evaluation by the US Californian Cancer Registry in 2011 found that the number of new cases of cancer occurring in the Hinkley Census Tract to be slightly, but not statistically significantly higher than the number of new cases expected for an average risk population with the same demographic characteristics as the Hinkley Census Tract population (CCR, 2011).

The California EPA has been monitoring the occurrence of chromium (VI) in California since the late 1990's. This is in response to the concerns from incidents such as Hinkley, to monitor

and determine to what extent chromium (VI) exists in California and to help develop a MCL for the state. In 2001, the California Department of Public Health adopted chromium (VI) into the list of “unregulated chemicals for which monitoring are required” or UCMR. The regulation was repealed in 2002, but some water systems continued to monitor for chromium (VI) to the present day. The data collected in California is summarised in Table 6.3, results that did not detect chromium (VI) are not included. Typically the majority of sample points that detected chromium (VI) appear to have a concentration of 1-5 µg/l with concentrations reaching up to >50 µg/l. Of these sources not all are used for drinking water and the sampling regimen includes a mixture of treatment systems.

Table 6.3 Chromium (VI) peak detections in drinking water sources in California, USA. (CDPH, 2012)

Chromium (VI) concentration range (µg/l)	No. of sample points	% of total sample points
>50	7	<1
41-50	3	<1
31-40	17	<1
21-30	63	3
11-20	243	11
6-10	484	21
1-5	1493	65

In 2004, the American Water Works Association (AWWA) undertook a national boron and chromium (VI) survey in drinking water sources (before treatment). The study included 407 source waters, both groundwater and surface waters, which covered 41 states and 189 different water utilities. Chromium (VI) was typically found at concentrations below the limit of detection (<0.2 µg/l) (Table 6.4).

Table 6.4 Drinking water source average across USA. (AWWA, 2004)

Parameter	Average (µg/l)	Median (µg/l)	Minimum (µg/l)	Maximum (µg/l)
Total chromium	2.0	0.8	<0.6	47.1
Chromium (VI)	1.1	<0.2	<0.2	52.6

The presence of chromium (VI) was recorded in approximately the same proportion of groundwaters as in surface waters. However groundwaters typically had higher concentrations than surface waters. The study looked particularly at the proportion of total chromium that is chromium (VI) versus the proportion that is chromium (III). The study found that the proportion of total chromium that was chromium (VI) was higher in groundwaters than it was in surface waters.

It was found that source waters that serve between 10 001 and 50 000 people had the highest concentrations of total chromium and chromium (VI); this could be attributed to the fact that groundwaters are most commonly used to serve this population size. They also evaluated the regional occurrence trends across the USA and deduced that the region including California generally had the highest concentrations of total chromium and chromium (VI). It also found that the variability between total chromium concentrations was greater for surface waters than ground waters. There was no clear pattern of occurrence of total or hexavalent chromium in surface waters between regions however in groundwaters clear regional patterns were identified whereby the region containing California had the highest concentrations of total chromium and chromium (VI).

The AWWA (2004) also undertook a profiling exercise looking at the occurrence and shifts in speciation of chromium across supply networks, from the source water to treatment and then within the distribution system (Table 6.5). This aimed to provide insight into the transformation or removal of chromium species from source to tap. Seventeen utilities were selected (all had been included in the initial occurrence survey) to sample water from three points in the distribution system from the source water (surface water or ground water), after treatment and from the distribution system. There was a mixture of treatment types within the survey group which included:

- no treatment;
- disinfection only;
- iron/manganese removal;
- granular activated carbon (GAC); and
- Nanofiltration membrane treatment.

Initially the AWWA (2004) compared the concentrations of total chromium and chromium (VI) in the source waters for each utility this then represented the difference between two sampling events. This showed that the ratio of total chromium to chromium (VI) had remained the same as in the original survey but the magnitude of the concentrations had changed between the two sampling events.

Table 6.5 Chromium speciation in treatment profile utilities (AWWA, 2004)

Source water Treatment type	Raw water Cr (VI) µg/l	Finished water Cr (VI) µg/l	Distribution water Cr (VI) µg/l	Raw water Cr (VI): Total Cr (%)	Finished water Cr (VI): Total Cr (%)	Distribution water Cr (VI): Total Cr (%)
Groundwater Disinfection	22.2	-	0.4	71%	-	>100%
Groundwater Disinfection	0.7	0.8	0.7	51%	135%	106%
Groundwater Disinfection	14.0	13.8	8.4	106%	107%	127%
Groundwater Disinfection	1.3	4.2	11.9	62%	99%	104%
Groundwater Iron/manganese removal	<0.2	<0.2	<0.2	0%	0%	0%
Groundwater Iron/manganese removal	<0.2	<0.2	0.4	-	-	95%
Groundwater Iron/manganese removal	<0.2	<0.2	<0.2	0%	-	0%
Groundwater GAC	11.3	10.8	<0.2	122%	106%	0%
Groundwater GAC	4.9	5.5	3.4	98%	114%	103%
Groundwater Nanofiltration membrane	0.6	<0.2	-	200%	-	-
Groundwater No treatment	17.8	-	17.6	107%	-	109%

Source water Treatment type	Raw water Cr (VI) µg/l	Finished water Cr (VI) µg/l	Distribution water Cr (VI) µg/l	Raw water Cr (VI): Total Cr (%)	Finished water Cr (VI): Total Cr (%)	Distribution water Cr (VI): Total Cr (%)
Surface water Alum treatment	<0.2	<0.2	<0.2	0%	-	-
Surface water Alum treatment	1.8	1.5	1.7	80%	117%	109%
Surface water Alum treatment	<0.2	<0.2	<0.2	0%	0%	0%
Surface water Alum treatment	<0.2	<0.2	0.2	0%	-	55%
Surface water Ferric treatment	<0.2	<0.2	<0.2	0%	0%	0%

Of the sixteen profiled systems, ten had detectable levels of chromium (VI). Of these, six showed a decrease in chromium (VI) concentration between the source and the distribution system, three showed no change and one showed an increase in chromium (VI). Chromium (VI) was the dominant species of chromium in source waters and in the distribution supplies. The effects of oxidants used during the treatment process were inconclusive and could not be deduced from the results of this study. It appears chromium (III) is effectively removed from water treated using conventional systems (coagulation and filtration) but chromium (VI) is not. The study concluded that all of the treatment systems examined were ineffective at removing chromium (VI).

In 2009, the Environmental Working Group, an independent campaign group, conducted a survey of tap water quality in 35 cities across the USA. The tap water was collected from homes or public buildings such as hospitals, libraries or shopping malls. The EWG reported the presence of chromium (VI) in the tap water of 31 out of the 35 cities surveyed. The concentrations of chromium (VI) ranged from 0.03 µg/l to 12.90 µg/l. The report compares this value with the PHG issued by the California EPA of 0.06 µg/l and found that 25 of the 35 cities had tap water with levels higher than the PHG. It should be noted however that this report was written by a campaign group, and although they briefly describe the methods that they used, they do not go into any detail about the number of samples taken per city or whether these samples were taken over a period of time or sampled as a single spot samples. This report has encouraged more debate in the USA about public protection and the presence of chromium (VI) in drinking water.

Recently, the MWH Laboratories in California has reported on their survey of chromium (VI) in drinking water. These results are presented in Table 6.6.

Table 6.6 Monitoring programme conducted by MWH Laboratories, California (AWWA, 2011)

Concentration (ng/l)	MWH total data	MWH non-California monitoring programme	MWH targeted national sampling set
<0.02	10%	20%	25%
>0.05	80%	70%	57%
>0.1	75%	50%	35%
>1	50%	20%	10%
>10	10%	1%	<1%

6.5 Asia

Studies in Pakistan and Taiwan both looked at the risk posed to populations from various pollutants in their respective countries. In Taiwan, a study was conducted whereby the amount of chromium entering the environment from various human activities was modelled.

The authors modelled its transportation and fate with the environment and the exposure of humans to the chromium in the environment which included inhalation, consumption of food and drinking water. They estimated that the concentrations of chromium (VI) in the sixteen most polluted rivers ranged from 12 µg/l to 646 µg/l. This pollution mainly originated from upstream activities including chrome plating, dyeing, leather tanning, and waste metal reprocessing. They also estimated the release of chromium to the atmosphere through refractory production, stainless steel production and fossil fuel combustion. Releases to soil were from four major sources, bottom ash and fly ash from incineration, industrial sludge and municipal solid waste (Hwong-Wen *et al.*, 2007).

Pakistan particularly has a problem with chromium pollution in water supplies. Azizullaha *et al.* (2010) found that levels of total chromium in various groundwaters used for drinking water ranged from 10 µg/l to 9800 µg/l, levels in surface waters ranged from 10 µg/l to 300 µg/l.

In Liaoning Provence, China, studies have been undertaken after it was reported that some of the drinking water wells were producing water so polluted with chromium (VI) the water was yellow. The source of the pollution was the Jinzhou Iron Alloy plant which was smelting large amounts of metallic chromium. This plant released chromium (VI) in vast amounts of wastewater which entered a ditch and a dry riverbed and this then entered the groundwater. Solid waste from the plant was stockpiled on open ground which leached chromium (VI) into the ground water and atmospheric emissions also affected the area (Jiandong & Xilin, 1987). Contamination started in the late 1950's and continued into the 1970's. In 1965 a number of studies were undertaken in the area quantifying the extent of the chromium (VI) pollution. Initially 266 drinking water wells were sampled with 28% of them containing chromium (VI) at levels between 600-10 000 µg/l, with 15% of the 266 wells containing chromium (VI) >2000 µg/l. In 1974, levels of chromium (VI) in drinking water wells in the province reached up to 20 000 µg/l. These water sources were not subject to any treatment prior to being used for drinking water. Cancer mortality rates in the Liaoning Provence have been linked to the chromium (VI) levels in the local drinking waters (Beaumont *et al.*, 2008; Smith, 2008). In other parts of rural China levels of chromium (VI) ranged between 5 µg/l to 50 µg/l (Ni *et al.*, 2009). Generally, it is noted that China has low water consumption per person and pollution like Pakistan has affected a large amount of the water sources, especially in urban industrialised areas where waste water is discharged directly to water courses with little regulation (Wen-Qing *et al.*, 2008).

6.6 Conclusions

From these sources it appears that chromium (VI) has been linked with numerous pollution incidents especially in newly industrialised countries where legislation for environmental protection may be less stringent than the USA or Europe. It is also noted that these studies have centralised around drinking water sources that rarely undergo treatment prior to use for drinking water. The USA, and particularly California, has the most data gathered on the occurrence of chromium (VI) in their drinking water supplies especially since the Hinkley, California case against PG&E. This issue has had more attention in the USA than anywhere

else in the world. Generally, it appears that background concentrations of chromium (VI) are <1 µg/l, whereas levels can be many times that especially where industrial pollution occurs. The main industrial influences for the presence of chromium (VI) in the environment appear to be chromium ore smelting, leather tanning, industrial cooling waters and dyeing wastewater.

7. Development of a Method for the Determination of Total Chromium, Chromium (III) and Chromium (VI) Concentrations in Drinking Water

7.1 Introduction

Inductively-coupled plasma-mass spectrometry (ICP-MS) is a highly sensitive technique capable of determining the concentration of a range of metals at levels approaching 1 ng/l (1 ppt). ICP-MS uses inductively-coupled plasma as a means of producing ions that are then separated and quantified in a mass spectrometer. The method has the advantage of speed, precision and sensitivity although the potential does exist with specific analytes for there to be interferences which may require additional effort to ensure reliable results. The US Environmental Protection Agency have a published method for the determination of trace elements in waters and wastes by ICP-MS (Method 200.8 Revision 5.4) (US EPA, 1994) and this has been adopted as the starting point for the establishment of the chromium analytical method which is described in this report. A specific objective of this study is to attempt to measure both the total chromium and chromium (VI) and chromium (III) concentrations in drinking water samples and it is anticipated that this will be possible through the use of ion chromatography to separate the two chromium species before measurement.

7.2 Method Development - Results and Discussion

7.2.1 Calibration

Identifying a suitable calibration range for this method was a challenge as the concentration of chromium in the samples to be analysed is unknown. The importance, however, of attempting to measure very low concentrations of chromium (VI) in samples was recognised and so initially a range of 0.05 – 50 µg/l was selected as it was anticipated that this will be adequate for the analysis of the majority of samples received without the need for dilution. Selection of a suitable internal standard is a critical factor and ideally, the same element should be used for both the total chromium determination and the chromium (VI) and chromium (III) species determinations. As the latter requires the use of ion chromatography to first separate the species, options for the introduction of the internal standard are limited to either in-line addition via a T-connector post-column or through spiking of the mobile phase used in the ion chromatography. In-line addition was quickly dismissed as the increased dead volume created by the introduction of the T-connector destroyed the separation between the two chromium species as a result of peak broadening.

Identifying a suitable element that could be introduced into the mobile phase as an internal standard was a challenge as it would need to have an atomic weight similar to chromium to

be relevant and yet it should not interfere with the separation of the two chromium species using the ion chromatographic conditions employed. The proprietary kit (ChromFast™, Thermo Scientific) used for the chromium speciation work recommends the addition of thulium to the mobile phase at a concentration of 10 mg/l to enhance the separation of the two chromium species. During the work reported here, acceptable separation was possible between the two chromium species without this addition. However, as the addition of thullium would not detriment the chromatography, thulium was used at a lower concentration of 10 µg/l as the internal standard.

7.2.2 Total chromium determination

For total chromium determinations, spectrum mode was used and the regression analysis of the calibration data generated using this protocol mode were performed using the Agilent Mass Hunter software employing a standard linear fit algorithm with the contribution from the blank, which determines the Y intercept, being given no special significance through the selection of the “ignore” option in the software. Standards were prepared separately for both chromium (III) (in 1% aqueous nitric acid) and chromium (VI) (in deionised water) (Elemental Scientific, Omaha, USA) using an initial 1000 mg/l standard stock solution of each. Although data generated using the two calibration sets were essentially identical (Table 7.1), based upon data that were generated subsequently (Table 7.2), the chromium (III) calibration standard set was adopted for all total chromium determinations as this showed no deterioration over a period of four weeks.

Table 7.1 Cr calibration standards covering the range 0.05 - 50 µg/l (w/v)

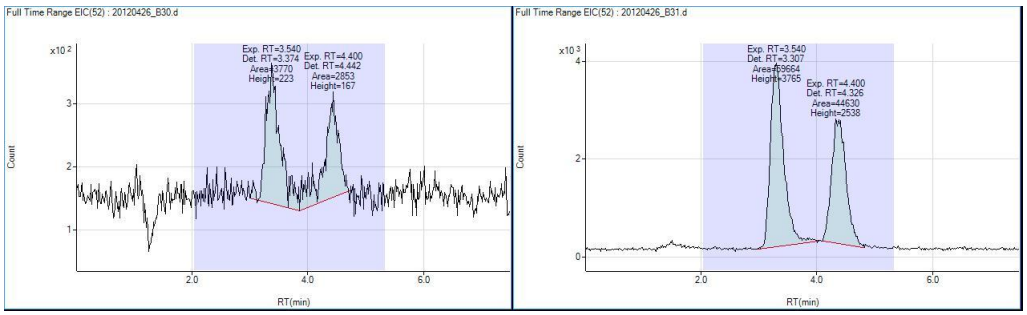
Standard	52 Chromium (III)		52 Chromium (VI)	
	Conc. [µg/l]	Conc. RSD	Conc. [µg/l]	Conc. RSD
Blank	<0.000	N/A	0.006	10.977
Cr 0.05 µg/l	<0.000	N/A	0.039	16.849
Cr 0.1 µg/l	0.060	9.576	0.096	6.261
Cr 0.5 µg/l	0.459	1.811	0.514	0.899
Cr 1.0 µg/l	0.970	2.152	0.985	1.906
Cr 5.0 µg/l	5.087	1.890	5.084	1.637
Cr 10.0 µg/l	10.080	0.330	9.918	1.412
Cr 50.0 µg/l	49.976	0.264	50.008	1.452

7.2.3 Chromium (VI) and chromium (III) determinations

For the chromium (VI) and chromium (III) determinations, it was necessary to carry out the analysis using the time resolved analysis mode with data being collected in chromatogram format. Processing of these data proved to be a challenge due to severe limitations

associated with the auto-integration package incorporated within the Mass Hunter software. When processing data generated over the entire calibration range, it was not possible to identify a set of integration parameters that could deal with standards at either end of the range. At low concentrations, the integration parameters tended to overestimate peak areas due to the relative high level of background noise present whilst at high concentrations, peak areas were underestimated due to the inability of the integration application to perform a suitable baseline drop integration (Figure 7.1). To overcome this problem, all peak areas were manually integrated and the peak area data exported into an Excel spread-sheet for subsequent processing along with the unknowns.

Figure 7.1 Chromium (III) and chromium (VI) at 0.5 µg/l and 10 µg/l, respectively



Initially, the option of creating a calibration standard set containing both chromium (VI) and chromium (III) species covering the concentration range 0.05 – 50 µg/l was explored. Using 1% v/v aqueous nitric acid, standards containing both chromium (VI) and chromium (III) at 1, 5, 10 and 50 µg/l were prepared and the ratio of the two species determined at Day 0 (Figure 7.2) and Day 6 (Figure 7.3).

Figure 7.2 Ratio of chromium (VI) and chromium (III) species at Day 0 in 1% aq. nitric acid

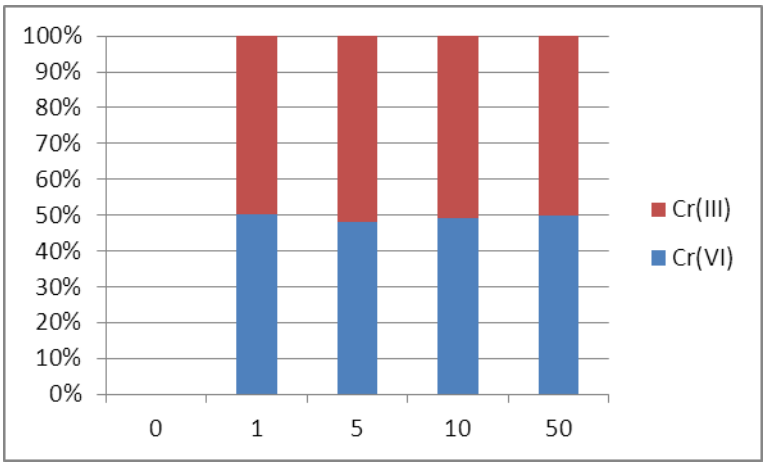
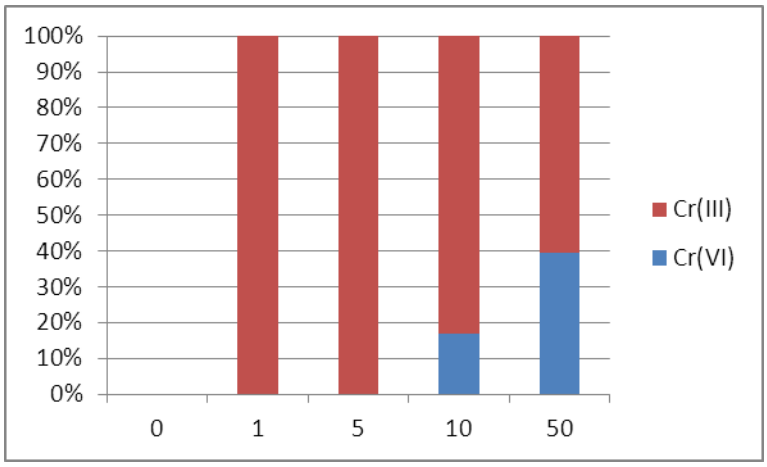


Figure 7.3 Ratio of chromium (VI) and chromium (III) species at Day 6 in 1% aq. nitric acid



Although the total chromium levels remained constant, as can be seen, over six days the chromium (VI) present was slowly reduced to chromium (III) under these conditions such that at low concentrations, no chromium (VI) remained. A repeat of the same experiment in deionised water revealed the opposite behaviour with the chromium (III) present being slowly oxidised to chromium (VI).

Claims in a series of technical notes issued by the California Dept. of Public Health that chromium (VI) and chromium (III) could be stabilised in the presence of each other through the use of aqueous buffers for up to 28 days were explored. Two buffer systems were cited each based upon borate, although the critical factor in both cases was claimed to be their buffering ability and achieving a pH of >9 in samples. Unfortunately, the use of boron in ICP-MS is problematic due to extensive memory effects that are encountered in the detector through its use. However, it was felt that use of an alternative buffer system based upon aqueous sodium carbonate and sodium hydrogen carbonate solutions and capable of achieving pH of >9 in samples was be worthy of further investigation.

The likely lower limit of detection, a range of 0.1 – 50 µg/l was selected for the calibration standard sets of chromium (VI) (prepared in deionised water) and chromium (III) (prepared in 1% aq. nitric acid) and chromatograms were generated for each on Days 0, 1 and 4. Following manual integration of the peak areas, export of the data into an Excel spread-sheet and regression analysis over the three time points, good agreement was obtained over the time period of this study.

Table 7.2 Concentration of chromium (III) and chromium (VI) standards over 4 days

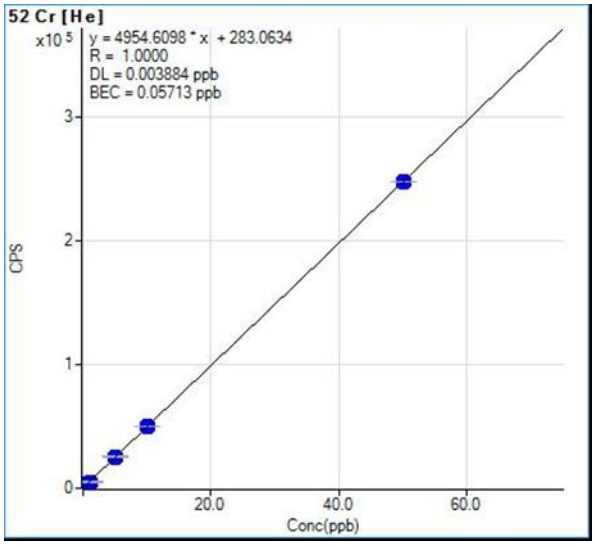
Standard (µg/l)	Day 0		Day 1		Day 4	
	Cr(III)	Cr(VI)	Cr(III)	Cr(VI)	Cr(III)	Cr(VI)
0.1	0.24	0.14	0.20	0.13	0.20	0.08
0.5	0.51	0.53	0.56	0.55	0.44	0.48
1.0	0.92	1.01	1.01	1.05	0.99	1.06
5.0	5.26	5.11	5.11	5.05	4.91	4.98
10.0	9.63	9.79	9.67	9.78	10.07	*
50.0	50.05	50.03	50.06	50.05	49.99	50.00

* Sample missed

7.3 Linearity

Over the calibration range selected (Figure 7.5 and Figure 7.6), the total chromium method exhibited excellent linearity with regression analysis giving a value of R = 1 (Figure 7.4). This had been achieved by eliminating some of the instrument performance variables that can impact upon the raw data obtained. Specifically, three replicate data points were obtained for each sample using an integration time of 1 second as opposed to the more usually used 0.1 second and a mean of the three data points taken for the sample. By using this extended integration time much greater precision was obtained, as shown by the conc. RSD% values (typically <5%) when compared with those obtained using an integration time of 0.1 second (conc. RSD <10%).

Figure 7.4 Calibration curve for total chromium using chromium (III) standard set



For the chromium (VI) and chromium (III) species determinations, an integration time of 0.5 seconds was selected. This helped considerably with smoothing out measurements and

suppressing the baseline noise without adversely affecting the separation of the two chromium species; regression analysis gave R values of typically 0.9999.

Figure 7.5 Calibration curve for chromium (VI) standard data on day 1

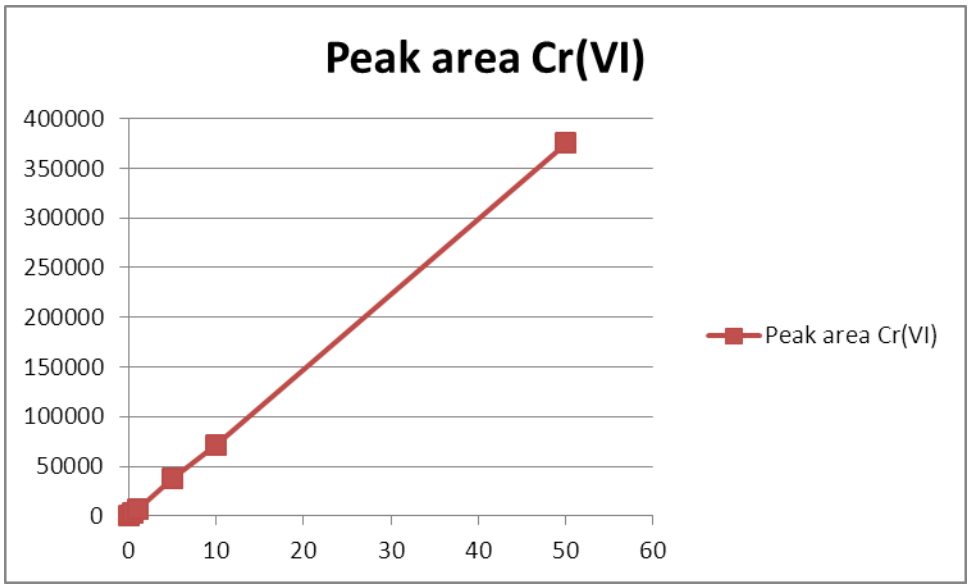
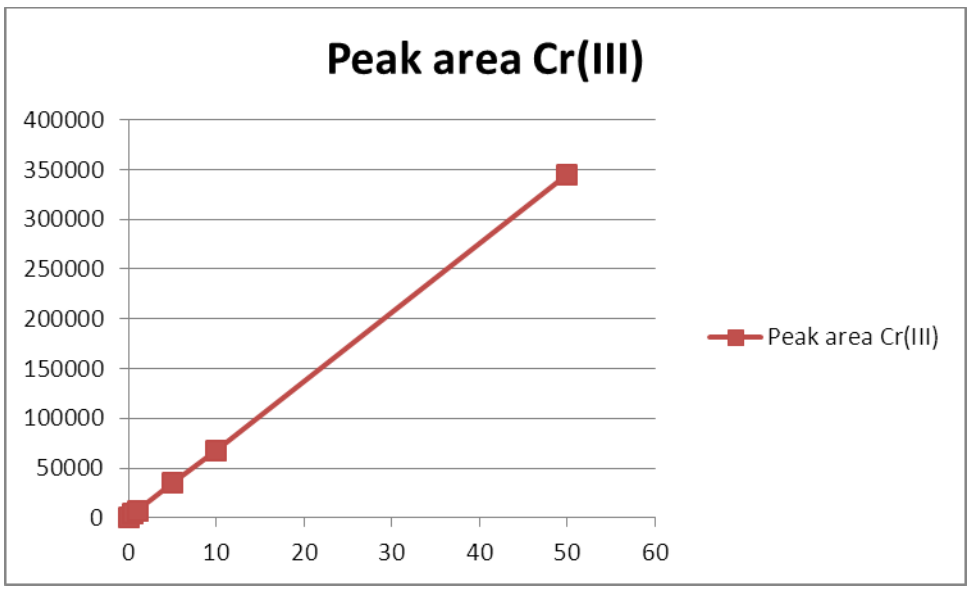


Figure 7.6 Calibration curve for chromium (III) standard data on day 1



7.4 Precision of measurements, limits of detection and quantitation

For the total chromium method, the limit of detection for the method was set at 0.1 µg/l based upon a signal to noise ratio of 3:1. The use of 1% v/v aqueous nitric acid as the matrix for all samples, standards and blanks being primarily responsible for the majority of the noise

observed during analysis. The limit of quantitation was set at 0.5 µg/l based upon a signal to noise ratio of 10:1. The precision of the measurements was very good and above the limit of quantitation the coefficient of variation (conc. RSD%) is <2%. Below the limit of quantitation the precision appeared to still be good (conc. RSD% <6%) and so all data above 0.1 µg/l were be reported. Data below the limit of detection were be reported as <0.1 µg/l.

