PUBLIC HEALTH GOALS FOR CHEMICALS IN DRINKING WATER

HEXAVALENT CHROMIUM (Cr VI)

July 2011

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Public Health Goal for
Hexavalent Chromium (Cr VI)
in Drinking Water

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PREFACE

Drinking Water Public Health Goals
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This Public Health Goal (PHG) technical support document provides information on health effects from contaminants in drinking water. PHGs are developed for chemical contaminants based on the best available toxicological data in the scientific literature. These documents and the analyses contained in them provide estimates of the levels of contaminants in drinking water that would pose no significant health risk to individuals consuming the water on a daily basis over a lifetime.

The California Safe Drinking Water Act of 1996 (Health and Safety Code, Section 116365) requires the Office of Environmental Health Hazard Assessment (OEHHA) to perform risk assessments and adopt PHGs for contaminants in drinking water based exclusively on public health considerations. The Act requires that PHGs be set in accordance with the following criteria:

1. PHGs for acutely toxic substances shall be set at levels at which no known or anticipated adverse effects on health will occur, with an adequate margin of safety.
2. PHGs for carcinogens or other substances that may cause chronic disease shall be based solely on health effects and shall be set at levels that OEHHA has determined do not pose any significant risk to health.
3. To the extent the information is available; OEHHA shall consider possible synergistic effects resulting from exposure to two or more contaminants.
4. OEHHA shall consider potential adverse effects on members of subgroups that comprise a meaningful proportion of the population, including but not limited to infants, children, pregnant women, the elderly, and individuals with a history of serious illness.
5. OEHHA shall consider the contaminant exposure and body burden levels that alter physiological function or structure in a manner that may significantly increase the risk of illness.
6. OEHHA shall consider additive effects of exposure to contaminants in media other than drinking water, including food and air, and the resulting body burden.
7. In risk assessments that involve infants and children, OEHHA shall specifically assess exposure patterns, special susceptibility, multiple contaminants with toxic mechanisms in common, and the interactions of such contaminants.
8. In cases of insufficient data for OEHHA to determine a level that creates no significant risk, OEHHA shall set the PHG at a level that is protective of public health with an adequate margin of safety.

9. In cases where scientific evidence demonstrates that a safe dose response threshold for a contaminant exists, then the PHG should be set at that threshold.
10. The PHG may be set at zero if necessary to satisfy the requirements listed above in items seven and eight.
11. PHGs adopted by OEHHA shall be reviewed at least once every five years and revised as necessary based on the availability of new scientific data.

PHGs are not regulatory requirements, but instead represent non-mandatory goals. Using the criteria described above, PHGs are developed for use by the California Department of Public Health (DPH) in establishing primary drinking water standards (State Maximum Contaminant Levels, or MCLs). Thus, PHGs are not developed as target levels for cleanup of ground or ambient surface water contamination, and may not be applicable for such purposes, given the regulatory mandates of other environmental programs.

Whereas PHGs are to be based solely on scientific and public health considerations, drinking water standards adopted by DPH are to consider economic factors and technical feasibility. Each primary drinking water standard adopted by DPH shall be set at a level that is as close as feasible to the corresponding PHG, with emphasis on the protection of public health. Each primary drinking standard adopted by DPH is required to be set at a level that is as close as feasible to the corresponding PHG, with emphasis on the protection of public health. MCLs established by DPH must be at least as stringent as the federal MCL, if one exists.

Additional information on PHGs can be obtained at the OEHHA web site at www.oehha.ca.gov.

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PUBLIC HEALTH GOAL FOR HEXAVALENT CHROMIUM IN DRINKING WATER

SUMMARY

The Office of Environmental Health Hazard Assessment (OEHHA) is publishing a Public Health Goal (PHG) for hexavalent chromium of 0.02 parts per billion (ppb) or micrograms per liter (μg/L) in drinking water. OEHHA has reviewed the available data on the toxicity of hexavalent chromium and has identified the PHG level as protective against all identified toxic effects from both oral and inhalation exposure to hexavalent chromium that may be present in drinking water.

While hexavalent chromium has long been recognized as a potent carcinogen via inhalation, there is now sufficient evidence that hexavalent chromium is also carcinogenic by the oral route of exposure, based on studies in rats and mice conducted by the National Toxicology Program (NTP, 2008). To calculate the PHG, OEHHA utilized an oral cancer slope factor of 0.5 $(mg/kg\text{-}day)^{-1}$, based on a dose-related increase of tumors of the small intestine in male mice (NTP, 2008). While this approach follows the default approach described in OEHHA guidelines (OEHHA, 2009a), it is also consistent with the proposed mutagenic mode of action (McCarroll *et al.*, 2010). OEHHA also used an inhalation cancer slope factor of 510 $(mg/kg\text{-}day)^{-1}$, based on occupational studies, with an exposure assessment (Keating and McKone, 1993) to estimate inhalation of waterborne hexavalent chromium during showering, for estimating inhalation risk. The combined-route cancer risk is dominated by the oral exposure despite the much higher inhalation potency, because the inhalation of water droplets during showering is very small. The PHG was adjusted to account for increased sensitivity associated with early-in-life exposures.

A health-protective level of 2 ppb for non-carcinogenic effects is also identified based on liver toxicity (mild chronic inflammation, fatty changes) in female rats in the NTP study (2008). Other studies have indicated adverse effects in the liver and blood forming tissues.

Chromium is a heavy metal that occurs throughout the environment. The soluble hexavalent form is relatively toxic, while the less-soluble trivalent form has very low toxicity and is a required nutrient. The two forms are inter-convertible in the environment.

Available studies characterized the carcinogenic and non-carcinogenic activity of hexavalent chromium resulting from inhalation or oral exposure in both experimental animals and humans. Most of the toxicity studies investigated carcinogenic activity, because hexavalent chromium has been identified as a carcinogen. Other studies focused on the pharmacokinetics of hexavalent and trivalent chromium. The findings of these studies are very important in understanding the toxic actions of this metal.

Following oral administration of hexavalent chromium to humans and experimental animals, increased levels of total chromium in whole blood and plasma were observed, while little change was observed following trivalent chromium administration. Increases in blood/plasma total chromium levels following oral hexavalent chromium administration demonstrate bioavailability of the hexavalent form of the metal. Demonstrating bioavailability for orally administered products through increases in plasma and/blood levels is a routine method (required, for example, in submitting new drug applications).

It has been suggested that because nearly all ingested hexavalent chromium is converted to trivalent chromium in the acidic environment of the stomach, hexavalent chromium poses a negligible risk of toxicity (carcinogenic or non-carcinogenic) by the oral route (De Flora *et al*. 1997; Proctor *et al.*, 2002b). Complete conversion of hexavalent chromium to trivalent chromium in the stomach would result in the two forms behaving identically with respect to absorption, distribution, and toxic effects. However, studies in animals and humans have revealed that orally administered hexavalent chromium results in differences in blood/plasma and tissue-total chromium levels and increased urinary half-life compared to trivalent chromium. Increased toxicity following oral exposure to hexavalent chromium (compared to trivalent chromium) also suggests that hexavalent chromium is not completely converted to trivalent chromium in the stomach. After absorption into the body, the hexavalent form is eventually reduced to the trivalent form.

Given the abundant evidence that hexavalent chromium is not completely converted to trivalent chromium in the stomach and that a fraction of orally administered hexavalent chromium is bioavailable, the evidence of potential carcinogenic and non- carcinogenic effects of the hexavalent form of the metal needed to be evaluated.

Evidence on carcinogenic effects of hexavalent chromium has been summarized by others, principally for the inhalation route (IARC, 1990). Evaluation of carcinogenic risk for this assessment focused on the evidence of systemic availability and the resulting risk of carcinogenic effects after oral exposure. Studies of the mechanism of action of hexavalent chromium suggest a carcinogenic response if hexavalent chromium enters cells, regardless of the exposure route. Based on available evidence a mutagenic mode of action (MOA) has been fully described and justified (McCarroll *et al.*, 2010). Orally administered hexavalent chromium results in genotoxicity at sites distal to the site of entry, the gut, which indicates that chromium reaches those sites in the hexavalent form. Administration via drinking water of hexavalent chromium to mice (Borneff *et al.*, 1968) resulted in a statistically significant increase in stomach tumors compared to controls (OEHHA analysis). Administration of hexavalent chromium in drinking water to male and female F344 rats resulted in statistically significant increases in papillomas or carcinomas (combined) of the oral cavity in the high dose groups of both sexes, compared to controls (NTP, 2008). Administration of hexavalent chromium in drinking water to male and female $B6C3F_1$ mice resulted in statistically significant and dose-related increases in adenomas or carcinomas (combined) of the small intestine in both sexes (NTP, 2008).

Exposure of a human population to hexavalent chromium in drinking water resulted in a statistically significant increase in stomach tumors compared to rates in the surrounding province (Zhang and Li, 1987). More recently, citizens of the Oinofita municipality of Greece exposed to Cr VI in their drinking water (five highest concentrations ranged from 44 to 156 µg/L) exhibited a statistically significant increase in primary liver cancer mortality compared to the population of the surrounding prefecture (Linos *et al.*, 2011). Review of occupational studies in which humans were exposed to hexavalent chromium primarily by the inhalation route identified a significantly increased risk of stomach cancer in 3 of 25 studies. An examination of this evidence provides further support to consider hexavalent chromium to be carcinogenic by the oral exposure route.

The existing California and U.S. Environmental Protection Agency (U.S. EPA) Maximum Contaminant Levels (MCLs) of (total) chromium in drinking water are 50 ppb and 100 ppb (50 µg/L and 100 µg/L), respectively. Neither of these regulatory levels is specific for hexavalent chromium, and neither involves the assumption of potential carcinogenicity of hexavalent

chromium. The California Detection Limit for the Purposes of Reporting, or DLR, is 10 ppb for total chromium in drinking water. Hexavalent chromium was detected in 1,997 out of over 6,400 water sources analyzed as of April 6, 2004 (CDHS, 2004), with a DLR of 1 ppb. About 10 percent of the samples had reported levels of 5 ppb or more. In a February 2009 update, 2208 California water sources reported detection of hexavalent chromium above 1 ppb.

In 1987, chromium (hexavalent compounds) became one of the first substances identified as a carcinogen under California's Safe Drinking Water and Toxic Enforcement Act of 1986, more commonly known as Proposition 65. In November 2008, the state's Developmental and Reproductive Toxicant Identification Committee (DARTIC) determined that chromium (hexavalent compounds) was clearly shown to cause developmental toxicity, male reproductive toxicity and female reproductive toxicity (OEHHA, 2009c; OEHHA, 2010). The DARTIC's action added chromium (hexavalent compounds) to the Proposition 65 list of chemicals known to cause reproductive toxicity.

The PHG is intended to help guide the California Department of Public Health in developing a Maximum Contaminant Level for hexavalent chromium in drinking water, as defined in the Safe Drinking Water Act. PHGs are not developed as target levels for cleanup of contamination of ground or ambient surface water or other environmental media, and may not be applicable for such purposes, given the regulatory mandates and constraints of other environmental programs.

INTRODUCTION

Chromium is an industrially important metal that has the potential to contaminate drinking water sources. The hexavalent ionic form of chromium, also known as Cr VI, is more water soluble, more easily enters living cells, and is much more toxic than the trivalent ionic form, known as Cr III. Trivalent chromium is an essential trace element in the human diet. Chromium in this form is thought to potentiate the action of insulin, acting in combination with the glucose tolerance factor (ATSDR, 2000). Hexavalent chromium is a human carcinogen, as determined by the National Toxicology Program (NTP), the International Agency for Research on Cancer (IARC), the U.S. Environmental Protection Agency (U.S. EPA), and OEHHA (NTP, 1998; IARC, 1980b, 1990; U.S. EPA, 1998; CDHS, 1985).

A critical issue for determination of a health-protective concentration of Cr VI in drinking water is the extent to which this chromium form may be absorbed as such through the gastrointestinal tract and pose a carcinogenic hazard, versus being reduced to Cr III, which is very poorly absorbed and has very low toxicity. This document provides a literature review and an extensive analysis of the exposure issues, and the resulting toxic potential of Cr VI.

CHEMICAL PROFILE

Chemical Identity

Chromium is a metallic element with an atomic number of 24. It is a member of group VIB on the periodic table, along with molybdenum and tungsten. Chromium possesses one electron in its outer electron shell. There are four naturally occurring isotopes of chromium. The most

common ones are ^{52}Cr (83 percent) and ^{53}Cr (9.5 percent). None of the natural isotopes is radioactive (Weast *et al*., 1988).

Physical and Chemical Properties

Chromium generally occurs in small quantities associated with other metals, particularly iron. The atomic weight of chromium is 51.996. Metallic chromium melts at 1,875° C, and boils at 2,680° C; its specific gravity is 7.19. The most common valences of chromium are +3 and +6. Chromium salts are characterized by a variety of colors, solubilities and other properties. The name "chromium" is from the Greek word for color. The most important chromium salts are sodium and potassium chromates and dichromates, and the potassium and ammonium chrome alums (Hodgman *et al*., 1961).

Production and Uses

The metal is usually produced by reducing the chromite ($FeCr_2O_4$) ore with aluminum (Weast *et al*, 1988). The combined production of chromium metal and chromium ferroalloys in the United States in 1988 was 120,000 metric tons (ATSDR, 1993). Chromium is used to harden steel, in the manufacture of stainless steel, and in the production of a number of industrially important alloys (Weast *et al*., 1988). Chromium is used in making of pigments, in leather tanning and for welding. Chromium plating produces a hard mirror-like surface on metal parts that resists corrosion and enhances appearance.

Sources

The principal ore of chromium is chromite ($FeCr_2O_4$), found in Zimbabwe, Russia, Transvaal, Turkey, Iran, and other countries (Weast *et al*., 1988). The ore has not been mined in the United States since 1961 (ATSDR, 2000). Ore is imported into the U.S. from the above-mentioned countries, and refined in the U.S. into chromium metal and alloys. In California there are over a hundred industrial facilities that process imported chromium (ATSDR, 2000).

ENVIRONMENTAL OCCURRENCE AND HUMAN EXPOSURE

Air

Chromium is present in the atmosphere in particulate form, usually as very small particles (approximately 1 μm in diameter). Chromium can enter the ambient air from anthropogenic point sources such as smelters, or from windblown soil, road dust or seawater. Cigarette smoke contributes chromium to indoor air. Chromium levels in the air in the U.S. are typically <0.01 $\mu g/m^3$ in rural areas, and in the range of 0.01 to 0.03 $\mu g/m^3$ in urban areas (ATSDR, 2000).

Soil

Chromium occurs naturally in crustal rocks, but an important source of chromium in soil is probably disposal of commercial products. Chromium is present in rock (basalts and serpentine) and soil primarily in the form of the insoluble oxide, Cr_2O_3. Chromium is generally not mobile in soil (ATSDR, 2000).

Water

Chromium enters environmental waters from anthropogenic sources such as electroplating factories, leather tanneries and textile manufacturing facilities. Chromium also enters groundwater by leaching from soil. Chromium can exist in water as either Cr III or Cr VI. Rivers in the U.S. have been found to have from <1 to 30 μg/L of chromium. U.S. lakes usually have < 5 μg/L of chromium. When high levels are present, they can usually be related to sources of pollution. A survey of drinking water sources in the U.S. conducted for 1974 to 1975 found chromium levels ranging from 0.4 to 8.0 μg/L, with a mean of 1.8 μg/L (ATSDR, 2000).

California water monitoring data from 1984 to 1996 (CDHS, 1997) show that chromium (as total chromium) was detected in 822 of 9,604 drinking water sources, or approximately 9 percent of the sources surveyed. The practical detection limit was 10 μg/L. The range of total chromium levels in the samples where chromium was detected was from 10 μg/L up to a maximum of 1,100 μg/L, with a mean of 23 μg/L and a median of 17 μg/L. The chromium was not speciated, so we do not know how many of these sources would have had detectable amounts of Cr VI.

In January 2001 the California Department of Health Services (CDHS), now California Department of Public Health (CDPH), adopted a regulation adding Cr VI to the list of unregulated chemicals requiring monitoring. As of February 2002, 483 systems that collectively serve approximately 19.6 million of the state's 34 million people had sampled 32 percent of their sources (CDHS, 2002). Hexavalent chromium was detected in 59 percent of the sources (detection limit of 1 ppb). Thirty-eight percent of the sources had Cr VI levels between 1 and 5 ppb, and 13 percent of the sources detected Cr VI concentrations from 6 to 10 ppb. Six percent of the sources had Cr VI levels between 11 and 20 ppb.

CPDH (2010) reported 2208 sources of drinking water with detections above 1 ppb in the most recent update (February 17, 2009). Seven sources had Cr VI levels above 50 ppb, 5 sources had levels between 41 and 50 ppb, 14 sources with levels between 31 and 40 ppb, 61 sources had levels between 21 and 30 ppb. Hexavalent chromium levels in 456 sources were between 6 and 10 ppb and 1434 sources had levels between 1 and 5 ppb.

Food

Virtually all foods contain some chromium, ranging from 20 to 590 μg/kg (U.S. EPA, 1985). The chromium is generally in the trivalent form, although the analytical measurements do not usually provided speciation (distinction between Cr III and Cr VI). The foods with the highest levels of chromium are meats, mollusks, crustaceans, vegetables, and unrefined sugar (U.S. EPA, 1985). Analysis of samples of bread in Portugal for both total chromium and Cr VI revealed that roughly 10 percent of the total chromium in bread was Cr VI (Soares *et al.*, 2010). Mean levels of Cr VI in bread were 3.8 and 4.6 μg/kg for white and whole bread, respectively. The author estimated mean daily Cr VI intakes of 0.57 and 0.69 μg/day from bread.

Chromium is only slightly bioconcentrated in fish. Trout exhibit a bioconcentration factor (BCF) for chromium of 1. Mollusks bioconcentrate chromium to a much greater extent, with BCFs from 86 to 192 (ATSDR, 2000). Dietary intake of chromium by humans has been estimated to range from 5 to 500 μg/day, with a typical value of approximately 100 μg/day (U.S. EPA, 1985).

There is no known physiological or nutritional role for Cr VI. Trivalent chromium is an essential element, with an estimated adequate daily intake of 20-45 μg/day for various population groups, from adolescents to adults (IOM, 2001).

Other Exposure Sources

Workers in chromium production, stainless steel production and welding, chromium plating, ferrochrome and chromium pigment industries may have occupational exposures to Cr III and Cr VI (ATSDR, 2000). Ingestion exposures could occur in industry if industrial hygiene rules are not followed. See ATSDR (2000) for a complete list of industries that may contribute to sources of chromium exposure.

EXPOSURE ASSESSMENT

In addition to the ingestion of drinking water, exposure to Cr VI in a domestic water supply can occur due to inhalation of water droplets and dermal contact with water during bathing.

Inhalation Route

Exposure to toxicants in tap water due to inhalation in the shower can occur due to the movement of the agent from water into indoor air or the inhalation of the water droplets generated during showering. Because of the low volatility and high water solubility of Cr VI, the assessment of exposure to Cr VI in water focuses on the inhalation of aerosols during showering. Showerheads produce aerosols with a range of droplet sizes. Keating and McKone (1993) measured the range of aerosol droplet sizes produced by three showerheads. Only one of these was a commercially available showerhead intended for home use. Droplet sizes were measured using a hot-wire anemometer. When water droplets hit the hot wire in the instrument's probe, they cool the wire. This causes a momentary change in conductivity of the wire, which is registered by the electronics of the instrument. These momentary fluctuations in conductivity are recorded and used to calculate the distribution of droplet sizes. The home-use showerhead tested in this way (made by Teledyne WaterPic) had a median aerosol droplet diameter of 7.1 μm. The aerosol concentration in a shower chamber where this showerhead was used was 1022 aerosol particles/cm^3. From these data and employing estimates of breathing volumes (U.S. EPA, 1997), the amount of aerosol water that is inhaled by an adult taking a shower is calculated.

To determine the dose to a showering adult we must first determine the mass of the liquid phase of the aerosol they will inhale. The first step is to calculate the total volume of aerosol liquid (V_L) in a cubic centimeter of air.

$$V_L = V_d \times \text{number of droplets}$$

$$= 187.4\ \mu m^3\ \text{/droplet} \times 1022\ \text{droplets/cm}^3\ \text{air}$$

$$= 191{,}500\ \mu m^3\ \text{liquid/cm}^3\ \text{air}$$

In this equation Vd represents the volume of a "volume median aerosol droplet" (droplet volume at which accumulated liquid volume of aerosols is one-half of the total volume of the droplets; see Keating and McKone, 1993), and V_L is the total volume of aerosol liquid in a cubic centimeter of air. Vd and the number of droplets per cubic centimeter of air in the shower are taken from Keating and McKone (1993).