For the chromium (VI) and chromium (III) determinations, integration was performed manually and, therefore, the limits of detection and quantitation were somewhat more subjective. However, it was possible to identify the presence of both species at concentrations between 0.1 µg/l and 0.5 µg/l although it was only possible to make an estimate of the concentration. Above 0.5 µg/l, manual integration of the peak presented little problem thereby allowing a more accurate determination of the actual concentration present to be made.

Figure 7.7 Chromium (III) standards at 0.1 and 0.5 µg/l, respectively

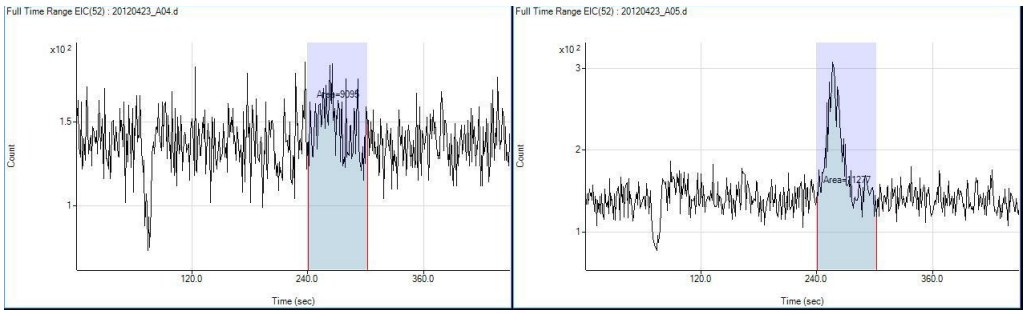
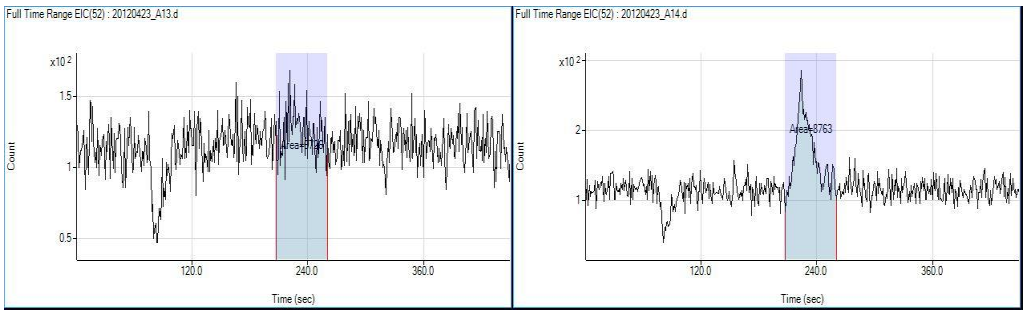


Figure 7.8 Chromium (VI) standards at 0.1 and 0.5 µg/l, respectively



7.5 Reproducibility of the measurements

As demonstrated in Table 7.2, both chromium (VI) and chromium (III) calibration standards showed good agreement over a four-day period when analysed by ion chromatography followed by ICP-MS thereby establishing the reproducibility of the chromium species method. For the total chromium method, the chromium (III) calibration standard set appeared to be the more robust of the two.

7.6 Instrument performance and potential interferences

ICP-MS is an ideal technique to measure concentrations of trace metals in aqueous matrices. However, the potential for interferences in using this technique must be recognised and corrected for and should include compensation for isobaric elemental interferences as well as those arising from polyatomic ions derived from the plasma gas, reagents or sample matrix. Chromium has two main isotopes at mass 52 (relative abundance 83.8%) and 53 (relative abundance 9.5%) that lend themselves to quantitation by ICP-MS. The greater abundance of the chromium-52 isotope obviously makes it an attractive choice to measure because of the greater sensitivity it offers. However, there is a known isobaric interference arising at mass 52 due to the possible presence of ArC+ and ArO+ in the plasma matrix.

Fortunately, the Agilent 7700x ICP-MS was equipped with an Octopole Reaction System which, when used in helium mode, was capable of overcoming this potential interference should it present. Moreover, the expected absence of any significant organic material in the samples being analysed supported by the absence of a background signal at mass 52 when the ICP-MS was operated in both standard “No gas” mode and “Helium” mode suggested that any possible interference from ArC+ species in the plasma matrix was likely to be minimal. Nevertheless, a comparison was made of the mass response for the two chromium isotopes at m/z 52 and m/z 53 over a range of concentration standards when measured in “No gas” and “Helium” modes. Examining the ratio of the ^{52}chromium to the ^{53}chromium signals showed no enhancement over and above what would be expected for the ratio derived from the natural abundance of the two chromium isotopes of 8.82 thereby supporting the argument for the absence of additional contributions at mass 52 arising from the presence of isobaric polyatomic species. In fact, the actual ratios found (Table 7.3) were lower than expected although this is more likely to be attributable to the poor sensitivity with which the mass at 53 was detected.

Table 7.3 Ratio of 52-Cr to 53-Cr in No gas and helium modes

Standard (µg/l)	No gas mode			Helium mode		
	m/z 52	m/z 53	Ratio	m/z 52	m/z 53	Ratio
5	27117	3509	7.73	24107	3024	7.97
10	47029	5930	7.93	48132	5896	8.16
50	173790	20578	8.44	246760	30374	8.12

The use of an internal standard in ICP-MS method development was essential to ensure that any instrumental drift as well as suppressions or enhancements of instrument response caused by the sample matrix were corrected. Selection of an appropriate metal for use as an internal standard has been discussed already as well as how it has been implemented in the two methods.

7.7 Stability of chromium species

Ensuring the integrity of the samples prior to conducting the chromium analysis has been a critical part of this study and much has been published and claims made as to the effectiveness of various stabilisation systems for chromium (VI) and chromium (III) in drinking water samples. It is important that any chromium (VI) present is not reduced to chromium (III) whilst any chromium (III) present is not oxidised to chromium (VI). During the course of the investigation reported here, many preservation systems were investigated to establish whether the claims made in the literature were supported. In only one case were we able to generate data that partially supported those claims and then, only for a period of up to 4 days and not the 14 days claimed in the paper.

As described earlier, in acidic pH (i.e. pH <2), any chromium (VI) present is reduced to chromium (III) whilst at neutral pH, chromium (III) is oxidised to chromium (VI) over relatively short time periods i.e. 1-3 days.

In EPA 218.7, two preservative systems have been developed based upon an ammonium-based buffer mixture. One is liquid based comprising ammonium sulphate (3.3 g) and ammonium hydroxide (6.5 ml; 30% w/v) in deionised water (100 ml) which is employed at a level 1% v/v per sample i.e. 1 ml per 100 ml sample. The second is a solid based system comprising nominally ammonium sulphate (33 mg), sodium carbonate (13.3 mg) and sodium hydrogen carbonate (10.5 mg) per 100 ml sample. It is claimed that the ammonium ions present in the preservative systems complex any free chlorine present resulting in the formation of chloramines which in turn minimise the oxidation of chromium (III), although they do not completely prevent oxidation. Data generated in EPA 218.7 suggest that use of the liquid preservative minimises the oxidation of chromium (III) to chromium (VI) to <3% over for 14 days whilst for the solid preservative the value obtained is <1%. In the work reported here, both liquid and solid preservative systems did not afford the same level of protection as reported and of the two, the solid preservative offered the greatest potential for minimising the oxidation of chromium (III) to chromium (VI) with this being kept to <25% over 4 days (Table 7.4). Consequently due to shelf life considerations and ease of use, the EPA 218.7 solid preservative system was recommended for use.

Table 7.4 Stabilised tap water samples spiked with chromium (III) and chromium (VI) at 0.5 and 10 µg/l

		EPA Liquid Preservative				EPA Solid Preservative			
		0.5 µg/l		10 µg/l		0.5 µg/l		10 µg/l	
		Measured concentration	% composition	Measured concentration	% composition	Measured concentration	% composition	Measured concentration	% composition
pH		**9.28**		**9.30**		**8.42**		**8.39**	
Day 0 TW	Chromium (VI)	0.24	23%	8.36	48%	0.61	56%	9.69	55%
	Chromium (III)	0.82	77%	8.90	52%	0.48	44%	7.89	45%
	Total chromium	1.06		17.26		1.09		17.58	
Total chromium measured		0.87		17.24		0.98		17.92	
Ratio 6/3		0.29		0.94		1.29		1.23	
Day 1 TW	Chromium (VI)	0.34	36%	8.70	51%	0.62	67%	9.97	59%
	Chromium (III)	0.61	64%	8.48	49%	0.31	33%	6.91	41%
	Total chromium	0.95		17.18		0.93		16.88	
Ratio 6/3		0.57		1.03		2.01		1.44	
Day 4 TW	Chromium (VI)	0.31	42%	10.31	56%	0.59	67%	10.87	68%
	Chromium (III)	0.42	58%	8.15	44%	0.28	33%	5.15	32%
	Total chromium	0.73		18.46		0.87		16.02	
Ratio 6/3		0.73		1.27		2.07		2.11	

7.8 Quality controls

A range of quality control procedures have been employed during the development of this method and have included the analysis of blanks, spiked samples and analyses of replicates. In the absence of any suitable external reference material for this purpose, an internally prepared reference standard comprising 5 µg chromium (III)/l has been used and this has been sampled at appropriate points in the sample sequence during each analytical run to confirm the absence of drift. In addition, for the total chromium determinations, at the end of each run, the set of calibration samples was re-run to confirm the stability of instrument performance. Laboratory blanks and field blanks were also prepared in the same way as the samples and included in the sample sequence of the analytical run to demonstrate the absence of any contamination arising during either sample collection, preparation or during the analysis.

7.9 Assessment of errors in the measurements

Volumetric flasks, glass pipettes, graduated sample containers and air displacement pipettes are calibrated using water to demonstrate that the errors on any measurements are within ± 5%.

8. Drinking Water Treatment Sites

Table 8.1 provides details on the drinking water treatment procedure and their source of abstraction at the surveyed sites.

Table 8.1 Drinking Water Treatment of Surveyed Sites

Site	Source Water	Treatment
A	Surface water - reservoir, a highly coloured soft upland source which has been known to stratify seasonally.	Coagulation with aluminium sulphate, clarification through DAF, filtration through sand RGFs, pH elevation using kalic and oxidation with chlorine gas through second stage sand filters and finally further pH conditioning with kalic, disinfection with chlorine gas and plumbosolvency control using orthophosphoric acid.
B	Groundwater - two boreholes, which are situated 45 m apart and water is abstracted from the Chalk/Greensand aquifer.	Treated with sodium hypochlorite solution currently producing approximately 0.9 Ml/day.
C	Surface water - reservoirs, lakes, streams.	Preozonation, pH adjustment, PAC, Coagulation, DAF, RGF, pH correction, super- and de-chlorination.
D	Surface water - river.	Coagulation/clarification, filtration, GAC adsorption and aeration. Disinfection at the site is achieved using a combination of chlorine and UV followed by chloramination. Plumbosolvency using phosphoric acid.
E	Groundwater - greensand boreholes.	Aeration and chlorination prior to filtration, super-chlorination and then chloramination. Plumbosolvency using phosphoric acid.
F	Surface water - upland reservoir water, which is highly coloured and contains high concentrations of manganese. Can be very high organic loading.	Pretreatment with MIEX before coagulation with ferric sulphate, and then DAFs. pH correction pre RGF, and further pH correction and sodium hypochlorite prior to manganese contactors. Final dose of sodium hypochlorite for disinfection, and contact in a relatively large (but variable) volume clean water tank.
G	Surface water mainly - Impounding Reservoir (IR) which receives river water and also a supply from a number of other impounding reservoirs, an upland River source and a borehole.	Pre-treatment with ferric sulphate for coagulation and sodium hydroxide for pH correction, then treated with a polyelectrolyte coagulant aid prior to entering the accelerators for clarification. The water then enters the primary RGF. Primary filtered water will be treated with sodium hypochlorite and pH corrected with sodium hydroxide before entering the second stage filters. Water is pH corrected with sodium hydroxide and dosed with orthophosphate prior to the contact tank and onto distribution.

Site	Source Water	Treatment
H	Ground water - two greensand boreholes (25-30 m deep).	Treatment involves raw water pumped to a GAC unit for pesticide removal and flows into a balancing tank. Partial flow is fed to an ion exchange treatment plant for nitrate removal and blended back with the raw water. The flow is marginally chlorinated to residual using a liquid sodium hypochlorite dosing system.
I	Groundwater - two wells.	Pressurised membranes followed by marginal chlorination.
J	Groundwater - combination of greensand and chalk boreholes.	Chlorination, aeration, softening and coagulation with lime and ferric, filtration, superchlorination and then chloramination. Plumbosolvency using phosphoric acid.
K	Surface water – river with high colour and turbidity when in spate, generally a high quality source.	Coagulation with ferric sulphate followed by direct filtration through a set of sand pressure filters. Filtered water is dosed with lime, carbon dioxide and chlorine (hypo) before filtration through secondary sand pressure filters and finally ultrafiltration through a membrane plant.
L	Surface water - upland reservoir water, which is highly coloured and contains high concentrations of manganese.	Blended and then coagulated at pH 4.5 with ferric sulphate and dosed with polyelectrolyte, followed by upflow clarification. There is further pH correction to approx. pH 6.2 on RGFs, and then approx. pH 8.0 and 0.5 mg chlorine/l on manganese contactors, before final treatment with sodium hypochlorite and the a dedicated two compartment relatively small (fixed) volume contact tank.
M	Surface water - impounding reservoirs under normal operation, also river if required.	The works applies ferric sulphate ahead of flat-bottomed clarification. Lime is then added for manganese removal and the water then passes through rapid gravity filters (sand/anthracite) before being chlorinated. Phosphoric acid and fluoride is also added at this point.
N	Surface water - river.	The process consists of two separate streams, each with clarification and rapid gravity filtration stages. They differ by the type of clarifier, flat-bottomed and Passovant Turbo-LME lamellas, using alum and ferric salts respectively. Some minor pH adjustment is made upstream of the RGFs for manganese removal. The two partially treated streams combine after RGFs and then pass through GAC adsorbers before chlorine, phosphoric acid and fluoride is added ahead of a combined contact / treated water storage tank.
O	Surface water - river.	Three-stage treatment plant. After abstraction, the water is pumped into three parallel streams and the pH of the raw water is adjusted with either sulphuric acid or lime and dosed with aluminium sulphate and polyelectrolyte for coagulation. The water passes to Accentrifloc (Streams 1 and 2) or Superpulsator clarifiers (Stream 3). The clarified water then passes through GAC primary filters. The filtered water from streams 1, 2 and 3 combines and flows via the IPS to the 2nd stage sand RGFs. At this point the water is pH adjusted using lime. Chlorine is dosed downstream of the lime to aid oxidation on the 2nd stage RGFs. The filtered water is chlorinated for final disinfection and flows into two contact tanks. De-

Site	Source Water	Treatment
		chlorination with sulphur dioxide occurs on the outlets of the contact tanks. The water is then pumped into distribution.
P	Surface and Groundwater - Impounding reservoir (IRs) receiving supplies from springs, two boreholes and other IRs.	Raw water entering the works is pH adjusted with with sodium hydroxide or sulphuric acid and dosed with a ferric sulphate coagulant. The dosed water then enters the DAF tank for clarification. Following clarification, the partially treated water is pH adjusted with sodium hydroxide before entering the primary filters (sand filters). Following filtration the water is pH adjusted and dosed with sodium hypochlorite for manganese removal. The water is then filtered through the second stage RGFs. Filtered water is then disinfected with sodium hypochlorite solution before entering the Contact Tank. Water is dosed with Sodium dihydrogen orthophosphate for plumbosolvency control on the inlet to the contact tank and disinfected with chlorine gas to reach final set point on the outlet main.
Q	Groundwater - private borehole.	No details available
R	Groundwater - the current site has two boreholes	No details available
S	Groundwater - has twenty on-site and off-side boreholes.	Oxidation of dissolved iron and manganese before dosing using sodium hypochlorite and particulate removal. The outlet flow is mixed with raw water from one off-site source and enters six GAC filters. Following GAC treatment is dosed with sodium hypochlorite and flows into disinfection contact tanks. Chlorine, sodium bisulphite and ammonium sulphate are used before entering the on-site clean water tank.
T	Groundwater - private borehole.	No details available
U	Surface water - river.	Flat-bottom clarification using aluminium sulphate, followed by rapid gravity filtration (sand/polarite), GAC adsorbers, chlorination, plumbosolvency using phosphoric acid, contact and storage, and artificial fluoridation.
V	Groundwater - greensand boreholes.	Aeration and chlorination prior to filtration, superchlorination and then chloramination. Plumbosolvency using phosphoric acid.
W	Groundwater - two deep chalk boreholes.	Treatment required is minimal so there is a UV unit for disinfection and then a small chlorine residual is added.

Site	Source Water	Treatment
X	Groundwater - main aquifer is chalk and there are two boreholes which operate on standby.	Raw water is superchlorinated by chlorine gas (daily licence of 8.2 Ml/day) and dosed using sulphur dioxide gas.
Y	Groundwater – several boreholes, drawn from chalk	Raw water from two boreholes flow through four GAC filters and into the blending tank with the additional raw water from the other boreholes. The water is disinfected using sodium hypochlorite and then flows into contact tanks. Sodium dihydrogen orthophosphate and sodium bisulphite are added to the water and distributed to two on-site pumping stations.
Z	Groundwater - two boreholes	Raw water is superchlorinated by chlorine gas (daily licence 19.1 Ml/day) and dosed using sulphur dioxide gas. A support supply is available from a combination of sources situated some 5-10 km of the site. The raw water is treated using a variety of methods including marginal chlorination, a combination of UV disinfection for *cryptosporidium* deactivation with super chlorination and dosing of sulphur dioxide gas.
A1	Groundwater - several boreholes, drawn from chalk	Raw water from two boreholes flow through four GAC filters and into the blending tank with the additional raw water from the other boreholes. The water was disinfected using sodium hypochlorite and then flows into contact tanks. Sodium dihydrogen orthophosphate and sodium bisulphite are added to the water and distributed to two on-site pumping stations.
B1	Groundwater - borehole supplied by four different groundwater sources	The raw water from the boreholes mixes immediately and a proportion of the water goes through ion exchange process to reduce nitrate levels. The water then passes through an in-line mixer, which further reduces the nitrate concentrations. Sodium hypochlorite is used as a disinfectant prior to the in-line mixer. The dosed water then enters the raw water inlet blending tank. Membrane filters are used as part of the main treatment proccess, which are divided into four skids each containing three racks of filters. Each filter rack contains eight individual membrane filters. Following the membrane filtration, the water receives a further dose of sodium hypochlorite and sodium dihydrogen orthophosphate for plumbosolvency control.
C1	Groundwater - two boreholes, which are situated 20 m apart and water is abstracted from the Forest Marble Formation/Great Oolite aquifer.	Treated with chlorine gas in solution producing up to approximately 8 Ml/day.
D1	Groundwater – greensand borehole	Following aeration and chlorination, the raw water is filtered, superchlorinated and chloraminated. Water is also dosed with phosphoric acid for plumbosolvency control.

DAF: Dissolved air flotation;

GAC: Granular activated carbon;

IPS: Interstage pumping station;

PAC: Powdered activated carbon;

RGF: Rapid gravity filter;

UV: Ultra-violet;

MIEX: Magnetic ion exchange.

9. First Sampling Survey (2012-2013)

Drinking water treatment works in England and Wales were selected for a 12-month sampling survey (one survey per season) on the basis that they were anticipated to contain measureable levels of chromium in the finished drinking water. This assumption was based on communication with water quality officers within the water companies that identified sites where chromium had previously been detected, and geological survey data that indicated chromium was present in river sediment samples.

9.1 Overall Results

Consolidated results for the entire survey are provided in Table 9.1. The results presented are averages of duplicate analysis, and where chromium was not detected by chromium analysis, further analysis for chromium speciation to calculate chromium (III) and (VI) concentrations were not conducted. Therefore, where the chromium concentrations are reported to be <0.1 µg/l, the inferred concentrations of <0.1 µg/l are reported for chromium (III) and (VI).

9.1.1 Chromium Analysis

Despite selecting sites on the basis that they were anticipated to contain measureable levels of chromium in the finished drinking water, in general, chromium concentrations in drinking water at these sites were low. Most sites either had no detectable chromium in finished water, or the concentration was just above the analytical limit of detection (0.1 µg/l).

9.1.2 Chromium (VI) and Chromium (III) Analysis

Of the 23 sites surveyed, 11 had at least one sample containing a detectable level of chromium (VI), and the majority of these samples were only slightly above the analytical limit of detection of 0.1 µg/l.

The analyses of chromium speciation indicate that chromium (VI) is the dominant form of chromium in drinking water, accounting for 50-100% of the total chromium detected. There is no evidence of any seasonal effect for chromium (VI) concentrations in finished drinking water at any of these sites.

Chromium (III) was only detected above the limit of detection at four sites, at a maximum concentration of 0.15 µg/l (Site V). It is noteworthy that chromium (III) was only detectable in these sites during the summer survey, albeit at a concentration that is only just above the limit of detection. Unfortunately the limited number of analytical detections of chromium (III) prevents any statistical determination of the significance of seasonal effects.

9.1.3 Summary

With the exception of one site (Site W), there is no evidence from this survey to suggest that concentrations of chromium in drinking water supplies are any higher than levels detected in other studies internationally, and the data presented here are consistent with typical background concentrations of chromium (VI) of <1 µg/l.

Table 9.1 Summary of chromium concentrations detected in finished drinking water at survey sites (Summer 2012-Spring 2013)

Site	Concentration (µg/l)															
	Summer				Autumn				Winter				Spring			
	Cr (VI)	Cr (III)	Cr (III) + Cr (VI)	Cr	Cr (VI)	Cr (III)	Cr (III) + Cr (VI)	Cr	Cr (VI)	Cr (III)	Cr (III) + Cr (VI)	Cr	Cr (VI)	Cr (III)	Cr (III) + Cr (VI)	Cr
A	0.14	<0.10	0.14	0.23	0.22	<0.10	0.22	0.27	0.13	<0.10	0.13	0.18	0.21	<0.10	0.21	0.23
B	*	*	*	*	*	*	*	*	*	*	*	*	<0.10	<0.10	<0.10	<0.10
C	<0.10	0.1	0.1	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	0.16	<0.10	<0.10	<0.10	<0.10
D	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	**	**	**	**
E	*	*	*	*	*	*	*	*	*	*	*	*	<0.10	<0.10	<0.10	<0.10
F	0.11	<0.10	0.11	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
G	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	**	**	**	**
H	*	*	*	*	*	*	*	*	*	*	*	*	0.4	<0.10	0.4	0.39
I	0.12	0.12	0.24	0.46	0.12	<0.10	0.12	0.16	<0.10	<0.10	<0.10	0.16	0.19	<0.10	0.19	<0.10
J	0.45	0.11	0.56	0.48	0.66	<0.10	0.66	0.73	NS	NS	-	-	0.17	<0.10	0.17	<0.10
K	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	0.15	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
L	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
M	NS	NS	NS	NS	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
N	0.1	<0.10	0.1	0.14	<0.10	<0.10	<0.10	0.15	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
O	0.11	<0.10	0.11	<0.10	0.13	<0.10	0.13	0.27	0.11	<0.10	0.11	0.28	<0.10	<0.10	<0.10	<0.10
P	<0.10	<0.10	<0.10	<0.10	NS	NS	NS	NS	<0.10	<0.10	<0.10	<0.10	**	**	**	**
Q	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	0.1	<0.10	<0.10	<0.10	<0.10
R	0.13	<0.10	0.13	<0.10	NS	NS	NS	NS	<0.10	<0.10	<0.10	0.16	0.18	<0.10	0.18	<0.10
S	*	*	*	*	*	*	*	*	*	*	*	*	0.2	<0.10	0.2	0.13
T	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
U	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
V	0.11	0.15	0.26	<0.10	0.49	<0.10	0.49	0.54	0.15	<0.10	0.15	0.16	0.12	<0.10	0.12	<0.10
W	6.65	<0.10	6.65	6.9	6.74	<0.10	6.74	6.87	8.82	<0.10	8.82	7.6	8.08	<0.10	8.08	6.99

* This site was not originally part of the survey; it was added for the final survey in an attempt to locate additional sites with detectable levels of chromium.

** This site was not included in the spring survey; due to the consistent lack of detectable levels of chromium in the finished water, this site was substituted with samples from a new location.

NS: Not sampled due to logistical reasons.

9.2 Site A

The results of the chromium analysis at Site A are presented in Table 9.2, Figure 9.1 and Figure 9.2. These data indicate that chromium was present in the finished water at all measured time points, and that the concentration of chromium was relatively consistent.

Chromium (III) was not detected above the limit of detection in any sample at Site A, indicating that chromium (VI) was the dominant form in finished drinking water.

Table 9.2 Summary of chromium concentrations detected in finished drinking water at Site A

Season	Concentration (µg/l)			
	Chromium	Chromium (VI)	Chromium (III)	Chromium (VI) + Chromium (III)
Summer	0.23	0.14	<0.10	0.14
Autumn	0.27	0.22	<0.10	0.22
Winter	0.18	0.13	<0.10	0.13
Spring	0.23	0.21	<0.10	0.21

Figure 9.1 Summary of chromium speciation results in finished drinking water at Site A

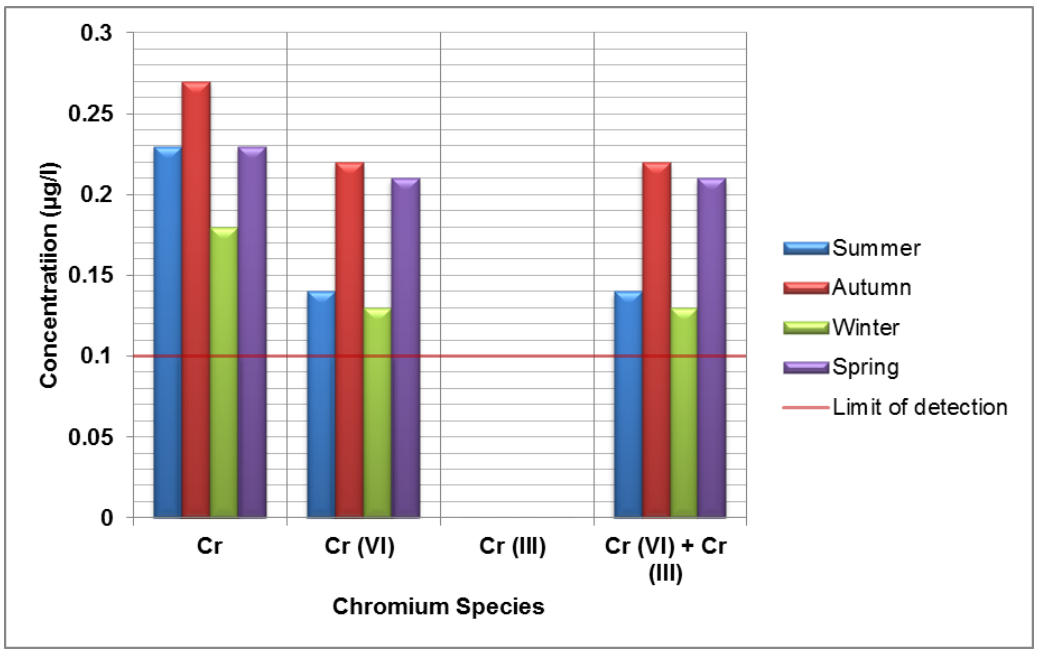
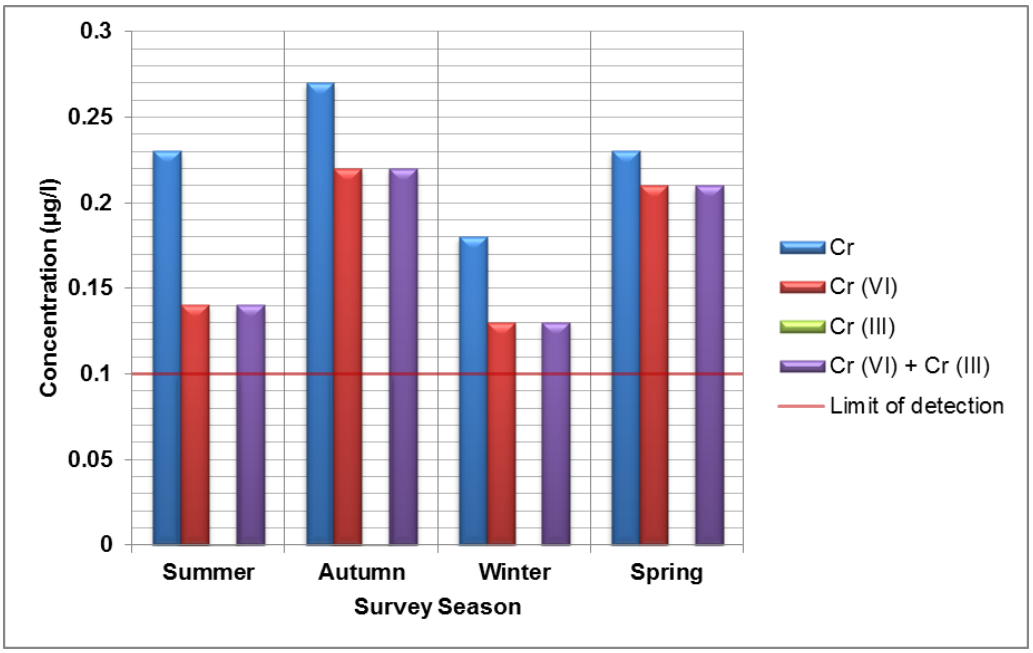


Figure 9.2 Summary of chromium seasonal results in finished drinking water at Site A



9.3 Site B

Site B was added to the sampling programme for the final survey (spring survey) in an attempt to locate additional sites that were suspected of containing measurable concentrations of chromium in finished water. However, chromium was not detectable at this site at a concentration above the limit of detection (0.1 µg/l).

9.4 Site C

The results of the chromium analysis at Site C are presented in Figure 9.3 and Figure 9.4. These data indicate that chromium (III) was only detected in the summer survey (at the limit of detection).

Interestingly, a chromium concentration of 0.16 µg/l was detected in the winter survey, however, on this occasion, neither chromium (III) nor chromium (VI) were detected above the limit of detection. This may indicate the presence of other forms of chromium in the sample (which are generally considered to be unstable intermediates in the formation of chromium (III) and (VI)). However, caution must be applied when considering this result. The raw data provided by the analytical laboratory indicates that chromium was also found in the control sample for this site. Therefore, the possibility that this result may be an analytical artefact cannot be discounted.

Figure 9.3 Summary of chromium speciation results in finished drinking water at Site C

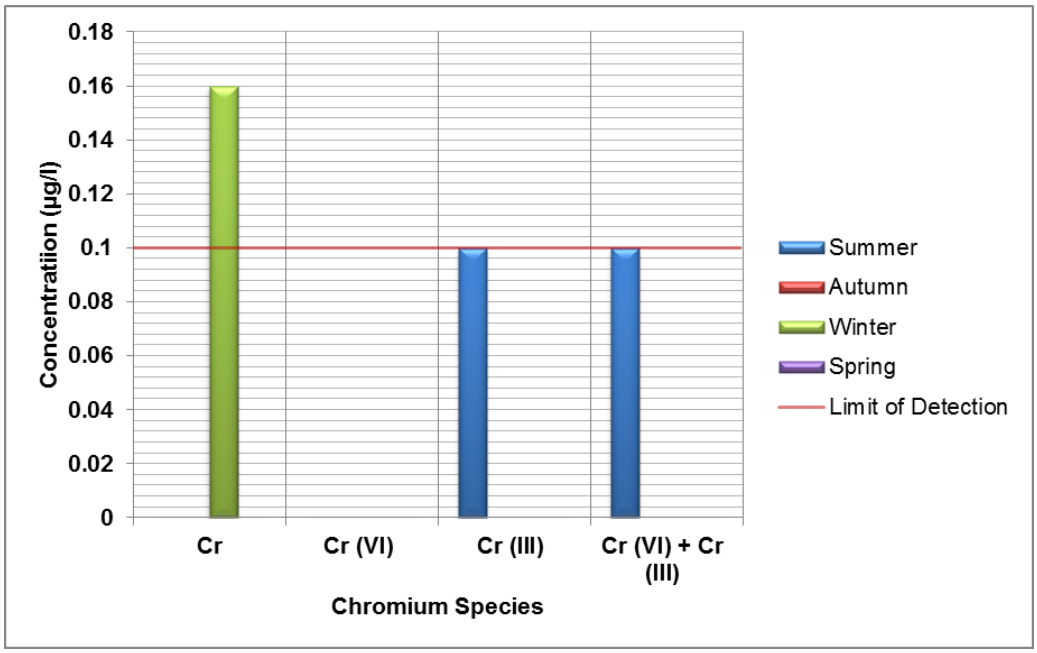
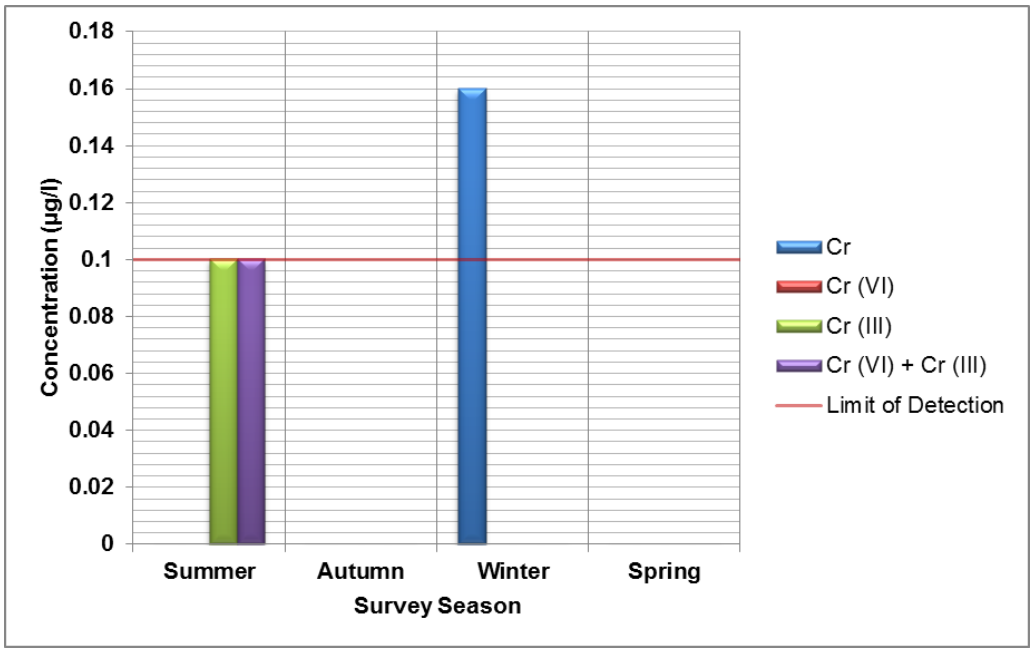


Figure 9.4 Summary of chromium seasonal results in finished drinking water at Site C



9.5 Site D

Chromium was not detected in any sample of finished water from Site D above the analytical limit of detection (0.1 µg/l). Due to the absence of detectable levels of chromium in the first three surveys, a survey was not conducted at Site D during the spring. Instead this site was substituted for a new location in an attempt to located additional sites with detectable levels of chromium.

9.6 Site E

Site E was added to the sampling programme for the final survey (spring survey) in an attempt to locate additional sites that were suspected of containing measurable concentrations of chromium in finished water. However, chromium was not detectable at this site at a concentration above the limit of detection (0.1 µg/l).

9.7 Site F

The results of the chromium analysis at Site F are presented in Figure 9.5 and Figure 9.6. These data indicate chromium was only detectable above the limit of detection in the summer survey as chromium (VI), when measured as individual species, however, the chromium concentration was reported to be below the limit of detection.

Figure 9.5 Summary of chromium speciation results in finished drinking water at Site F

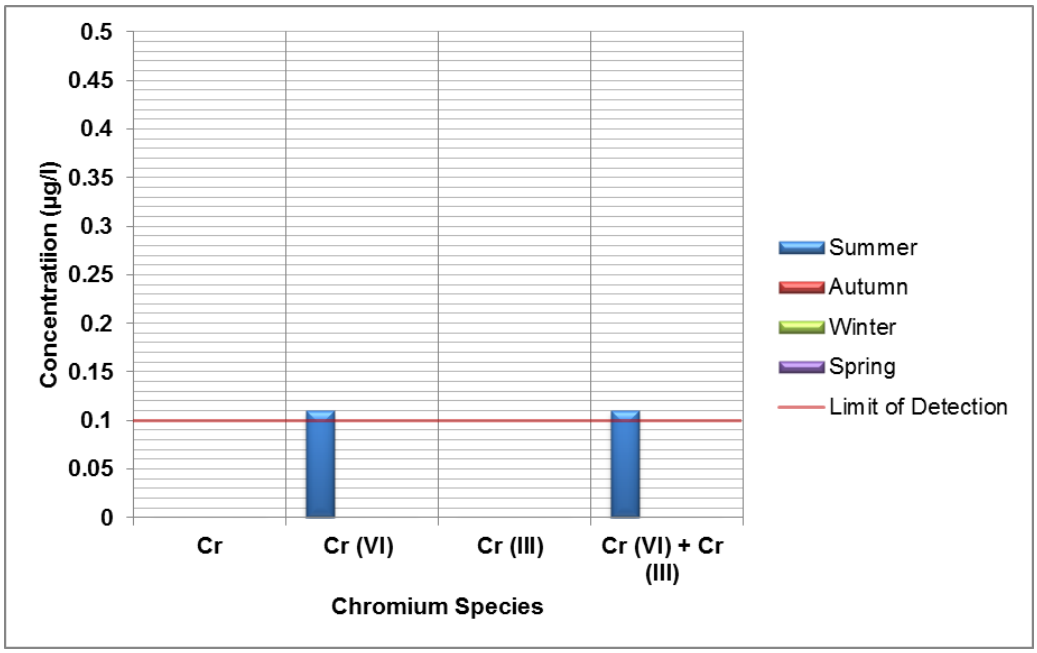
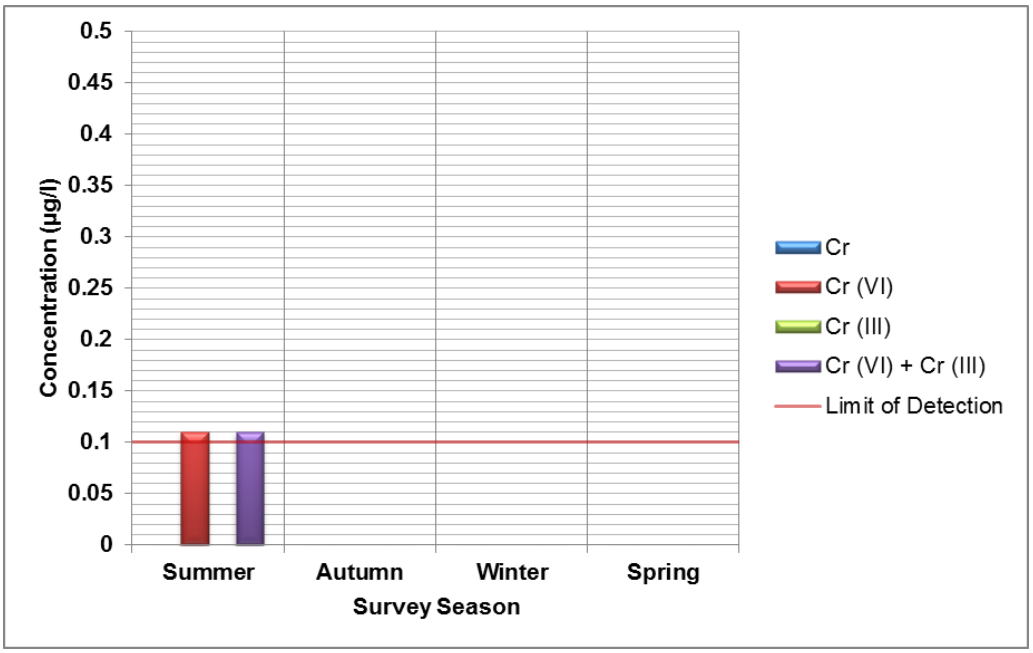


Figure 9.6 Summary of chromium seasonal results in finished drinking water at Site F



9.8 Site G

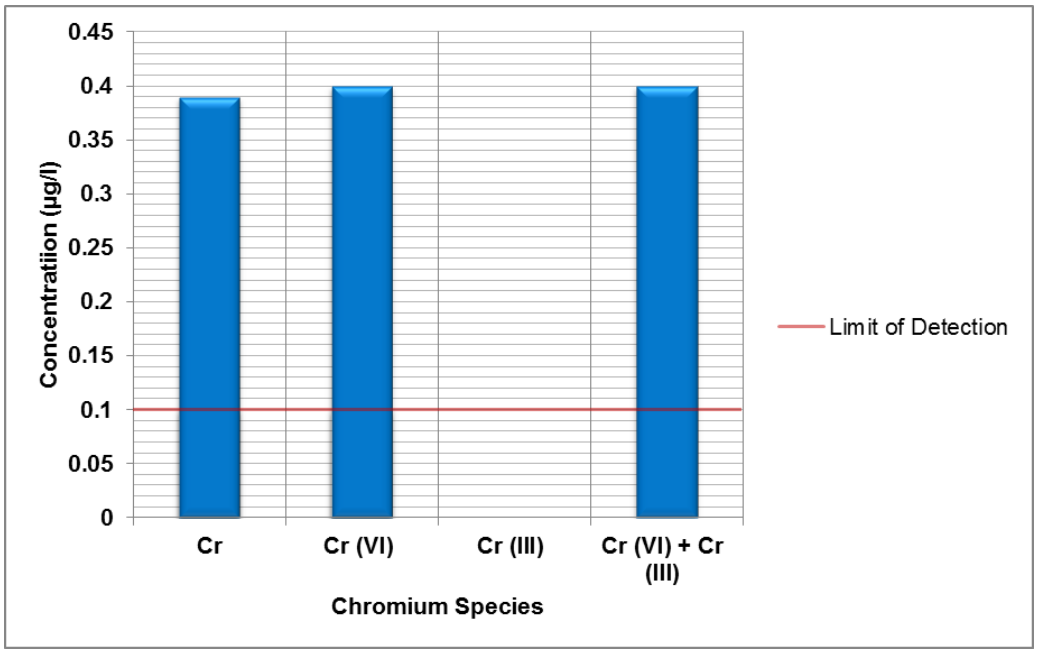
Chromium was not detected in any sample of finished water from Site G above the analytical limit of detection (0.1 µg/l). Due to the absence of detectable levels of chromium in the first three surveys, a survey was not conducted at Site G during the spring. Instead this site was substituted for a new location in an attempt to located additional sites with detectable levels of chromium.

9.9 Site H

Site H was added to the sampling programme for the final survey (spring survey) in an attempt to locate additional sites that were suspected of containing measurable concentrations of chromium in finished water.

The results of the chromium analysis at Site H are presented in Figure 9.7. These data indicate that chromium was present in the finished water entirely in the form of chromium (VI), with the results for chromium measurements and chromium (VI) measurements essentially identical.

Figure 9.7 Summary of chromium speciation results in finished drinking water at Site H



9.10 Site I

The results of the chromium analysis at Site I are presented in Table 9.3, Figure 9.8 and Figure 9.9. These data indicate that chromium was present in the finished water at all measured time points.

Chromium analysis indicated detectable levels of chromium in the summer, autumn and winter surveys, with the concentration in the summer approximately three times higher than the autumn and winter surveys.

Chromium (VI) was detected in the summer, autumn and spring surveys. The results were generally consistent, although the concentration in the spring survey was slightly higher than the summer and autumn surveys.

Chromium (III) was only detected in the summer survey. It may be of note that the sum concentration of chromium (III) and chromium (VI) in the summer survey only accounts for half of the chromium detected by chromium analysis. The consistency between the duplicate samples at this site would indicate that this result is not due to an analytical artefact; therefore, this may indicate the presence of other forms of chromium in the sample.

It is also interesting to note that this is one of only four sites where chromium (III) has been detected above the limit of detection, and while there is no clear trend for a seasonal effect for total chromium or chromium (VI) concentrations, all four of those sites only had detectable levels of chromium (III) in the summer survey.

Table 9.3 Summary of chromium concentrations detected in finished drinking water at Site I

Season	Concentration (µg/l)			
	Chromium	Chromium (VI)	Chromium (III)	Chromium (VI) + Chromium (III)
Summer	0.46	0.12	0.12	0.24
Autumn	0.16	0.12	<0.10	0.12
Winter	0.16	<0.10	<0.10	<0.10
Spring	<0.10	0.19	<0.10	0.19

Figure 9.8 Summary of chromium speciation results in finished drinking water at Site I

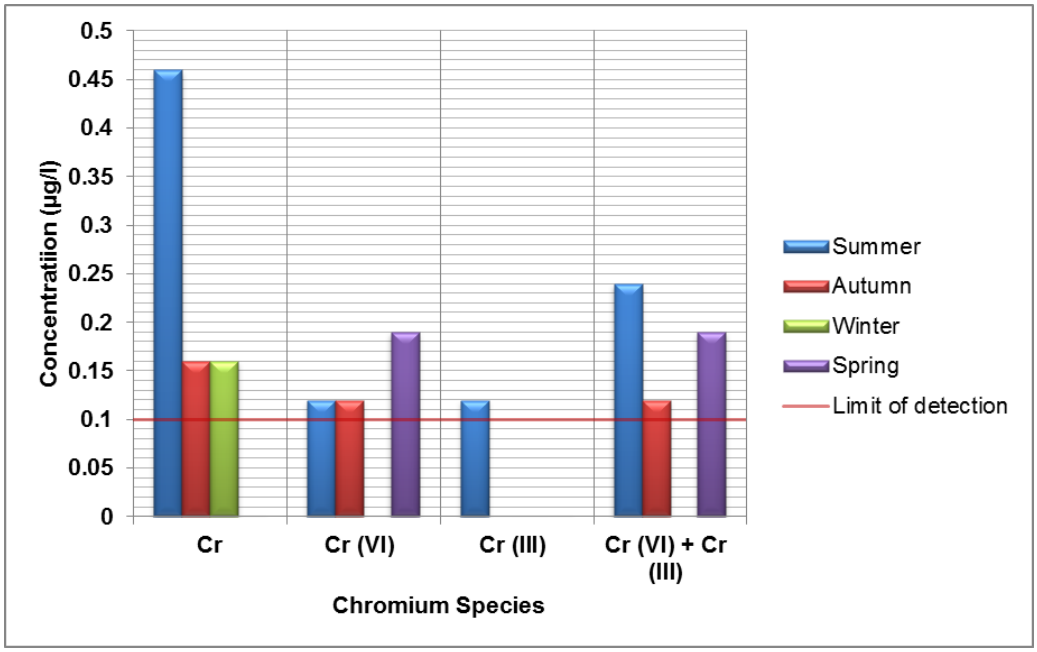
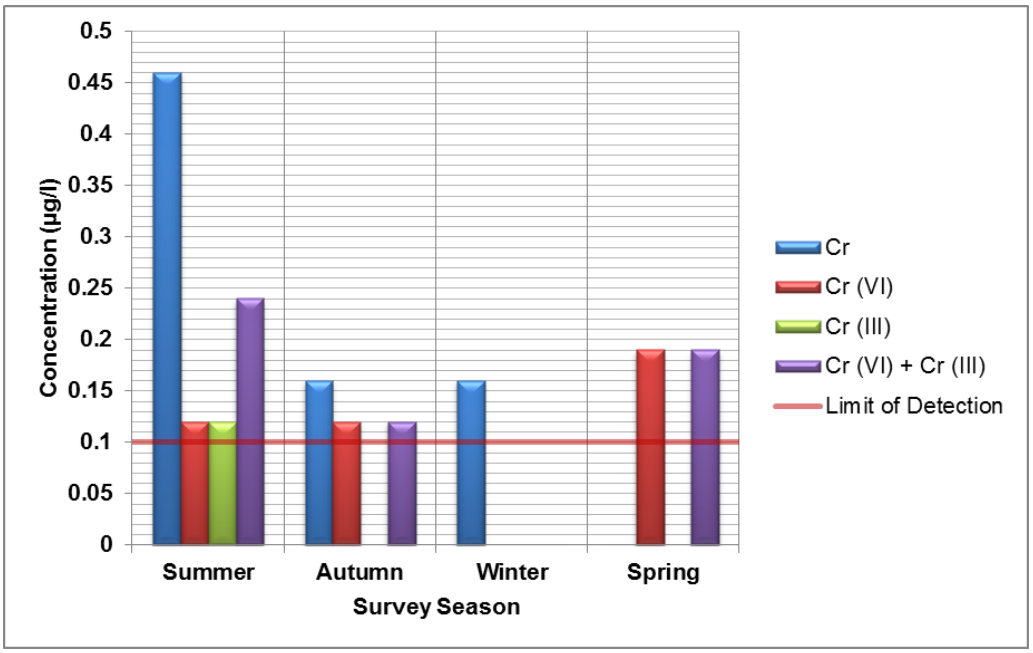


Figure 9.9 Summary of chromium seasonal results in finished drinking water at Site I



9.11 Site J

The results of the chromium analysis at Site J are presented in Table 9.4, Figure 9.10 and Figure 9.11. These data indicate that chromium (VI) was the dominant form of chromium present in the water samples, with detectable levels in all seasons that were surveyed at this site. Unfortunately due to unforeseeable circumstances, this works was shut down over the winter, and it was therefore not possible to survey this site during the winter period. The levels of chromium (VI) were also generally consistent with the results from the chromium analysis.