The next step is to determine the mass in milligrams of the liquid phase of the aerosol in each cubic centimeter of air in the shower (ML). This equation involves a unit conversion factor of 10^{-9} mg/μm^3 water.

$$ML = 191{,}500\ \mu m^3 \text{ liquid/cm}^3 \text{ air} \times (10^{-9} \text{ mg/}\mu m^3 \text{ water})$$

$$= 1.92 \times 10^{-4} \text{ mg liquid/cm}^3 \text{ air}$$

The volume (Vra) of air respired in a single showering episode, defined by convention as 10 minutes in duration, is calculated as:

$$Vra = (20\ m^3\text{/day}) \times (\text{l day/24 hrs}) \times (\text{l hr/60 min}) \times (10 \text{ min/shower})$$

$$= 0.14\ m^3\text{/shower}$$

The 20 m^3/day is the standard respiratory rate for an adult that was used in calculating the inhalation cancer potency for Cr VI. The result of this equation is the volume of air inhaled during a 10 minute shower (U.S. EPA, 1997).

The mass of liquid (Mrl) that an individual would inhale during a 10 minute shower is calculated as:

$$Mrl = ML \times Vra$$

$$= 1.92x10^{-4} \text{ mg liquid/cm}^3 \text{ air} \times 0.14\ m^3 \text{ air} \times 10^6\ cm^3/m^3$$

$$= 27 \text{ mg of water that is inhaled, or } 27x10^{-6} \text{ L}$$

A 70-kg adult breathing 20 m^3 of air per day, taking a 10-minute shower (U.S. EPA, 1997) would inhale 27 mg of liquid per shower per day, or $3.86x10^{-7}$ L/kg-day. This represents the average daily exposure to water by the inhalation route.

Finley *et al*. (1996a) also estimated chromium exposure to showering individuals based on air samplers set up in a typical home shower stall. They measured Cr VI levels in air in the breathing-zone height ranging from 87 to 324 ng Cr VI/m^3 when the water concentration of Cr VI was 0.89 to 11.5 mg/L. A serious drawback in this study was that the shower water was not heated. (The shower water in the Keating and McKone (1993) study was heated to 40 to 50°C.)

The indoor ambient temperatures are not given in the report, nor does the report state whether the indoor air was heated or cooled during the shower experiments. The outdoor ambient temperatures ranged from 21 to 79°F (-6°C to 26°C). Temperature affects the viscosity and volatility of water, so the formation and dissolution of aerosol droplets would be affected by temperature. The water temperature of the shower cannot be determined from the report, nor can one determine whether the air temperature was held constant during repetitions of the experiment. Therefore, the health-protective concentration will be derived using the results of Keating and McKone (1993).

The PHG for Cr VI will address both the inhalation and ingestion routes of exposure to water using the estimate of $3.86x10^{-7}$ L/kg-day exposure to inhaled water droplets in the shower (the reason for the preceding calculations) and an estimate of ingested tap water.

Dermal route

Dermal exposure to Cr VI in the water during showering is also a factor to be considered. The assessment of dermal exposure in the shower is based on studies that measured the rate of dermal

absorption of Cr VI in humans. Four subjects were submersed in water containing 22 parts per million (ppm) of Cr VI for three hours (Corbett *et al.*, 1997). Following exposure, a total of 6.1 μg of chromium (average, standard deviation of 7.7 μg [OEHHA calculation]) was recovered in the urine (above background) over the next four days. Based on the height and weight of the subjects, it was determined that on average 13,440 cm^2 of skin surface area had been exposed to water containing Cr VI.

Using these data, a dermal penetration rate constant (Kp) was determined for Cr VI, starting with the observation of 6.1 μg absorbed in three hours of exposure, or 2.03 μg in 1 hour. Then:

$$\text{Kp (cm/hr)} = \frac{\text{Absorbed dose (}\mu\text{g/hr)}}{\text{Concentration (}\mu\text{g/cm}^3\text{)} \times \text{surface area (cm}^2\text{)}}$$

$$\text{Kp} = \frac{2.03}{22 \times 13{,}400} = 7\text{x}10^{-6}\ \text{cm/hr}$$

Comparison of the dermal to ingestion dose of Cr VI

Drinking water ingestion rate (for this analysis) = 2 L/day
Surface area of whole body in shower = 20,000 cm^2
Time in shower = 10 minutes or 1/6 hr/day

Assume a concentration of Cr VI in water of 10 μg/L and one percent absorption from the gut (Kerger *et al.*, 1996a; Finley *et al.*, 1997; Paustenbach *et al.*, 1996).

Absorbed dose (dermal) = Kp × concentration × surface area × 1/6 hr

= $7\text{x}10^{-6}$ cm/hr × 0.01 μg/cm^3 × 20,000 cm^2 × 1/6 hr = $1.5\text{x}10^{-4}$ μg/day

Absorbed dose (ingestion) = concentration × ingestion rate × 0.01 (absorbed)

= 10 μg/L × 2 L/day × 0.01 = 0.20 μg/day

Absorbed dose from dermal exposure < 0.1 percent of the absorbed oral dose. Dermal exposure therefore does not appear to contribute significantly to the overall exposure, and will not be further considered.

METABOLISM AND PHARMACOKINETICS

Substantial information regarding the toxicokinetics of chromium began to be collected in the 1950s as the result of the use of radiolabeled chromium as a marker for measuring red blood cell turnover in humans. In addition, impetus to investigate the toxicokinetics of chromium in humans and animals resulted from the well-known carcinogenic effects of inhaled Cr VI. Most of the toxicokinetic research that was conducted to address inhalation exposure to Cr VI is relevant to the evaluation of exposure to Cr VI via the oral route. The findings of these studies are very useful in gaining an understanding of whether, or under what conditions, exposure to Cr VI may pose a significant risk to public health. Careful consideration of the experimental methods employed, the form of chromium administered, the route of administration, the doses used, particularly in how these parameters are reflected in chromium blood/plasma levels, is necessary when trying to sort out the findings of these studies.

Hexavalent chromium is highly reactive in biological systems and is rapidly converted to Cr III. In biological environments, little Cr III is converted to the hexavalent form of the metal. Once inside the cell, highly reactive Cr VI is thought to directly damage macromolecules or generate reactive metabolites that damage macromolecules, thereby producing toxicity. The rapid uptake of Cr VI into cells may also play a role in its toxicity. While administered Cr III does not result in toxicity comparable to that of Cr VI, once Cr VI has penetrated the cell, it is possible that Cr III produced by intracellular reduction is also a proximal toxicant. The evidence that Cr VI gets into tissues following oral exposure is a concern regardless of whether the toxicity is due to the reaction of Cr VI with macromolecules inside the cell or due to its rapid uptake by the cell.

In most studies, it is unclear which form(s) of chromium occurred in the tissues because most investigators did not attempt to or could not differentiate between the hexavalent and trivalent forms of the metal in tissues (total chromium levels are reported). Because of its reactivity, it is very difficult to resolve which form(s) of the metal actually occurred in a tissue. For example, any Cr VI that occurred within erythrocytes (red blood cells, RBC) may be reduced during the time that whole blood is centrifuged to obtain the RBC fraction. However, since Cr VI and not Cr III readily crosses biological membranes, the two forms of the metal behave differently in biological systems. The ability to move across membranes may explain differences in the amount of absorption between the two forms of the metal. Suggestions of a theoretical possibility of an absorbable form of Cr III have been discounted by O'Flaherty and associates (2001) "because no known complexes of Cr(III) are absorbed to the extent that Cr(VI) is." In any event, observed differences in behavior act as "fingerprints" that can be employed to identify the presence of a particular form of chromium.

Hexavalent Chromium Reduction by Saliva and Gastric Fluids

Several investigators have studied the capacity and speed of Cr VI reduction to Cr III by saliva and stomach fluids because this reduction would markedly reduce or eliminate chromium absorption into the body. Complete conversion of Cr VI to Cr III would prevent toxicity, as little toxicity has been ascribed to the trivalent form of the metal. Any saturation or exhaustion of the reducing capacity of saliva and gastric fluids by high doses of Cr VI would be expected to result in increased absorption, elevated blood levels and the appearance of toxicity that may not occur at lower doses (which are more consistent with environmental exposures).

De Flora and Wetterhahn (1989), De Flora *et al*. (1997), and De Flora (2000) estimated that saliva has the capacity to reduce 0.7 to 2.1 mg of Cr VI per day and that gastric juices have the ability to reduce at least 80.3 to 84 mg of Cr VI per day. These investigators indicate that the reaction is complete within 10-20 minutes, with at least half accomplished within one minute. Proctor and coworkers investigated the reducing capacity of stomach secretions using human gastric fluid and a simulated stomach fluid (Proctor *et al.*, 2002a). The findings of these investigators appear to be consistent with estimates of De Flora and others that gastric fluids are capable of rapidly reducing large quantities of Cr VI. Both human stomach fluid and simulated stomach fluid reduced from 300 to 1,000 μg/L (gastric fluid) to 10,000 μg/L (simulated fluid) of Cr VI within minutes. Dilution of the stomach fluid by 10 fold had little effect. From the authors: "Thus, diluted stomach fluid reduces approximately the same amount of Cr (VI) as full strength stomach fluid when put in terms of actual gastric fluid/enzymes" (Proctor *et al.*, 2002a). Changing pH from 1.5 to 4.5 had some effect on Cr VI reduction. At a pH of 1.5, 60 μg/L of Cr VI was reduced in the first two minutes, while at a pH of 4.5, 40 μg/L of Cr VI was reduced

within the first two minutes. The addition of an antacid did not tangibly alter the reducing properties of the simulated stomach fluid. Finally, varying the starting concentration of Cr VI from 100 to 400 μg/L had no material effect on the rate of reduction, indicating zero order kinetics, or that reduction of Cr VI is not a function of initial concentration (was saturated) in the range of concentrations tested.

Kerger and associates investigated the reducing capacity of various beverages such as coffee, tea, lemonade and orange juice (Kerger, 1996a). They identified a level of Cr VI in water (roughly 5 mg/L or greater), which is not likely to be ingested due to organoleptic considerations. Based on this level, Cr VI was added to various beverages at 50 mg/L and 10 mg/L (2 to 10 times the level that would in all probability be rejected by consumers). Reducing capacity of these beverages was observed over time. Virtually all Cr VI added to orange juice was reduced in a few minutes, while coffee, tea and lemonade were somewhat slower. After 15 minutes, more than 97 percent of 10 mg/L Cr VI added to orange juice, coffee, tea or lemonade was reduced to Cr III.

Given that the maximum plausible levels of Cr VI in water that would likely be ingested by humans has been estimated to be less than 5 mg/L (Kerger *et al*., 1996a), exhaustion of the capacity of saliva and gastric fluids to reduce Cr VI appears unlikely. Moreover, evidence of Cr VI absorption and/or toxicity observed at 10 mg/L or less, and perhaps up to 50 mg/L, would not appear to be a consequence of the exhaustion of the capacity of saliva and stomach fluids to reduce the metal.

On the other hand, having the capacity to reduce Cr VI to Cr III does not necessarily mean that complete reduction always occurs. If complete reduction were to occur, then Cr VI administration would be expected to behave as if Cr III had been administered. Evidence summarized below indicates that this is frequently untrue.

Absorption

Most studies that have investigated oral absorption of Cr VI or Cr III have measured changes in chromium blood/plasma levels or changes in urinary excretion. Analysis of changes in blood levels are the "gold standard" for demonstrating the bioavailability of xenobiotics. Measures such as the area under the plasma/serum/whole blood concentration-time curve and maximum blood/plasma concentration are employed by the U.S. Food and Drug Administration to establish equivalent bioavailability of different products (FDA, 2002). Urinary recovery of administered chromium provides a reasonable estimate of oral absorption of chromium because most chromium is excreted in the urine and little is retained in the carcass (Yamamoto *et al*., 1981; Bryson and Goodall, 1983; Hopkins, 1965). Two percent or less of a dose of Cr III was recovered in the carcass of mice seven days post-administration (Gonzalez-Vergara *et al.*, 1981).

Urinary recovery

Trivalent chromium is very poorly absorbed from the gastrointestinal tract. Typically, one percent or less of an orally administered dose of Cr III is recovered in the urine of humans or experimental animals (Febel *et al*., 2001; Donaldson and Barreras, 1966) or humans (Donaldson and Barreras, 1966; Kerger *et al.*, 1996a; Gargas *et al.*, 1994; Anderson *et al.*, 1983 Aitio *et al.*, 1984; Doisy *et al.*, 1971, Garcia *et al*. 2001). Oral absorption of Cr III complexed with an organic ligand was also very low and no better than inorganic forms **(**Gonzalez-Vergara *et al.*, 1981**;** Anderson *et al*,. 1996). Bypassing the stomach by infusing Cr III into the duodenum or jejunum resulted in at most one to two percent of the dose being absorbed in humans (Donaldson

and Barreras, 1966), or one percent (Febel *et al.*, 2001) to four percent in the rat (Donaldson and Barreras, 1966). Hexavalent chromium is also poorly absorbed from the gut. Less than ten percent of the administered dose of Cr VI was recovered in the urine in humans (6.9 percent, Kerger *et al.*, 1996a; 3.4 percent, Finley *et al*., 1996b; 1 to 4 percent, Finley *et al*., 1997; 2 percent, Paustenbach *et al.*, 1996); or in the rat (2 percent, Febel *et al.*, 2001). This is probably due to the substantial reduction of Cr VI to Cr III in the stomach. While the absorption of Cr VI was low, these studies do indicate significantly greater oral absorption of Cr VI than Cr III (Donaldson and Barreras, 1966; Finley *et al.*, 1996b; Kerger *et al.*, 1996a; Febel *et al*., 2001).

The range of doses of Cr VI administered to humans in different studies was considerable. Donaldson and Barreras (1996) gave 20 ng of radiolabeled Cr VI, Kerger *et al*. (1996a) gave 5 mg of Cr VI, Finley *et al*. (1996) gave 0.005 mg/kg-day of Cr VI for three days.

Finley *et al*. (1997) gave 0.1, 0.5, 1.0, 5.0 or 10 mg/day of Cr VI for four days, which enables an estimate of dose-related recovery. The urinary recovery of administered dose (as Cr III) was 1.7 % at 100, 1.2 % at 500, 1.4 % at 1,000, 1.7 % at 5,000 and 3.5 % at 10,000 µg/day. Linear regression (OEHHA analysis) of the urinary recoveries of chromium in the five subjects in Finley *et al*. (1997) revealed a slope that was not different from zero, indicating that urinary recovery of chromium was not dose-related. Neither the results of this study nor the others, at a single dose, indicate that oral absorption of Cr VI only begins to occur when the reducing capacity of the stomach is exhausted.

Infusion of Cr VI into the duodenum or jejunum (bypassing the stomach) resulted in marked increase in absorption in humans (Donaldson and Barreras, 1966) and experimental animals (Febel *et al*., 2001; Donaldson and Barreras, 1966). Donaldson and Barreras (1966) recovered 11 to 30 percent of the administered dose of Cr VI in human urine when the metal was infused into the intestine (only one to two percent of the dose of Cr III was absorbed). Fifty seven percent of the dose of Cr VI administered into the ligated jejunum of rats was recovered in the jejunum after 60 minutes while approximately 98 percent of the dose of Cr III was recovered in the jejunum under the same experimental conditions (Febel *et al*., 2001). Following the oral administration of Cr VI to humans, increased recovery of chromium in the urine was observed under conditions of low stomach acidity (pernicious anemia) compared to control (eight percent vs. two percent) (Donaldson and Barreras, 1966).

Kerger *et al*. (1996a) administered Cr VI to humans mixed with orange juice to determine to what degree the acidic-organic environment (somewhat analogous to the stomach) reduces oral absorption of the metal. The addition of Cr VI to orange juice prior to its ingestion was a *de facto* reductive pretreatment of Cr VI. In spite of this, the fraction of the dose of chromium recovered in the urine appeared to be greater for Cr VI than when Cr III was administered (0.6 percent versus 0.13 percent). However, the absorbed fraction was considerably less than when Cr VI was administered in water (6.9 percent).

Blood/plasma and tissue levels of chromium

Finley and associates observed marked increases in RBC chromium levels in some individuals (but not in others) that ingested three daily doses of Cr VI at total doses as low as 0.1 mg/day, while plasma chromium levels were less affected (Finley *et al.*, 1997). Increases in plasma chromium were also observed in individuals that ingested 1, 5 or 10 mg/day for three days. Paustenbach *et al*. (1996) observed elevated plasma chromium levels in one individual who ingested 4 mg/day of Cr VI. Both plasma and red blood cell levels of chromium (peak levels and

the area under the plasma time curve) appeared to be much higher in individuals ingesting one 5 mg dose of Cr VI than when the trivalent form of the metal was ingested (Kerger *et al.*, 1996a).

Increased concentrations of chromium in the blood, kidney and femur were detected in rats, mice and guinea pigs administered 1, 3, 10, 30, 100 or 300 ppm of Cr VI as sodium dichromate in their drinking water for 21 days (Anderson *et al*., 2002). Levels of chromium in the tissues increased linearly with dose below 80 ppm. Increased levels of chromium with dose were also observed in the liver and kidney of male and female mice (NTP, 2008). A more detailed analysis of the findings of this study is found in Appendix A. Thus, the difference in absorption of Cr VI versus Cr III does not appear to be the result of the exhaustion of the reducing capacity of saliva and gastric fluids because absorption was observed across all doses.

Chromium levels were measured in the urine, plasma and red blood cells (RBCs) of four human volunteers submersed below the shoulders in water containing Cr VI (22 ppm) for three hours (Corbett *et al.*, 1997). Chromium levels in urine substantially increased in three of the four individuals on the day of exposure and then returned to background levels in two of these individuals by the day after the exposure. Levels of chromium in the plasma and RBCs also increased on the day of exposure. Interestingly, plasma and RBC chromium levels in one individual remained elevated for three days after the exposure, and urine chromium levels remained elevated at four days after the exposure when the study ended.

Distribution

Distribution of chromium in the blood - When Cr VI is incubated with washed isolated RBCs, almost the entire dose is taken up by the cells. It is reduced inside the cells to Cr III, essentially trapping it inside the RBC. In contrast, little Cr III appears to be taken up by RBCs in *in vitro* incubations (Gray and Sterling, 1950; Donaldson and Barreras, 1966; Bentley, 1977; Aaseth *et al.*, 1982). When Cr VI is incubated with whole blood or RBCs plus plasma, only a fraction (depending on conditions) of the Cr VI is taken up by the RBC (Lewalter *et al.*, 1985; Coogan *et al.*, 1991b; Corbett *et al.*, 1998; Wiegand *et al.*, 1985). This is probably due to the reduction of a portion of the administered Cr VI to Cr III outside of the RBC (Korallus *et al*., 1984; Richelmi *et al*., 1984; Capellmann and Bolt, 1992). The converted trivalent component of chromium is then largely excluded from the RBC.

Negligible amounts of Cr III were associated with RBC in many *in vivo* studies (Doisy *et al.*, 1971; Onkelinx, 1977; Sayato *et al.*, 1980; Wiegand *et al.*, 1984; Suzuki *et al.*, 1984; Minoia and Cavalleri, 1988; Coogan *et al.*, 1991b). However, in other studies there is some evidence that Cr III is taken up by RBCs, particularly at higher concentrations (Venezia and Karol, 1984; Lewalter *et al.*, 1985; Merritt *et al.*, 1984; Kortenkamp *et al.*, 1987; Suzuki *et al.*, 1984). While the amount of Cr III uptake by the RBC appears to be substantially less than that of Cr VI, it could be noticeable when a large dose of Cr III is administered or when Cr VI is mostly absent. Some of the Cr III associated with the RBC fraction can be washed free, implying it is loosely bound to sites on the outside of the RBC.

While most of the Cr VI that is taken up by the RBC remains there for the lifetime of the RBC, a portion of the radiolabel is eluted. When *in vitro*-labeled RBCs are reinjected into their donors, about two percent of the labeled chromium is lost from the RBCs during the first 24 hours. This is followed by a slow steady elution or "leakage" of chromium from cells at a rate of about one percent a day (ICSH, 1980). This leakage must be accounted for when determining the RBC

survival rates clinically. The International Committee for the Standardization of Haematology (ICSH) developed a correction table that accounts for the elution of chromium from the RBC, facilitating more accurate estimates of RBC survival rates.