Table 9.4 Summary of chromium concentrations detected in finished drinking water at Site J

Season	Concentration (µg/l)			
	Chromium	Chromium (VI)	Chromium (III)	Chromium (VI) + Chromium (III)
Summer	0.48	0.45	0.11	0.56
Autumn	0.73	0.66	<0.10	0.66
Winter	NS	NS	NS	NS
Spring	<0.1	0.17	<0.1	0.17

NS: Not Sampled

Figure 9.10 Summary of chromium speciation results in finished drinking water at Site J

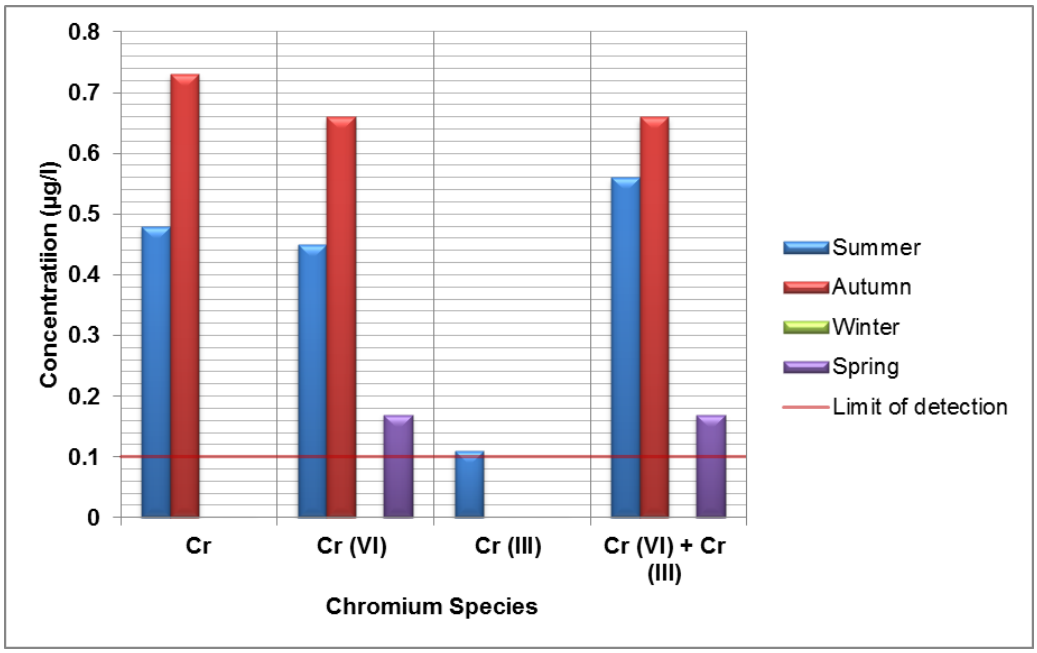
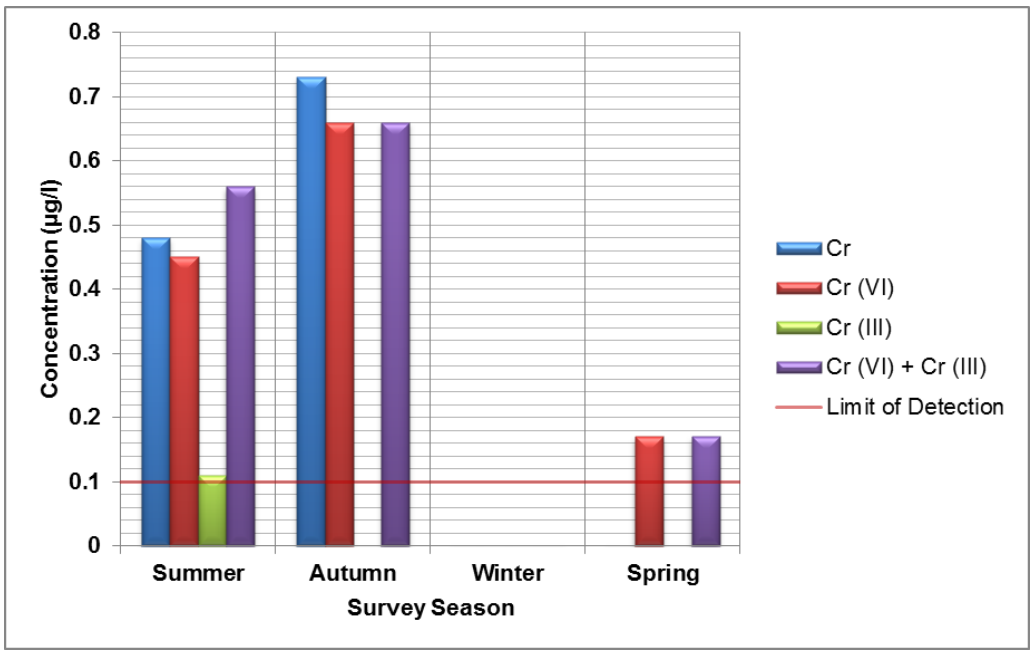


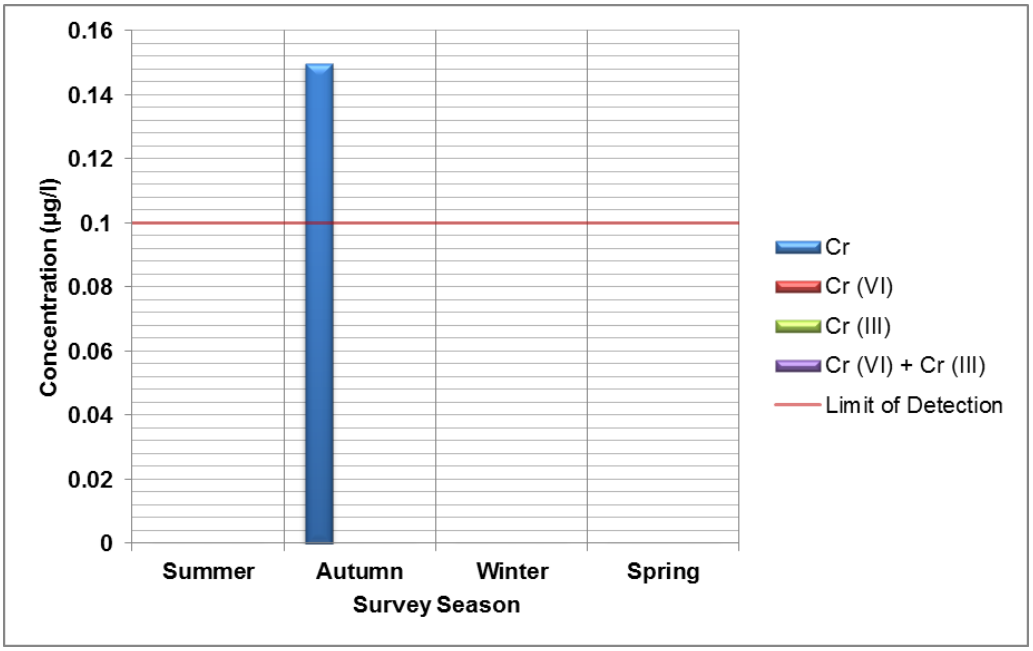
Figure 9.11 Summary of chromium seasonal results in finished drinking water at Site J



9.12 Site K

The results of the chromium analysis at Site K are presented in Figure 9.12. These data indicate that chromium was only detected in the autumn survey by chromium analysis.

Figure 9.12 Summary of chromium seasonal results in finished drinking water at Site K



9.13 Site L

Chromium was not detected in any sample of finished water from Site L above the analytical limit of detection (0.1 µg/l).

9.14 Site M

Chromium was not detected in any sample of finished water from Site M above the analytical limit of detection (0.1 µg/l).

9.15 Site N

The results of the chromium analysis at Site J are presented in Figure 9.13 and Figure 9.14. These data indicate that chromium was detected by chromium analysis in the summer and autumn surveys and chromium (VI) was only detected in the summer survey. Chromium (III) was not detected above the limit of detection in any of the surveys at this site.

Figure 9.13 Summary of chromium speciation results in finished drinking water at Site N

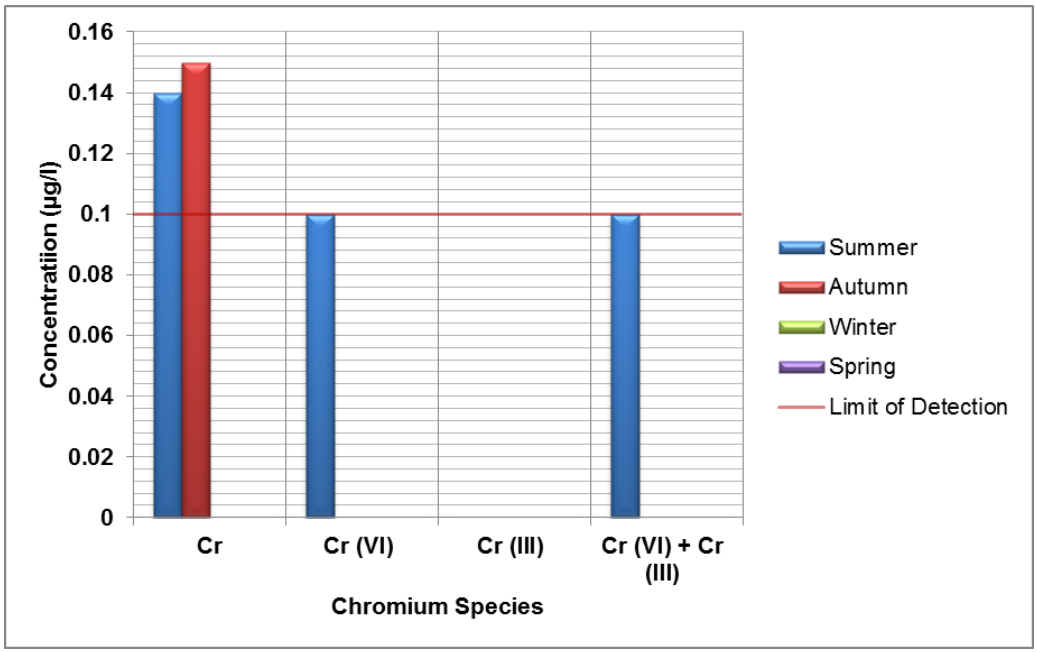
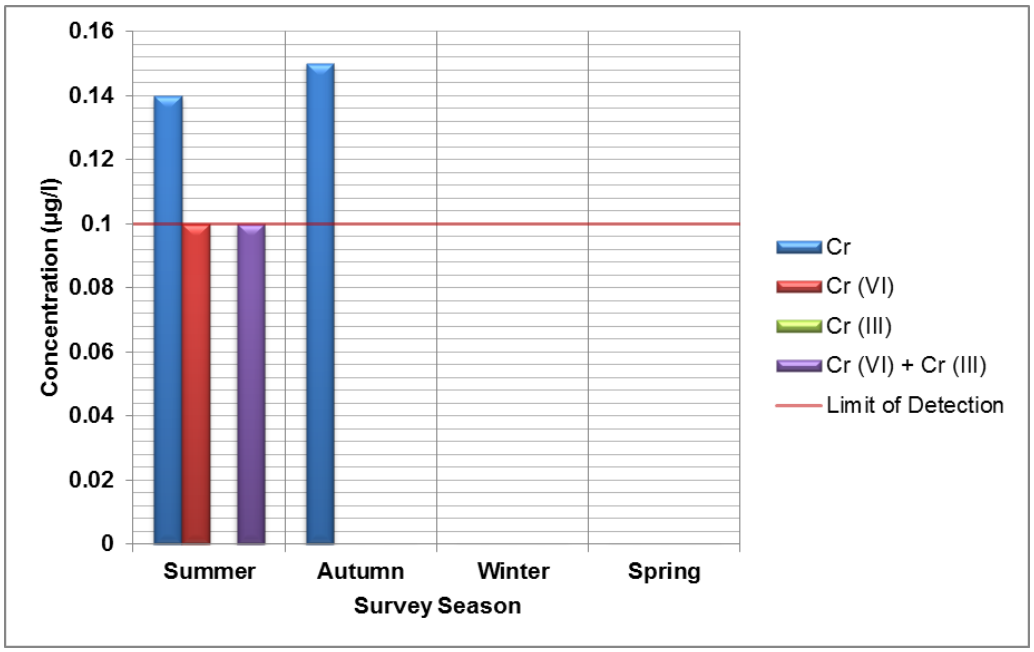


Figure 9.14 Summary of chromium seasonal results in finished drinking water at Site N



9.16 Site O

The results of the chromium analysis at Site O are presented in Table 9.5, Figure 9.15 and Figure 9.16. These data indicate that chromium (VI) was detected just above the limit of detection in the summer, autumn and winter surveys, but was not detectable in the spring survey. Chromium (III) was not detected above the limit of detection in any of the surveys.

Chromium was detected by chromium analysis in the autumn and winter surveys. Interestingly, in both of these surveys, the chromium concentration was more than twice the concentration of chromium (VI). The consistency between the duplicate samples in these surveys would indicate that this result is not due to an analytical artefact; therefore, this may indicate the presence of other forms of chromium in the sample.

Table 9.5 Summary of chromium concentrations detected in finished drinking water at Site O

Season	Concentration (µg/l)			
	Chromium	Chromium (VI)	Chromium (III)	Chromium (VI) + Chromium (III)
Summer	<0.10	0.11	<0.10	0.11
Autumn	0.27	0.13	<0.10	0.13
Winter	0.28	0.11	<0.10	0.11
Spring	<0.10	<0.10	<0.10	<0.10

Figure 9.15 Summary of chromium speciation results in finished drinking water at Site O

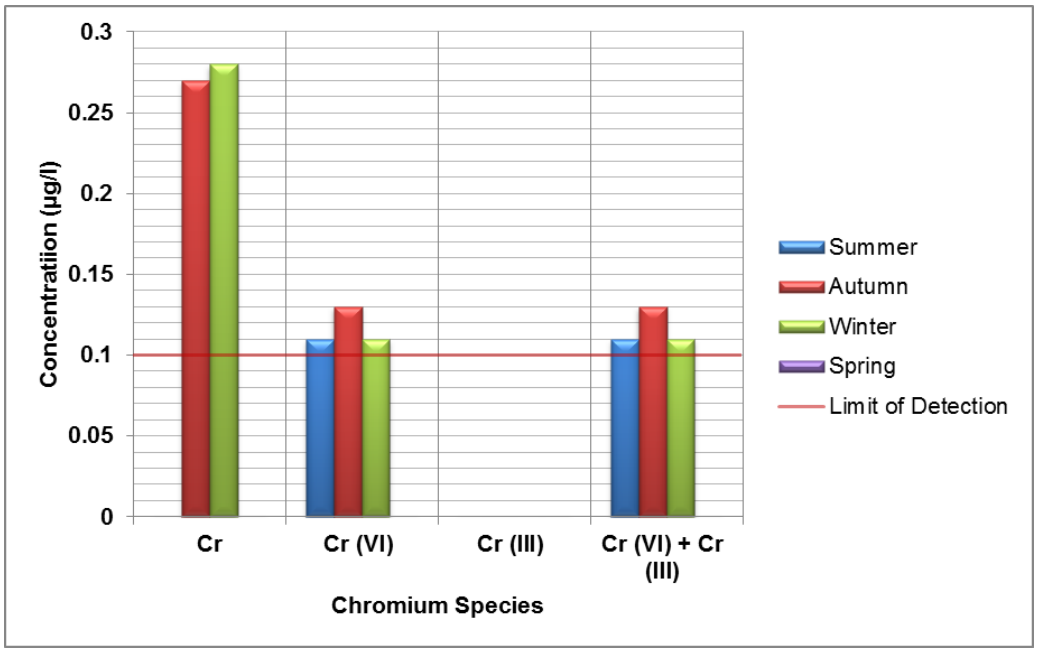
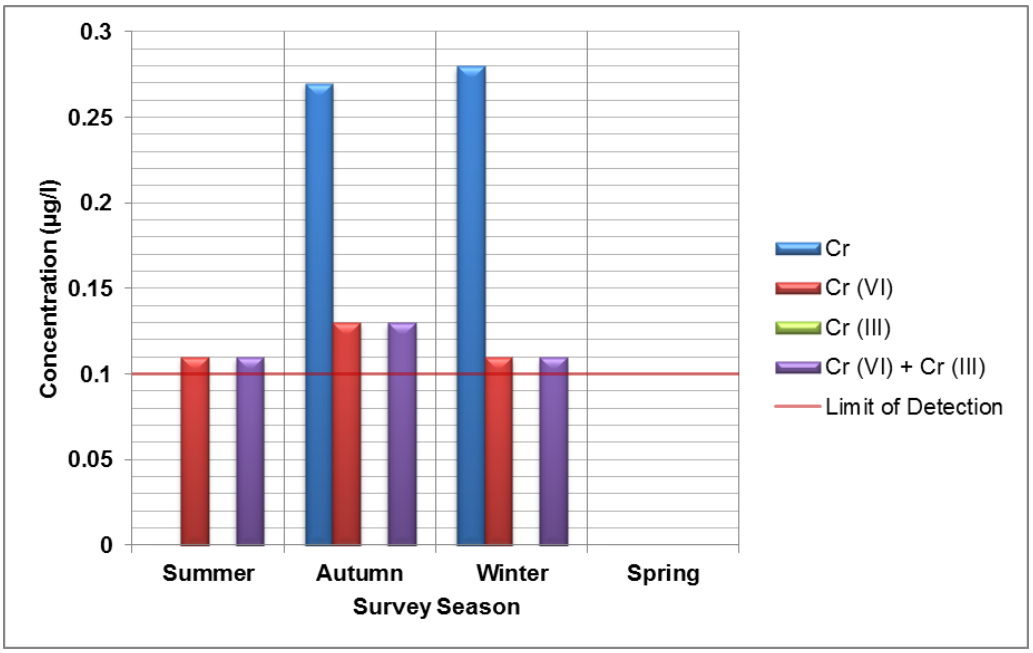


Figure 9.16 Summary of chromium seasonal results in finished drinking water at Site O



9.17 Site P

Chromium was not detected in any sample of finished water from Site P above the analytical limit of detection (0.1 µg/l).

9.18 Site Q

Chromium was not detected in any sample of finished water from Site Q above the analytical limit of detection (0.1 µg/l).

9.19 Site R

The results of the chromium analysis at Site R are presented in Table 9.6, Figure 9.17 and Figure 9.18. These data indicate that chromium (VI) was detected in both the summer and spring surveys, but not the winter survey (sampling did not take place at this site during the autumn survey). Chromium was detected by chromium analysis during the winter and spring surveys.

Table 9.6 Summary of chromium concentrations detected in finished drinking water at Site R

Season	Concentration (µg/l)			
	Chromium	Chromium (VI)	Chromium (III)	Chromium (VI) + Chromium (III)
Summer	<0.10	0.13	<0.10	0.13
Autumn	NS	NS	NS	NS
Winter	0.16	<0.10	<0.10	<0.10
Spring	0.11	0.18	<0.10	0.18

NS: Not Sampled

Figure 9.17 Summary of chromium speciation results in finished drinking water at Site R

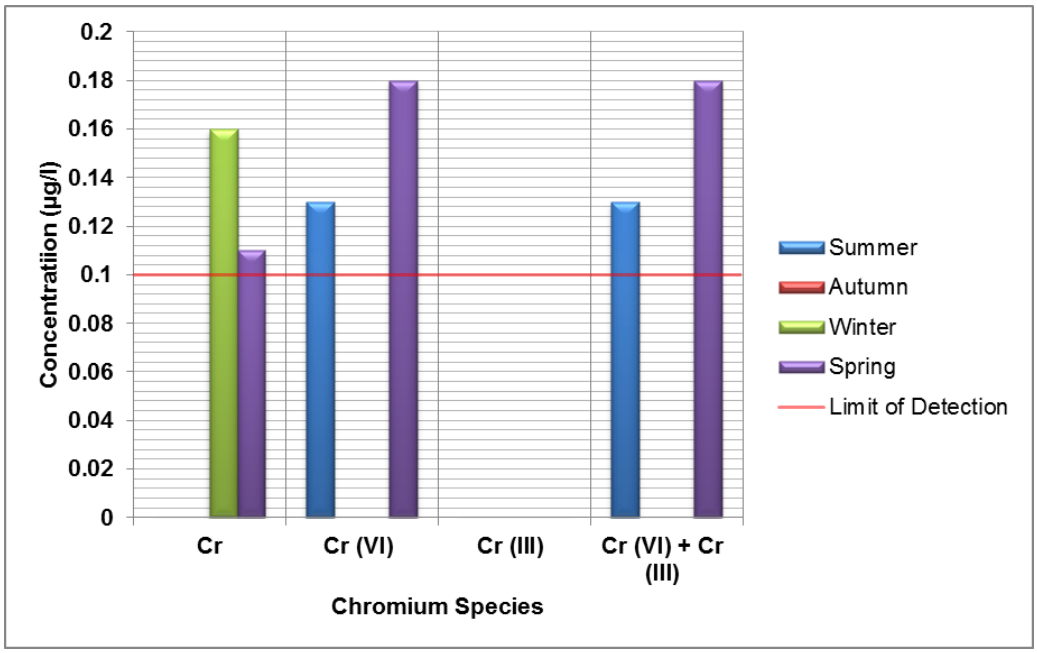
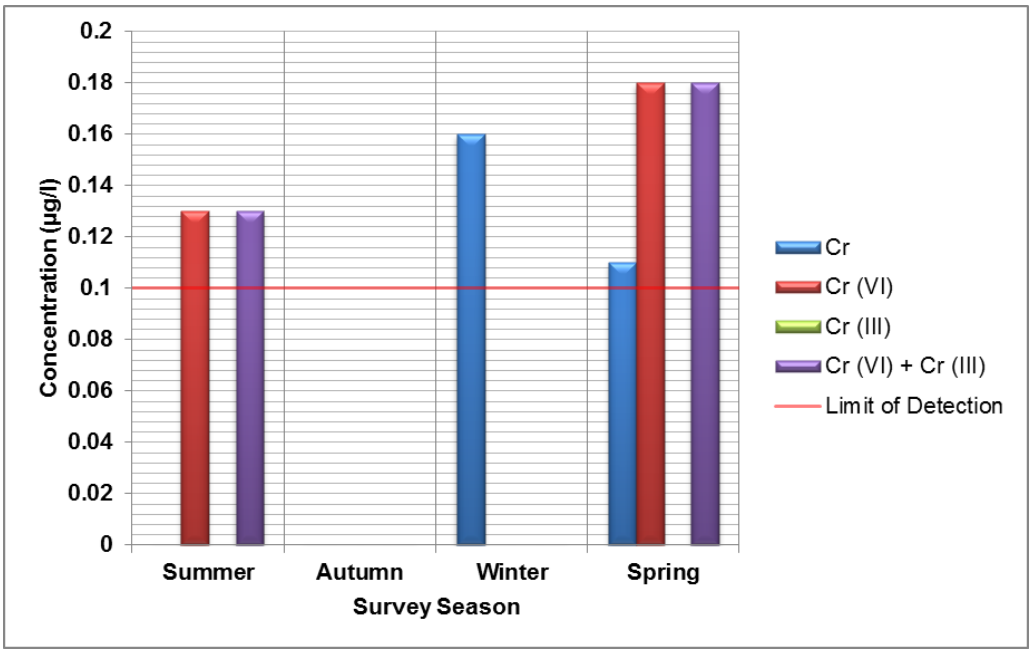


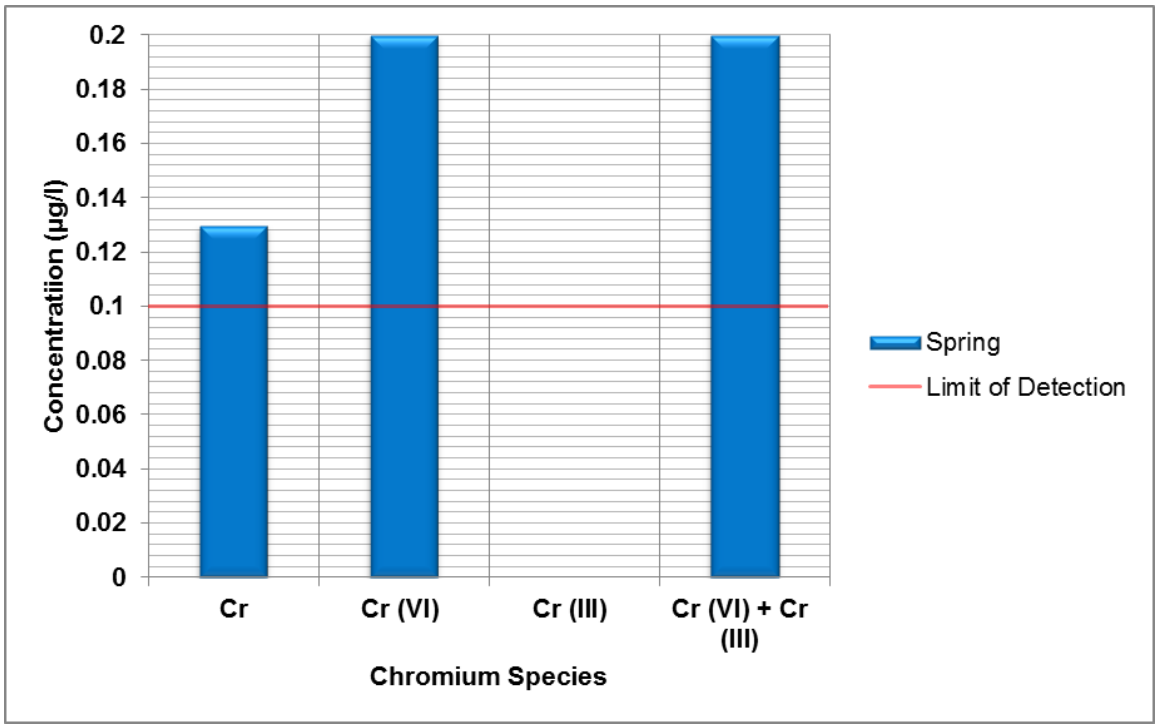
Figure 9.18 Summary of chromium seasonal results in finished drinking water at Site R



9.20 Site S

Site S was added to the sampling programme for the final survey (spring survey) in an attempt to locate additional sites that were suspected of containing measurable concentrations of chromium in finished water. The results of the chromium analysis at Site S are presented in Figure 9.19. These data indicate that chromium (VI) was the dominant form of chromium in the water supply.

Figure 9.19 Summary of chromium speciation results in finished drinking water at Site S



9.21 Site T

Chromium was not detected in any sample of finished water from Site T above the analytical limit of detection (0.1 µg/l).

9.22 Site U

Chromium was not detected in any sample of finished water from Site U above the analytical limit of detection (0.1 µg/l).

9.23 Site V

The results of the chromium analysis at Site V are presented in Table 9.7, Figure 9.20 and Figure 9.21. These data indicate that chromium (VI) was detected during all surveys, with the concentration relatively consistent during the summer, winter and spring surveys, but higher in the autumn survey. This site also had detectable levels of chromium (III) in the summer survey, although no chromium (III) was reported in the autumn, winter or spring surveys. Chromium was detected by chromium analysis during the autumn and winter surveys at a concentration similar to that of chromium (VI). However, chromium was not detected by chromium analysis during the summer survey, where the combined concentration of chromium (III) and chromium (VI) by individual species analysis was 0.26 µg/l.

Table 9.7 Summary of chromium concentrations detected in finished drinking water at Site V

Season	Concentration (µg/l)			
	Chromium	Chromium (VI)	Chromium (III)	Chromium (VI) + Chromium (III)
Summer	<0.10	0.11	0.15	0.26
Autumn	0.54	0.49	<0.10	0.49
Winter	0.16	0.15	<0.10	0.15
Spring	<0.10	0.12	<0.10	0.12

Figure 9.20 Summary of chromium speciation results in finished drinking water at Site V

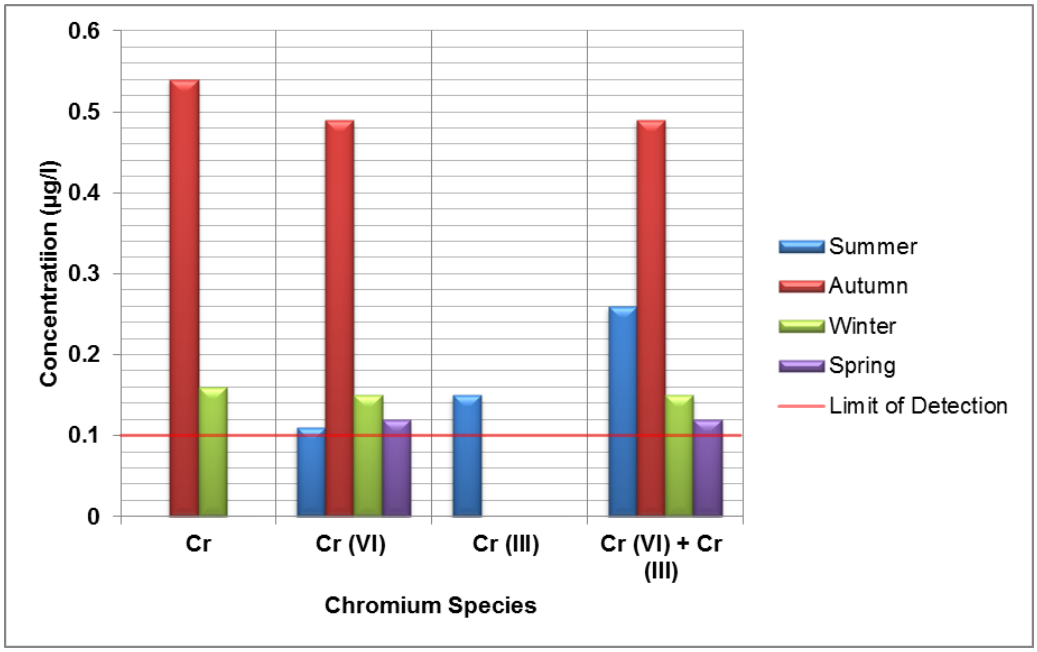
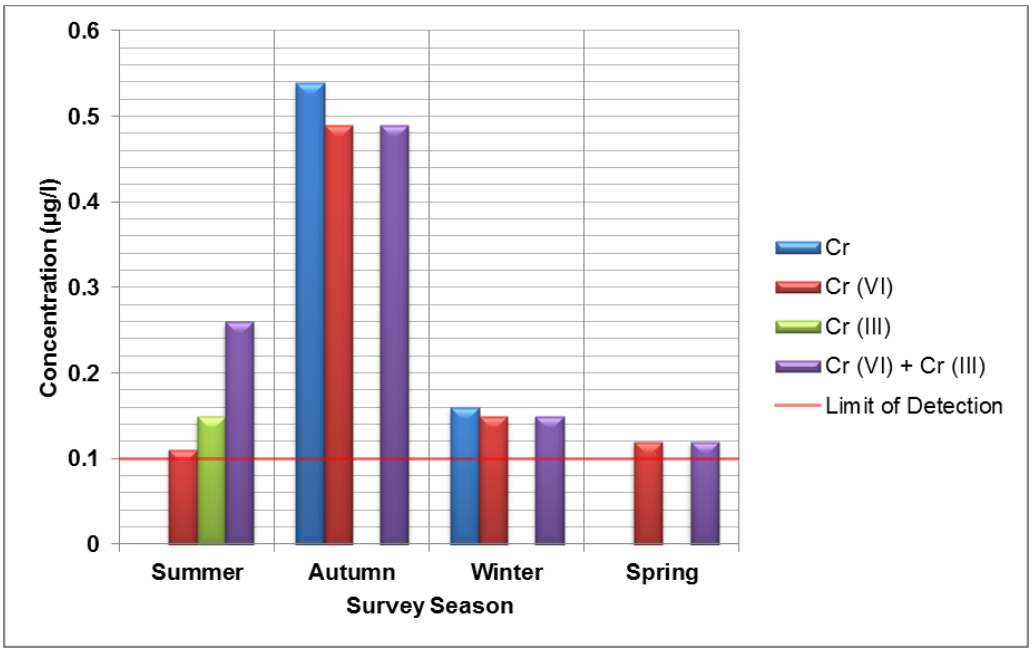


Figure 9.21 Summary of chromium seasonal results in finished drinking water at V



9.24 Site W

The results of the chromium analysis at Site W are presented in Table 9.8, Figure 9.22 and Figure 9.23. Site W consistently contained the highest levels of chromium and the highest levels of chromium (VI) throughout the survey. Concentrations of chromium (VI) were lowest in the summer survey, at 6.65 µg/l, and highest in the winter at 8.82 µg/l. Concentrations of chromium were highest in the summer at 9.1 µg/l, and lowest in the autumn at 6.87 µg/l. Chromium (III) was not detected in any of the surveys above its limit of detection.

It should be noted that the pH of samples at this site was often below 8. The polypropylene digi-tubes used in all the surveys contained a solid preservative that was intended to raise the pH of the water samples to >8 to maintain sample stability, i.e. to prevent reduction of chromium (VI) to chromium (IIII). Even with this preservative, a pH of >8 was not always obtained. However, lack of detectable chromium (III) in these samples would suggest that despite the lower pH, the samples were sufficiently stable for the duration of the sampling and analysis period.

Table 9.8 Summary of chromium concentrations detected in finished drinking water at Site W

Season	Concentration (µg/l)			
	Chromium	Chromium (VI)	Chromium (III)	Chromium (VI) + Chromium (III)
Summer	9.1	6.65	<0.10	6.65
Autumn	6.87	6.74	<0.10	6.74
Winter	7.6	8.82	<0.10	8.82
Spring	6.99	8.08	<0.10	8.08

Figure 9.22 Summary of chromium speciation results in finished drinking water at Site W

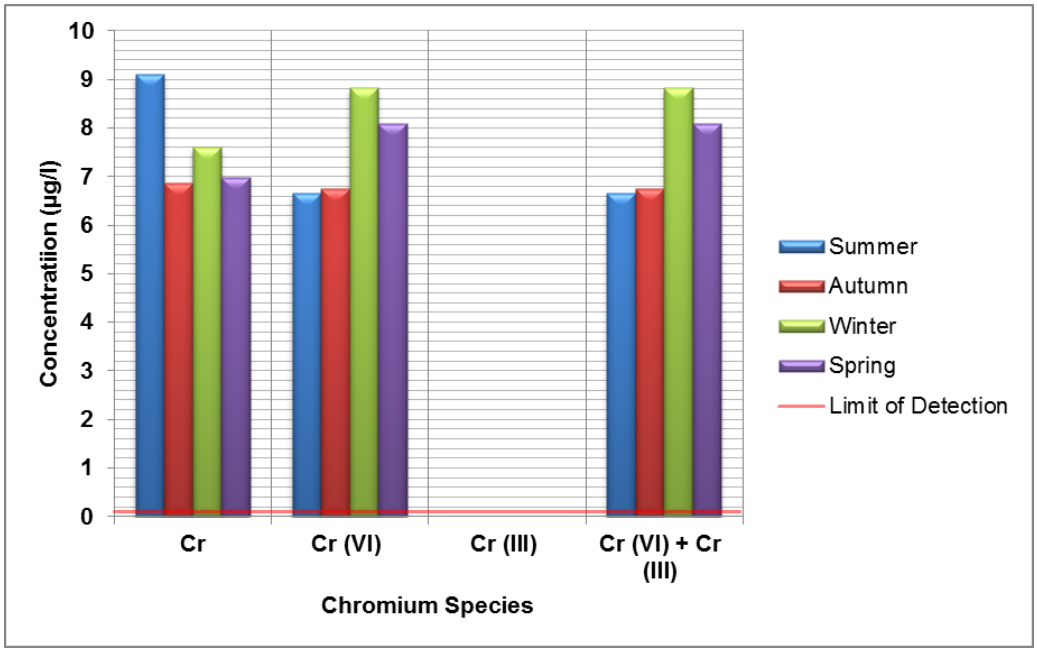
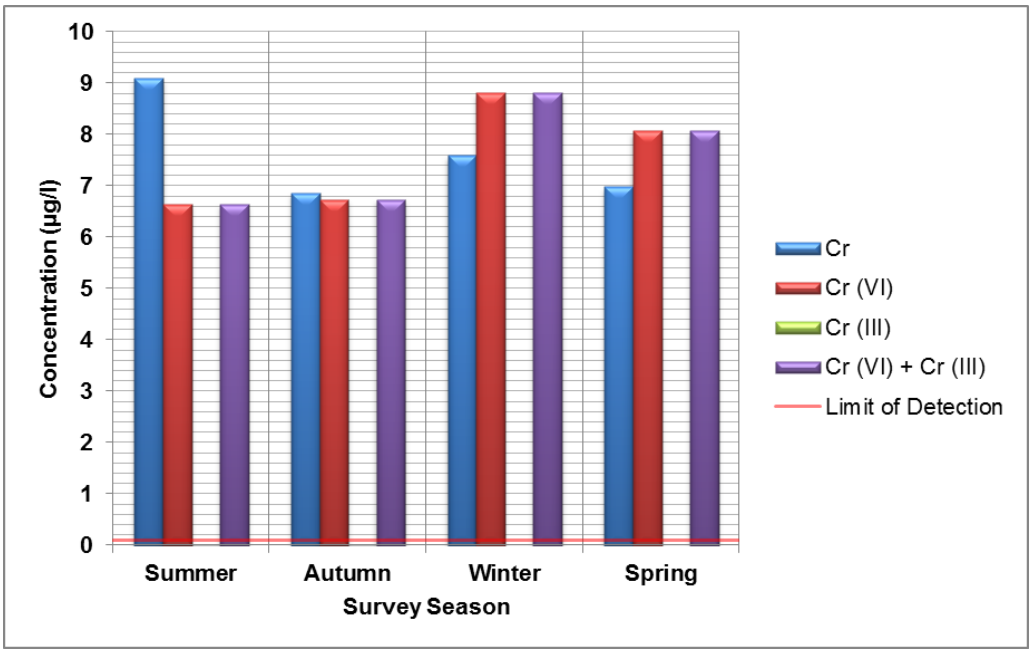


Figure 9.23 Summary of chromium seasonal results in finished drinking water at Site W



9.24.1 Comparison of Raw and Finished Water

During the winter and spring surveys at Site W, raw water samples were also collected from the site to provide an indication as to whether drinking water treatment processes were influencing the speciation of chromium in the finished water. Raw water is abstracted from two boreholes at Site W, and two measurements were made of the samples from each of these boreholes. Additionally, a further analysis was conducted on the one sample from each borehole with sample that was not treated with preservative in the winter survey (as described in Section 7.7).

While conclusive statements cannot be drawn from a single site, these results suggest that drinking water treatment does not alter the speciation of chromium in water (Table 9.9 and Figure 9.24). However, only minimal water treatment occurs at this site, so no conclusions can be drawn with respect to the actions of drinking water treatment and its effects on chromium speciation at other sites in this survey. An additional survey of raw water used for abstraction in England and Wales may be could provide further information on the environmental speciation of chromium and determine the effect of drinking water treatment on chromium speciation.

The concentration of chromium in the finished drinking water is also consistent with the concentrations that would be expected from a blending of equal concentrations of samples from the two untreated boreholes. This may indicate that the drinking water treatment processes in operation at this site are ineffective at removing chromium from the water supply.

Table 9.9 Summary of chromium speciation in untreated and finished water at Site W in the Winter Survey

Sample Type	Speciation	Concentration (µg/l)	
		Sample 1	Sample 2
Finished	Chromium	11.04	9.33
	Chromium (III)	<0.10	<0.10
	Chromium (VI)	8.83	8.8
Untreated Borehole 1	Chromium	3.76	3.41
	Chromium (III)	<0.10	<0.10
	Chromium (VI)	3.26	3.16
Untreated & unpreserved Borehole 1	Chromium	3.1	NS
	Chromium (III)	<0.10	NS
	Chromium (VI)	3.25	NS
Untreated Borehole 2	Chromium	15	14.72
	Chromium (III)	<0.10	<0.10
	Chromium (VI)	14.52	14.9
Untreated & unpreserved Borehole 2	Total	15.75	NS
	Chromium (III)	<0.10	NS
	Chromium (VI)	14.72	NS

NS: Not Sampled.

Table 9.10 Summary of chromium speciation in untreated and finished water at Site W in the Spring Survey

Sample Type	Speciation	Concentration (µg/l)	
		Sample 1	Sample 2
Finished	Chromium	8.33	10.3
	Chromium (III)	<0.10	<0.10
	Chromium (VI)	8.1	8.07
Untreated & unpreserved Borehole 1	Chromium	3.08	2.78
	Chromium (III)	<0.10	<0.10
	Chromium (VI)	2.94	2.85
Untreated & unpreserved Borehole 2	Chromium	7.23	7.15
	Chromium (III)	<0.10	<0.10
	Chromium (VI)	13.83	14.22

Figure 9.24 Summary of chromium speciation in untreated and finished water at Site W in the Winter Survey

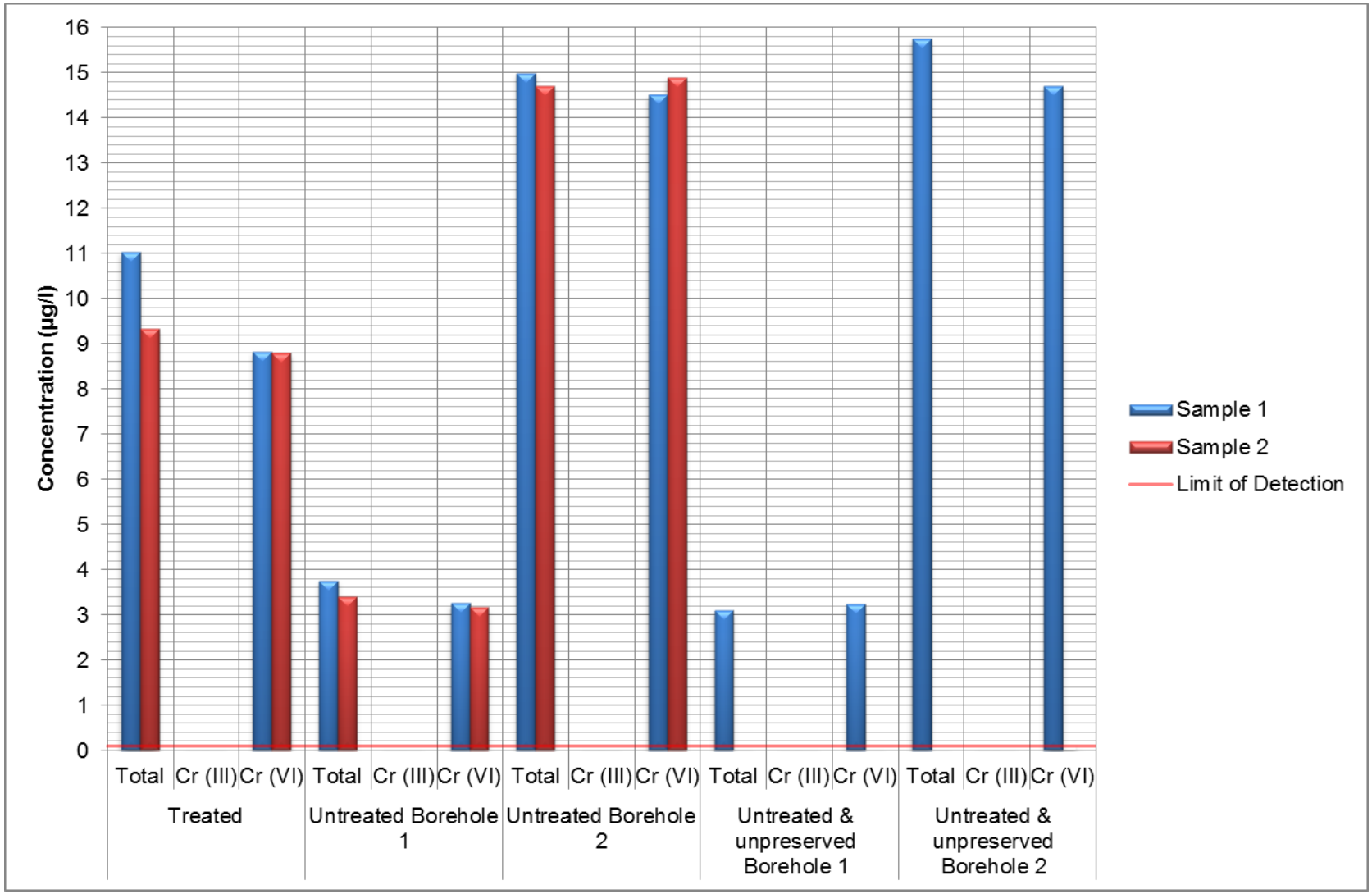
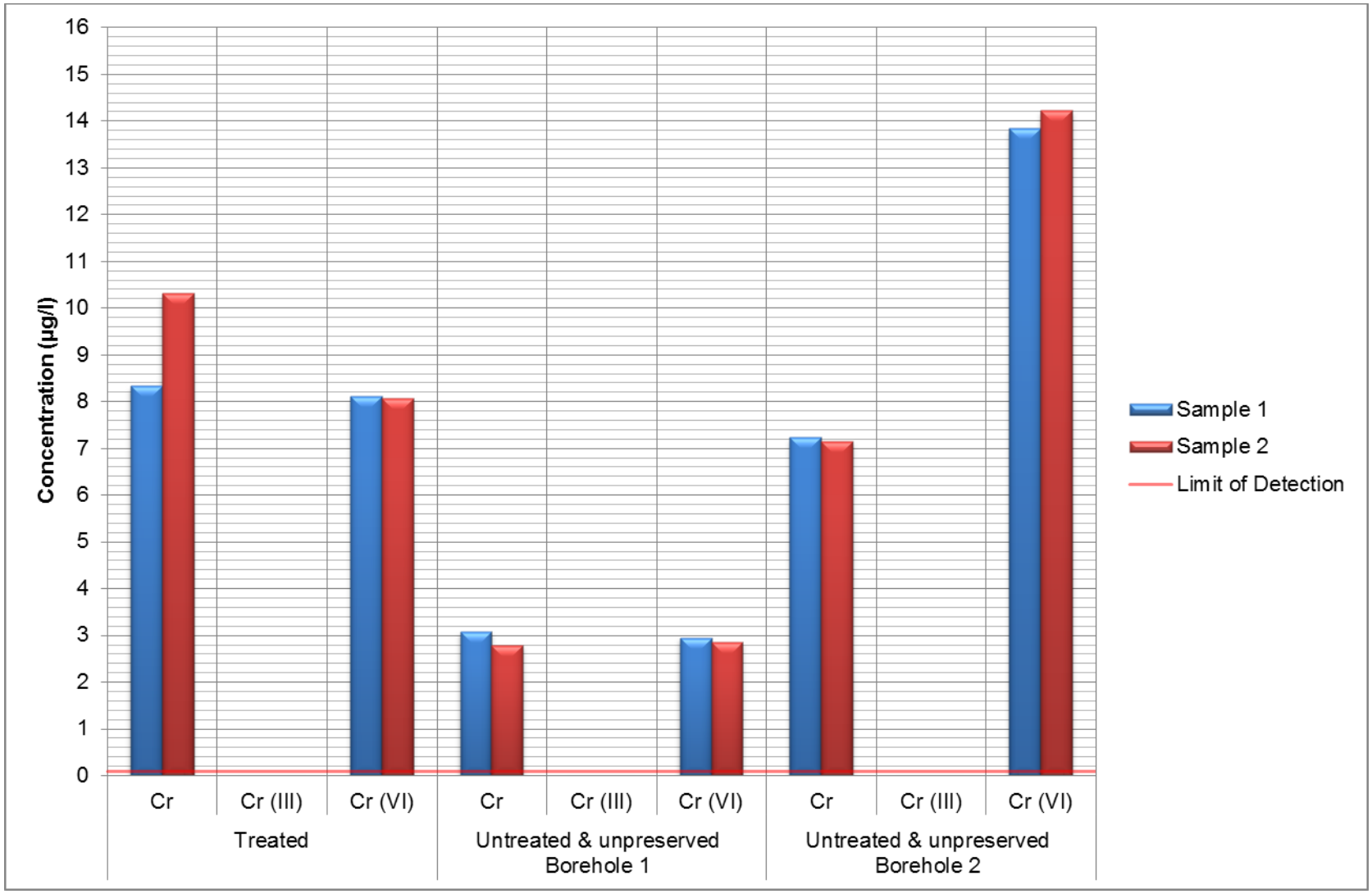


Figure 9.25 Summary of chromium speciation in untreated and finished water at Site W in the Spring Survey



10. Second Sampling Survey (2013-2014)

An additional sampling survey was conducted analysing levels of chromium, chromium (VI) and chromium (III) in raw and finished drinking water over autumn and winter 2013/2014. In the sampling survey, some of the original drinking water treatment works were used (Sites A, B, H, I, J, R, S and W) as well as other new drinking water treatment works (Sites X, Y, Z, A1, B1, C1 and D1). These new sites were chosen based on discussions with DWI and water companies who identified them as possible sites of potential detectable chromium levels in raw and finished drinking water.

10.1 Overall Results

Consolidated results for the entire survey are provided in Table 10.1. The results presented are averages of duplicate analysis, and where chromium was not detected by chromium analysis, further analysis for chromium speciation to calculate chromium (III) and (VI) concentrations were not conducted. Therefore, where the chromium concentrations are reported to be <0.1 µg/l, the inferred concentrations of <0.1 µg/l are reported for chromium (III) and (VI).

10.1.1 Chromium Analysis

Most of the selected drinking water treatment work sites reported a concentration of chromium in raw and finished drinking above the analytical limit of detection (0.1 µg/l) in both autumn and winter. Of the sites sampled, only three of these sites during autumn and three sites during winter, detected chromium concentrations below the limit of detection either in raw or finished water, or both. At Site W (reported in raw and finished drinking water) during autumn and winter and Site D1 (reported in finished drinking water) during winter, total chromium concentrations were detected at concentrations >1 µg/l.

There is no clear indication of any seasonal effects on the concentration of total chromium in raw and finished drinking water in the surveyed sites in 2013/2014. There appears to be no definitive difference after comparison with Sites A, B, H, I, J, S and W between autumn 2012 and autumn 2013. However, when comparing winter 2013 with winter 2014, Sites I and W there was an increase of 0.21 and 0.99 µg/l, respectively, of chromium in finished drinking water.

10.1.2 Chromium (VI) and Chromium (III) Analysis

During the autumn and winter, most sites surveyed detected concentrations of chromium (VI) <1 µg/l, with the exception of Site W (reported in raw and finished drinking water) and Site D1 (reported in finished drinking water) during winter at concentrations >1 µg/l.

Although this was only a limited survey of raw and finished waters from primarily groundwater sites with limited treatment, sites that were tested during the autumn and winter showed small or no decreases between the concentration of chromium (VI) in raw and finished drinking water, therefore indicating that very little to no chromium (VI) is being removed during the treatment of raw water.

Except at Sites H and D1, there is very little seasonal variation of the concentration of chromium (VI) in raw and finished drinking water in the other sites that were surveyed. From the surveyed results there is little evidence of variation in chromium (VI) concentrations in finished drinking water between sites during autumn 2012 with autumn 2013 and winter 2012/2013 with winter 2013/2014. The largest differences of chromium (VI) concentrations in final drinking water occur at Sites J and I during autumn 2012 and 2013 and winter 2012/2013 and 2013/2014, respectively.

Chromium (VI) was detected as the most dominant species, when comparing with chromium (III) in raw and finished drinking water. This could be due to the increased solubility and mobility of chromium (VI) and the adsorption of chromium (III) to sediment and is therefore potentially not available in the water column (WHO, 2009).

Chromium (III) was only reported above the limit of detection at Sites J and W at concentrations of 0.13 and 0.19 µg/l, respectively. These concentrations were only detected in autumn, suggesting a possible seasonal effect, however these effects were not observed in the previous year.

10.1.3 Summary

Generally the data presented is consistent with typical background concentrations of chromium (VI) which is <1 µg/l. The data also suggests that very little chromium (VI) is removed during the treatment of raw water to final drinking water.

Table 10.1 Summary of chromium concentrations detected in raw and finished drinking water at survey sites

Site	Sample Source	Concentration (µg/l)							
		Autumn				Winter			
		Cr(VI)	Cr(III)	Cr(III) + Cr(VI)	Cr	Cr(VI)	Cr(III)	Cr(III) + Cr(VI)	Cr
A	Raw	0.2	<0.10	0.2	0.53	0.15	<0.10	0.15	0.14
	Finished	0.22	<0.10	0.22	0.17	0.19	<0.10	0.19	0.13
B	Raw	NS	NS	NS	0.1	NS	NS	NS	<0.10
	Finished	NS	NS	NS	0.22	NS	NS	NS	<0.10
H	Raw	0.39	<0.10	0.39	0.34	0.44	<0.10	0.44	0.38
	Finished	0.41	<0.10	0.41	0.42	<0.10	<0.10	<0.1	<0.10

Site	Sample Source	Concentration (µg/l)							
		Autumn				Winter			
		Cr(VI)	Cr(III)	Cr(III) + Cr(VI)	Cr	Cr(VI)	Cr(III)	Cr(III) + Cr(VI)	Cr
I	Raw	NS	NS	NS	0.13	0.19	<0.10	0.19	0.14
	Finished	NS	NS	NS	0.15	0.29	<0.10	0.29	0.37
J	Raw	NS	NS	NS	NS	NS	NS	NS	NS
	Finished	0.21	0.13	0.34	0.14	NS	NS	NS	NS
R	Raw	NS	NS	NS	NS	NS	NS	NS	NS
	Finished	NS	NS	NS	NS	NS	NS	NS	NS
S	Raw	NS	NS	NS	<0.10	NS	NS	NS	NS
	Finished	NS	NS	NS	0.2	NS	NS	NS	NS
W	Raw (borehole 1)	3.12	<0.10	3.12	2.68	3.62	<0.10	3.62	3.09
	Raw (borehole 2)	13.58	0.19	13.77	11.62	16.18	<0.10	16.18	15.1
	Finished	7.97	<0.10	7.97	6.65	9.95	<0.10	9.95	8.59
X	Raw	0.18	<0.10	0.18	0.2	0.23	<0.10	0.23	0.25
	Finished	0.19	<0.10	0.19	0.21	0.22	<0.10	0.22	0.27
Y	Raw	NS	NS	NS	NS	NS	NS	NS	NS
	Finished	NS	NS	NS	NS	NS	NS	NS	NS
Z	Raw	0.3	<0.10	0.3	0.21	0.21	<0.10	0.21	0.22
	Finished	0.32	<0.10	0.32	0.29	0.24	<0.10	0.24	0.26
A1	Raw	0.14	<0.10	0.14	0.36	0.2	<0.10	0.2	0.2
	Finished	0.16	<0.10	0.16	0.12	0.13	<0.10	0.13	0.13
B1	Raw	0.27	<0.10	0.27	0.17	0.2	<0.10	0.2	0.2
	Finished	0.21	<0.10	0.21	0.16	0.23	<0.10	0.23	0.21
C1	Raw	NS	NS	NS	<0.10	NS	NS	NS	<0.10
	Finished	NS	NS	NS	<0.10	NS	NS	NS	<0.10
D1	Raw	0.21	<0.10	0.21	<0.10	0.87	<0.10	0.87	0.74
	Finished	0.23	<0.10	0.23	0.32	1.03	<0.10	1.03	1.06

NS: Not sampled

10.2 Site A

The chromium concentration results from Site A are reported in Table 10.2 and Figure 10.1. The chromium (VI) concentrations detected in raw and finished drinking water are relatively consistent, ranging from 0.15 – 0.22 µg/l. The concentrations of chromium (III) were reported

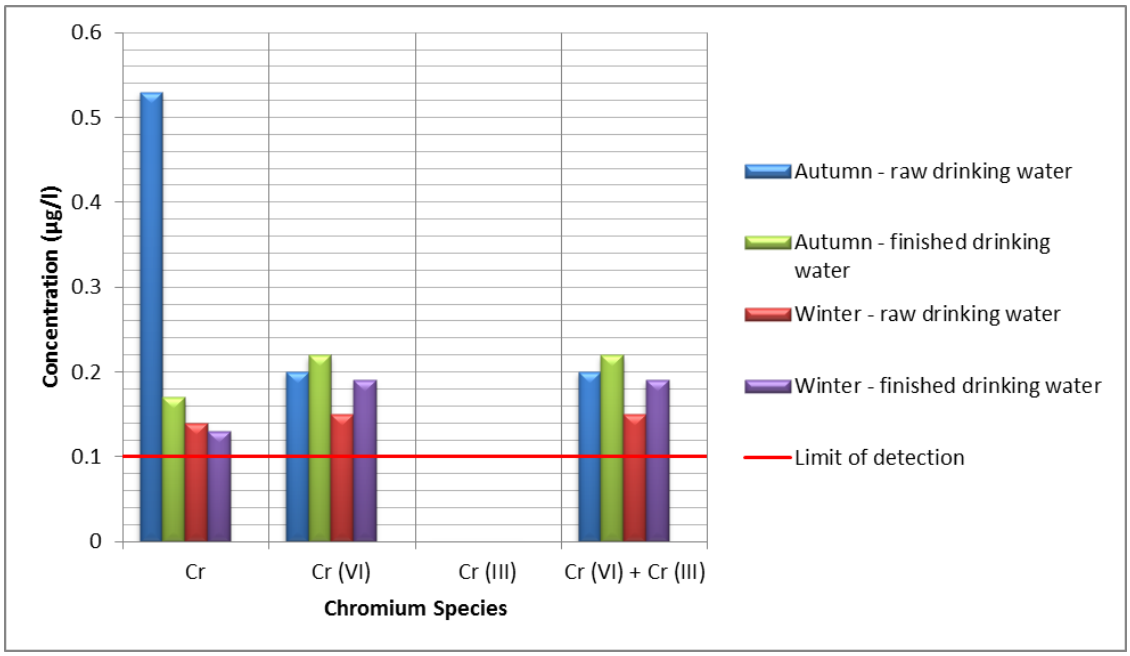
below the limit of detection for all samples at Site A during the autumn and winter. In the autumn a total chromium concentration of 0.53 µg/l was reported in raw water, however there is only a total chromium (VI) and (III) of 0.2 µg/l, which indicates a possible mixture of several chromium species with several other oxidation states (e.g. Cr(IV), Cr(V))present in the raw water at the time of sampling.