When Cr VI was inhaled or administered intratracheally, intraperitoneally, or intravenously, much of the chromium in the blood (25 to 70 percent) was taken up by RBCs (Sayato *et al.*, 1980; Weber, 1983; Wiegand *et al.*, 1984; Edel and Sabbioni, 1985; Minoia and Cavalleri, 1988; Gao *et al.*, 1993). At the same time, a sizable portion of the amount in blood remained in the plasma fraction (30 to 75 percent). The reduction of Cr VI to Cr III at the site of administration as well as in the plasma probably accounts for this result (Korallus *et al.*, 1984; Cavalleri *et al.*, 1985; Richelmi and Baldi, 1984; Lewalter *et al.*, 1985; Suzuki, 1988). The amount accumulated by RBCs compared to how much remains in the plasma is a function of the rate of absorption from the site of administration (which is a function of its solubility and the blood flow at the site of administration). In addition, the size of the dose, which will determine if the reducing capacity of the tissue and plasma are exhausted, will also influence whether chromium uptake into RBCs is favored.

Trivalent chromium binds to large proteins and smaller peptides (Aisen *et al.*, 1969; Yamamoto *et al.*, 1981; Aaseth *et al.*, 1982). There is little evidence that Cr VI binds to the various proteins and peptides that bind Cr III. In the plasma, Cr III preferentially binds to transferrin but as transferrin binding sites become saturated, a greater fraction of Cr III begins to bind to other molecules such as albumin (Aisen *et al.*, 1969; Frankendal and Stigbrand, 1973; Lim *et al.*, 1983; Ani and Moshtaghie, 1992; Moshtaghie *et al.*, 1992; Yang and Black, 1994). At higher levels, more chromium also occurs in the ultrafiltrate of plasma, also indicating transferrin-binding sites have become saturated (Frankendal and Stigbrand, 1973; Onkelinx, 1977). An apparently non-specific binding of chromium to proteins on the outside of RBCs can also be significant, particularly at higher concentrations. Edel and Sabbioni (1985) observed that 15 percent of Cr III in the blood was associated with RBCs 24 hours post-administration. Up to 35 percent of the Cr III in the blood was associated with RBCs in the study of Gao *et al.*, 1993. Increased blood levels of chromium following oral administration of Cr III to humans were associated with the plasma fraction (Kerger *et al*., 1996a). Increased levels of chromium also occurred in the RBCs in one of four individuals in the study.

Distribution of chromium into organs and tissues - The ability of Cr VI to penetrate the cell membrane is believed to be due to its uptake through anion channels in the plasma membrane. It should be noted that the structures responsible for the uptake of Cr VI into RBCs are present in other cells. Therefore, other cells would be expected to readily take up Cr VI, while little Cr III would be expected to be taken up by most cells. Indeed, oral, intratracheal, intravenous, or intraperitoneal administration of Cr VI results in increased chromium levels in a number of tissues, while little uptake occurs following the administration of Cr III (MacKenzie *et al.*, 1958; Baetjer *et al.*, 1959; Yamaguchi *et al.*, 1983; Edel and Sabbioni, 1985; Wiegand *et al.*, 1984; NTP, 2008). The uptake of Cr VI was very rapid in the isolated perfused rat liver (Wiegand *et al*., 1986). Relative to Cr VI, little uptake of Cr III occurred even when it was administered intravenously, which ensured that the metal was immediately available for tissue and cellular uptake (Visek *et al*., 1953; Baetjer *et al*., 1959; Sayato *et al*., 1980).

The widespread distribution of chromium into tissues following Cr VI administration by inhalation, intratracheal installation, subcutaneous injection, intraperitoneal injection and ingestion indicates that although reduction is likely to be occurring in the blood, it does not occur

at a fast enough rate to prevent Cr VI from reaching and being taken up by tissues. While chromium was detected in high levels in the kidney, spleen, RBCs, and liver when Cr VI was administered, little chromium was detected in these tissues following the administration of Cr III except at the site of its excretion, the kidney (and at much lower levels than when Cr VI was administered) (Yamamoto *et al.*, 1981; Weber, 1983; Yamaguchi *et al.*, 1983; Suzuki *et al.*, 1984; Costa, 1997). Substantial uptake of Cr VI by the liver is indicated by elevated levels of chromium in the bile following intravenous administration of Cr VI, compared to Cr III administration (Cikrt and Bencko, 1979; Manzo *et al.*, 1983; Cavalleri *et al.*, 1985). One particularly notable finding was the detection of Cr VI in bile for two hours after it was administered to animals (Cavalleri *et al.*, 1985). Increased levels of chromium were detected in the fetuses of female mice exposed to Cr VI in their drinking water (Trivedi *et al.*, 1989; Junaid *et al*., 1996a,b).

Oral administration of Cr VI revealed a slightly different pattern of distribution compared to other exposure routes, with high levels of chromium in the liver, spleen, and kidney but much lower levels in the RBC (Witmer *et al.*, 1989; Thomann *et al.*, 1994; Sutherland *et al.*, 2000; NTP, 2008). Higher levels of chromium in the liver are consistent with the immediate passage of blood from the gut to the liver. The reduced levels in the RBC relative to other routes of exposure may be due to uptake in the liver. Little chromium was detected in these tissues following oral administration of Cr III. If Cr VI were rapidly and completely reduced to Cr III, it should have been distributed in a manner that is virtually identical to that observed following Cr III administration. This is not apparent in any study regardless of the route of administration.

In humans, there have been no direct observations on the distribution of absorbed chromium. However, findings that suggest that patterns observed in animals also occur in humans include a marked difference in the urinary half-lives of chromium following the administration of Cr VI and Cr III to humans, with an average half-life of 10 hours following Cr III administration versus an average half-life of 39 hours following administration of Cr VI (Kerger *et al.*, 1996a). The prolonged urinary half-life following Cr VI administration suggests that there is a pool(s) of chromium that is slowly being released. This release or elution is reminiscent of the slow release of chromium from RBCs that occurs when labeled RBCs are introduced into humans in nuclear medicine (ICSH, 1980).

Because the half-life of chromium in RBCs was quite short after oral administration of Cr VI to humans (Kerger *et al.*, 1996a), any retention and slow release of chromium from the RBC does not appear to be responsible for the prolonged urinary half-life. This observation appears consistent with studies in animals in which Cr VI administered by the oral route resulted in elevated chromium levels in the liver, kidney, and spleen, while RBC and plasma chromium levels were only modestly elevated (Witmer *et al.*, 1989; Thomann *et al.*, 1994; Costa, 1997). Given that the circulation of blood is from the gut to the liver, accumulation by the liver would be expected. Observed accumulation of Cr VI in the liver following intravenous administration by Sayato *et al.* (1980) also suggests that liver is a site of Cr VI uptake. The half-life of chromium in various tissues (other than plasma) of rats administered Cr VI exceeded 20 days (Weber, 1983). The slow release (elution or "leakage") of chromium from the liver and other tissues in humans would explain the prolonged urinary half-life observed by Kerger *et al.* (1996a). Furthermore, the uptake of chromium into these tissues after administration of Cr VI would be consistent with the behavior of Cr VI but not Cr III.

In the experiment of Kerger and associates involving administration of Cr VI mixed with orange juice (Kerger *et al.*, 1996a), presumably reducing much of the Cr VI, the urinary half-life of the absorbed chromium was still prolonged (15 hours versus 10 hours for Cr III controls). This finding provides additional evidence that mixing chromate with food in an acidic environment somewhat analogous to the stomach does not completely reduce Cr VI to Cr III.

For some of the subjects in the human studies, changes in chromium levels in RBCs following Cr VI behaved as if Cr III had been administered (Kerger *et al.*, 1996a; Paustenbach *et al*., 1996; Finley *et al*, 1997). The levels of chromium in the RBC fraction rose rapidly and declined rapidly. However, chromium RBC levels did remain elevated in a couple of individuals as expected for Cr VI, unlike the pattern observed following Cr III administration. In other individuals, RBC and plasma chromium levels remained essentially unchanged following Cr VI administration.

That changes in the RBC chromium level following Cr VI administration appeared as if Cr III had been administered is not surprising if most of the chromium in the blood was Cr III. The pattern of rapid increase and decrease in RBC chromium levels does not exclude the presence of Cr VI, but only indicates that the Cr III predominates. At the high doses administered in these studies Cr III may have adsorbed onto the RBC surface proteins. Thus, a sizable portion of the increase of chromium levels in the plasma and RBC following oral administration of Cr VI to humans is probably Cr III. This is due to: 1) extensive reduction of absorbed Cr VI in the plasma and RBC as the result of gradual absorption when the metal is administered by the oral route; 2) the absorption of some small proportion of the Cr III formed in the stomach. A lack of analytical sensitivity may have prevented detection of changes in chromium levels in RBCs (changes in the half-life) after the large pulse of Cr III had cleared from the blood.

Elimination

Administered Cr III is rapidly cleared from the blood, RBCs, and plasma (Onkelinx, 1977; Sayato *et al.*, 1980; Gao *et al.*, 1993). Rapid declines of urinary chromium levels have also been observed (Aitio *et al.*, 1984). By contrast, following intratracheal, intravenous, or inhalation administration of Cr VI, RBC chromium levels or the ratio of RBC to plasma chromium either did not decline as rapidly or remained elevated for quite some time (Langard *et al.*, 1978; Sayato *et al.*, 1980; Weber, 1983; Suzuki *et al.*, 1984; Coogan *et al.*, 1991b; Gao *et al.*, 1993). Some of the initial decline in RBC chromium levels following Cr VI administration probably reflects the portion of the dose that was immediately converted to Cr III.

One notable exception to the pattern of a slow rate of decrease in RBC chromium levels following Cr VI administration was a rapid decrease following the oral administration of Cr VI in the rat (Coogan *et al.*, 1991b). Due to its slow rate of absorption, an oral dose of Cr VI would be expected to be largely converted to Cr III in the stomach and plasma. As such, the toxicokinetics would have the appearance as if Cr III had been administered. Thus, the apparent contradiction may simply reflect the predominance of Cr III.

Pharmacokinetics of Trivalent versus Hexavalent Chromium

Kerger *et al.* (1996b), De Flora *et al.* (1997), De Flora (2000), O'Flaherty *et al.* (2001), Proctor *et al.* (2002b) and others have suggested that at plausible maximum levels of Cr VI in drinking water, the saliva, stomach and blood have an abundant ability to rapidly convert Cr VI to Cr III.

O'Flaherty *et al*. (2001) noted, "De Flora *et al*., 1987 calculated a total daily gastric Cr(VI) reduction capacity of greater than 85 mg/day, assuming 3 meals per day. Even if all of the maximum single or multiple 5-mg doses had been ingested instantaneously in the studies on which the model calibration is based, the total reducing capacity of gastric juice should not have been exceeded." Proctor *et al*. (2002b) state: "Thus, endogenous reducing agents within the upper gastrointestinal tract and in plasma offer sufficient reducing potential to practically eliminate systemic absorption of Cr(VI) following drinking water exposures at 5-10 ppm. … It is likely that Cr(VI) ingested in drinking water is completely reduced to Cr(III) prior to systemic absorption at concentrations at least as high as 1 ppm."

This abundance of reductive capacity prompted one investigator to propose a threshold for Cr VI carcinogenesis. "The issue of thresholds in carcinogenesis, especially in the case of genotoxic carcinogenesis, is quite controversial. In the case of chromium(VI), even disregarding possible mechanisms occurring after occurrence of DNA damage, e.g. at the level of DNA repair, apoptosis, cell replication or promotion, there seems to be no doubt that toxicokinetic patterns, which restrict the availability of chromium (VI) to certain tissues, and metabolic patterns, which affect the availability of chromium(VI) to DNA, imprint a threshold character to the carcinogenesis process" (De Flora, 2000).

Based on this belief that orally administered Cr VI is rapidly converted to Cr III in the stomach and saliva, no differences in absorption, distribution, or elimination should be apparent for Cr VI versus Cr III. However, the results of the toxicokinetic studies in humans (Donaldson and Barreras, 1966; Kerger *et al.*, 1996a; Finley *et al.*, 1997; Paustenbach *et al.*, 1996) and animals (MacKenzie *et al*., 1958; Costa, 1997) do not support the conviction that Cr VI is completely converted to Cr III. Orally administered Cr III does not behave as if Cr VI had been administered in humans or experimental animals. O'Flaherty *et al*. (2001) commented, "Nonetheless it is clear based on total urinary chromium excretion, that a consistently greater percentage of Cr(VI) than of the Cr(III) was absorbed. This observation, consonant with other observations in humans (Donald and Barreras, 1966), implies that some Cr(VI) escaped reduction in the stomach and entered portal venous blood. (An alternative, theoretical possibility, that Cr(VI) is reduced in the stomach to a particularly absorbable form of Cr(III) is considered implausible because no known complexes of Cr(III) are absorbed to the extent that Cr(VI) is.)"

OEHHA proposes two models that account for the differences in behavior of Cr VI and Cr III observed in animals and humans (Figures 1 and 2). The increase in absorption, as reflected by increased plasma and erythrocyte levels, increased amount excreted in the urine, and prolonged plasma and urinary half-lives, appears to indicate that the hexavalent form of the metal is orally absorbed, distributed to tissues and then taken up by cells. Based on the findings in animals, the liver is likely to be an important site of cellular uptake of Cr VI (Witmer *et al.*, 1989; Thomann, *et al.*, 1994; Costa, 1997; Sutherland *et al.*, 2000; NTP, 2008). The prolonged plasma and urinary half-life appear to result from chromium being taken up and then eluted from cells. The behavior of administered Cr III - low plasma, erythrocyte and urinary levels, rapid decreases in plasma, and erythrocyte levels and short urinary half-life - indicate that this form of the metal is largely excluded from cells.

The differences in the distribution of Cr VI and Cr III in tissues and the difference in the urinary half-life of the two forms of the metal are indicative of the reason for concern about Cr VI exposure. If the absorbed Cr VI was rapidly reduced to Cr III in the plasma, then the pattern of

tissue distribution and rate of urinary elimination should be essentially identical to what is observed for the trivalent form of the metal. Following Cr VI administration, the findings of a prolonged plasma and urinary half-life and its distribution to the liver and other tissues (relative to Cr III) indicate that Cr VI moves into cells prior to its reduction to Cr III.

One finding that at first glance appears to contradict the aforementioned models is that following the administration of Cr VI, the half-life of chromium in the erythrocyte, while prolonged compared to when Cr III was administered, was still relatively short compared to the rate of erythrocyte turnover (Kerger *et al.,* 1996a). However, studies in animals have revealed that orally administered Cr VI is distributed more to the liver and other tissues and less to erythrocytes. Minimal amounts of the absorbed Cr VI appear to be taken up by the erythrocytes. Therefore, it appears that the bulk of the chromium in the blood in the Kerger *et al.* (1996a) study was probably Cr III. The chromium associated with the erythrocytes probably was bound to macromolecules on the outside of the cell. Non-specific binding of Cr III to erythrocytes has been observed at high concentrations by other investigators (Edel *et al.*, 1985; Gao *et al.*, 1993).

Relatively insensitive analytical methods were employed in the Kerger *et al.* (1996a) study, so a small pool of chromium inside the cell would probably not have been detectable (especially in the presence of Cr III bound to the outside). Thus, the prolonged urinary half-life in the Kerger *et al.* (1996a) study does not appear to be due to elution from erythrocytes, but probably resulted from elution from other tissues.

The toxicokinetics of Cr VI associated with dermal exposure of humans are also notable (Corbett *et al.*, 1997). Dermal transport would be expected to be rather slow, allowing more time for the conversion of Cr VI to Cr III before any uptake into tissues might occur. However, a prolonged urinary elimination of chromium observed in one individual as well as the prolonged levels in the RBC suggest that some portion of the hexavalent form of the metal was being absorbed in this case, taken up by tissues and then slowly released into the urine.

Figure 1. Toxicokinetic Model - Hexavalent Chromium

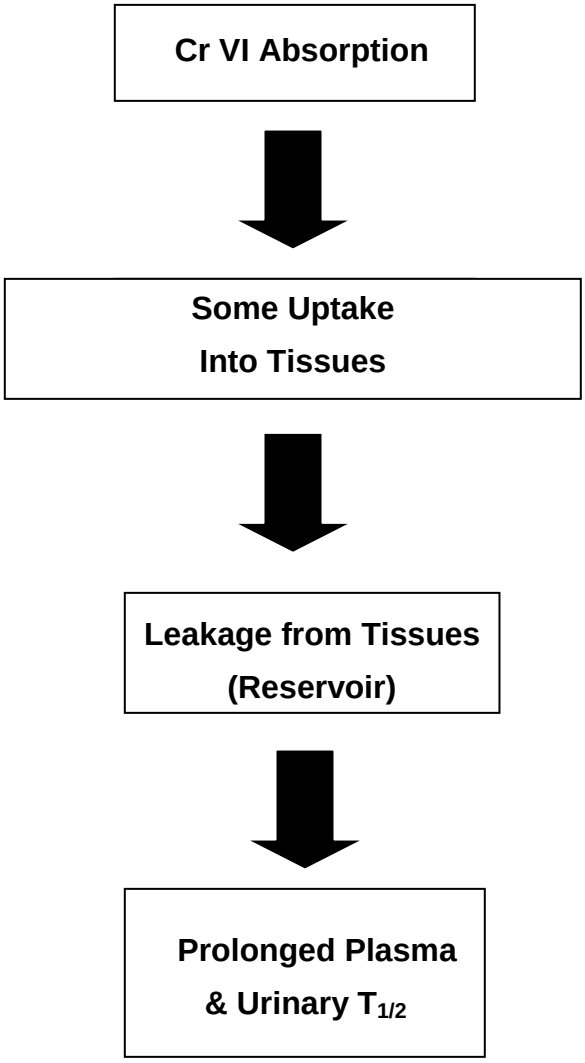
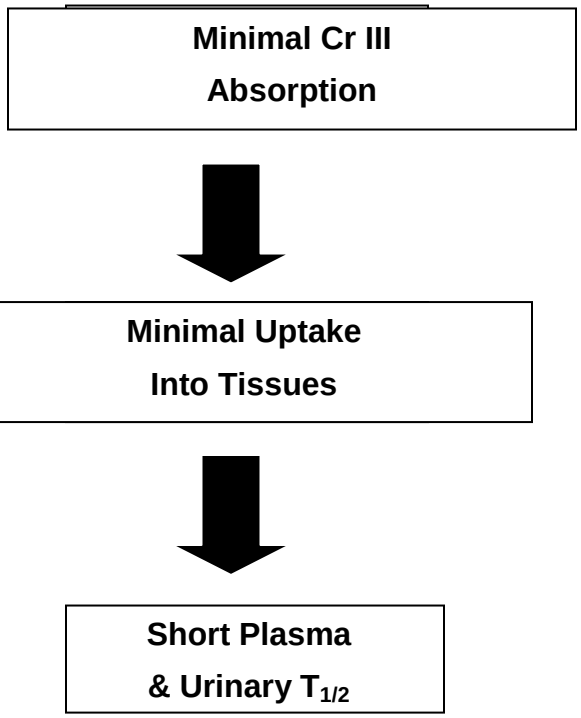


Figure 2. Toxicokinetic Model - Trivalent Chromium



Summary - Trivalent and hexavalent chromium behave differently in humans, experimental animals and *in vitro*. Differences in the amount of chromium associated with RBC and the pattern of chromium distribution among the various tissues appear to be largely due to the uptake of Cr VI into cells, while Cr III is largely excluded from cells. The difference in the amount of absorption from the gut may also reflect the uptake of Cr VI but not Cr III by cells. Quantitative differences in the propensity of Cr VI and Cr III to associate with RBCs and differences in other characteristics such as the rate of decline of chromium in RBC following uptake of Cr III (rapid) and Cr VI (delayed) allow one to identify which form of chromium occurred in tissues.

Variability of the Human Toxicokinetics of Chromium

Remarkable differences in the behavior of chromium were evident between individuals in different studies, in the same study, and within the same individual in multiple administration study designs (Kerger *et al.*, 1996a; Finley *et al.*, 1997). Following the administration of Cr VI, plasma and RBC levels were markedly elevated in certain individuals while they were essentially unchanged in other individuals. Within the same individual, chromium levels sometimes markedly increased at one dose, but no response was observed at a higher dose (Finley *et al.*, 1997). In one individual, no change in RBC and plasma levels of chromium was observed following the administration of 5 milligrams of Cr VI. Three days later the same dose in the same individual (subject 1) resulted in markedly elevated blood and plasma chromium levels.

Likely sources of this variability are differences in the contents and pH of the stomach and rate of gastric emptying, which would influence how much chromium reduction occurs in the

stomach. Differences in the ability to reduce Cr VI in the plasma would also be expected to substantially affect the levels of Cr VI in the plasma and RBC (Lewalter *et al.*, 1985; Corbett *et al.*, 1998). Also, the size of the dose may affect how much reduction occurs in the plasma because of depletion of the plasma reducing capacity. All these factors would influence the amount of uptake of Cr VI into tissues as well as the amount of non-specific binding of trivalent Cr III to the outside of the RBC.

TOXICOLOGY

Toxicological Effects in Animals

Acute Toxicity

Oral LD_{50}s for Cr VI compounds (sodium chromate, sodium dichromate, potassium dichromate, and ammonium dichromate) ranged from 13 to 19 mg Cr/kg in female rats, and 21 to 28 mg Cr/kg in male rats (Gad *et al*., 1986). In general Cr VI salts had greater acute toxicity than Cr III salts, and female rats appeared to be more sensitive than males to Cr VI salts (ATSDR, 2000).

Developmental and Reproductive Toxicity

BALB/c mice were administered Cr VI (potassium dichromate) or Cr III (chromium sulfate) (100, 200, or 400 ppm (15, 28 or 63 mg/kg-day of Cr VI)) in their feed for 35 days (Zahid *et al*., 1990). Epididymal sperm counts obtained from homogenized tissue were significantly decreased in mice receiving Cr III or Cr VI. The decrease in sperm counts appeared dose-related. Other effects were also reported including marked decreases in spermatogonia, increases in resting spermatocytes, and alteration in proportions of germ cells in different mitotic phases (decreases in leptotene and zygotene and marked increases in pachytene). These findings appeared internally inconsistent (decreases in all forms would be expected if spermatogonia had dramatically decreased unless adverse effects were delayed until the toxicant accumulated in the testis. Even then it is difficult to explain the marked decrease in spermatogonia, leptotene and zygotene with little effect on resting spermatocyte levels and a marked increase in pachytene).

The reproductive effects of Cr VI were evaluated in nine-week studies in the BALB/c mouse (NTP, 1997a) and Sprague-Dawley rat (NTP, 1996). Potassium dichromate was administered to males and females in the feed at concentrations of 0, 15, 50, 100 or 400 ppm. Based on measured food consumption, the average doses of Cr VI for male mice were 1, 3.5, 7.4 or 32 mg/kg-day and for female mice were 1.8, 5.6, 11.9 or 48 mg/kg-day. The average Cr VI doses for male rats were 0.4, 1, 2.1 or 8.4 mg/kg-day and for female rats were 0.4, 1, 2.5 or 9.8 mg/kg-day. There was no treatment related effect on preleptotene spermatocyte counts (normalized to number of Sertoli cells) in Stage X or XI tubules in BALB/c mice or Sprague-Dawley rats exposed to Cr VI for three, six or nine weeks.

Male and female BALB/c mice were exposed to Cr VI for 13 weeks, one week during pre-cohabitation followed by 12 weeks of cohabitation exposure in a continuous breeding study that yielded several litters (F_1 generation) (NTP, 1997b). The F_1 generation was exposed to the same level of potassium dichromate in their diet that their parents received after weaning at day 21 until day 74. The final F_1 litter was mated and pregnant females were allowed to deliver.

Potassium dichromate was administered in the feed at concentrations of 0, 100, 200 and 400 ppm. Based on feed consumption of the F_0 generation, week 1 doses of Cr VI for males were 7.9, 15.5 and 32.3 mg/kg-day and for females were 10.7, 21.6 and 51.2 mg/kg-day. Doses for the F_1 generation were: males (week 2) 7.9, 13.1, 33.3 mg/kg-day and (week 4) 9.1, 16.6 and 36.1 mg/kg-day and females (week 2) 8.5, 19.2 and 42.0 mg/kg-day and (week 4) 6.0, 14.9 and 35.5 mg/kg-day.

Both the F_0 and F_1 generations were evaluated for reproductive effects. No treatment related effects on fertility or reproductive performance were observed. No differences in the average number of litters per mating pair, nor pups per litter, pup sex ratio, the number of pups born alive or the adjusted weights of pups born to the F_0 generation were observed. No effects were observed on the weight of the right testis, prostate, and right epididymis. No differences were observed on the mean epididymal sperm density, percent of abnormal sperm, total number of spermatids per testis and various measures of sperm motility.

Measures of fertility and reproductive performance of the F_1 generation were also unaffected. There were no treatment-related effects on the proportion of pups born alive or mean average pup weight (combined male and females, although there was a decrease in the weight of female pups (F_2 generation) born to the F_1 females receiving 400 ppm of potassium dichromate). No significant differences on mean epididymal sperm density, percent abnormal sperm, spermatids per testis or measures of sperm motility were observed in the F_1 generation. The body weights of F_1 male and female mice administered 400 ppm of potassium dichromate were decreased by about 9 percent on 74 day. The body weights of F_1 female mice that were administered 200 ppm of potassium dichromate in the diet were slightly decreased (four percent) on day 74.

Epididymal sperm counts were significantly decreased in Wistar rats orally administered 10 or 20 mg/kg-day of Cr_2O_3 (5 or 10 mg/kg-day of Cr VI) for six days and then sacrificed six weeks later (Li *et al*., 2001). Increased sperm abnormality was also reported. Reported effects on seminiferous tubules (decreased diameters) are equivocal given the uncertainty in the methods used in sampling and sectioning of tissue.

Exposure of female mice to high levels (250, 500, 1,000 ppm (48, 99, 234 mg/kg-day)) of Cr VI (as potassium dichromate) in drinking water on day 0 though day 19 of gestation resulted in numerous embryotoxic and fetotoxic effects (Trivedi *et al*., 1989). The mice were sacrificed on day 19 and their uterine contents examined. Increased resorptions and post-implantation losses, and reduced fetal weight and crown to rump length were observed in animals receiving 250 and 500 ppm of potassium dichromate. In addition, reduced litter size, and pre-implantation losses were observed in animals administered 500 ppm of potassium dichromate. Maternal weight was significantly reduced at the 500 and 1,000 ppm levels, indicating maternal toxicity. At 1,000 ppm of potassium dichromate, no implantations were observed. Increases in gross and skeletal abnormalities were notable in fetuses of animals administered 500 ppm and reduced cranial ossification was observed in the fetuses of animals receiving 250 ppm of potassium dichromate.

Male and female mice were exposed for 12 weeks to very high levels of Cr VI (as potassium dichromate) in drinking water and then mated (cohabitation for 10 days) with unexposed mice (Elbetieha and Al-Hamood, 1997). Seven days following cohabitation, the female mice were sacrificed and their uterine contents examined. When male mice were exposed to 1,000, 2,000, 4,000 or 5,000 ppm potassium dichromate (60, 120, 230 or 300 mg/kg-day of Cr VI) for 12 weeks and then mated with untreated mice, the number of pregnant females appeared to be

affected, but only in the high dose group. Estimates of the dose associated with this high concentration of Cr VI in water are problematic because of evidence of aversion to drinking of the water in other studies at concentrations well below that in this study. The number of implantations and viable fetuses were decreased in animals exposed to 2,000 and 4,000 ppm of potassium dichromate. When female mice were exposed to 2,000 or 5,000 ppm of potassium dichromate (120, 300 mg/kg-day Cr VI) for 12 weeks and then mated with untreated males, the number of pregnant females appeared to be unaffected but the number of implantations and viable fetuses decreased and the number of mice with resorptions increased in both dose groups. In a separate group of males and females exposed to 5,000 ppm of potassium dichromate in drinking water for 12 weeks and then sacrificed, effects on body weight (male), testis weight, seminal vesicle weight, preputial gland weight and ovary weight were reported.

Female mice were exposed to potassium dichromate (1,000 ppm, 72 mg/kg-day of Cr VI) in drinking water from day 12 of gestation through day 20 of lactation (Al-Hamood *et al.*, 1998). The male and female offspring at 60 days of age were then bred with unexposed mice for 10 days. The female mice were then sacrificed one week later and their uterine contents examined. No statistically significant effects were reported in female mice mated with males exposed to Cr VI pre- and postnatally (although the numbers of pregnant females may have been reduced). Reduced numbers of pregnant females, implantations and viable fetuses were observed in female mice exposed to Cr VI pre- and postnatally.

Female rats were administered Cr VI in drinking water (250, 500 or 750 ppm (31, 60, 75 mg/kg-day) as potassium dichromate) for 20 days prior to mating with untreated male rats (Kanojia *et al.*, 1996). The rats were sacrificed on day 19 of gestation and their uterine contents examined. Significant reductions in mating and fertility indices, the number of implantations, live fetuses, and number of corpus lutea were observed principally in the two highest dose groups. Increases in pre-implantation and post-implantation losses and the number of resorptions were also reported. There was a significant decrease in the weight gain of the dams indicating maternal toxicity. Significant increases in gross abnormalities and skeletal abnormalities were observed in animals treated with 750 ppm of potassium dichromate.

Hexavalent chromium was administered in drinking water (250, 500 or 750 ppm (45, 89, 124 mg/kg-day) as potassium dichromate) to female Druckrey rats for 90 days (Kanojia *et al.*, 1998). Fifteen percent of animals treated with 500 ppm died and 10 percent treated with 750 ppm died during the first 14 days of treatment. All treated animals were acyclic but within 15 to 20 days after the treatment ended when placed with a male the animals began to mate. The females were then sacrificed after 19 days of gestation and their uterine contents examined. Significant decreases in implantations and the number of live fetuses per litter (500 and 750 ppm) and increases in the number of resorptions (700 and 750 ppm) and pre- and post-implantational losses (all doses) were observed. Decreases in fetal (all doses) and placental weights and crown-to-rump length (500 and 750 ppm) were also observed in the treated animals compared to control. Decreased body weight of the dams (500 and 750 ppm) indicated maternal toxicity. Numerous abnormalities (gross structural and skeletal) were observed in pups born to rats exposed to 500 and 750 ppm of potassium dichromate.

Female mice were administered Cr VI in drinking water (250, 500 or 750 ppm (52, 98, 169 mg/kg-day) as potassium dichromate) for 20 days prior to mating with untreated male mice (Junaid *et al.*, 1996b). The mice were sacrificed on day 19 of gestation and their uterine contents examined. Significant reductions in the number of implantations and live fetuses per mouse

(500 ppm), fetal and placental weight, and crown-to-rump length (250 and 500 ppm) were observed. Increases in pre-implantation (500 ppm) and post-implantation (250 and 500 ppm) losses and the number of resorptions per mouse (500 ppm) were also reported. No implantations were observed in animals receiving 750 ppm of Cr VI in their drinking water. There was a marked reduction in the weight gain of the dams in the 750 ppm group and three animals died. Significant increases in gross abnormalities and skeletal abnormalities were observed in the fetuses of animals receiving 500 ppm of potassium dichromate.

The administration of Cr VI in drinking water (250, 500 or 750 ppm Cr VI as potassium dichromate (53, 101, 152 mg/kg-day) to female mice from day 6-14 of gestation resulted in significant toxicity to the conceptus (Junaid *et al.*, 1996a). The mice were sacrificed on day 19 of gestation and their uterine contents examined. Significant decreases in number of fetuses per litter, fetal weight (500 and 750 ppm), and increases in numbers of dead fetuses (500 and 750 ppm), resorption sites (all doses), and post-implantational losses (500 and 750 ppm) were observed. There was a significant decrease in the weight gain of the dams receiving 500 or 750 ppm of Cr VI indicating maternal toxicity. Increases in both gross and skeletal abnormalities were apparent in the fetuses of animals in the high dose group. In a similar study where Cr VI was administered from day 15 through day 19 of gestation (250, 500 or 750 ppm Cr VI as potassium dichromate, roughly 50, 100, 150 mg/kg-day), decreases in placental weight (500 and 750 ppm), fetal weight and crown-to-rump length (all doses) and increases in post-implantation loss (500 and 750 ppm) were observed (Junaid *et al.*, 1995). Increases in gross and skeletal abnormalities were observed in the two high-dose groups. There was a significant decrease in the weight gain of the dams receiving 500 or 750 ppm of potassium dichromate, indicating maternal toxicity.

Hexavalent chromium was administered (500 ppm of K_2CrO_4, 11 mg/kg-day Cr VI) in drinking water to male and female mice in a three-generation long-term study (Borneff *et al*., 1968). The F_0 generation was exposed for six weeks prior to mating and during pregnancy and postnatally. From 120 female mice bred with 10 males, 1105 offspring were reported. From each litter two mice were kept alive. After three weeks, the mice were weaned and separated. Hexavalent chromium (500 ppm of K_2CrO_4) was administered to the F_1 generation in their drinking water. During this study, an ectromelia epidemic occurred during the eighth month, which resulted in the death of numerous animals. All animals that survived were vaccinated, which effectively ended the epidemic. The investigators reported that they then resumed breeding but do not say when the breeding commenced. Based on the results reported in Figure 2 of the study, the F_2 generation occurred in the twelfth month of the study, indicating that the breeding of the F_1 generation commenced around the eleventh month. Only 364 offspring resulted from breeding 220 F_1 females, indicating reproductive toxicity in the F_1 generation.

This finding is consistent with the reproductive toxicity observed by other investigators. The difference in the number of offspring between the F_0 and F_1 generations may be related to a substantial difference in the length of exposure of the mice (six weeks exposure prior to breeding in the F_0 generation as opposed to 11 months of exposure in the F_1 generation). In other studies, rats exposed to Cr VI for 90 days were much more severely impacted by exposure to Cr VI compared to rats exposed at the same doses for only for 20 days (Kanojia *et al.*, 1996, 1998).

Summary - At very high oral doses of Cr VI, embryotoxic and fetotoxic effects have been observed in rodents. At lower doses the picture is less clear. Zahid and associates (Zahid *et al*., 1990) and Li and coworkers (Li *et al*., 2001) observed reduced sperm counts and/or increased

abnormalities in mice or rats. In the National Toxicology Program studies, no effects were observed on spermatogenesis or reproductive outcome in mice and rats exposed under similar conditions (NTP, 1996, 1997a,b).

Immunotoxicity

Daily exposure of rats to K_2CrO_4 (100 mg/L) in drinking water for three weeks led to sensitization of the animals as evidenced by increased proliferation of T and B lymphocytes in response to the mitogens concanavalin A and liposaccharide (Snyder and Valle, 1991). Reduced (T lymphocytes) or no change in response (B lymphocytes) was observed in animals receiving 200 mg/L of K_2CrO_4 in their drinking water.

Exposure of male Wistar rats to a chromium VI ($Na_2Cr_2O_7$) aerosol (25, 50, 100 or 200 $\mu g/m^3$ chromium) 22 hr/day for 90 days resulted in the stimulation of a humoral response at lower exposure levels and a reduced response at higher exposure levels (Glaser *et al*, 1985). *In vitro* T-lymphocyte response stimulated by 30 μg/mL of concanavalin A was increased in spleen cells harvested from animals exposed to 200 $\mu g/m^3$ chromium compared to control. Macrophage numbers in bronchioalveolar lavage fluid decreased in animals exposed to chromium. Clearance of iron oxide from the lung was reduced in animals exposed to high levels of Cr VI in air.

Exposure of male Wistar rats to a Cr VI ($Na_2Cr_2O_7$) aerosol (50, 100, 200 or 400 $\mu g/m^3$ chromium) for 30 or 90 days (22 hr/day) resulted in significant increases in lung weight and number of leucocytes in the blood for all dose groups compared to control (Glaser *et al*., 1990). The investigators also observed bronchioalveolar hyperplasia and lung histiocytosis but lung fibrosis appeared to be mostly absent. Increased albumin and total protein levels and increased macrophage levels were observed at 200 and 400 $\mu g/m^3$ in bronchioalveolar lavage fluid.

Immunomodulary effects of inhalation exposure to soluble and non-soluble forms of Cr VI were evaluated in a series of studies in male F344 rats (Cohen *et al*., 1998; 2006; 2010). Levels of macrophages, neutrophils and monocytes (combined) in lavage fluids were increased in animals treated with soluble (K_2CrO_4) or insoluble ($BaCrO_4$) Cr VI for two weeks when compared to control (Cohn *et al*., 1998). Neutrophils comprised 31 percent of the immune cells in lavage fluids from animals treated with soluble Cr VI while over 94 percent of the cells were macrophages in control or animals treated with insoluble Cr VI. *Ex vivo* interleukin-1 production in lipopolysaccharide stimulated pulmonary macrophages was decreased in rats treated with soluble but not insoluble Cr VI while interleukin 6 production was not affected by either treatment. *Ex vivo* tumor necrosis factor (TNFα) production in lipopolysaccharide stimulated macrophages was decreased by both treatments.

Effect of Cr VI treatment on immunological function as indicated by Listeria burden in the rat lung was assessed in Listeria infected rats treated with soluble or insoluble Cr VI or Cr III for five days. Lung levels of Listeria on day three post-infection were significantly increased in animals exposed to insoluble or soluble Cr VI with a much more marked effect in animals treated with insoluble Cr VI (unlike the aforementioned effects on lung immune cells) (Cohen *et al*., 2010). Cr III treatment had little effect of lung burden of Listeria (Cohen *et al*., 2006).

Single dose or repeated (a dose every two weeks; last dose on day 64) administration of Cr VI (zinc chromate) by the inhalation route to female BALB/c mice resulted in inflammation, as evidenced by increased levels of leukocytes and levels of interleukin-6 in pulmonary lavage fluid

(Beaver *et al*., 2009a,b). After a single Cr VI dose, neutrophil levels were significantly increased after six hours and remained elevated at 24 hours. Neutrophils levels were also elevated in animals receiving multiple doses of Cr VI. After a single dose of Cr VI, macrophage levels were significantly decreased after 24 hours but were elevated by day 8. Repeated exposures to Cr VI resulted in increased levels of macrophages in lavage fluid.

Repeated administration of Cr VI resulted in inflammation with "degenerative changes and the sloughing of the epithelial cells (Beaver, 2009b)." Cr VI treatment (single dose or repeated doses) upregulated the phosphorylation of Akt in the airway epithelial cells, an effect associated with lung inflammation and neutrophil migration.

Subchronic Toxicity

Eight subchronic animal studies were identified (Kumar and Rana, 1982, 1984; Kumar *et al*., 1985; Vyskocil *et al*, 1993; Chopra *et al.*, 1996; NTP, 1996, 1997a,b, 2007; Acharya *et al*., 2001) in which Cr VI was administered by the oral route.

NTP mouse and rat studies (1996 and 1997a)

Potassium chromate was administered in the diet (15, 50, 100 and 400 ppm) to male and female BALB/c mice and Sprague-Dawley rats for nine weeks followed by a recovery period of eight weeks. Animals were housed individually in these studies and analysis of the feed revealed that Cr VI levels remained stable under test conditions.

Groups of animals were sacrificed on study weeks 3, 6, 9 and 17. Changes in body and organ weight, food, and water consumption were measured. The following observations are based on animals in the terminal sacrifice (animals that were sacrificed after 17 weeks). Six males/dose group and 12 females/dose group were necropsied and various organs were examined for macroscopic changes. Samples of liver, kidney, testis, and ovaries were examined microscopically. Hematological parameters evaluated include erythrocyte and leukocyte count, hemoglobin, hematocrit, platelet count, mean corpuscular hemoglobin concentration, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean platelet volume.

No treatment-related mortality was observed in these studies. Cytoplasmic vacuolization of hepatocytes was observed in both male and female mice at concentrations of 50, 100 and 400 ppm. In the male mice, 1 of 6 animals exhibited mild cytoplasmic vacuolization in hepatocytes at a concentration of 50 ppm, 2 of 5 mice exhibited minimal or mild vacuolization at 100 ppm, and 2 of 6 exhibited mild or moderate vacuolization at 400 ppm.