Most of the data presented in Table 10.2 and Figure 10.1 suggest that chromium (VI) species are not removed during treatment of raw water.

Table 10.2 Summary of chromium concentrations detected in raw and finished drinking water at Site A

Season	Sample Source	Concentration (µg/l)			
		Chromium	Chromium (VI)	Chromium (III)	Chromium (VI) + Chromium (III)
Autumn	Raw	0.53	0.2	<0.10	0.2
	Finished	0.17	0.22	<0.10	0.22
Winter	Raw	0.14	0.15	<0.10	0.15
	Finished	0.13	0.19	<0.10	0.19

Figure 10.1 Summary of chromium speciation results in raw and finished drinking water at Site A



10.3 Site B

The chromium concentration results from Site B are reported in Table 10.3. The data presented indicates a decrease in the concentration of chromium in finished drinking water from 0.22 µg/l in the autumn to below the limit of detection during the winter, however no information was available on the concentrations of chromium (VI) and chromium (III) to compare alongside total chromium concentrations.

The chromium of both raw and finished drinking water during the winter were both reported below the limit of detection.

Table 10.3 Summary of chromium concentrations detected in raw and finished drinking water at Site B

Season	Sample Source	Concentration (µg/l)			
		Chromium	Chromium (VI)	Chromium (III)	Chromium (VI) + Chromium (III)
Autumn	Raw	0.1	NS	NS	NS
	Finished	0.22	NS	NS	NS
Winter	Raw	<0.10	NS	NS	NS
	Finished	<0.10	NS	NS	NS

NS: Not sampled

10.4 Site H

The chromium concentration results from Site H are reported in Table 10.4 and Figure 10.2. The concentrations of chromium (VI) in raw and finished drinking water strongly correlate with the concentrations of chromium in both autumn and winter, suggesting that the dominant chromium species in the total chromium sample at Site H was chromium (VI).

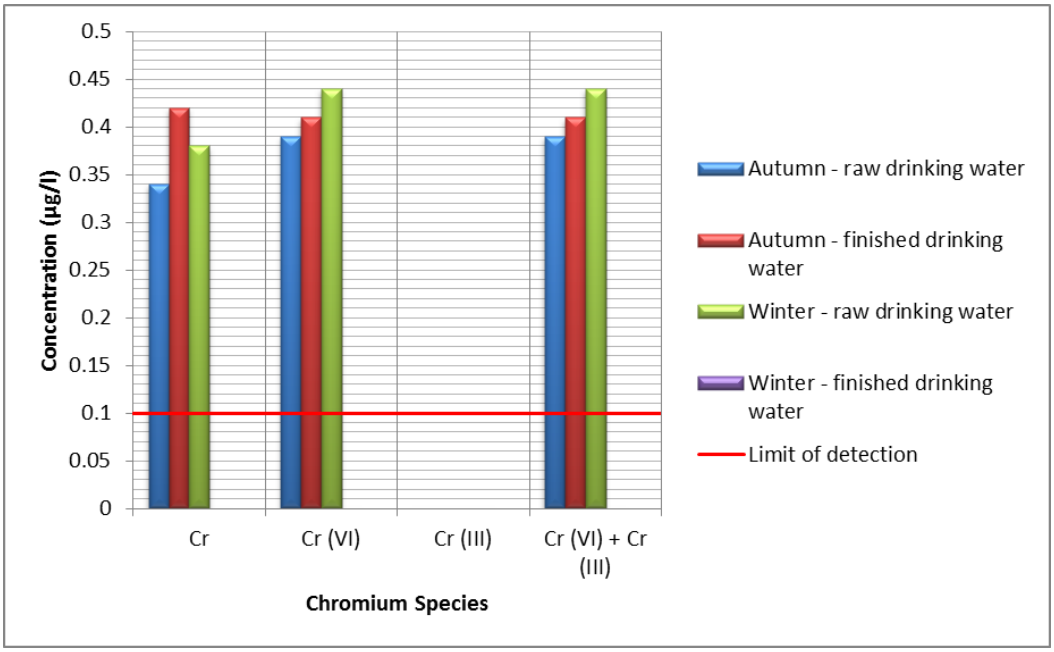
The concentrations of chromium (III) were reported below the limit of detection for all samples at Site H during the autumn and winter.

During winter, the concentration of total chromium and chromium (VI) is reduced from 0.38 µg/l to below the analytical detection limit and 0.44 µg/l to below the detection limit, respectively. This suggests that either there is possible dilution of chromium in the water due rain or that the treatment of the water in the drinking water treatment works is effective in removing chromium from the raw water. However, since there is an increase in total chromium and chromium (VI) from raw to finished drinking water during autumn it is more likely to be the former reason of dilution of chromium.

Table 10.4 Summary of chromium concentrations detected in raw and finished drinking water at Site H

Season	Sample Source	Concentration (µg/l)			
		Chromium	Chromium (VI)	Chromium (III)	Chromium (VI) + Chromium (III)
Autumn	Raw	0.34	0.39	<0.10	0.39
	Finished	0.42	0.41	<0.10	0.41
Winter	Raw	0.38	0.44	<0.10	0.44
	Finished	<0.10	<0.10	<0.10	<0.1

Figure 10.2 Summary of chromium speciation results in raw and finished drinking water at Site H



10.5 Site I

The chromium concentration results from Site I are reported in Table 10.5. The data indicate that the treatment of raw water at Site I has no effect in the removal of chromium from the raw drinking water.

The concentrations of chromium (III) were reported below the limit of detection for the winter samples in raw and finished drinking water.

Table 10.5 Summary of chromium concentrations detected in raw and finished drinking water at Site I

Season	Sample Source	Concentration (µg/l)			
		Chromium	Chromium (VI)	Chromium (III)	Chromium (VI) + Chromium (III)
Autumn	Raw	0.13	NS	NS	NS
	Finished	0.15	NS	NS	NS
Winter	Raw	0.14	0.19	<0.10	0.19
	Finished	0.37	0.29	<0.10	0.29

NS: Not sampled

10.6 Site J

The chromium concentration results from Site J are reported in Table 10.6.

Site J is one of two sites that detected chromium (III) above the limit of detection at a concentration of 0.13 µg/l.

It is unclear as to why the chromium concentration in finished drinking water during autumn is below the concentration of individual chromium species (total chromium (VI) and chromium (III) concentration of 0.34 µg/l). Some possible explanations could be due to either fluctuation of analytical instruments or detection of individual chromium species in analytical methods is more sensitive compared to measuring chromium.

Table 10.6 Summary of chromium concentrations detected in raw and finished drinking water at Site J

Season	Sample Source	Concentration (µg/l)			
		Chromium	Chromium (VI)	Chromium (III)	Chromium (VI) + Chromium (III)
Autumn	Raw	NS	NS	NS	NS
	Finished	0.14	0.21	0.13	0.34
Winter	Raw	NS	NS	NS	NS
	Finished	NS	NS	NS	NS

NS: Not sampled

10.7 Site R

Concentrations of chromium (VI), chromium (III) and total chromium in autumn and winter sampling were not analysed.

10.8 Site S

The total chromium concentration in raw water during autumn is below the limit of detection however, a concentration of 0.2 µg/l was detected in finished drinking water suggesting that the water treatment at Site S is not effectively removing chromium from raw drinking water.

10.9 Site W

The chromium concentration results from Site W are reported in Table 10.7, Figure 10.3 and Figure 10.4.

Throughout the autumn and winter survey, Site W consistently reported the highest concentrations of chromium in the raw and finished drinking water. There was also a notable difference in chromium concentrations detected in borehole 2, compared to borehole 1. Assuming the water is sourced in equal proportions from the two boreholes, the levels of chromium (VI) detected in the finished water would indicate very little, if any, removal of chromium (VI) is occurring at this site.

The lowest chromium (VI) concentration detected in raw water was in autumn at 3.12 µg/l from borehole 1. The highest concentration detected in raw water was in winter at 16.18 µg/l from borehole 2. The lowest chromium (VI) concentration detected in finished drinking water was in autumn at 7.97 µg/l from borehole 1. The highest concentration detected in final drinking water was in winter at 9.95 µg/l from borehole 2. Chromium concentrations in raw and final drinking water were lowest during the autumn. The chromium concentrations detected are almost equivalent to the chromium (VI) concentrations reported, indicating that it is likely that chromium (VI) is the most dominant chromium species at Site W.

Chromium (III) was detected in raw water from borehole 2 at a concentration of 0.19 µg/l during autumn but was below the limit of detection in all other samples.

Table 10.7 Summary of chromium concentrations detected in raw and finished drinking water at Site W

Season	Sample Source		Concentration (µg/l)			
			Chromium	Chromium (VI)	Chromium (III)	Chromium (VI) + Chromium (III)
Autumn	Raw	Borehole 1	2.68	3.12	<0.10	3.12
		Borehole 2	11.62	13.58	0.19	13.77
	Finished		6.65	7.97	<0.10	7.97
Winter	Raw	Borehole 1	3.09	3.62	<0.10	3.62
		Borehole 2	15.1	16.18	<0.10	16.18
	Finished		8.59	9.95	<0.10	9.95

Figure 10.3 Summary of chromium speciation results in raw and finished drinking water at Site W

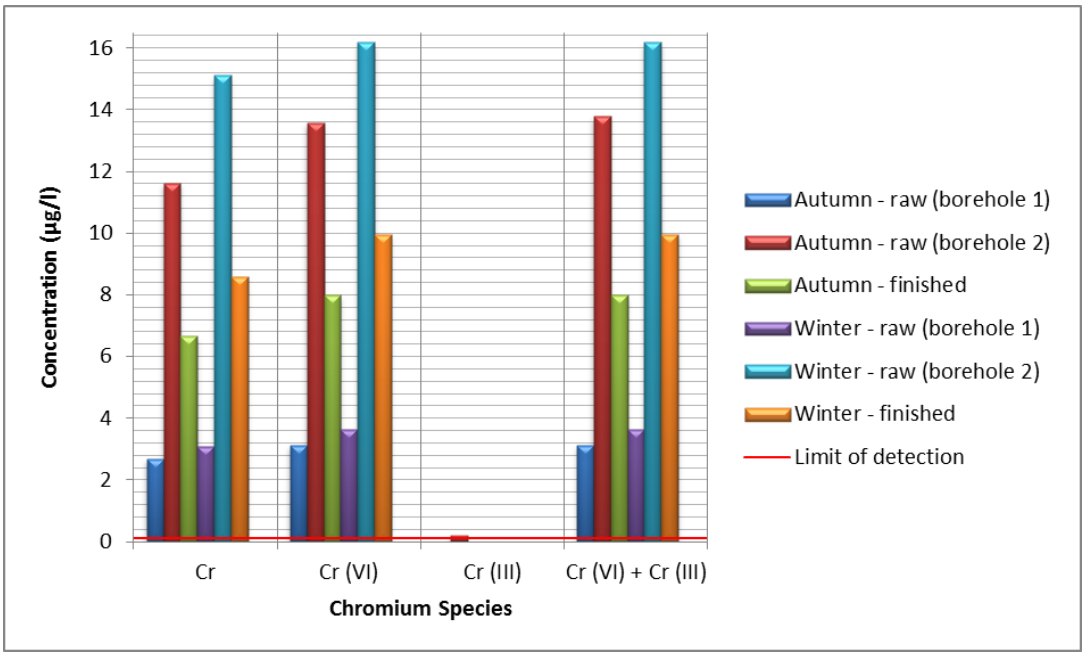
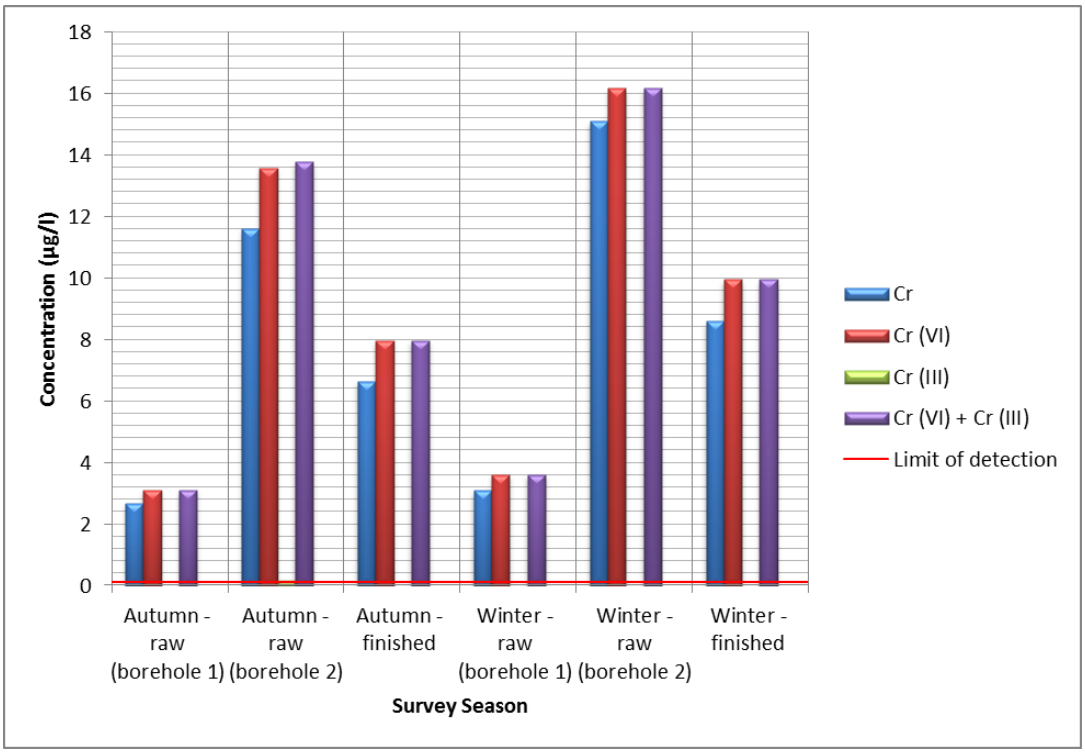


Figure 10.4 Summary of chromium seasonal results in finished drinking water at Site W



10.10 Site X

The chromium concentration results from Site X are reported in Table 10.8. The chromium concentrations detected are almost equivalent to the chromium (VI) concentrations reported, indicating that chromium (VI) is likely to be the most dominant chromium species at Site X and that it is not removed during the water treatment process.

No seasonal effects on the concentration of chromium in raw and finished drinking water were observed. Additionally there were no significant differences between the concentrations of chromium in raw and finished drinking water in either autumn or in winter.

The concentrations of chromium (III) were reported below the limit of detection for all samples at Site X during the autumn and winter.

Table 10.8 Summary of chromium concentrations detected in raw and finished drinking water at Site X

Season	Sample Source	Concentration (µg/l)			
		Chromium	Chromium (VI)	Chromium (III)	Chromium (VI) + Chromium (III)
Autumn	Raw	0.2	0.18	<0.10	0.18
	Finished	0.21	0.19	<0.10	0.19
Winter	Raw	0.25	0.23	<0.10	0.23
	Finished	0.27	0.22	<0.10	0.22

10.11 Site Y

Concentrations of chromium (VI), chromium (III) and chromium in autumn and winter sampling were not analysed.

10.12 Site Z

The chromium concentration results from Site Z are reported in Table 10.9. In each chromium (VI) and chromium sample in autumn and winter an increased concentration of chromium and chromium (VI) from raw to finished drinking water was detected.

The concentrations of chromium (III) were reported below the limit of detection for all samples at Site Z.

Table 10.9 Summary of chromium concentrations detected in raw and finished drinking water at Site Z

Season	Sample Source	Concentration (µg/l)			
		Chromium	Chromium (VI)	Chromium (III)	Chromium (VI) + Chromium (III)
Autumn	Raw	0.21	0.3	<0.10	0.3
	Finished	0.29	0.32	<0.10	0.32
Winter	Raw	0.22	0.21	<0.10	0.21
	Finished	0.26	0.24	<0.10	0.24

10.13 Site A1

The chromium concentration results from Site A1 are reported in Table 10.10 and Figure 10.5.

In the autumn there is a difference of 0.22 µg/l between the concentration of chromium and chromium (VI) in raw water, as chromium (III) is negligible, this suggests that in the raw water

there are other chromium species present at the time of sampling. However, there is a slight increase in the concentration of chromium (VI) in the raw water sample to the finished drinking water sample. This suggests possible oxidation of the other chromium species that were present in raw water to chromium (VI) in finished drinking water.

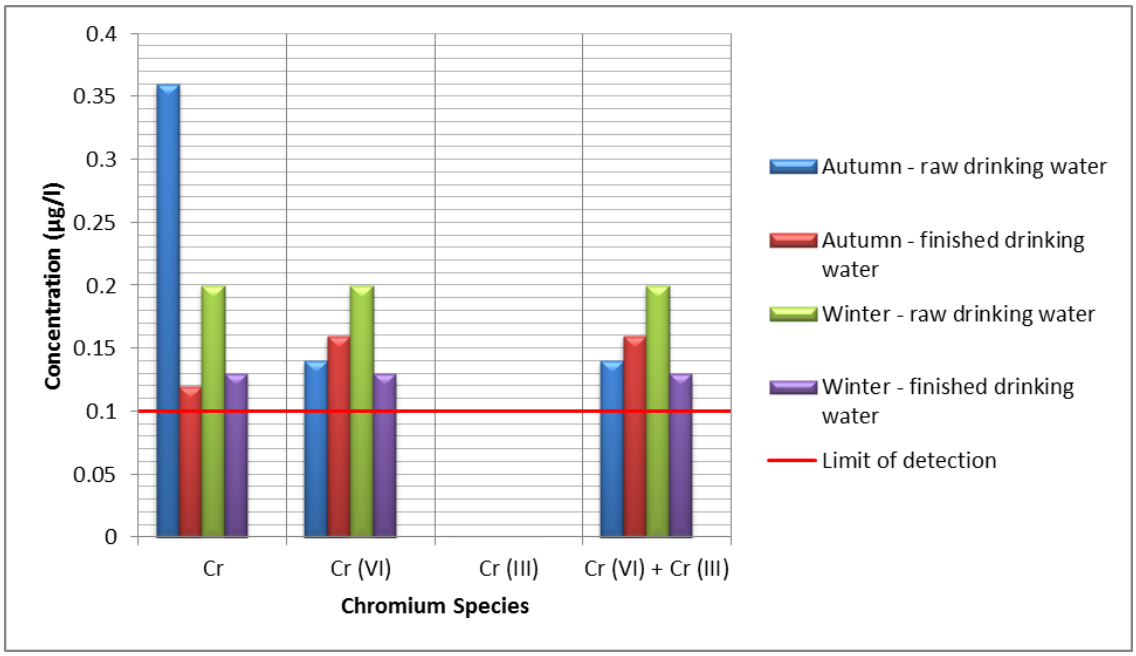
The winter data indicate a different scenario compared to the autumn data. The chromium present in raw water and finished drinking water are equivalent to the concentration of chromium (VI) in the same samples, suggesting that chromium (VI) is the dominant chromium species in the water samples.

The concentrations of chromium (III) were reported below the limit of detection for all samples at Site A1.

Table 10.10 Summary of chromium concentrations detected in raw and finished drinking water at Site A1

Season	Sample Source	Concentration (µg/l)			
		Chromium	Chromium (VI)	Chromium (III)	Chromium (VI) + Chromium (III)
Autumn	Raw	0.36	0.14	<0.10	0.14
	Finished	0.12	0.16	<0.10	0.16
Winter	Raw	0.2	0.2	<0.10	0.2
	Finished	0.13	0.13	<0.10	0.13

Figure 10.5 Summary of chromium speciation results in raw and finished drinking water at Site A1



10.14 Site B1

The chromium concentration results from Site B1 are reported in Table 10.11.

There is very little variation between the concentrations of total chromium and chromium (VI) in raw and finished drinking water within each season, suggesting that chromium is not removed during the treatment of water. Additionally, there is little variation of chromium in raw and finished drinking water between each season, suggesting there is no seasonal effect on the concentration of chromium at Site B1. The concentrations of chromium (III) were reported below the limit of detection for all samples at Site B1.

Table 10.11 Summary of chromium concentrations detected in raw and finished drinking water at Site B1

Season	Sample Source	Concentration (µg/l)			
		Chromium	Chromium (VI)	Chromium (III)	Chromium (VI) + Chromium (III)
Autumn	Raw	0.17	0.27	<0.10	0.27
	Finished	0.16	0.21	<0.10	0.21
Winter	Raw	0.2	0.2	<0.10	0.2
	Finished	0.21	0.23	<0.10	0.23

10.15 Site C1

The chromium concentration results from Site C1 are reported in Table 10.12.

Concentrations of chromium (VI) and chromium (III) in autumn and winter sampling were not analysed. However, chromium was sampled in both raw and finished drinking water for both autumn and winter and was reported below the limit of detection for all samples.

Table 10.12 Summary of chromium concentrations detected in raw and finished drinking water at Site C1

Season	Sample Source	Concentration (µg/l)			
		Chromium	Chromium (VI)	Chromium (III)	Chromium (VI) + Chromium (III)
Autumn	Raw	<0.10	NS	NS	NS
	Finished	<0.10	NS	NS	NS
Winter	Raw	<0.10	NS	NS	NS
	Finished	<0.10	NS	NS	NS

NS: Not sampled

10.16 Site D1

The chromium concentration results from Site D1 are reported in Table 10.13 and Figure 10.6.

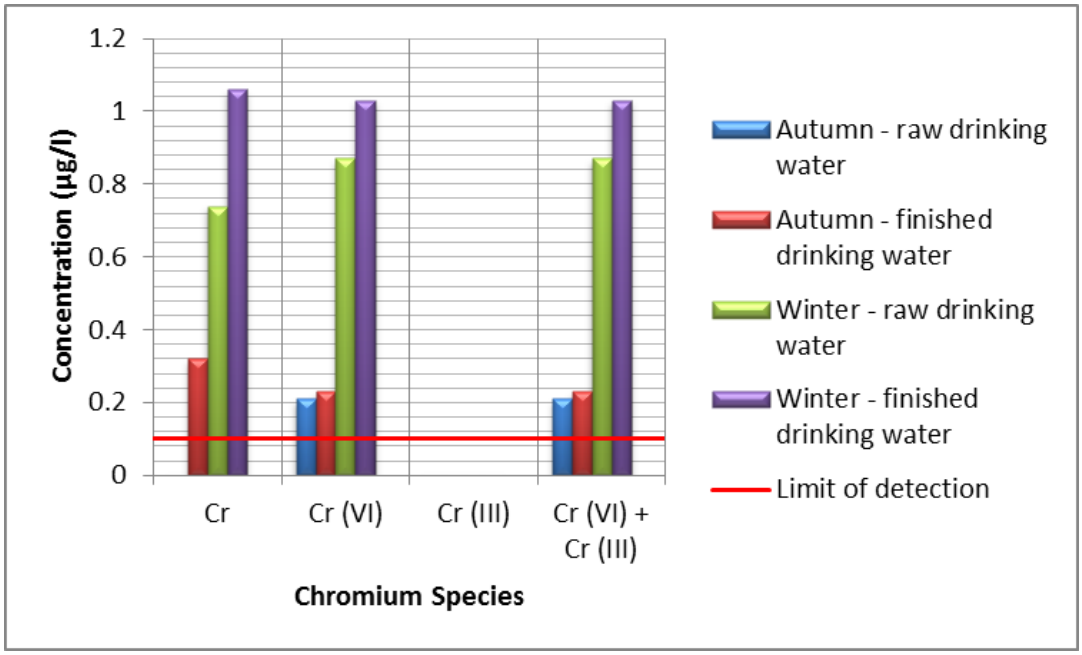
The concentrations of chromium and chromium (VI) in finished drinking water during the winter show an increase of >1 µg/l (1.06 and 1.03 µg/l, respectively).

The concentrations of chromium (III) were reported below the limit of detection for all samples at Site D1. Additionally a concentration of chromium in raw water during autumn was also reported below the limit of detection, however it is unclear as to why the chromium concentration is below the concentration of individual chromium species (chromium (VI) concentration of 0.21 µg/). Some possible explanations could be due to either fluctuation of analytical instruments or detection of individual chromium species in analytical methods is more sensitive compared to measuring chromium.