In female mice, 3 of 12 exhibited cytoplasmic vacuolization, one minimal, one mild and one moderate, at a concentration of 50 ppm. At 100 ppm, 2 of 12 exhibited mild vacuolization, and at 400 ppm 4 of 12 animals exhibited cytoplasmic vacuolization (one minimal, one mild, one moderate, and one marked). As indicated in the report and confirmed by discussions with the study pathologist, this type of change is well-defined and readily apparent (personal communication with Lynda Lanning). It should be noted that the effects were still evident after an eight-week recovery period (no Cr VI administration). Cytoplasmic vacuolization of mouse hepatocytes was not observed in either a two-year drinking water study (NTP, 2008) or another subchronic feeding study (NTP, 1997b).

The NTP designated a NOAEL of 15 ppm (1.1 mg/kg-day as Cr VI in male mice and 1.8 mg/kg-day in the female mice). At the 400 ppm level, reduced body weight, decreased MCV, and MCH

values compared to control were observed in males and females (in addition to cytoplasmic vacuolization in hepatocytes). The NTP designated 400 ppm as the MTD in male and female mice. In rats, the only signs of toxicity were changes in MCV and MCH values in both males and females at the 400 ppm level after 9 weeks of exposure. These values returned to normal after the recovery period of 8 weeks. The NTP designated 100 ppm as a NOAEL in the rat.

The NTP studies were limited in the number of animals per dose group and the microscopic pathology examination was limited to a few tissues, which reduces the sensitivity to detect adverse effects. In addition, the exposure period was only 9 weeks and the animals were allowed to recover for eight weeks prior to sacrifice. In spite of these limitations, hepatotoxicity was observed in these animals. Interestingly, the observed effects on the liver and perhaps the bone marrow/erythroid tissues are consistent with toxicokinetic studies that indicate these tissues are important sites of uptake of orally administered Cr VI.

Chopra et al., 1996

Potassium dichromate was administered in drinking water at 25 ppm (1.4 mg/kg-day of Cr VI, based on U.S. EPA, 1988) to female Wistar rats for 22 weeks. The control group received untreated water. A third group was given 10 percent ethanol in their drinking water and a fourth group was given both ethanol and Cr VI. Each group contained five or six animals. Food and water consumption was monitored daily and each animal was weighed once a week. After 22 weeks, the rats were sacrificed, and serum was analyzed for enzyme activity, triglycerides, cholesterol and glucose. Liver and kidney samples were taken for histological examination and to determine cholesterol, glycogen, and glutathione content.

Histopathological examination of liver of rats receiving Cr VI revealed "degeneration with reticular arrangement of hepatocytes, widened sinusoidal spaces, vacuolation and necrosis," which was more pronounced in the periportal region. Similar significant histology changes were observed in rats receiving alcohol, but in both the centrilobular and periportal areas of the liver. Serum levels of aspartate aminotransferase and alanine aminotransferase were significantly increased above controls, consistent with the liver damage in chromium-treated animals. Histopathological examination of the kidney revealed "diffused glomerulus, due to the damage inflicted on the basement membrane of the Bowman's capsule. Renal tubular lesions in the form of degeneration and syncytial appearance of epithelial cell of renal tubules were also evident." No information was provided on the number of animals examined or the number displaying histopathology. Serum cholesterol levels were reduced while serum triglyceride and glucose levels were significantly increased above control in rats given Cr VI. Liver glycogen levels decreased but cholesterol and glutathione levels were not significantly different from control.

Acharya et al., 2001

Potassium dichromate was administered in drinking water at 25 ppm (1.1 mg/kg-day as Cr VI based on U.S. EPA, 1988) to male Wistar rats for 22 weeks. The control group received water. A third group was given 10 percent ethanol in their drinking water and a fourth group was given both ethanol and Cr VI. Each group contained five or six animals. Food and water consumption was monitored daily and each animal was weighed once a week, although these results were not reported. After 22 weeks, the rats were sacrificed, samples of serum were collected and analyzed for serum enzyme activity, and liver and kidney samples were taken for histological examination and to determine lipid, glutathione, and glycogen content.

Histopathological examination of liver of animals receiving Cr VI revealed "degeneration, vacuolation, increased sinusoidal space, and necrosis," which was more pronounced in the periportal region. Animals that received ethanol revealed similar findings but in both the centrilobular and the periportal areas. Serum levels of aspartate aminotransferase and alanine aminotransferase were significantly increased above control, confirming the liver damage in chromium treated animals. Histopathological examination of the kidney revealed "vacuolation in glomeruli, degeneration of the basement membrane of Bowman's capsule, and renal tubular epithelial degeneration in the form of the syncytial appearance of nuclei of the epithelium." No information was provided on the number of rats examined or the number displaying histopathology. Decreased levels of triglycerides and glycogen, and increased levels of cholesterol compared to control, were observed in livers of rats treated with Cr VI.

NTP, 1997b

Using a continuing breeding protocol, potassium chromate was administered in the diet at 100, 200 or 400 ppm to male and female BALB/c mice (20 animals/group/sex) for 13 weeks, 1 week of pre-cohabitation exposure followed by 12 weeks of cohabitation exposure (see reproductive effects section). Based on feed consumption of the F_0 generation, week 1 doses of Cr VI for males were 7.9, 15.5 and 32.3 mg/kg-day and for females were 10.7, 21.6 and 51.2 mg/kg-day. Doses for the F_1 generation were: males (week 2) 7.9, 13.1, 33.3 mg/kg-day and (week 4) 9.1, 16.6 and 36.1 mg/kg-day and females (week 2) 8.5, 19.2 and 42.0 mg/kg-day and (week 4) 6.0, 14.9 and 35.5 mg/kg-day.

Necropsies were performed on control and treated animals and samples of liver and kidney were examined for histopathology. Hematology determinations were also conducted on F_1 mice prior to necropsy. Statistically significant decreases in mean MCV were observed in males receiving 200 and 400 ppm potassium chromate and females receiving 100, 200 and 400 ppm potassium chromate in their diet. No other significant effects on hematology were observed. No NOAEL was identified in this study because of hematopoietic changes in the 100 ppm F_1 female mice.

Kumar et al., 1985; Kumar and Rana, 1982, 1984

Potassium chromate was given by gavage to 10 male rats/group at 0.05 mg/kg-day for 20 days. Lipid accumulation was observed using histochemical methods in the liver and kidney. Increased lipid content (percent of organ that is lipid) was also observed using chemical analysis (Kumar and Rana, 1982). Changes in the distribution and the enzyme activity of alkaline phosphatase, acid phosphatase, glucose-6-phosphatase and cholinesterase in the liver were observed in animals treated with Cr VI compared to control (Kumar *et al.*, 1985). Changes in the distribution and enzyme activity of alkaline phosphatase, acid phosphatase and glucose-6-phosphatase in the liver were also observed in Cr VI-treated rats by Kumar and Rana (1984).

Vyskocil et al., 1993

Male and female Wistar rats were given Cr VI (25 ppm potassium dichromate) in drinking water for six months. Chromium intake was 2.47 mg/kg-day during the first three months and 1.76 mg/kg-day during the second three months in female rats. In male rats, chromium intake was 2.18 mg/kg-day during the first three months and 1.40 mg/kg-day during the second three months. Significant increases in urinary albumin at three and six months and β_2-microglobulin at three but not six months were found in female rats. No changes in kidney weight or urinary

lactate dehydrogenase, lysozyme, total protein, or β-*N*-acetyl-D-glucosaminidase were observed. No statistically significant changes in any of these parameters were observed in male rats.

NTP, 2007

The NTP reported findings of a 3 month-study in which F344 rats and $B6C3F_1$ mice were administered sodium dichromate in their drinking water (NTP, 2007). Sodium dichromate dihydrate (0, 62.5, 125, 250, 500 or 1,000 ppm) was administered in drinking water and based on average water consumption, the mean effective doses were 0, 1.6, 3.1, 5.8, 11.0 or 21.1 mg/kg-day of Cr VI for male rats and 0, 1.8, 3.5, 6.2, 11.5 or 21.4 mg/kg-day of Cr VI for females.

Mean body weights of both male and female rats were reduced in the high dose group. As with other studies, water consumption was reduced at higher concentrations, which may be responsible for the reduced body weight. Absolute and relative weights (normalized to body weight) of liver were significantly reduced in males in the two high dose groups while relative spleen weights (in the two highest dose groups) and relative kidney weights (at all but the lowest dose) were significantly increased in females. Numerous effects on hematological parameters were observed, some appearing to be transitory while others occurred for the study's duration and appeared to be dose-dependent. Most notable effects were decreases in erythrocyte levels, mean cell volume, mean cell hemoglobin (total and concentration) and platelet concentrations in male rats. Reductions in platelet, erythrocyte, and reticulocyte levels, and decreases in mean cell volume and cell hemoglobin concentrations were also observed in female rats.

Clinical chemistry findings included reduced serum cholesterol and triglycerides and increased levels of alanine aminotransferase and sorbitol aminotransferase in male rats. Similar finds were observed in female rats. Urinalysis revealed reduced urine volume and increased specific gravity and creatinine concentration in males and females, both consistent with reduced water intake in the higher dose groups. Histopathology revealed stomach lesions including irritation and focal ulcerations, which occurred at the junction of the glandular and non-glandular stomach in the high dose male and female groups, on the glandular side (personal communication, NTP chromium review panel meeting, July, 2002). Chronic liver inflammation was reported in the high dose female rats.

Based on their water consumption, the mean dose to the mice was 0, 3.1, 9.1, 15.7 or 30.0 mg/kg-day of Cr VI for males and 0, 3.1, 9.4, 15.4 or 26.2 mg/kg-day of Cr VI for females. Water consumption and body weight were reduced in both males and females in a dose-dependent manner. In the high dose groups, absolute but not relative liver weights were affected in male and female mice. Relative thymus weights were increased in male and female mice. Relative testis weights were increased in all but the low dose group males. In the duodenum, increases in minimal to mild epithelial hyperplasia were observed in both male and female mice at all dose levels.

Erythrocyte levels were increased in all but the lowest dose group in female mice. Mean cell volume and cell hemoglobin were reduced in both male and female mice in the higher dose groups. Compound-related stomach lesions were observed in the high dose male and the two highest female dose groups. Histiocytic infiltration was observed in the duodenum and histiocytic hyperplasia was noted in the mesenteric lymph nodes in both male and female mice. No clinical chemistry or urinalysis was performed in the mice.

The findings of this 90-day study are consistent with those observed in the earlier nine-week NTP study. Effects were observed in the blood-forming tissues and in clinical chemistry that possibly reflect effects on the stomach and the reduced weight gain observed in these animals. Higher doses were administered because the focus of this study was to identify doses for a two-year carcinogenic bioassay. Thus, a NOAEL was not identified in this study.

Chronic Toxicity

MacKenzie et al., 1958

Potassium chromate was administered in drinking water at 0, 0.45, 2.2, 4.5, 7.7, or 11.2 ppm to male and female Sprague-Dawley rats for one year in one experiment and 0 and 25 ppm in a second experiment. Each dose group was composed of 8 male and 8 female rats except the control groups, which consisted of 10 males and 10 females. At the end of six months, one male and one female rat in each dose group was sacrificed, and liver, kidneys and femur were analyzed for chromium. Few other details of the protocol were provided. No information was provided that suggests that the investigators attempted to analyze chromium concentration or the stability of Cr VI in the test article (other investigators had found that Cr VI is unstable in water (Borneff *et al*., 1968; NTP, 1996, 1997a)). The authors noted that "the rats were then grown and examined for pathological changes in both blood and tissues as described in the preceding paper," in which cadmium was administered to rats. Consequently, the methods used in the Cr VI study can only be inferred from the study on cadmium. It should be noted that the reported results focused on the uptake of chromium into various tissues.

Experimental details from the earlier cadmium study indicated that body weight, food and water consumption were recorded weekly. The investigators noted that samples of kidney, adrenal gland, liver, spleen, heart, brain, stomach, duodenum, ileum, colon and cross sections of bone marrow were preserved and stained with hematoxylin and eosin. Blood red and white cell counts, differential white cell counts and hemoglobin were analyzed at monthly intervals on half of the animals in each group. No information regarding the number of samples taken for pathological examination was provided (including if samples were examined from each animal). The authors reported that "Rats which died during the experimental period were examined for gross, and in some cases, microscopic pathological changes."

While the authors reported that mortality occurred from respiratory infection during the study, no information on how many animals were affected was provided. The authors concluded that there was no evidence that chromium influenced the prevalence of respiratory infection. The investigators found no differences in weight gain or food consumption among various groups, although no data were provided nor were details of the statistical analysis described. They also reported that neither gross changes in appearance nor pathological changes in blood or other tissues were observed. They did observe a decrease in water intake (84 percent in males and 77 percent in females compared to controls) in animals receiving 25 ppm of potassium chromate.

It is not clear how thorough the pathological examination was in this study. In the earlier cadmium study, no effects on growth, food consumption or pathological changes were observed in animals exposed to up to 10 ppm of cadmium in drinking water (Decker *et al.*, 1958). At a cadmium level of 50 ppm, changes in weight gain and food and water consumption were evident. Effects on hemoglobin and adverse effects on blood cells were also evident upon microscopic

examination. These animals were sacrificed after three months, presumably because of significant toxicity, but no other pathological effects were reported.

When evaluating the results of this chromium study it is important to acknowledge that the reported results focused on the uptake of chromium in various tissues. Very limited information was provided concerning what toxicological endpoints were actually assessed in this study. The lack of reported pathology in a parallel study in which cadmium was administered reinforces this concern. The reported intercurrent mortality is also an important confounding factor that complicates assessment of the effects of Cr VI on these animals.

NTP, 2008

Groups of 50 male and female rats (F-344) and mice ($B6C3F_1$) were administered sodium dichromate in drinking water (male and female rats and female mice: 14.3, 57.3, 172 or 516 mg/L; male mice: 14.3, 28.6, 85.7 or 257.4 mg/L) for two-years (NTP, 2008). Based on measured water consumption rates and body weights, male rats received a time weighted average dose of 0.21, 0.77, 2.1, or 5.9 mg/kg-day of Cr VI, while female rats received 0.24, 0.94, 2.4 or 7.0 mg/kg-day of Cr VI (NTP, 2008). Based on measured amounts of water consumption, male mice received an average dose of 0.38, 0.91, 2.4, or 5.9 mg/kg-day of Cr VI, while female mice received 0.38, 1.4, 3.1 or 8.7 mg/kg-day of Cr VI (NTP, 2008).

Survival of male and female rats was good. Significant reductions in mean weight gains were observed in the high dose group, in both male and female rats. Reduced water consumption due to poor palatability of high concentrations of Cr VI probably accounts, in part, for the decreases in weight gain in the high dose groups (NTP, 2008).

Similar to what has been observed in other studies (NTP, 1996, 2007), erythrocyte microcytosis was observed in male rats receiving 57.3, 172 and 516 mg/L. Decreased red blood cell volume was observed on day 4, day 22, and at 3 and 6 months. Mean cell volume appeared to increase with time indicating the rats were adapting to the insult. Anemia that appeared to be compound-related was observed at day 22 in male rats exposed to 57.3, 172 and 516 mg/L as evidenced by decreased hematocrit, hemoglobin and erythrocyte counts. The animals appeared to be recovering from the anemia by 12 months.

No treatment related non-neoplastic lesions were observed in the male rat. No adverse effects were reported in oral mucosa, forestomach, glandular stomach, small intestine or liver. Interestingly, irritation/ulcers observed in the stomach in the 3 month study were not observed in the rats after 2 years of exposure. However, the high dose in the two-year study (516 mg/L) was substantially lower than the high dose in the three month study (1,000 mg/L).

Administration of Cr VI to female rats for two years resulted in a dose-related increase in liver toxicity as shown by increased fatty changes and chronic inflammation. Statistically significant increases in the number of rats exhibiting chronic inflammation were observed in all dose groups. The chronic inflammation in females also exhibited increased severity at the two highest drinking water concentrations. The incidences of increased fatty changes were significantly increased at the three highest concentrations, while the increase at the lowest dose level was not statistically significant. Another possible indication of liver damage was that of increased serum alanine aminotransferase (ALT at > 57.3 mg/L) in males at day 4, day 22, month 3, month 6 and month 12; however, this may have been due to enzyme induction rather than liver damage, since no other serum markers of liver damage were observed. NTP noted that Cr VI appeared to

increase the incidence of chronic liver inflammation, commonly observed in aged rats. No treatment related non-neoplasm toxicity was observed in the oral mucosa, forestomach, glandular stomach or duodenum. Hematology, considered a special study and not routinely performed in two-year NTP studies, was not done in the female rat. A LOAEL of 14.3 mg/L was identified in the female rat, based on chronic inflammation, which is below exposure levels associated with hematological effects in the male rat.

The survival of both male and female mice was good. There was no evidence of reduced survival in animals receiving Cr VI. Body weight gains were largely unaffected by Cr VI in the mouse except in the high dose groups. As in the rat, water consumption was reduced in mice in the high dose groups and the reduced body weight was partly attributed (by NTP) to the reduced water consumption.

Comparable to the male rat, female mice exhibited a compound-related microcytosis (decreased cell volume), although the mouse appeared to be less affected than the rat. Mean cell hemoglobin levels and erythrocyte counts were significantly decreased at 12 months in female mice that received 172 or 516 mg/L Cr VI. No hematology was performed in male mice.

No notable exposure related adverse effects were reported in oral mucosa, forestomach, glandular stomach, small intestine or liver in male or female mice. A dose-related increase in diffuse hyperplasia of the epithelium was observed in the duodenum in female and male mice.

Strengths and weakness of subchronic and chronic animal studies

Much has been written on the elements of a good long-term animal bioassay to evaluate the safety of a chemical (U.S. EPA, 1984a, 1996a; NTP, 1984). Generally, a good rodent study should include sufficient numbers of both male and female animals (50 animal/sex/dose) maintained using good animal husbandry practices. The study should include at least three dose groups spaced to produce a gradation of effects plus a control(s) group. Doses should be selected so that the low dose group shows no evidence of toxicity while the high dose group should (in cancer bioassays) "elicit signs of toxicity without substantially altering the normal life span due to effects other than "tumors." The vehicle and route of exposure should be appropriate and the concentration of the test substance analyzed to determine the actual doses. The animals should be observed daily and body weight, food consumption, and clinical signs recorded. Clinical examination should include hematological and urinary determinations, and gross necrosis on all animals including those that died during the study. Tissues should also be examined for histopathology. Reporting requirements are numerous, and include detailed information on the results of the study.

Because bioassay protocols have evolved over the years, the results of bioassays conducted in years past are not summarily rejected because they fail to meet modern requirements. However, shortcomings in these bioassays do introduce considerable uncertainty when interpreting the findings or the lack of findings. This uncertainty must be treated accordingly.

No animal bioassay was identified that comprehensively evaluated the toxicity of orally administered Cr VI. All of the bioassays contained important deficiencies, as summarized in Table 1. These deficiencies introduced substantial uncertainty in assessing the risks associated with human exposure to Cr VI in drinking water.

Table 1. Strengths and Weaknesses of Available Hexavalent Chromium Bioassays

Study	Strengths	Weaknesses
MacKenzie *et al.*, 1958	-5 dose levels in one study, 1 dose level in second study -Food and water intake monitored -Body weights monitored -Drinking water vehicle	-Little information on animal care and QA/QC -Only one year study -Small number of animals/treatment group (8-10 at start of study) -Infection caused early mortality, and number of surviving animals not reported. -No individual animal data -No Cr VI analysis in the drinking water -Little documentation of histopathology; number of animals examined is unknown
Borneff *et al.*, 1968	-Drinking water vehicle -Analysis of Cr VI levels in administered solution -Monitored food and water intake -High number of female mice/treatment group -Vehicle and positive control groups -Chronic study, multigenerational exposure -Animal body weight was monitored	-Little information on animal care and QA/QC -Vehicle included detergent -Low number of males/treatment group -Intercurrent infection with early mortality -No individual animal tumor data -No tracking of animal relationships between generations -No indication of preneoplastic lesions -Only one Cr VI dose administered
National Toxicology Program, 1996; 1997a	-Animal husbandry and QA/QC -Analysis of Cr VI levels in feed -Monitored food and water intake -Individual animal data available -Three Cr VI dose levels -Extensive necropsy	-Cr VI in feed -Small number of animals group (6 males and 12 females) -Length of study only 9 weeks -Histopathology examination limited to a few tissues
National Toxicology Program, 1997b	-Animal husbandry and QA/QC -Analysis of Cr VI levels in feed -Monitored food and water intake -Individual animal data available -Three Cr VI dose levels	-Cr VI in feed -Small number of animals/treatment group (20) -Limited histopathology examination -Length of study only 90 days
National Toxicology Program, 2007	Animal husbandry and QA/QC -Analysis of Cr VI levels in administered solution -Monitored food and water intake -Individual animal data available -Cr VI in drinking water	-Small number of animals/treatment group (10) -Length of study only 90 days -Limited histopathology
Chopra *et al.*, 1996	-Drinking water vehicle -Animal husbandry and QA/QC -Food and water intake monitored	-Small number of animals/treatment group (5 or 6) -Length of study only 22 weeks -Limited histopathology -Unclear if Cr VI levels in administered solution were analyzed -Only one Cr VI dose administered
National Toxicology	-Animal husbandry and QA/QC	-Cancer bioassays with relatively high doses.

Study	Strengths	Weaknesses
Program, 2008	-Analysis of Cr VI levels in administered solution -Monitored food and water intake -Individual animal data available -Cr VI in drinking water -Chronic two year study	-Limited data on clinical chemistry (male rats) and hematology (male rats, female mice).
Acharya *et al.*, 2001	-Drinking water vehicle -Animal husbandry and QA/QC -Food and water intake monitored	-Small number of animals/treatment group (5 or 6) -Length of study only 22 weeks - Limited histopathology examination -Unclear if Cr VI levels in administered solution were analyzed -Only one Cr VI dose administered
Kumar *et al*., 1985 Kumar and Rana, 1982; 1984	-Water vehicle	-Small number of animals/treatment group (10) -dose administered by gavage -Length of study only 20 days -Limited histopathology -Unclear if Cr VI levels in administered solution were analyzed -Only one Cr VI dose administered
Vyskocil *et al*., 1993	-Drinking water vehicle	-Length of study only 6 months -Study limited to kidney, no histopathology -Unclear if Cr VI levels in administered solution were analyzed -Only one Cr VI dose administered

Genetic Toxicity

The genotoxic potential of Cr VI compounds has been evaluated in short-term test systems, in animals *in vivo*, and in workers occupationally exposed (IARC, 1990). Hexavalent chromium is genotoxic without exogenous activation in bacteria, and in human and other mammalian cells in culture. This information was previously reviewed in De Flora *et al.* (1990), IARC (1990) and ATSDR (2000). More recent reviews of Cr VI genotoxicity are included in Sedman *et al*. (2006), ATSDR (2008), Salnikow and Zhitkovich (2008), U.S. EPA (2010) and Nickens *et al*. (2010). Hexavalent chromium compounds induced gene mutations in multiple species and strains of bacteria, and DNA adducts, DNA-protein crosslinks, DNA strand breaks and crosslinks, abasic sites, oxidized bases, gene mutations, chromosomal aberrations, sister chromatid exchanges, and other forms of genomic damage in mammalian cells *in vitro*.

The genotoxicity of Cr VI compounds associated with *in vivo* exposures of humans and animals has been comprehensively described in the reviews listed above. The following summarizes the evidence of genotoxicity of Cr VI, emphasizing studies by the oral route because of the importance of this route in assessing the potential risk associated with Cr VI in drinking water. Studies of exposure via other routes are also described.

Inhalation, intratracheal, intraperitoneal and intravenous exposures

Human

IARC (1990) reviewed the studies of DNA damage in peripheral blood lymphocytes of workers exposed to Cr VI. IARC noted that "Elevated levels of sister chromatid exchange were observed in workers exposed to Cr VI compounds in electroplating factories in four out of six studies. Chromosomal aberrations were found in all three studies of exposed workers." More recent studies have tended to demonstrate genotoxicity in workers exposed to Cr VI. Gao *et al*. (1994) found no evidence of lymphocyte DNA damage in dichromate production workers exposed to 1 – 5.5 μg/m^3 Cr VI. Benova *et al*. (2002) did not observe increases in peripheral lymphocyte chromosomal aberrations or sister chromatid exchanges isolated from chrome plating workers exposed to total chromium concentrations of 7.5 – 25 μg/m^3. In contrast, Wu *et al*. (2001) found increased chromosomal aberrations and sister chromatid exchanges in whole blood from workers exposed to 600 μg/m^3 Cr VI. Benova *et al*. (2002) observed increased peripheral lymphocyte micronucleus formation in chrome plating workers at the concentrations described above. Gambelunghe *et al*. (2003) reported increased peripheral lymphocyte DNA strand breaks in Italian chrome platers. Airborne Cr VI concentrations were not listed in this study, but urine chromium concentrations were reported. Maeng *et al*. (2004) found a dose-related increase in chromosomal aberrations in Korean chrome plating workers exposed to airborne Cr VI concentrations of 1 - 50 μg/m^3.

Animal

Relatively few *in vivo* genotoxicity studies of Cr VI following exposures to the respiratory system were located. Bigaliev *et al*. (1977), as reported by IARC (1990) and De Flora *et al*. (1990) observed increases in chromosomal aberrations in rat bone marrow cells following intratracheal administration of potassium dichromate (1 to 15 mg/kg) to white non-inbred rats. Cheng *et al*. (2000) administered to C57Bl/6 Big Blue mice (a strain containing the *lacI* reporter transgene) a single dose (6.75 mg/kg) of an aqueous solution of potassium chromate in the trachea. Mutation frequency in the *lacI* gene relative to background rates was significantly elevated in the lung and kidney ($p < 0.001$) and elevated but not statistically significant in the liver ($p = 0.085$). The mutation frequencies in the lung correlated closely with the concentration of chromium deposited in this tissue (Cheng *et al.*, 2000). Izzotti *et al*. (1998) intratracheally dosed Sprague-Dawley rats with sodium dichromate (0.25 mg/kg) for three consecutive days and observed increases in DNA fragmentation, DNA-protein crosslinks and oxidized DNA bases in the lung, but not the liver.

Data from these inhalation and intratracheal studies suggest that the greatest degree of DNA damage occurs in the respiratory tract (i.e., the portal of entry), and some smaller amount of DNA damage occurs at distant tissues following absorption of chromium by the lungs and distribution to those tissues.

Several genotoxicity studies in which rodents were administered soluble Cr VI compounds (e.g., sodium dichromate, potassium dichromate, potassium chromate) either intraperitoneally (i.p.) or intravenously (i.v.) were reviewed by De Flora (1990), IARC (1990), ATSDR (2000, 2008) and U.S. EPA (2010). The majority of the studies reported positive genotoxicity in tissues distant to the site of administration. No genotoxicity studies employing subcutaneous or intramuscular injection were described in the published reviews. In rodents administered Cr VI via i.p. injection, significant increases were observed in mutations of the bone marrow and liver;

chromosomal aberrations, micronuclei and sister chromatid exchanges of the bone marrow, polychromatic erythrocytes or lymphocytes; DNA single strand breaks of the liver; and DNA-protein crosslinks of the liver, lung and kidney. In rodents administered Cr VI compounds via i.v. injection, significant increases in chromosomal aberrations in bone marrow and lymphocytes were reported (as reviewed by De Flora *et al.*, 1990).

Oral exposures

Fifteen primary studies of the potential genotoxic effects following ingestion of Cr VI by humans or other mammalian species were located. A summary of these studies is provided in Table 2. Nine of the fifteen studies reported positive genotoxicity findings in various tissues. DNA-protein crosslinks of the liver, DNA single strand breaks of the liver and brain, bone marrow chromosomal aberrations, or DNA deletions in retinal pigment epithelium were observed following exposure of rodents via drinking water or chronic dosing by gavage. The only study to date that has looked for genotoxicity in the oral cavity or gastrointestinal tract following oral administration of Cr VI was published by De Flora *et al*. (2008). The authors did not find evidence of DNA-protein crosslinks or 8-OH-dG adducts (indicative of oxidative DNA damage) in the mouse forestomach, glandular stomach or duodenum. However, judging from the responses of their positive controls, it is likely that their methodology lacked the sensitivity to measure DNA damage at the dose levels tested. In addition, McCarroll *et al*. (2010) noted that the levels tested in this study may in part explain the negative results because they are below the exposure levels used in the NTP 2-year drinking water study. The data described above are generally consistent with the idea that, following low to moderate bolus doses (gavage) or higher concentrations in drinking water, Cr VI is absorbed by the intestines and is transported to distant tissues where it damages DNA. Additional studies of genotoxicity of the oral cavity and gastrointestinal tract following oral ingestion of Cr VI would be useful.

There is some concern that high doses of Cr VI, such as those received by oral gavage or by rapidly drinking a large glass of contaminated water, may overwhelm the reducing capacity of the stomach. Indeed, the reductive capacity of the oral cavity and stomach and the dose rate in which Cr VI is ingested are important factors to consider in determining risk. Data summarized by De Flora (2000) suggest that the saliva and stomach have the capacity to completely reduce the dose that a human would receive from rapid ingestion of Cr VI -containing drinking water at concentrations typically found in California water supplies. However, genotoxic effects in distant tissues (i.e., bone marrow, liver and brain) have been observed in rodents chronically administered Cr VI by gavage at doses (1.0 mg/kg-d, Bigaliev *et al*., 1977; 2.5 mg/kg-d, Bagchi *et al.*, 1997; 0.59, 1.19 and 2.38 mg/kg-day, Dana Devi *et al*., 2001) not likely to overwhelm the reductive capacities of the stomach, intestines and blood.

Table 2. Summary of *In Vivo* Genotoxicity Studies of Hexavalent Chromium by the Oral Route

Study	Species /Strain	Method of Administration	Dose and Dose Regimen	Response	Genotoxic Endpoint and Site
Shindo *et al.*, 1989	MS/Ae mice	gavage	20 to 320 mg/kg, potassium chromate, single dose, measured 24 hr after dosing	-	micronuclei in polychromatic erythrocytes
	CD-1 mice	gavage	20 to 320 mg/kg, potassium chromate, single dose, measured 24 hr after dosing	-	micronuclei in polychromatic erythrocytes
Coogan *et al.*, 1991a	F344 rats	drinking water	100 or 200 ppm (6.1 or 8.7 mg/kg-d) potassium chromate, three weeks	+ -	DNA-protein crosslinks in liver DNA-protein crosslinks in lymphocytes
Sarkar *et al.*, 1993	Swiss mice	gavage	20 mg/kg, Cr VI oxide, single dose, measured 24 hr after dosing	+	chromosomal aberrations in bone marrow
Bagchi *et al.*, 1995a	Sprague-Dawley rats	gavage	25 mg/kg, sodium dichromate, single dose, measured 48 hr after dosing	+	DNA single strand breaks in liver
Bagchi *et al.*, 1995b	Sprague-Dawley rats	gavage	10 mg/kg-d, sodium dichromate, 15, 30, 45, 60, 75 or 90 days	+	DNA single strand breaks in liver
Kuykendall *et al.*, 1996	humans	drinking water	5 mg (~0.007 mg/kg), potassium dichromate, in 0.5 L water	_	DNA-protein crosslinks in leukocytes
Mirsalis *et al.*, 1996	Swiss-Webster mice	drinking water	1 to 20 ppm (~0.2 to 3.5 mg/kg-d) potassium dichromate, two days, measured 24 hr after dosing	_	micronuclei in polychromatic erythrocytes
	Swiss-Webster mice	gavage	0.02 to 0.4 mg/kg, potassium dichromate, two days, measured 24 hr after dosing	_	micronuclei in polychromatic erythrocytes

Table 2. Summary of *in vivo* Genotoxicity Studies of Hexavalent Chromium by the Oral Route (continued)

Study	Species	Vehicle	Dose and Dose Regimen	Response	Genotoxic Endpoint and Site
Mirsalis *et al.*, 1996	F344 rats	drinking water	1 to 20 ppm (~0.05 to 1.0 mg/kg-d) potassium dichromate, two days, measured 24 hr after dosing	_	micronuclei in polychromatic erythrocytes
Bagchi *et al.*, 1997	Sprague-Dawley rats	gavage	2.5 mg/kg-d, sodium dichromate, 120 days	+	DNA single strand breaks in liver and brain
Bigaliev *et al.*, 1997	white rats	gavage[1]	1 mg/kg-d, potassium dichromate, one year	+	chromosomal aberrations in bone marrow
	white rats	gavage	15 mg/kg, potassium dichromate, single dose, measured 2, 4, 6, 8 or 12 hr after dosing	+	chromosomal aberrations in bone marrow
Dana Devi *et al.*, 2001	Swiss mice	gavage	0.59 – 76 mg/kg-day (7 doses), potassium dichromate	+	DNA strand breaks (comet assay) in leukocytes
De Flora *et al.*, 2006	BDF1 (C57BL× DBA_2) mice	gavage	10 and 20 mg/L (3 and 6 mg/kg-day) Cr VI as potassium dichromate, 20 days	-	micronuclei in polychromatic erythrocytes
	Swiss mice	gavage	5 and 10 mg/L Cr VI as potassium dichromate, 17 days	-	micronuclei in polychromatic erythrocytes
Kirpnick-Sobol *et al.*, 2006	C57BL/ 6Jp^{un}/p^{un} mice	drinking water	62.5 and 125 mg/L (12.5 and 25 mg/kg-day), potassium dichromate, prenatal dosing	+	DNA deletions in retinal pigment epithelium
De Flora *et al.*, 2008	SKH-1 hairless mice	drinking water	5 and 20 mg/L (1.2 and 4.7 mg/kg-day), 9 months	-	DNA-protein crosslinks, 8-oxo-dG adducts
NTP, 2007	$B6C3F_1$ mice	drinking water	62.5, 125 and 250 mg/L sodium dichromate dehydrate for 3 months	+/- (equivo-cal)	micronuclei in normochromatic erythrocytes

Study	Species	Vehicle	Dose and Dose Regimen	Response	Genotoxic Endpoint and Site
	Am3-C57-BL/6 mice	drinking water	62.5, 125 and 250 mg/L sodium dichromate dehydrate for 3 months	+	micronuclei in normochromatic erythrocytes
	BALB/c mice	drinking water	62.5, 125 and 250 mg/L sodium dichromate dehydrate for 3 months	-	micronuclei in normochromatic erythrocytes

1. In the Bigaliev *et al.*, 1997 study, for this dose group only, the methods translated from Russian state that the rats were chronically administered with a "...dosage 1 mg per 1 kg of live weight orally or inside trachea with 0.2 mL of 5 percent solution of $K_2Cr_2O_7$." It is difficult to interpret this statement, but it appears that the authors were not sure to what extent the dosing tube was passed into the stomach or the trachea over the year-long dosing period.

Summary

Hexavalent chromium has been shown to be genotoxic by all routes of administration in rodents treated with high doses of Cr VI. Hexavalent chromium also has been shown to cause DNA damage in the lymphocytes of workers occupationally exposed (i.e., via inhalation). However, due to the reductive capacities of the lung for inhalation exposures or the stomach for oral exposures (De Flora, 2000), it is unclear whether significant DNA damage is likely to result from low environmental exposures to Cr VI.

Based on genotoxicity data following direct exposure to the respiratory system, the greatest frequency of DNA damage was observed at the site of exposure (i.e., the lung), and lower frequencies of DNA damage were observed in the liver and kidney, correlating with the concentration of chromium measured in the lung, kidney and liver (Cheng *et al.*, 2000). These observations correlate well with observations from cancer studies in humans and rodents. Studies in rodents exposed to Cr VI compounds via inhalation, i.p., or intramuscular injections yielded tumors almost exclusively at the site of exposure.

In humans exposed via inhalation, chromium-induced cancers are predominantly at the site of exposure (i.e., the sinuses and lung). It is unclear whether inhalation exposures among workers are also associated with cancers of the digestive system and other non-respiratory sites. Given what is known about the toxicokinetics of Cr VI, the likelihood of detecting a carcinogenic response at non-respiratory sites in workers exposed via inhalation is uncertain, because a relatively small portion of the inhaled dose would be expected to reach non-respiratory sites. An important question surrounding the potential risks posed by Cr VI is whether it causes DNA damage to the oral cavity or gastrointestinal tract following oral ingestion. Additional studies analyzing a variety of types of DNA damage are needed to answer this.

In summary, Cr VI is reduced to Cr III to a considerable extent at the site of entry and in blood (De Flora, 2000). However, several oral genotoxicity studies observed DNA damage at sites distant from the site of application (i.e., bone marrow, liver, or brain), which suggests that portions of Cr VI can evade reduction in the oral cavity, gastrointestinal tract and blood. Currently, it is uncertain whether significant portions of lower oral doses of Cr VI evade *in situ* reduction and cause DNA damage in the oral cavity and gastrointestinal tract.

Mechanism of Genotoxicity and Carcinogenicity

Although Cr VI has been extensively studied for its genotoxic and carcinogenic potential, there is not a consensus as to the precise mechanism(s) of carcinogenesis. Hexavalent chromium induces a wide range of DNA damage, including DNA adducts, DNA-protein crosslinks, DNA-DNA crosslinks, mutations, DNA strand breaks, abasic sites, oxidized DNA bases, chromosomal aberrations, sister chromatid exchanges, and micronuclei (De Flora and Wetterhan, 1989; Cohen *et al.*, 1993; Sugden and Stearns, 2000; Zhitkovich, 2005; Salnikow and Zhitkovich, 2008; Wise *et* al., 2008; ATSDR, 2008; U.S. EPA, 2010; Nickens *et al*., 2010. The wide spectrum of genotoxic effects likely reflects multiple mechanisms of DNA damage (Sugden and Stearns, 2000).

Hexavalent chromium may not itself be the active species that causes DNA damage. Hexavalent chromium is readily taken up by cells, likely because it is a tetrahedral anion that mimics phosphate and sulfate salts that are taken up into cells via active transport systems (Sugden and Stearns, 2000). Once taken up by cells, Cr VI is reduced from a +6 oxidation state to a +3 electron oxidation state, i.e., Cr III. Cr III is stable and far less toxic than Cr VI (IARC, 1990). It is during the reduction of Cr VI to Cr III that many DNA-reactive species are formed, including the high-valency species Cr IV and Cr V, as well as free radicals such as hydroxyl radical, singlet oxygen, superoxide anion (O_2^-), glutathione and other thiyl radicals, and organic- or carbon-based radicals (De Flora and Wetterhan, 1989; Cohen *et al.*, 1993; Sugden and Stearns, 2000).

The relative contribution of these species to the DNA damage is unknown (De Flora and Wetterhan, 1989; Cohen *et al.*, 1993; Sugden and Stearns, 2000), and is probably variable (Salnikow and Zhitkovich, 2008). Additionally, the newly formed Cr III may build up to high concentrations within the cell, and may be itself an important mediator of Cr VI carcinogenicity (Costa, 1997). Cr III has been shown to bind to isolated nuclei and DNA, and to cause DNA-protein crosslinks (Cohen *et al.*, 1993). These properties of rapid uptake into cells and intracellular generation of free radicals in the course of reduction to the directly genotoxic trivalent state, have led to the characterization of Cr VI as a compound that “functions as a sort of Trojan horse" (De Flora, 2000). It is widely believed that DNA damage from Cr VI is a result of intracellular reduction, whereas extracellular reduction is considered a detoxification process (Cohen *et al.*, 1993; Sugden and Stearns, 2000). The contribution of reductive enzymes within the cell to the overall reduction of Cr VI and DNA damage is not well understood (Sugden and Stearns, 2000).

The postulated mechanisms of Cr VI -induced DNA damage include: (1) indirect free radical DNA damage, (2) direct metal-mediated oxidative DNA damage, and (3) direct metal-DNA binding. Hexavalent chromium carcinogenesis is thought to be mediated through this DNA damage.

In support of the first mechanism, there is extensive evidence to suggest that reactive oxygen species, especially hydroxy radicals, and other free radical species are involved in the genotoxicity of Cr VI (reviewed in De Flora and Wetterhahn, 1989; Cohen *et al.*, 1993; Sugden and Stearns, 2000, Slade *et al*. 2005, 2007). This evidence includes the measurement of reactive oxygen species in *in vitro* tests of Cr VI genotoxicity, observations of lesions consistent with damage caused by reactive oxygen species and other free radicals (e.g., oxidized DNA bases, abasic sites, DNA strand breaks and DNA-DNA and DNA-protein crosslinks) following Cr VI treatment *in vitro* and *in vivo*, and observations that Cr VI toxicity is reduced in the presence of free radical scavengers (reviewed in ATSDR, 2000, 2008; Sugden and Stearns, 2000).