Table 10.13 Summary of chromium concentrations detected in raw and finished drinking water at Site D1

Season	Sample Source	Concentration (µg/l)			
		Chromium	Chromium (VI)	Chromium (III)	Chromium (VI) + Chromium (III)
Autumn	Raw	<0.10	0.21	<0.10	0.21
	Finished	0.32	0.23	<0.10	0.23
Winter	Raw	0.74	0.87	<0.10	0.87
	Finished	1.06	1.03	<0.10	1.03

Figure 10.6 Summary of chromium speciation results in raw and finished drinking water at Site D1



11. Discussion and Comment on Limitations of the Available Data

No studies were located on the measurement of absorption of chromium (VI) in humans from water. Differences in absorption have been reported between administration via oral gavage and via the diet in humans in volunteer studies, with absorption being significantly higher following gavage administration. Information on the absorption of chromium (VI) via drinking water may provide a better understanding of any potential health risks from exposure via this route.

The data indicate that significant reduction of chromium (VI) to chromium (III) occurs in gastric juices, acting to limit the absorption of chromium. If this process completely reduces chromium (VI) to chromium (III), which decrease absorption and effectively prevent chromium (VI) toxicity. Studies in rodents conducted with high (mg/l) concentrations of chromium (VI) appear to demonstrate that this reduction process can be overwhelmed, resulting in release of chromium (VI) to the small intestine, where the most significant absorption occurs, resulting in the carcinogenic effects in mice described by the US National Toxicology Program (NTP, 2008). However, there is significant uncertainty in the threshold for this reductive capacity.

The recent study by Proctor *et al.* (2012) determined thresholds for reducing capacities for rats and mice, based on extraction of stomach contents and spiking with chromium (VI). However, it is important to note that the approach adopted by Proctor *et al.* (2012) only provides a static estimate of the reducing capacity of the stomach. As the authors of this study themselves note, the stomach is a dynamic environment, with continual excretion of gastic fluids that will serve to provide additional reductive capacity. Additionally, even at concentrations below the threshold for the reductive capacity of the stomach, reduction of chromium (VI) although substantial, was not complete. Therefore, even at low concentrations, it is likely that some chromium (VI) will reach the duodenum following oral administration, where it may potentially be absorbed. Therefore, additional data are required to determine whether the concentrations suggested by Proctor *et al.* (2012) are appropriate for extrapolation to *in vivo* systems.

Further understanding is required in the differences in toxicokinetic behaviour between species. Kirman *et al.* (2012) note that there were differences in chromium concentrations in the small intestines of mice and rats that suggested a greater tendency to absorb chromium in mice. However, there are also substantial differences between the rodent and human gastrointestinal system. The rodent forestomach has two defined areas, a non-glandular forestomach and a glandular stomach. This forestomach helps maintain a consistent volume of GI lumen contents and reduces the occurrence of significant peaks in gastric acid production. In contrast, humans have no forestomach and therefore gastic acid production is subject to far greater variation, with post-prandial peaks (increases after consumption of a meal), and more acidic conditions during times of fasting. Therefore, there are likely to be

significant differences in the capacity of the human stomach to reduce chromium (VI) to chromium (III) than in rodents. This is particularly important when considering that many of the toxicokinetic studies indicate that the duodenum, the part of the small intestine into which the gastric contents are emptied, represents the most significant region for absorption of chromium into the portal system. These differences introduce significant uncertainty into the data that may mean that the use of typical uncertainty factors in the extrapolation of animal-to-human data for the purpose of risk assessment is inappropriate. However, a study on a mono-gastric species, such as the dog, may reduce the uncertainty and allow animal-to-human extrapolations to be conducted.

Additional information is required on the formation of DNA adducts, particularly with regards to any epigenetic effects they may produce (i.e. whether they produce changes in gene expression), and the significance of these potential effects. Further studies on the oxidative damage produced by chromium (VI), and in particular, identification of the threshold above which cells can no longer mitigate against the oxidative effects, is also required to provide a better understanding of any potential health risks from exposure. Therefore, until such time as sufficient information is available to address these issues, it would be prudent to assume a genotoxic mode of action may be involved in the carcinogenicity of chromium (VI).

12. Outcomes and Conclusions

12.1 Review of Chromium (VI) Toxicokinetic and Toxicodynamic Data

Chromium (VI) is reported to undergo reduction in saliva and gastric juices to chromium (III). This reduction is reported to be rapid, with at least half of a chromium (VI) dose reduced within 1 minute and complete reduction occurring within 10-20 minutes. Intestinal bacteria are also reported to reduce chromium (VI) to chromium (III). Chromium (VI) is more readily absorbed than chromium (III), and as such reduction by saliva and in the gastrointestinal tract will decrease the availability of chromium (VI) to be absorbed.

Administration of highly soluble chromium (VI) compounds via oral gavage or inhalation to humans results in approximately 20-30% absorption. However, absorption via food is much lower due to reduction of chromium (VI) to chromium (III) (1-3% in rats and mice).

Once absorbed into the cell, chromium (VI) undergoes a series of reduction reactions to yield chromium (III). This process does not require enzymes but involves direct electron transfer from ascorbate and non-protein thiols. Ascorbate is the dominant chromium (VI) reducer, accounting for greater than 90% of chromium (VI) metabolism *in vivo*. This reduction can form chromium (V) and chromium (VI) intermediates, which may be capable of inducing DNA damage. Once metabolised to chromium (III), chromium-DNA adducts can be formed. These adducts are reported to be the predominant form of chromium (VI)-induced genetic effect in mammalian cells, with the major adducts being glutathione-chromium-DNA, cysteine-chromium-DNA, histidine-chromium-DNA and ascorbate-chromium-DNA complexes.

Chromium compounds are usually excreted via the faeces, due to their poor absorption rate. However, absorbed chromium is excreted via the urine.

12.2 Toxicological Review of Chromium (VI)

The toxicology of chromium is highly complex and dependent upon the form of chromium. In general, the data indicate that chromium (III) compounds are far less toxic than chromium (VI) compounds.

The available literature indicates that there is evidence of chromium (VI)-induced carcinogenicity via the oral route. In studies conducted by the National Toxicology Program (NTP), rats and mice were administered chromium (VI) via drinking water. Rats displayed significant increases in the incidence of tumours in the oral mucosa, while mice displayed significant increases in the duodeneum and jejunum. It is worth noting that in both these species, these tumours were only observed at the higher doses, and were restricted to the upper parts of the gastro-intestinal system.

This restriction of tumours to the upper parts of the gastro-intestinal system is consistent with the reported data on toxicokinetics of chromium, as chromium (VI) undergoes significant reduction to chromium (III) as it passes through the gastro-intestinal system, effectively reducing its ability to be absorbed by tissues in the latter part of the gastro-intestinal system. However, it is somewhat unexpected that tumours formed in the oral cavity of male and female rats, as the transit time through the oral cavity is expected to be very brief, and therefore, significant absorption of material would not normally be anticipated, which may support the argument for a genotoxic mode of action.

A number of *in vitro*, and some *in vivo* studies have examined the effects of chromium exposure on the expression of proteins, such as glutathione reductase (GR) and glutathione peroxidase (GPx), which respond to oxidative stress, and p53 and other proteins involved in the regulation of the cell cycle and apoptosis. These data suggest that following exposure to low concentrations of chromium (VI), cells adapt by increasing the production of proteins that respond to oxidative stress by arresting the cell cycle and/or inducing apoptosis. However, at higher concentrations, chromium (VI) either saturates the process that respond to oxidative stress, or inhibits the production of proteins which control the regulation of the cell cycle.

Co-administration of chromium (VI) with ascorbate (vitamin C) appears to mitigate many of the effects of chromium toxicity by protecting against some of the effects of oxidative stress.

The current EU and English and Welsh drinking water standard for total chromium is 0.05 mg/l (50 µg/l). This standard is based on the WHO guideline value which was initially based on chromium (VI) health concerns; however, the guideline was changed to total chromium due to difficulty in analysing chromium (VI).

A significant body of data on the toxicological properties of chromium (VI) have become available subsequent to the setting of this standard. However there remain gaps in the data, and areas of uncertainty. Nonetheless a range of authoritative international bodies have established TDIs, RfDs or other health values, from which, lifetime health-based levels for drinking water can be derived. These TDIs and RfDs may have limitations in term of the data considered and differences in interpretation, and consequently a wide range of values have been proposed. However, in general, most organisations have suggested health-values in the range of 0.9-6 µg/kg bw/day.

By assuming the same principles that WHO would adopt in the derivation of their lifetime Guidelines for Drinking-water Quality (GDWQ), lifetime health-based values for drinking water can be derived and are included in Table 12.1.

Table 12.1 Health-based values drinking water guidelines for chromium (VI) assuming allocations of 20% or 80% of the health-value to drinking water

Evaluating Organisation	Proposed health value (µg/kg bw/day)	Lifetime health-based value in drinking water, assuming a 20% allocation to water (µg/l)	Lifetime health-based value in drinking water, assuming a 80% allocation to water (µg/l)
ATSDR intermediate MRL	5	30	120
ATSDR chronic MRL	0.9	5.4	21.6
US EPA RfD	0.9	5.4	21.6
WHO CICAD	0.9	5.4	21.6
EFSA BDML10 (non-neoplastic effects)	110	660	2640
EFSA BDML10 (neoplastic effects)	1000	6000	24 000
Thompson *et al*., 2013	6	36	144

12.3 Fate of Chromium (VI) During Water Treatment

A number of technologies are available for the removal of chromium during drinking water treatment.

Chromium (VI) is not effectively removed by alum and ferric coagulants and needs to be reduced to chromium (III) for the removal to take place. The use of ferrous sulphate has been shown to remove nearly 100% of the chromium (VI) present through reduction to chromium (III). As the reduction of chromium (VI) is more effective at lower pH, chromium (VI) removal is usually carried out in two steps. The chromium (VI) is first reduced at low pH and then the pH is raised to precipitate chromium hydroxide. It should be noted that some disinfectants can oxidise chromium (III) to chromium (VI) and treatments such as pre-chlorination and pre-ozonation can make the chromium more mobile and difficult to remove. The addition of ferrous sulphate can under certain conditions increase the leaching of chromium (VI) from chromium-rich ore but these conditions are unlikely to be met during drinking water treatment.

Iron oxides, such as ferrihydrite and goethite, and iron oxide coated sand (IOCS) are effective in removing both anions and cations but the removal is highly pH-dependent; cations are generally more readily removed at high pH and anions more readily at low pH. Removal of both chromium (III) and chromium (VI) is therefore likely to require pH changes and the removal carried out in stages. These types of adsorbents can achieve a considerably lower aqueous chromium solution concentration than precipitation processes.

Ion exchange is effective in removing both chromium (III) and chromium (VI), between 80-96% of the ions can be expected to be removed. As chromium (III) generally forms cations and chromium (VI) forms anions, a cation exchanger is effective for chromium (III) removal and an anion exchanger is effective for chromium (VI).

Membrane technologies and particularly reverse osmosis (RO) are considered one of the best technologies available for chromium removal. The main drawbacks with the membrane technologies are that they are working at relatively high pressures that require energy to maintain and the fouling and subsequent cleaning of the membranes can also be costly.

Biological removal of chromium (VI) by bacteria has been shown to be effective by a number of researchers. The chromium (VI) is reduced to chromium (III) and precipitated within the biomass. However, all studies have shown that anaerobic conditions work best and this is not suitable for drinking water treatment.

12.4 Summary of Previous Monitoring Studies and Occurrence Data

Chromium (VI) has been linked with numerous pollution incidents, especially in newly industrialised countries where legislation for environmental protection may be less stringent than the USA or Europe. It is also noted that these studies have centralised around drinking water sources that rarely undergo treatment prior to use for drinking water. The USA, and particularly California, has the most data gathered on the occurrence of chromium (VI) in their drinking water supplies especially since the Hinkley, California case against PG&E. This issue has had more attention in the USA than anywhere else in the world. Generally, it appears that background concentrations of chromium (VI) are <1 µg/l, but levels can be many times this in areas where industrial pollution has occurred. The main industrial influences for the presence of chromium (VI) in the environment appear to be chromium ore smelting, leather tanning, industrial cooling waters and dyeing wastewater.

12.5 Development of a method for the determination of total chromium, chromium (III) and chromium (VI) concentrations in drinking water

ICP-MS is used to directly measure total chromium concentrations in water and, then in combination with an ion chromatographic separation to measure the individual chromium species (chromium (III) and chromium (VI). The method uses a thulium internal standard.

The method has been shown to be reproducible and precise, to have a linear range up to 50 µg/l (w/v) and a minimum detectable amount for chromium of 0.1 µg/l. The limit of quantitation is 0.5 µg/l. During development of the method, account was taken of EPA Method 218.7: Determination of hexavalent chromium in drinking water by ion chromatography with post-column derivatization and UV-visible spectroscopic detection.

12.6 First Sampling Survey

Despite selecting sites on the basis that they were anticipated to contain measureable levels of chromium in the finished drinking water, in general, chromium concentrations in drinking water at these sites were low. Most sites either had no detectable chromium in finished water, or the concentration was just above the analytical limit of detection (0.1 µg/l) and only one site had a total chromium concentration of >1 µg/l.

The analyses of chromium speciation indicate that chromium (VI), when present, is the dominant form of chromium in drinking water. There is no evidence of any seasonal effect for chromium (VI) concentrations in finished drinking water at any of these sites.

Eleven sites had at least one sample containing a detectable level of chromium (VI). The majority of these samples were only slightly above the analytical limit of detection of 0.1 µg/l. Only Site W had a total chromium concentration of >1 µg/l, at a maximum concentration of 8.82 µg/l.

Chromium (III) was only detected above the limit of detection at four sites, at a maximum concentration of 0.15 µg/l.

There is no evidence from this survey to suggest that concentrations of chromium in drinking water supplies are any higher than levels detected in other studies internationally, and the data presented here are consistent with typical background concentrations of chromium (VI) of <1 µg/l.

12.7 Second Sampling Survey

An additional sampling survey was conducted analysing concentrations of chromium species in raw and finished drinking water. In total 15 sites were surveyed, eight of the original drinking water treatment works from the first sampling survey were used, as well as seven new drinking water treatment works. Generally, chromium concentrations reported at the sites were <1 µg/l. Four of the sampled sites did not report total chromium concentrations above the limit of detection in either raw and/or finished water. Two sampled sites detected a total chromium concentration >1 µg/l. There is no clear indication of any seasonal effects on the concentration of total chromium in raw and finished drinking water in the surveyed sites.

Reviewing the data indicates that chromium (VI) is the dominant species in raw and drinking water. There is little evidence of seasonal effects on the concentrations of chromium (III) and chromium (VI) in raw and finished drinking water.

The data suggest that the drinking water treatment process is having little to no effect on the removal of chromium (VI) from raw to finished drinking water, although it should be acknowledged that most of these sites only had minimal treatment in place. Therefore, caution should be applied in extrapolating these results to other sites.

12.8 Conclusion

There remain uncertainties within the toxicokinetic data for chromium (VI), however, there are several conclusions that can be drawn from the available data:

1. The available toxicokinetic data indicate that chromium (VI) is likely to undergo significant reduction to chromium (III) in the gastrointestinal system, decreasing the amount available for absorption.

2. Co-administration of chromium (VI) with ascorbate (a reducing agent) appears to mitigate many of the effects of chromium toxicity by protecting against some of the effects of oxidative stress.

3. The concentrations of chromium (VI) in drinking water in England and Wales are very low, and are generally <1 µg/l, which appears to be consistent with typical background concentrations of chromium (VI).

4. It is likely that at the low concentrations found in drinking water, the concentrations of reducing agents are likely to be in excess of the absorbed dose of chromium (VI), and may therefore, mitigate against any potential toxicological effects.

Therefore, in the majority of cases, it is considered unlikely that significant exposure to chromium (VI) occurs via drinking water in England and Wales.

In addition, despite the uncertainties that remain in the data, authoritative international bodies have derived Tolerable Daily Intakes (TDIs) or equivalent levels using different interpretations and varying amount of the available information. Notably, the World Health Organization (WHO) has recently derived a TDI in their Concise International Chemical Assessment Documents (CICAD) of 0.9 µg/kg bw/day, Thompson *et al.* (2013) have derived an oral Reference Dose of 6 µg/kg bw/day and the European Food Safety Authority (EFSA) has derived values of 110 and 1000 µg/kg bw/day for non-neoplastic and neoplastic endpoints, respectively (EFSA, 2014).

Using these values and following WHO principles for drinking water guideline derivation a range of lifetime health-based concentrations in drinking water can be derived. This range of possible health-based guideline values serves to demonstrate the uncertainty that remains within the toxicological and toxicokinetic database for this chemical.

For the majority of sites considered in this survey, the concentrations of chromium (VI) were well below even the more conservative of these health-based values, and in the majority of cases were <1 µg/l. Therefore, adverse health effects from exposure to chromium (VI) would not be anticipated. However, concentrations of total chromium and chromium (VI) reported in raw and finished drinking water at Site W slightly would exceed the lowest health-based value of 5.4 µg/l, that can be derived using the most precautionary assumptions (20% allocation of

the TDI of 0.9 µg/kg bw/day to water) (chromium (VI) concentrations ranged from 6.65-9.95 µg/l). If a higher allocation of this TDI is applied to drinking water (80%), which is likely to be a more realistic reflection of the significance of water as a source of chromium (VI), a lifetime health-based drinking water value of 21.6 µg/l can be derived, which is above the concentration of chromium (VI) detected at site W. Use of other health-values with an 80% allocation to drinking water would result in even higher health-based drinking water values.

13. Suggestions and Considerations

This project was commissioned to provide an understanding of the significance of exposure to chromium in drinking water, and in particular the potential risks of exposure to chromium (VI). The results the survey suggests that concentrations of chromium in drinking water generally are very low. However, there are significant gaps within the data that mean that while health-based standards can be derived, there remains uncertainty around the mode of action. This means that these health-based values may need to be reconsidered when additional information becomes available.

Therefore, the following suggestions and considerations are made based on the findings of this report:

- The data indicate that significant reduction of chromium (VI) to chromium (III) occurs in gastric juices, acting to limit the absorption of chromium. This reductive capacity appears to have a threshold, above which the rate of absorption of chromium (VI) increases significantly. However, there is significant uncertainty in the threshold for this reductive capacity. Further information, such as the reductive capacity for chromium (VI) in a monogastric species following administration via drinking water, or an *in vitro* system that mimics the human system, is required to assess the potential health risks from exposure via this route.

- Chromium (VI) was detected as the most dominant species in the water samples in raw and finished drinking water, which may be considered atypical to what might be expected, as chromium (III) is generally considered to be the dominant form in the environment. This could be due to the increased solubility of chromium (VI) and the adsorption of chromium (III) to sediment. Consequently, potential future analysis could include the evaluation of chromium species in sediment and water samples taken simultaneously, to determine the potential dominant chromium species present.

- Despite the uncertainties and data gaps described above several authoritative organisations have derived tolerable daily intakes or similar values; for example, a World Health Organization (WHO) Tolerable Daily Intake (TDI), and an oral Reference Dose (RfD) derived by Thompson *et al*. (2013). Caution must be used in interpreting these values given the uncertainties and data gaps. Applying standard assumptions to these values, with allocations of the TDI to water ranging from 20% to 80%, this would yield lifetime health-based values ranging from 5.4 µg/l to 144 µg/l. This range of values straddles the current WHO Guideline for Drinking-water Quality (GDWQ) for lifetime exposure to total chromium. Based on the lower end value, the existing GDWQ may not be sufficiently protective against adverse effects of chromium (VI). Therefore, it may be appropriate to review or reconsider the current drinking water standard but the uncertainties described above make this difficult at present.

- It is noted that analysis for chromium is usually conducted on the basis of total chromium, rather than for different chromium species. However, as chromium (VI) is considered to be of the greatest toxicological concern, a standard based on chromium (VI) should be sufficiently protective for all species of chromium and therefore could be used as a surrogate value for total chromium.

References

Afolaranmi, G.A., Henderson, C. and Grant, M.H. (2011). Effect of chromium and cobalt ions on phase I and phase II enzymatic activities in vitro in freshly isolated rat hepatocytes. *Toxicol. In Vitro*, 25(1):125-30.

Akbar, M., Brewer, J.M. and Grant, M.H. (2011). Effect of chromium and cobalt ions on primary human lymphocytes *in vitro*. *J. Immunotoxicol*., 8(2):140-9.

American Chrome and Chemicals (1989). Chromic Acid. Material Safety Datasheets. Corpus Christi, TX, American Chrome and Chemicals, Inc.

Anderson, R.A., Polansky, M.M., Bryden, N.A., Patterson, K.Y., Veillon, C. and Glinsmann, W.H. (1983). Effects of chromium supplementation on urinary Cr excretion of human subjects and correlation of Cr excretion with selected clinical parameters. *J. Nutr.,* 113:276-281.

Andersson, M.A., Petersson Grawé, K.V., Karlsson, O.M., Abramsson-Zetterberg, L.A. and Hellman, B.E. (2007). Evaluation of the potential genotoxicity of chromium picolinate in mammalian cells *in vivo* and *in vitro*. *Food Chem Toxicol*., 45(7):1097-106.

Asatiani, N., Abuladze, M., Kartvelishvili, T., Kulikova, N., Asanishvili, L., Holman, H.Y. and Sapojnikova, N. (2010). Response of antioxidant defense system to chromium (VI)-induced cytotoxicity in human diploid cells. *Biometals*, 23(1):161-72.

ATSDR (2008). Draft Toxicological Profile of Chromium. US Department of Health and Human Services. Public Health Service. Agency for Toxic Substances and Disease Registry.

ATSDR (2012). Toxicological Profile of Chromium. US Department of Health and Human Services. Public Health Service. Agency for Toxic Substances and Disease Registry.

AWWA (2011). Chrome-6: Déjà vu All Over Again. American Water Works Association.

Azizullaha, A., Khattak, M.N.K., Richter, P. and Hädera, D.P. (2010). Water pollution in Pakistan and its impact on public health — A review, *Environ Int*., 37(2):479-97.

Bagchi, D., Stohs, S.J., Downs, B.W., Bagchi, M. and Preuss, H.G. (2002). Cytotoxicity and oxidative mechanisms of different forms of chromium. *Toxicology*, 180(1):5–22.

Bailey, M.M., Sturdivant, J., Jernigan, P.L., Townsend, M.B., Bushman, J., Ankareddi, I., Rasco, J.F., Hood, R.D. and Vincent, J.B. (2008). Comparison of the potential for developmental toxicity of prenatal exposure to two dietary chromium supplements, chromium picolinate and $[Cr_3O(O_2CCH_2CH_3)_6(H_2O)_3]^+$, in mice. *Birth Defects Res*. *B Dev Reprod*. *Toxicol.*, 83(1):27-31.

Bailey, R.P., Bennett, T., Benjamin, M.M. (1992). Sorption onto and recovery of Chromium (VI) using iron-oxide-coated sand. *Water Science & Technology*, 26(5-6):1239–1244.

Bailey, S.E., Olin, T.J., Bricka, R.M. and Adrian, D.D. (1999). A review of potentially low-cost sorbents for heavy metals. *Water Research*, 33 (11):2469-2479.

Banu, S.K., Samuel, J.B., Arosh, J.A., Burghardt, R.C. and Aruldhas, M.M. (2008). Lactational exposure to hexavalent chromium delays puberty by impairing ovarian development, steroidogenesis and pituitary hormone synthesis in developing Wistar rats. *Toxicol. Appl. Pharmacol.* 232(2):180-9.

Bayer (1972). Akute Toxizität von anorganischen Pigmenten. Wuppertal, Bayer, *Institut für Toxikologie*, 1-4.

Bayer (1978). Akute orale Toxizität. Wuppertal-Elberfeld, Bayer, *Institut für Toxikologie Wuppertal-Elberfeld*: 1.

Bataineh, H., Al-Hamood, M.H., Elbetieha, A. and Hani, I.B. (1997). Effect of long-term ingestion of chromium compounds on aggression, sex behaviour and fertility in adult male rat. *Drug and Chemical Toxicology*, 20(3):133-149.

Beaumont, J.J., Sedman, R.M., Reynolds, S.D., Sherman, C.D., Li, L.H., Howd, R.A., Sandy, M.S., Zeise, L. and Alexeeff, G.V. (2008). Cancer mortality in a Chinese population exposed to hexavalent chromium in drinking water. *Epidemiology*, 19(1):12-23.

Burastero, S.E., Paolucci, C., Breda, D., Ponti, J., Munaro, B. and Sabbioni, E. (2006). Chromium (VI)-induced immunotoxicity and intracellular accumulation in human primary dendritic cells. *Int. J. Immunopathol. Pharmacol*., 19(3):581-91.

CCR (2011). The Criterion, March 2011. California Cancer Registry. Available from http://library.constantcontact.com/download/get/file/1102141380567-58/20110323_Hinkley_3_2011.pdf

CDPH (2012). Chromium-6 in Drinking Water Sources: Sampling Results, Website: http://www.cdph.ca.gov/certlic/drinkingwater/Pages/Chromium6sampling.aspx. Accessed: 12/03/12

Chang, H.R., Tsao, D.A. and Tseng, W.C. (2011). Hexavalent chromium inhibited the expression of RKIP of heart *in vivo* and *in vitro*. *Toxicol. In Vitro,* 25(1):1-6.

Chen, J.M. and Hao, O.J. (1997). Biological removal of aqueous hexavalent chromium. *Journal of Chemical Technology and Biotechnology*, 69:70–76.

Chen, W.Y., Chen, C.J., Liao, J.W. and Mao, F.C. (2009). Chromium attenuates hepatic damage in a rat model of chronic cholestasis. *Life Sci*., 84(17-18):606-14.

Chen, Y. and Gu, G. (2005). Short-term batch studies on biological removal of chromium from synthetic wastewater using activated sludge biomass. *Bioresource Technology*, 96(15):1722–1729.

Chiu, A., Shi, X.L., Lee, W.K., Hill, R., Wakeman, T.P., Katz, A., Xu, B., Dalal, N.S., Robertson, J.D., Chen, C., Chiu, N. and Donehower, L. (2010). Review of chromium (VI) apoptosis, cell-cycle-arrest, and carcinogenesis. *J. Environ. Sci. Health C Environ. Carcinog. Ecotoxicol. Rev*., 28(3):188-230.

Clochesy, J.M. (1984). Chromium ingestion: A case report. *J. Emerg. Nurs.,* 10:281-282.

Costa, M. (1997). Toxicity and Carcinogenicity of Chromium (VI) in Animal Models and Humans. *Critical Reviews in Toxicology*, 27(5):431-442.

Davidson, T., Kluz, T., Burns, F., Rossman, T., Zhang, Q., Uddin, A., Nadas, A. and Costa, M. (2004). Exposure to chromium (VI) in the drinking water increases susceptibility to UV-induced skin tumors in hairless mice. *Toxicol. Appl. Pharmacol*., 196(3):431-7.