In support of the second mechanism, as proposed in a review paper by Sugden and Stearns (2000), a direct metal-mediated mechanism may be the predominant mechanism of oxidative DNA damage by Cr VI. This mechanism is consistent with observations from studies of Cr VI-induced effects on the expression of stress genes in human lung cells, studies of Cr VI reduction by ascorbate, glutathione, and hydrogen peroxide (without oxygen radical formation), and studies of DNA oxidation by model Cr V

complexes. A recent review of this mechanism was published by Salnikow and Zhitkovich (2008).

In support of the third mechanism, researchers have observed direct binding of chromium with DNA and other cellular macromolecules (reviewed in ATSDR, 2000, 2008). Chromium can interact with DNA to form chromium-DNA adducts and DNA-protein crosslinks and it can interact through other means that can also result in interference with DNA replication. Such interactions can give rise to effects such as mutation, aneuploidy or alteration of gene transcription (reviewed in Cohen *et al.*, 1993; ATSDR, 2000).

McCarroll *et al.* (2010) processed Cr VI through the U.S. Environmental Protection Agency's (EPA's) Cancer Guidelines Mode of Action (MOA) framework. The postulated key steps in tumor formation were: 1) interaction of DNA with Cr VI and reduction to Cr III; 2) mutagenesis; 3) cell proliferation and 4) tumor formation. They concluded that "the weight of evidence supports the plausibility that Cr VI may act through a mutagenic MOA." Based on the proposed MOA of Cr VI the U.S. EPA Cancer Guidelines recommend a linear extrapolation and the application of age sensitivity factors to protect children.

In summary, numerous studies demonstrate that Cr VI is both genotoxic and mutagenic. A mutagenic MOA has been fully described and justified. Unless there are data supporting an alternative mechanism of action, the standard approach for carcinogens operating via a genotoxic or mutagenic MOA is to apply a linearized multistage model to calculate cancer potency (U.S. EPA, 2005; OEHHA, 2009a).

Carcinogenicity

A number of reviews have summarized the evidence that links inhalation exposure to chromium to increases in cancer (IARC, 1980b, 1990; CDHS, 1985; U.S. EPA, 1998). Another summary of this extensive literature is not needed, and therefore will not be included in this PHG document. IARC (1980b) concluded there is sufficient evidence for carcinogenicity in humans for Cr VI compounds, and also stated, "The epidemiological data do not allow an evaluation of the relative contributions to carcinogenic risk of metallic chromium, chromium [III] and chromium [VI] or of soluble versus insoluble chromium compounds." IARC (1990) concluded "There is sufficient evidence in humans for the carcinogenicity of chromium [VI] compounds as encountered in the chromate production, chromate pigment production and chromium plating industries." IARC (1990) also stated: "…and several types of other relevant data which support the underlying concept that chromium [VI] ions generated at critical sites in the target cells are responsible for the carcinogenic action observed." U.S. EPA stated that "Epidemiological studies of chromate production plants in Japan, Great Britain, West Germany and the United States have revealed a correlation between occupational exposure to chromium and lung cancer, but the specific form of chromium responsible for the induction of cancer was not identified (U.S. EPA, 1998)."

The evidence for carcinogenicity of Cr VI by the oral route was less clear, but has become considerably stronger in recent years, with the completion of long-term drinking water studies in rats and mice by the NTP (NTP, 2008). The more relevant studies are described here.

Borneff *et al.*, 1968

Until the recent publication of the results of the NTP biossay for sodium dichromate (NTP 2008), only one long-term animal cancer bioassay where Cr VI was administered by the oral route was identified (Borneff *et al.*, 1968). Using a three-generation study design, Borneff *et al.* (1968) treated 120 female and 10 male NMRI mice with 1 mg K_2CrO_4 per day (500 ppm) in drinking water (containing 3 percent household detergent). A control group of animals received drinking water (3 percent detergent) only. An outbreak of mousepox (ectromelia) virus occurred during the eighth month of the experiment, and within three months, the majority (512) of the animals died. All animals received a mousepox vaccination two months after the outbreak. This effectively ended the epidemic and the study continued. Two carcinomas of the forestomach were observed in female mice exposed to K_2CrO_4. No malignant stomach tumors were found in control mice. Nine benign forestomach tumors were observed in female mice exposed to K_2CrO_4. Benign and malignant neoplasms were combined for the statistical analysis (McConnell *et al.*, 1986; U.S. EPA, 2005b). The combined incidence of malignant and benign forestomach tumors (11/66) in K_2CrO_4-exposed-female mice was significantly different than the combined incidence of tumors in control female mice (2/79) [Fisher's Exact test, $p<0.05$, (OEHHA analysis)]. A detailed evaluation of this study is found in Appendix B.

NTP, 2008

Groups of 50 male or female F-344 rats and $B6C3F_1$ mice were administered sodium dichromate dihydrate in drinking water (male and female rats and female mice: 14.3, 57.3, 172 or 516 mg/L; male mice: 14.3, 28.6, 85.7 or 257.4 mg/L) for two years (NTP, 2008). Based on measured water consumption rates and body weights, male rats received a time-weighted average dose of 0.21, 0.77, 2.1, or 5.9 mg/kg-day of Cr VI, while female rats received 0.24, 0.94, 2.4 or 7.0 mg/kg-day of Cr VI (NTP, 2008). Male mice received an average dose of 0.38, 0.91, 2.4, or 5.9 mg/kg-day of Cr VI, while female mice received 0.38, 1.4, 3.1 or 8.7 mg/kg-day of Cr VI (NTP, 2008).

Rat

The survival of rats (both male and female) was good. Survival in rats of both sexes receiving Cr VI was similar to that in the control groups (Figures 3 and 4). Body weight gains were largely not affected by Cr VI in rats except in high dose males and females (Figure 5 and 6). Drinking water consumption was reduced in the 172 and 516 mg/L sodium dichromate dihydrate groups of both sexes. However, there were no indications that the animals were dehydrated (NJDEP, 2009). The reductions in body weight and drinking water consumption in both sexes were partly attributed to poor palatability of the dosed water and not due to direct toxic effects of Cr VI exposure (NTP, 2008).

Neoplasms

The administration of Cr VI resulted in statistically significant increases in epithelial tumors of the oral cavity (oral mucosa or tongue) in male and female rats receiving the highest dose of Cr VI (Tables 3 and 4). The increases were observed for squamous cell carcinomas alone and for combined squamous cell carcinomas or papillomas. The tests

for trend were positive. NTP reported that squamous cell carcinomas of the oral mucosa of the rat were rarely observed in historical controls of either sex.

The increases in tumors of the oral cavity are consistent with these tissues being directly exposed to high levels of Cr VI in drinking water. But no other significant pathology was noted in the oral cavity indicating that the tumors were not secondary to tissue necrosis and subsequent tissue regeneration. Also, no increases in tumors were observed in the forestomach or stomach, organs which would be expected to be exposed to high levels of Cr VI in drinking water.

Other than the oral cavity, male rats exposed to Cr VI had an occasional statistically significant increase or decrease in tumors at a given site that did not appear to be compound-related. Increases in benign pheochromocytomas were observed in the adrenal medulla in animals receiving 14.3 or 57.3 mg/L of Cr VI. No increases were observed at the two highest dose levels and the test for trend suggested a significant decrease in tumors as a function of dose. This later observation probably reflects the significant increase in tumors only at the lower dose levels.

Figure 3. Survival curves for female rats

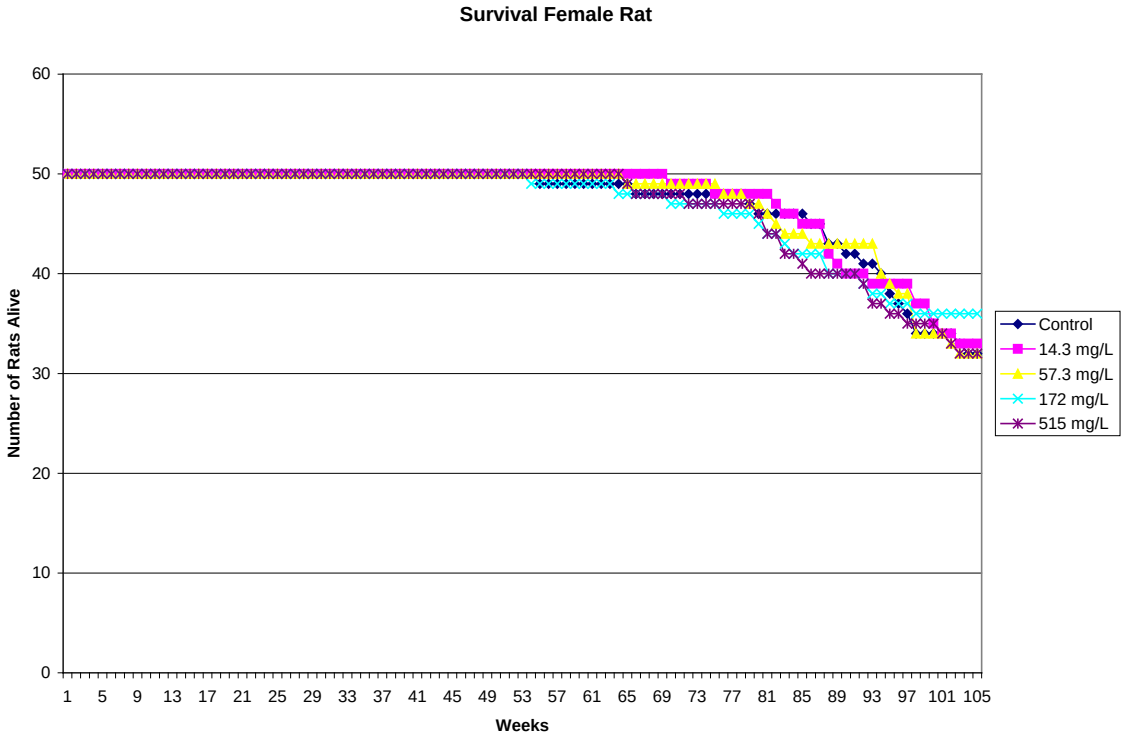


Figure 4. Survival curves for male rats

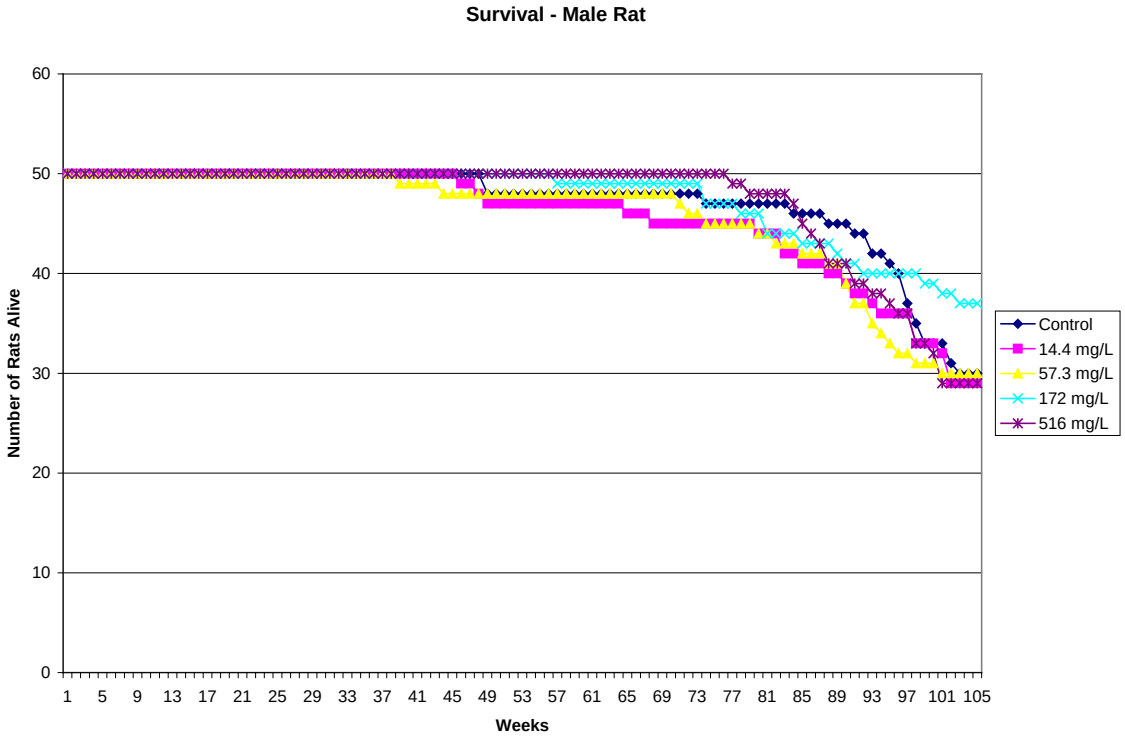


Figure 5. Female rats body weights, by week

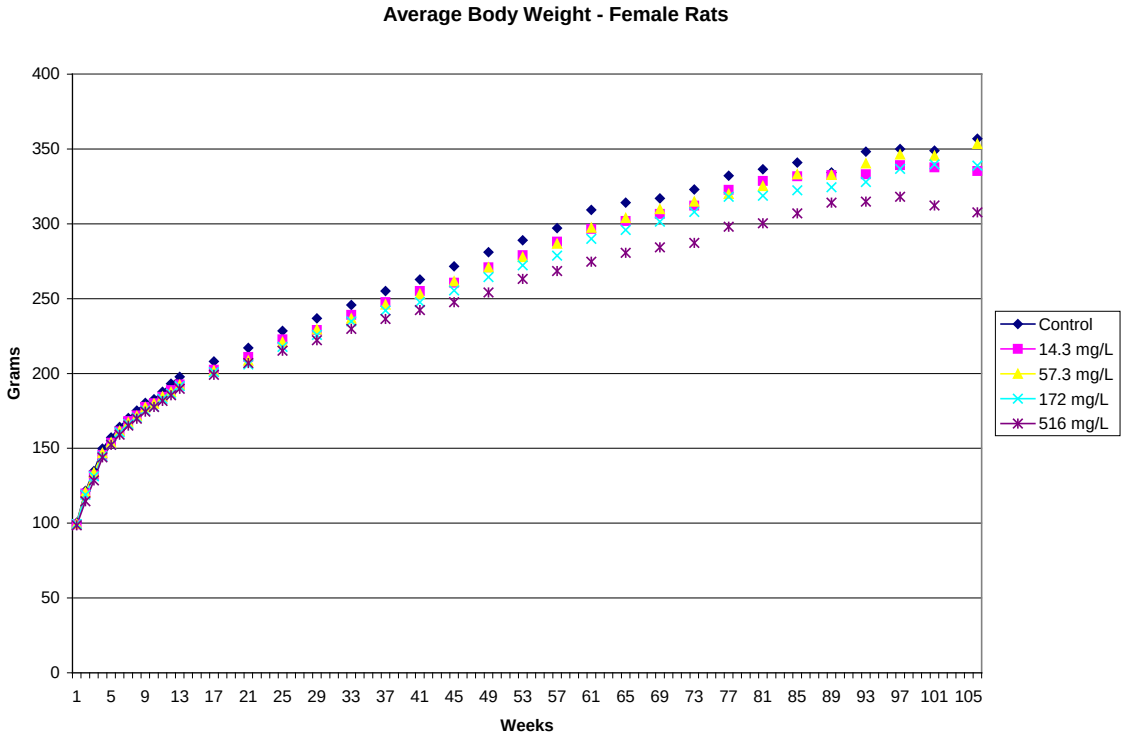
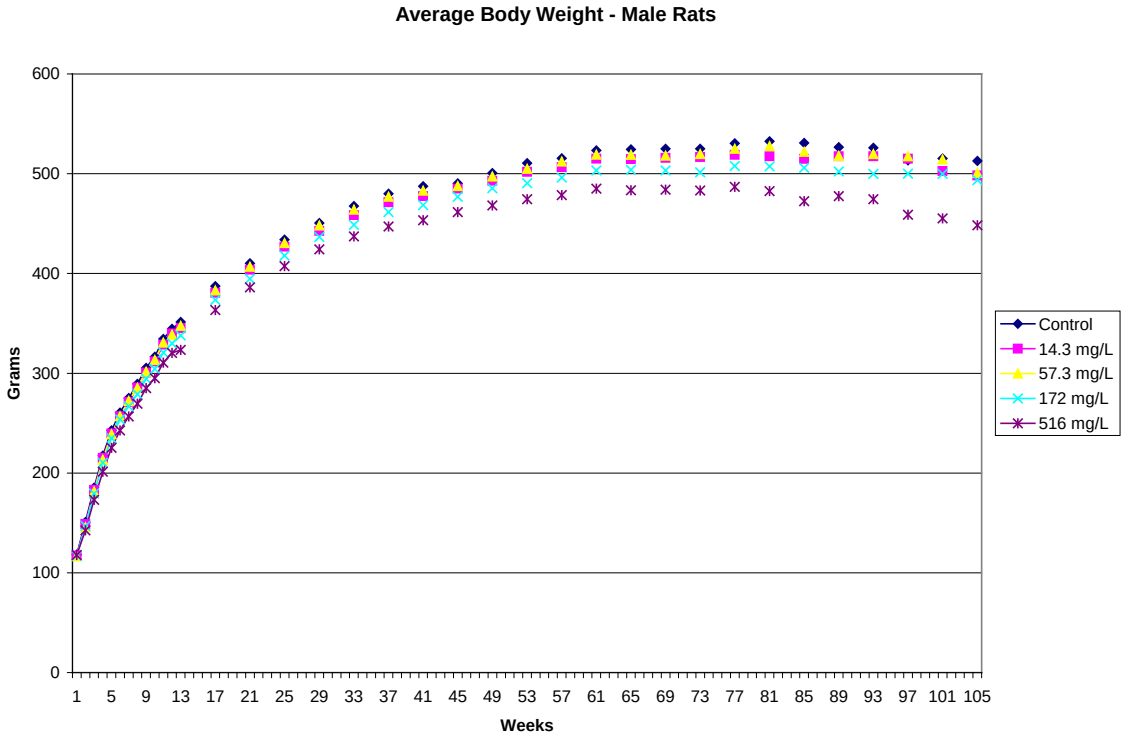


Figure 6. Male rat body weights, by week



Other than the oral cavity, female rats exposed to Cr VI had an occasional statistically significant increase or decrease in tumors at a given dose at a particular site. There were no notable increases that appeared to be compound-related. Statistically significant increases in adenomas were observed in the clitoral gland in animals that received 14.3 or 57.3 mg/L of Cr VI. Statistically significant increases in adenomas or carcinomas were observed in the 14.3 mg/L group. The tests for trend were not positive at this site.

Table 3. Tumors in the Oral Mucosa and Tongue in Male Rats Administered Hexavalent Chromium

Tumor	Concentration of Sodium Dichromate Dihydrate in Drinking Water				
	0 mg/L	14.3 mg/L	57.3 mg/L	172 mg/L	516 mg/L
Papillomas	0/47[a,b]	0/45	0/44	0/47	2/49
Carcinomas	0/47[c]	1/45	0/44	0/47	6/49[d]
Papillomas or Carcinomas	0/47[c]	1/45	0/44	0/47	7/49[e]

[a]Number of animals with tumors/number of animals at risk. Animals were considered to be at risk of developing a tumor if alive at the time of the first occurrence of tumors of the oral mucosa or tongue (day 543) and if tissues were examined.
[b]Statistically significant ($p<0.05$) Exact trend test.
[c]Statistically significant ($p<0.0005$) Exact trend test.
[d]Statistically significant ($p<0.05$) Fisher's Exact test.
[e]Statistically significant ($p<0.01$) Fisher's Exact test.

A decrease in carcinomas or adenomas was observed in the pituitary gland, pars distalis or unspecified site (pairwise comparison) in animals receiving the high dose. The tests for trend suggested a negative trend (decrease in tumors with dose) which appeared to be related to the decrease in tumors in the high dose group.

Table 4. Tumors in the Oral Mucosa and Tongue in Female Rats Administered Hexavalent Chromium.

Tumor	Concentration of Sodium Dichromate Dihyrate in Drinking Water				
	0 mg/L	14.3 mg/L	57.3 mg/L	172 mg/L	516 mg/L
Papillomas	1/49[a]	1/48	0/49	0/47	0/48
Carcinomas	0/49[b]	0/48	0/49	2/47	11/48[d]
Papillomas or Carcinomas	1/49[b]	1/48	0/49	2/47	11/48[c]

[a]Number of animals with tumors/number of animals at risk. Animals were considered to be at risk of developing a tumor if alive at the time of the first occurrence of tumors of the oral mucosa or tongue (day 506) and if tissues were examined.
[b]Statistically significant ($p<0.0001$) Exact trend test.
[c]Statistically significant ($p<0.005$) Fisher's Exact test.
[d]Statistically significant ($p<0.0005$) Fisher's Exact test.