DeFlora, S. (2000). Threshold mechanisms and site specificity in chromium(VI) carcinogenesis. *Carcinogenesis*, 21(4):533-41.

DeFlora, S., Iltcheva, M. and Balanskym R.M. (2006). Oral chromium(VI) does not affect the frequency of micronuclei in hematopoietic cells of adult mice and of transplacentally exposed fetuses. *Mutat. Res*. 610(1-2):38-47.

DeFlora, S., D'Agostini, F., Balansky, R., Micale, R., Baluce, B. and Izzotti, A. (2008). Lack of genotoxic effects in hematopoietic and gastrointestinal cells of mice receiving chromium(VI) with the drinking water. *Mutat. Res*., 659(1-2):60-7.

Deshmukh, N.S., Bagchi, M., Lau, F.C. and Bagchi, D. (2009a). Safety of a novel oxygen-coordinated niacin-bound chromium(III) complex (NBC): I. Two-generation reproduction toxicity study. *J. Inorg. Biochem*., 103(12):1748-54.

Deshmukh, N.S., Bagchi, M., Lau, F.C. and Bagchi, D. (2009b). Safety of an oxygen-coordinated niacin-bound chromium(III) complex (NBC): II. Developmental toxicity study in rats. *J. Inorg. Biochem*., 103(12):1755-60.

Donaldson, R.M. and Barreras, R.F. (1996). Intestinal absorption of trace quantities of chromium. *Journal of Laboratory and Clinical Medicine*, 68:484-493.

Eastmond, D.A., Macgregor, J.T. and Slesinski, R.S. (2008). Trivalent chromium: assessing the genotoxic risk of an essential trace element and widely used human and animal nutritional supplement. *Crit. Rev. Toxicol*., 38(3):173-90.

EFSA (2014). Scientific Opinion. 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.

Elbetieha, A. and Al-Hamood, M.H. (1997). Long-term exposure of male and female mice to trivalent and hexavalent chromium compounds: effect on fertility. *Toxicology,* 116(1-3):39-47.

El-Yamani, N., Zúñiga, L., Stoyanova, E., Creus, A., Marcos, R. (2011). Chromium-induced genotoxicity and interference in human lymphoblastoid cell (TK6) repair processes. *J*. *Toxicol*. *Environ*. *Health* 74(15-16):1030-9.

Environment Agency (EA) (2002). Contaminants in soil: collation of toxicological data and intake values for humans. Chromium. Environment Agency and DEFRA. R&D Publication TOX 4.

Environmental Working Group (EWG) (2010). Chromium-6 in U.S. Tap water. Report. Website: http://www.ewg.org/chromium6-in-tap-water

EU (2005). Chromium trioxide, sodium chromate, sodium dichromate, ammonium dichromate and potassium dichromate. European Union Risk Assessment Report. Institute for Health and Consumer Protection. European Chemicals Bureau.

Feuerman, E. (1971). Chromates as the cause of contact dermatitis in housewives. *Dermatologica*, 143(5):292-297.

Figgitt, M., Newson, R., Leslie, I.J., Fisher, J., Ingham, E. and Case, C.P. (2010). The genotoxicity of physiological concentrations of chromium (Cr(III) and Chromium (VI)) and cobalt (Co(II)): an *in vitro* study. *Mutat. Res*., 688(1-2):53-61.

Floqi, T., Vezi, D. and Malollari, I. (2007). Identification and evaluation of water pollution from Albanian tanneries, Desalination, Volume 213, Issues 1–3, 15 July 2007, Pages 56–64, New Water Culture of South East European Countries-AQUA 2005 — 21–23 October 2005, Athens, Greece Presented at the International Conference on New Water Culture of South East European Countries-AQUA 2005, 21–23 October 2005, Athens, Greece.

Gad, S.C., Powers, W.J., Dunn, B.J., Hoffman, G.M., Siino, K.M. and Walsh, R.D. (1986). Acute toxicity of four chromate salts. In: Serrone D.M. ed. Chromium Symposium 1986: An Update. Pittsburgh, PA: Industrial Health Foundation Inc., 43-58.

Galan, B., Castaneda, D., Ortiz, I. (2005). Removal and recovery of Chromium (VI) from polluted groundwater sources: a comparative study of ion-exchange technologies. *Water Research*, 39(18):4317-4324.

Gao, M., Levy, L.S., Braithwaite, R.A. and Brown, S.S. (1993). Monitoring of total chromium in rat fluids and lymphocytes following intratracheal administration of soluble trivalent or hexavalent chromium compounds. *Human and Experimental Toxicology*, 12:377-382.

Geelhoed, J.S., Meeusse, J.C., Roe, M.J., Hillier, S., Thomas, R.P., Farmer, J.G., Paterson, E. (2003). Chromium remediation or release? Effect of iron(I) sulfate addition on chromium(VI) leaching from columns of chromite ore processing residue. *Environmental Science and Technology*, 37(14):3206-3213.

Goh, C.L., Gan, S.L. and Ngui, S.J. (1986). Occupational dermatitis in a prefabrication construction factory. *Contact Dermatitis*, 15(4):235-240.

Goullé, J.P., Saussereau, E., Grosjean, J., Doche, C., Mahieu, L., Thouret, J.M., Guerbet, M. and Lacroix, C. (2011). Accidental potassium dichromate poisoning. Toxicokinetics of chromium by ICP-MS-CRC in biological fluids and in hair. *Forensic Sci. Int.* [Epub ahead of print].

Gudi, R., Slesinski, R.S., Clarke, J.J. and San, R.H. (2005). Chromium picolinate does not produce chromosome damage in CHO cells. *Mutat. Res*., 587(1-2):140-6.

Guo, Y.L., Wang, B.J., Yeh, K.C., Wang, J.C., Kao, H.H., Wang, M.T., Shih, H.C. and Chen, C.J. (1999). Dermatoses in cement workers in southern Taiwan. *Contact Dermatitis*, 40(1):1-7.

Hafianea, A., Lemordantb, D. and Dhahbi, M. (2000). Removal of hexavalent chromium by nanofiltration. *Desalination*, 130(3):305–312.

Hopkins, L.L. Jr. (1965). Distribution in the rat of physiological amounts of injected Cr^{51}(III) with time. *Am. J. Physiol.*, 209:731-735.

HPA (2007). Chromium Toxicological Overview. Health Protection Agency. Public Health England.

Huang, C.P. (1977). The removal of chromium(VI) from dilute aqueous solution by activated carbon. *Water Research*, 11(8):673–679.

Hwong-wen, M., Ming-Lung, H. and Pi-Cheng, C. (2007). A systemic health risk assessment for the chromium cycle in Taiwan, *Environment International*, 33(2):206-218.

IARC (2012). Arsenic, Metals, Fibres, and Dusts. IARC Monographs, Volume 100C. A Review of Human Carcinogens. International Agency for Research on Cancer.

IPSC (2011). Concise International Chemical Assessment Document. Chromium (VI). Post-Consultative Group Draft. September 2011. International Programme on Chemical Safety.

Janus, J.A. and Krajnc, E.I. (1990). Integrated criteria document for chromium: effects. Appendix. Bilthoven, Netherlands, National Institute of Public Health and Environmental Protection.

Jiandong, Z. and Xilin, L. (1987). Investigation and study of Chromium Pollution in Jingzhou. *Chinese Journal of Preventative Medicine*, 21(5).

Kanojia, R.K., Junaid, M. and Murthy, R.C. (1996). Chromium induced teratogenicity in female rat. *Toxicol*. *Lett*. 89(3):207-13.

Kaufman, D.B., DiNicola, W. and McIntosh, R. (1970). Acute potassium dichromate poisoning: Treated by peritoneal dialysis. *Am. J. Dis. Child*, 119:374-376.

Kerger, B.D., Finley, B.L., Corbett, G.E., Dodge, D.G. and Paustenbach, D.J. (1997). Ingestion of chromium(VI) in drinking water by human volunteers: absorption, distribution, and excretion of single and repeated doses. *J*. *Toxicol*. *Environ*. *Health*, 50:67-95.

Kiec-Swierczynska, M. (1990). Occupational dermatoses and allergy to metals in Polish construction workers manufacturing prefabricated building units. *Contact Dermatitis*, 23(1):27-32.

Kirman C.R., Hays S.M., Aylward L.L., Suh M., Harris M.A., Thompson C.M., Haws L.C. and Proctor D.M. (2012). Physiologically based pharmacokinetic models for rats and mice orally exposed to chromium. *Chemico-Biological Interactions*, 200:45-64.

Komori, K.A., Rivas, A., Toda, K. and Ohtak, H. (2004). Biological removal of toxic chromium using an *Enterobacter cloacae* strain that reduces chromate under anaerobic conditions. *Biotechnology and Bioengineering*, 35(9):951-954.

Komorowski, J.R., Greenberg, D. and Juturu, V. (2008). Chromium picolinate does not produce chromosome damage. *Toxicol*. *In Vitro*, 22(3):819-26.

Lalaouni, A., Henderson, C., Kupper, C. and Grant, M.H. (2007). The interaction of chromium (VI) with macrophages: depletion of glutathione and inhibition of glutathione reductase. *Toxicology*, 236(1-2):76-81.

Levina, A., Harris, H.H. and Lay, P.A. (2005). Binding of chromium(VI) to histones: implications for chromium(VI)-induced genotoxicity. *J*. *Biol*. *Inorg*. *Chem*., 11(2):225-34.

Lim, T.H., Sargent III, T. and Kusubov, N. (1983). Kinetics of trace element chromium(III) in the human body. *Am. J. Physiol.*, 244:445-454.

Linos, A., Petralias, A., Christophi, C.A., Christoforidou, E., Kouroutou, P., Stoltidis, M., Veloudaki, A., Tzala, E., Makris, K.C. and Karagas, M.R. (2011). Oral ingestion of hexavalent chromium through

drinking water and cancer mortality in an industrial area of Greece - an ecological study. *Environ*. *Health*, 10:50.

Marouani, N., Tebourbi, O., Mokni, M., Yacoubi, M.T., Sakly, M., Benkhalifa, M. and Ben Rhouma, K. (2011). Embryotoxicity and fetotoxicity following intraperitoneal administrations of hexavalent chromium to pregnant rats. *Zygote*, 19(3):229-35.

McNeill, L., McLean, J., Edwards, M. and Parks, J. (2011). State of the Science of Hexavalent Chromium in Drinking Water. Water Research Foundation.

Mignini, F., Tomassoni, D., Traini, E., Vitali, M., Scuri, S., Baldoni, E., Grappasonni, I. and Cocchioni, M. (2009). Immunological pattern alteration in shoe, hide, and leather industry workers exposed to hexavalent chromium. *Environ*. *Toxicol*., 24(6):594-602.

Mortazavi, B., Barikbin, B., Moussavi, G.H.R. (2010). Survey of nano filtration performance for hexavalent chromium removal from water containing sulfate. *Iranian Journal of Health and Environment*, 3(2).

Mozaffari, M.S., Abdelsayed, R., Liu, J.Y., Wimborne, H., El-Remessy, A. and El-Marakby, A. (2009). Effects of chromium picolinate on glycemic control and kidney of the obese Zucker rat. *Nutr*. *Metab*. *(Lond.)*, 10(6):51.

National Toxicology Program (2007). NTP 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. *Toxic Rep*. *Ser.*, 72:1-G4.

National Toxicology Program (2008). Toxicology and carcinogenesis studies of sodium dichromate dihydrate (CAS No. 7789-12-0) in F344/N rats and B6C3F1 mice (drinking water studies). *Natl*. *Toxicol*. *Program Tech*. *Rep*. *Ser*., 546:1-192.

National Toxicology Program (2010). 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). *Natl*. *Toxicol*. *Program Tech*. *Rep*. *Ser*., 556.

Ni, F., Liu, G., Ren, H., Yang, S., Ye, J., Lu, X. and Yang, M. (2009). Health Risk Assessment on Rural Drinking Water Safety - A Case Study in Rain City District of Ya'an City of Sichuan Province, *J. Water Resour. Protect*., 1(2):128-135.

Nielsen, N.H. and Menne, T. (1992). Allergic contact sensitization in an unselected Danish population. The Glostrup Allergy Study. Denmark. *Act Derm. Venereol.*, 72(6):456-460.

Nudler, S.I., Quinteros, F.A., Miler, E.A., Cabilla, J.P., Ronchetti, S.A. and Duvilanski, B.H. (2009). Chromium VI administration induces oxidative stress in hypothalamus and anterior pituitary gland from male rats. *Toxicol*. *Lett*., 185(3):187-92.

Oktor, K., Yilmaz, S., Tuerker, G. and Erkus, E. (2008). Speciative determination of Cr (III) and Cr (VI) in dyeing waste water of Dil Creek discharge to Izmit Gulf (Izmit-Kocaeli, Turkey) by ICP-AES, *Environ. Monit. Assess*., 141(1-3):97-103.

Patlolla, A.K., Barnes, C., Hackett, D. and Tchounwou, P.B. (2009). Potassium dichromate induced cytotoxicity, genotoxicity and oxidative stress in human liver carcinoma (HepG2) cells. *Int*. *J*. *Environ*. *Res*. *Public Health*, 6(2):643-53.

Perricone, N.V., Bagchi, D., Echard, B. and Preuss, H.G. (2010). Long-term metabolic effects of different doses of niacin-bound chromium on Sprague-Dawley rats. *Mol*. *Cell Biochem*., 338(1-2):91-103.

Petresin, E. (1996). Water quality problems in Slovenia. *European Water Pollution Control*, 6(3):31-35.

Philipot, J.M., Chaffange, F. and Sibony, J. (1985). Hexavalent chromium removal from drinking water. *Water Science & Technology*, 17(6/7):1121-1132.

Proctor, D.M., Suh, M., Aylward, L.L., Kirman, C.R., Harris, M.A., Thompson, C.M., Gürleyük, H., Gerads, R., Haws, L.C. and Hays, S.M. (2012). Hexavalent chromium reduction kinetics in rodent stomach contents. *Chemosphere*, 89:487-493.

Quievryn, G., Messer, J. and Zhikovich, A. (2006). Lower mutagenicity but higher stability of Cr-DNA adducts formed during gradual chromate activation of ascorbate. *Carcinogenesis*, 27(11):2315-2321.

Quinones, P.A. and Garcia Munoz, C.M. (1965). [Allergic contact sensitizations to nickel and chromium: Presence of these metallic elements in commercial detergents for household use.] *Ann. Dermatol. Syphiligr*. (Paris), 92(4):383-386.

Quinteros, F.A., Poliandri, A.H., Machiavelli, L.I., Cabilla, J.P. and Duvilanski, B.H. (2007). *In vivo* and *in vitro* effects of chromium VI on anterior pituitary hormone release and cell viability. *Toxicol*. *Appl*. *Pharmacol*., 218(1):79-87.

Raghunathan, V.K., Tettey, J.N., Ellis, E.M. and Grant, M.H. (2009a). Comparative chronic *in vitro* toxicity of hexavalent chromium to osteoblasts and monocytes. *J*. *Biomed*. *Mater*. *Res*. *A*, 88(2):543-50.

Raghunathan, V.K., Ellis, E.M. and Grant, M.H. (2009b). Response to chronic exposure to hexavalent chromium in human monocytes. *Toxicol*. *In Vitro*, 23(4):647-52.

Raja, N.S. and Nair, B.U. (2008). Chromium(III) complexes inhibit transcription factors binding to DNA and associated gene expression. *Toxicology*, 251(1-3):61-5.

Reichelderfer, T.E. (1968). Accidental death of an infant caused by ingestion of ammonium dichromate. *South Med*. *J.* 61:96-97.

Rowbotham, A.L., Levy, L.S. and Shuker, L.K. (2010). Chromium in the Environment: An Evaluation of Exposure of the UK General Population and Possible Adverse Health Effects. *Journal of Toxicology and Environmental Health, Part B*, 3(3):145-178.

Salnikow, K. and Zhitkovich, A. (2008). Genetic and epigenetic mechanisms in metal carcinogenesis and cocarcinogenesis: nickel, arsenic, and chromium. *Chem*. *Res*. *Toxicol*., 21(1):28-44.

Sharma, A. (2003). The Villain of Hinkley, California: Chromium-6 Takes Center Stage, *On Tap* 3 (1):28-29.

Sharma, S.K., Petrusevski, B. and Amy, G. (2008). Chromium removal from water: a review. *Journal of Water Supply: Research and Technology – AQUA*, 57(8):541-553.

Shelnutt, S.R., Goad, P. and Belsito, D.V. (2007). Dermatological toxicity of hexavalent chromium. *Crit*. *Rev*. *Toxicol*., 37(5):375-87.

Shubochin, L.N. and Pokhodzie, Y.I. (1980). [Toxic properties of strontium chromate.] *Gig. Sanit*., 45:76-77.

Slesinski, R.S., Clarke, J.J., San, R.H. and Gudi, R. (2005). Lack of mutagenicity of chromium picolinate in the hypoxanthine phosphoribosyltransferase gene mutation assay in Chinese hamster ovary cells. *Mutat*. *Res*., 585(1-2):86-95.

Smith, A.H. (2008). Hexavalent Chromium, Yellow Water, and Cancer: A Convoluted Saga. *Epidemiology*, 19(1).

Smyth, H.F., Carpenter, C.P. and Weil, C.S. (1969). Range finding toxicity data: List VII. *American Industrial Hygiene Association Journal*, 30:470-476.

Soudani, N., Sefi, M., Bouaziz, H., Chtourou, Y., Boudawara, T. and Zeghal, N. (2011). Nephrotoxicity induced by chromium(VI) in adult rats and their progeny. *Hum*. *Exp*. *Toxicol*., 30(9):1233-45.

Staniek, H., Krejpcio, Z. and Iwanik, K. (2010). Evaluation of the acute oral toxicity class of tricentric chromium(III) propionate complex in rat. *Food Chem*. *Toxicol*., 48(3):859-64.

Stern, A.H., Bagdon, R.E., Hazen, R.E. and Marzulli, F.N. (1993). Risk assessment of the allergic dermatitis potential of environmental exposure to hexavalent chromium. *J. Toxicol. Environ. Health*, 40(4):613-641.

Stout, M.D., Nyska, A., Collins, B.J., Witt, K.L., Kissling, G.E., Malarkey, D.E. and Hooth, M.J. (2009). Chronic toxicity and carcinogenicity studies of chromium picolinate monohydrate administered in feed to F344/N rats and B6C3F1 mice for 2 years. *Food Chem*. *Toxicol*., 47(4):729-33.

Subramanian, S., Rajendiran, G., Sekhar, P., Gowri, C., Govindarajulu, P. and Aruldhas, M.M. (2006). Reproductive toxicity of chromium in adult bonnet monkeys (*Macaca radiata* Geoffrey). Reversible oxidative stress in the semen. *Toxicol*. *Appl*. *Pharmacol*., 215(3):237-49.

Sugiyama, M., Tsuzuki, K., Lin, X. and Costa, M. (1992). Potentiation of sodium chromate (VI)-induced chromosomal aberrations and mutations by vitamin B2 in Chinese hamster V79 cells. *Mutat. Res*., 283:211-214.

Taleb-Ahmeda, M., Taha, S., Maachia, R., and Dorange, G. (2002). The influence of physico-chemistry on the retention of chromium ions during nanofiltration. *Desalination*, 145(1–3):103–108.

Tandon, S.K., Behari, J.R. and Kachru, D.N. (1979). Distribution of chromium in poisoned rats. *Toxicology*, 13:29-34.

Thomas, D.H., Rohrera, J.S., Jackson, P.E., Pak, T. and Scott, J.N. (2002). Determination of hexavalent chromium at the level of the California Public Health Goal by ion chromatography, *Journal of Chromatography A*, 956(1-2):255-259 14th International Ion Chromatography Symposium.

Thompson C.H.M., Proctor D.M., Haws L.C. Herbert C.D., Grimes S.D., Shertzer H.G., Kopec A.K., Hixon J.G., Zacharewski T.R. and Harris M.A. (2011). Investigation of the Mode of Action Underlying the Tumorigenic Response Induced in B6C3F1 Mice Exposed Orally to Hexavalent Chromium. *Toxicological Sciences*, 123(1):58-70.

Thompson C.M., Kirman C.R., Proctor D.M., Haws L.C., Suh M., Hays S.M., Hixon J.G. and Harris M.A (2014). A chronic oral reference dose for hexavalent chromium-induced intestinal cancer. *Journal of Applied Toxicology*, 34: 525-536.

US EPA (1994). Method 200.8. Determination of trace elements in waters and wastes by inductively coupled plasma-mass spectrometry. US Environmental Protection Agency.

US EPA (1998). Toxicology Review of Trivalent Chromium (CAS RN: 16065-83-1) In Support of Summary Information on the Risk Integrated Information System (IRIS). August 1998. US Environmental Protection Agency, Washington D.C.

US EPA (2003). Water treatment technology feasibility support document for chemical contaminants; in support of EPA six-year review of national primary drinking water regulations. EPA report 815-R-03-004, US Environmental Protection Agency.

US EPA (2010a). Chromium-6 in Drinking Water. Office of Water, EPA 815-F-10-005. US Environmental Protection Agency.

US EPA (2010b). Draft Toxicological Review of Hexavalent Chromium (CAS No. 18540-29-9) In Support of Summary Information on the Integrated Risk Information System (IRIS) EPA/635/R-10/004A. US Environmental Protection Agency.

US EPA (2012). Integrated Risk Information Service (IRIS). Chromium (III), Insoluble Salts. US Environmental Protection Agency.

Villalobos, M., Trotz, M.A. and Leckie, J.O. (2001). Surface complexation modeling of carbonate effects on the adsorption of Chromium (VI), Pb(II), and U(VI) on goethite. *Environmental Science and Technology*, 35(19):3849-3856.

Visek, W.J., Whitney, I.B., Kuhn III, U.S.G. and Comar, C.L. (1953). Metabolism of Cr-51 by animals as influenced by chemical state. *Proc. Soc. Exp. Biol. Med.*, 84:610-615.

Wakeman, T.P., Kim, W.J., Callens, S., Chiu, A., Brown, K.D. and Xu, B. (2004). The ATM-SMC1 pathways is essential for activation of chromium(VI)-induced S-phase checkpoint. *Mutation Research*, 554:241-251.

Wang, X.F., Xing, M.L., Shen, Y., Zhu, X. and Xu, L.H. (2006). Oral administration of Chromium (VI) induced oxidative stress, DNA damage and apoptotic cell death in mice. *Toxicology*, 228(1):16-23.

Wen-Qing, L., Shao-Hui, Z., Wen-Shan, Z., Shao-Hui, Z. and Ai-Lin, L. (2008). Water Pollution and health impact in China: a mini review, *Open Environmental Sciences*, 2(1-5).

WHO (2003). Chromium in Drinking-water. Background Document for Development of WHO Guidelines for Drinking-water Quality. WHO/SDE/WSH/03.04/04. World Health Organization.

WHO (2009). Inorganic chromium(III) compounds. Concise International Chemical Assessment Document 76. World Health Organization.

WHO (2011). Guidelines for Drinking-water Quality. Fourth Edition. World Health Organization

WHO (2013). Inorganic chromium(VI) compounds. Concise International Chemical Assessment Document 78. World Health Organization.

Wise, S.S., Holmes, A.L., Qin, Q., Xie, H., Katsifis, S.P., Thompson, W.D. and Wise, J.P. Sr. (2010). Comparative genotoxicity and cytotoxicity of four hexavalent chromium compounds in human bronchial cells. *Chem*. *Res*. *Toxicol*., 23(2):365-72.

Xia, B., Yang, L.Q., Huang, H.Y., Pang, L., Hu, G.H., Liu, Q.C., Yuan, J.H., Liu, J.J., Xia, Y.B., Zhuang, Z.X. (2011). Chromium(VI) causes down regulation of biotinidase in human bronchial epithelial cells by modifications of histone acetylation. *Toxicol*. *Lett*., 205(2):140-5. [Epub ahead of print].

Yao, H., Guo, L., Jiang, B.H., Luo, J. and Shi, X. (2008). Oxidative stress and chromium(VI) carcinogenesis. *J. Environ*. *Pathol. Toxicol*. *Oncol*., 27(2):77-88.

Zelga, J. (1964). [On clinical aspects and pathogenesis of chromate eczema.]. *Arch. Klin. Exp. Dermatol.,* 218:499-542.

Zhang, X.H., Zhang, X., Wang, X.C., Jin, L.F., Yang, Z.P., Jiang, C.X., Chen, Q., Ren, X.B., Cao, J.Z., Wang, Q. and Zhu, Y.M. (2011). Chronic occupational exposure to hexavalent chromium causes DNA damage in electroplating workers. *BMC Public Health*, 11:224.

Zhitkovich, A. (2011). Chromium in drinking water: sources, metabolism, and cancer risks. *Chem*. *Res*. *Toxicol*., 24(10):1617-29.

Zouboulisa, A.I., Sarasidisa, V. and Moussasa, P.A. (2010). Removal of copper from synthetic wastewaters by the hybrid coagulation–microfiltration process. *Separation Science and Technology*, 45(11):1658-1666.

Appendix A Literature search

An extensive literature search has been conducted for chromium, which has included searching by chemical name and CAS number and a range of search terms, including:

- Toxicokinetics
- Absorption
- Distribution
- Metabolism
- Excretion
- Toxicity
- Mutagenicity
- Genotoxicity
- Carcinogenicity
- Removal
- Drinking water
- Volatilisation
- Volatility
- GAC
- Activated carbon
- Ozone
- Chlorine
- Treatment
- Absorption
- Occurrence

Information was sought from a wide range of sources, including:

- SRC Physprop database: (http://esc.srcinc.com/fatepointer/search.asp)
- ChemID Plus: (http://chem.sis.nlm.nih.gov/chemidplus/)
- Organisation for Economic Co-operation and Development eChem Portal: (http://www.echemportal.org/echemportal/index?pageID=0&request_locale=en)
- European Chemicals Agency (http://echa.europa.eu/web/guest/home)
- European Food Safety Authority (http://www.efsa.europa.eu/)
- US Environmental Protection Agency (http://www.epa.gov/)

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- World Health Organization Guidelines for Drinking-water Quality background documents (http://www.who.int/water_sanitation_health/dwq/chemicals/en/)
- European Union Risk Assessment Reports (http://esis.jrc.ec.europa.eu/)
- US National Toxicology Program (http://ntp-server.niehs.nih.gov/)
- US Agency for Toxic Substances and Disease Registry (http://www.atsdr.cdc.gov/toxprofiles/index.asp)
- International Programme on Chemical Safety INCHEM database (http://www.inchem.org/)
- Health Canada (http://www.hc-sc.gc.ca/index-eng.php)
- PubMed (http://www.ncbi.nlm.nih.gov/sites/entrez)
- Scopus (http://www.scopus.com/)
- Science Direct (http://www.sciencedirect.com/)
- Committee on Toxicity (http://cot.food.gov.uk/)
- Google (https://www.google.co.uk)