Mouse

The survival of mice (both male and female) was good. Survival in mice of both sexes receiving Cr VI was similar to that in the control groups (Figures 7 and 8). Body weight gains were largely unaffected by Cr VI administration in mice in the low dose groups (Figures 9 and 10). Body weight in the high dose group in male mice was initially reduced but recovered to levels observed in control animals by the end of the study (Figure 11). This effect also appeared to be occurring in the female mouse (in the two highest dose groups (Figure 12), but body weight in high dose females never fully recovered to levels observed in the control group. As in the rat, water consumption was reduced in mice of both sexes in the high dose groups (Figures 13 and 14). However, there were no indications that the animals were dehydrated (NJDEP, 2009). The reduced body weights were partly attributed by the NTP to the reduced water consumption.

Neoplasms

The administration of Cr VI to male and female mice resulted in statistically significant and dose-related increases in adenomas or carcinomas (combined) in the duodenum (data not shown) and the entire small intestine (duodenum, jejunum and ileum) (Tables 5 and 6). Most adenomas and carcinomas occurred in the duodenum (data not shown). The dose-response relationship between Cr VI and tumors of the small intestine appeared to be quite similar in male and female mice.

Intestinal tissues reported undergoing autolysis were not examined microscopically but were grossly examined for tumors (supplemental information provided by the NTP pathologist, David Malarkey, and discussed in Stern, 2010). Essentially all intestinal tumors were observed upon gross examination. The effective number of mice in Tables 5 and 6 (the denominator) reflects animals alive at the time of the first occurrence of tumors in the small intestine in each experiment, excluding animals for which tissues of the small intestine were missing.

The intestinal tumors occurred during the second year of the studies, with the first tumor detected in males on day 451 and in females on day 625. Most of the tumors were detected at the time of the terminal sacrifice. In male mice, three tumors were detected in animals that lived less than 100 weeks. In female mice, only two tumors were detected in animals prior to terminal sacrifice. These findings are consistent with the survival curves in that the occurrence of tumors in the high dose groups did not result in an increase in mortality.

Figure 7. Survival curves for female mice

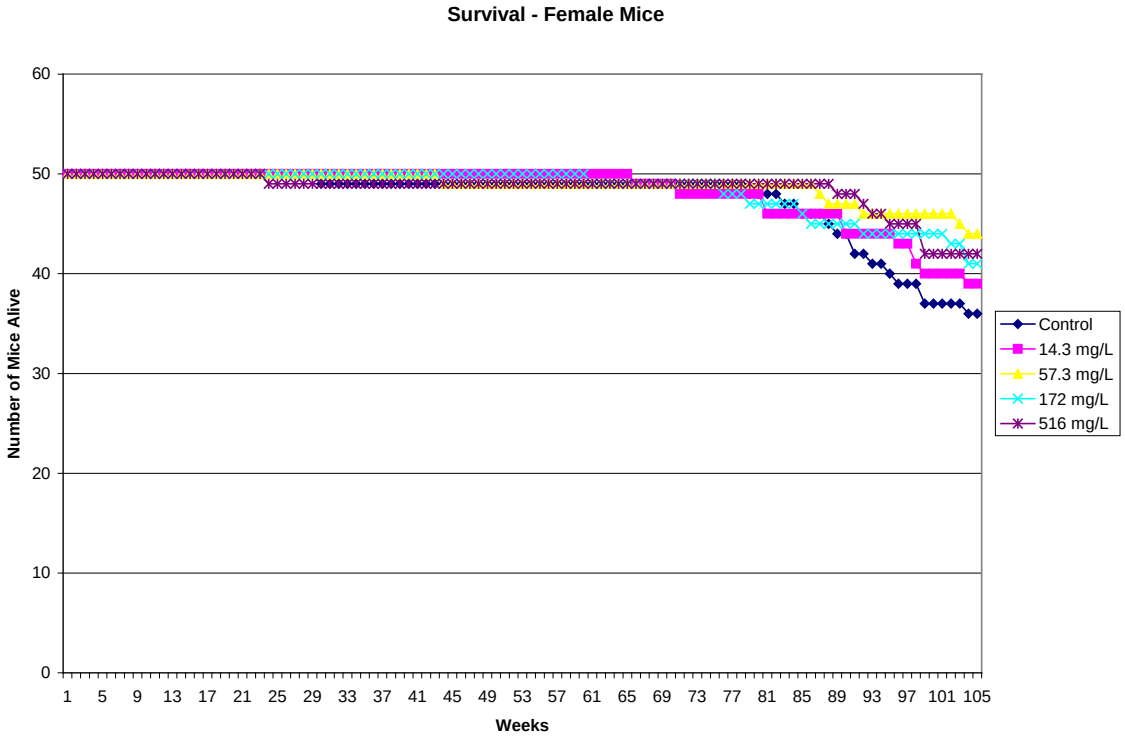


Figure 8. Survival curves for male mice

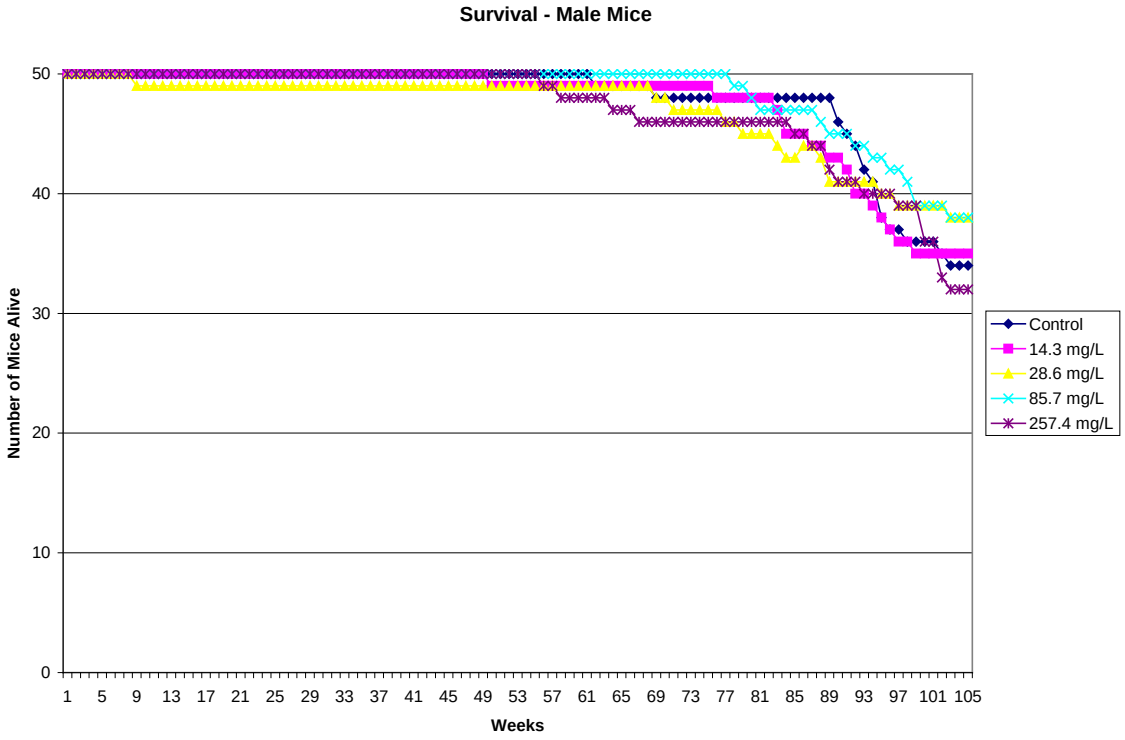


Figure 9. Male mouse body weights, by week

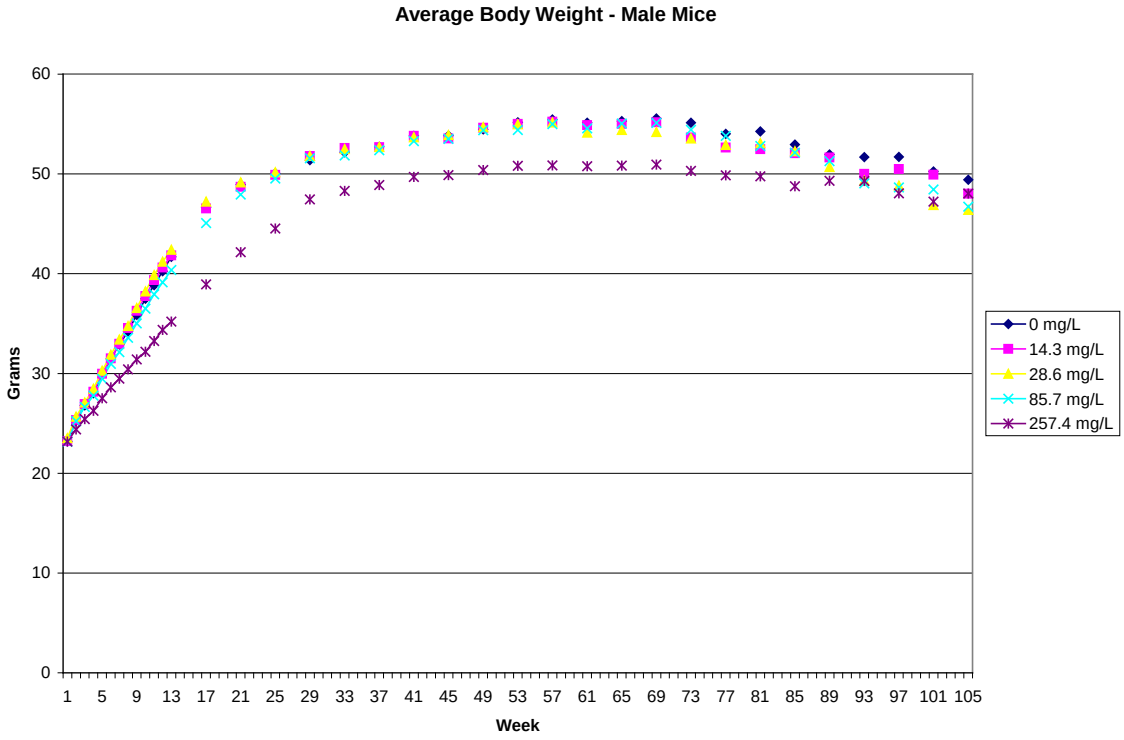


Figure 10. Female mouse body weights, by week

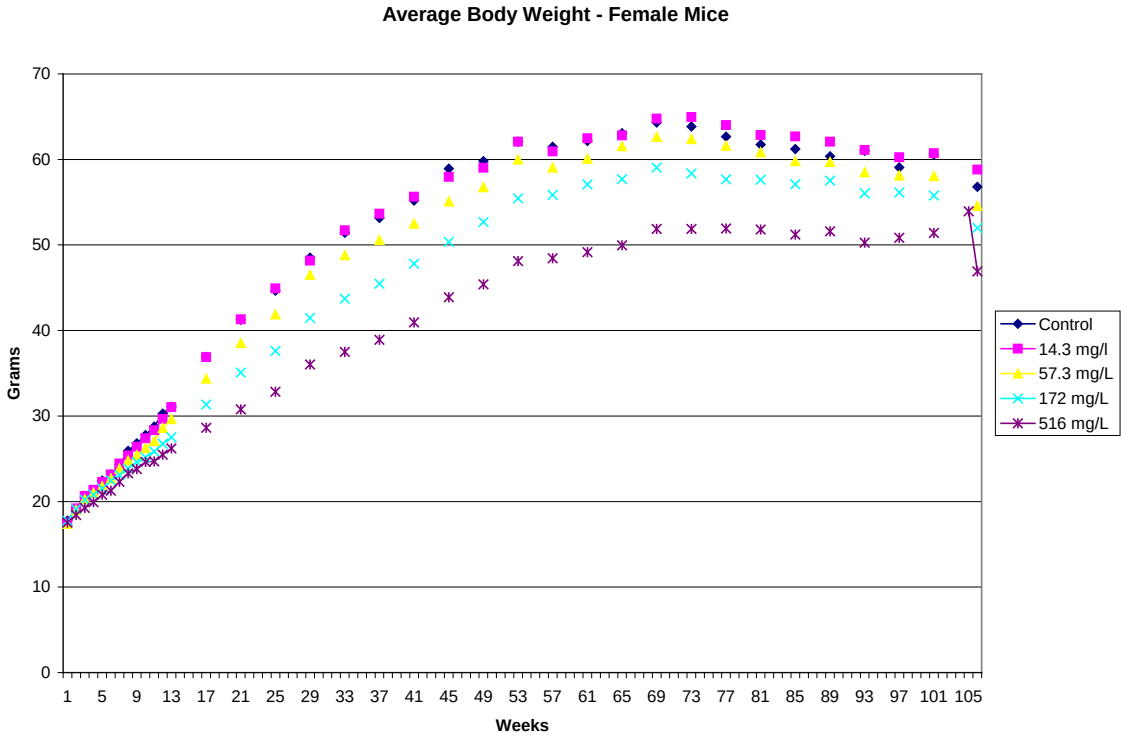


Figure 11. Body weights of male mice, compared to control

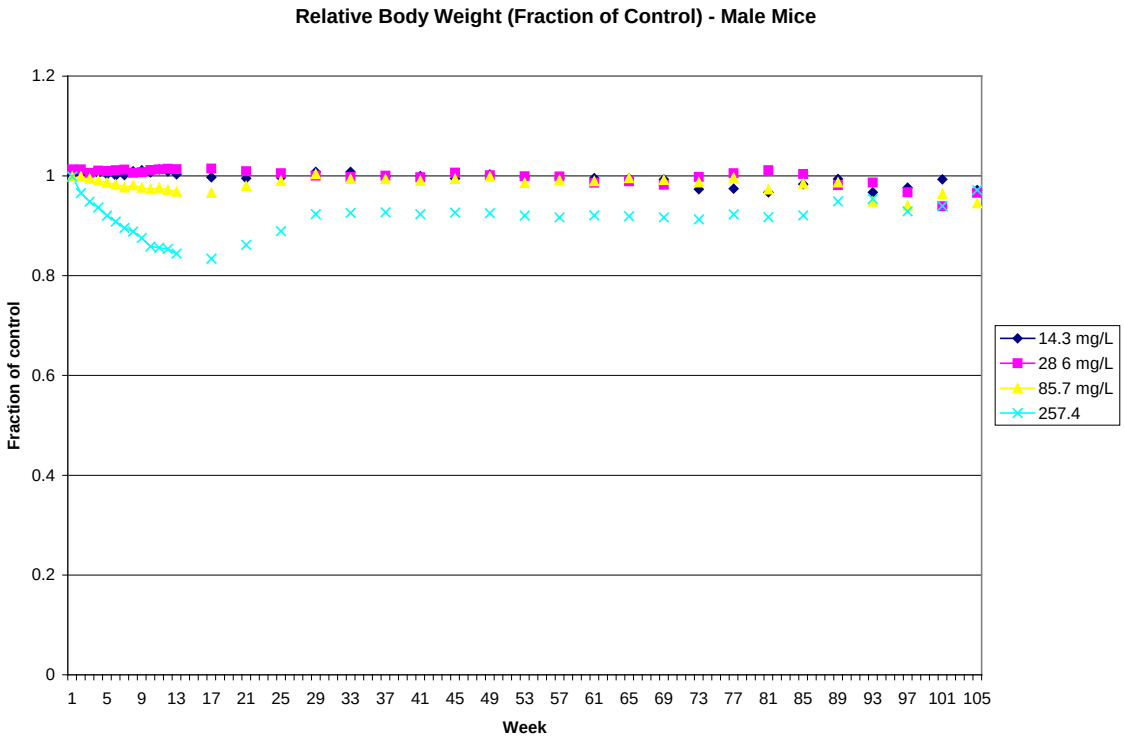


Figure 12. Body weights of female mice, compared to control

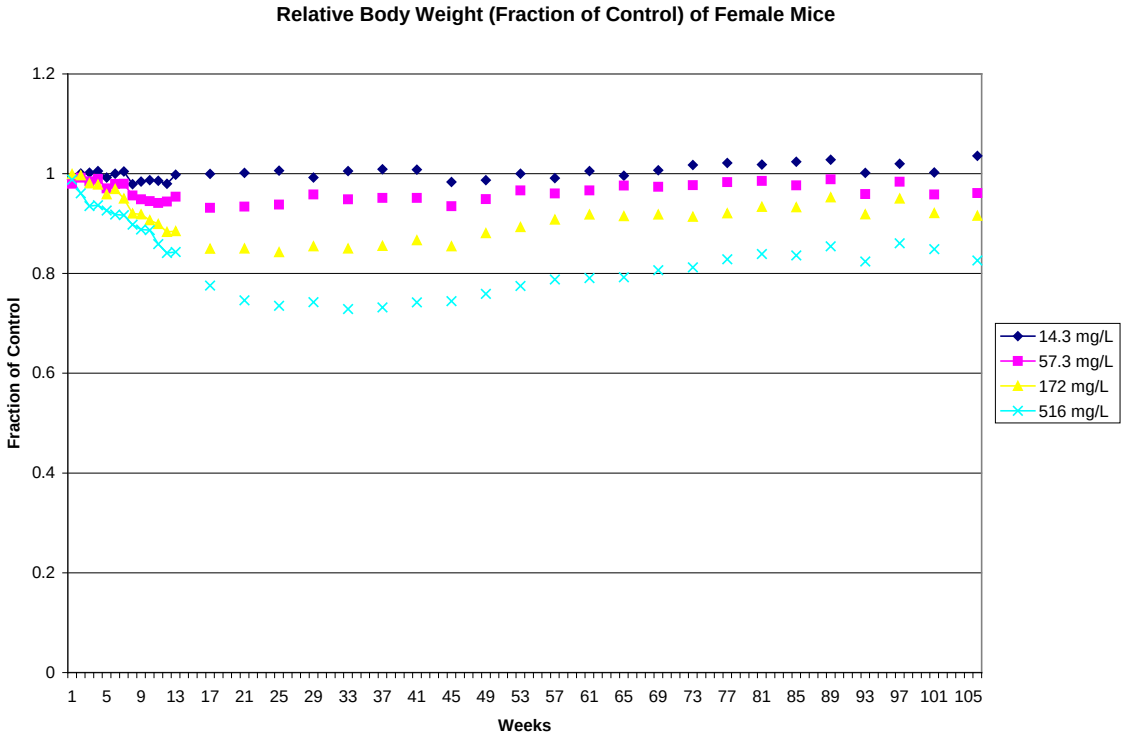


Table 5. Small Intestine Tumors in Male Mice Administered Hexavalent Chromium.

Organ	Tumor Type	Concentration of Sodium Dichromate Dihydrate in Drinking Water				
		0 mg/L	14.3 mg/L	28.6 mg/L	85.7 mg/L	257.4 mg/L
Small Intestine[a]	Adenomas	1/49[b,d]	1/49	1/49	5/50	17/48[f]
	Carcinomas	0/49[c]	2/49	1/49	3/50	5/48[e]
	Adenomas or Carcinomas	1/49[d]	3/49	2/49	7/50[e]	20/48[f]

[a]Includes duodenum, ileum and jejunum.
[b]Number of animals with tumors/number of animals at risk (alive at the time of the first occurrence of tumor (day 451)) and if tissue was available (not missing).
[c]Statistically significant (p=0.01) Exact trend test.
[d]Statistically significant (p<0.0001) Exact trend test.
[e]Statistically significant (p<0.05) Fisher's exact test.
[f]Statistically significant (p<0.0001) Fisher's exact test.

Table 6. Small Intestine Tumors in Female Mice Administered Hexavalent Chromium.

Organ	Tumor Type	Concentration of Sodium Dichromate Dihydrate in Drinking Water				
		0 mg/L	14.3 mg/L	57 mg/L	172 mg/L	516 mg/L
Small Intestine[a]	Adenomas	0/44[b,d]	1/45	2/47	15/45[f]	16/49[f]
	Carcinomas	1/44[c]	0/45	2/47	3/45	7/49[e]
	Adenomas or Carcinomas	1/44[d]	1/45	4/47	17/45[f]	22/49[f]

[a]Includes duodenum, ileum and jejunum.
[b]Number of animals with tumors/number of animals at risk (alive at the time of the first occurrence of tumor (day 625)) and if tissue was available (not missing).
[c]Statistically significant (p<0.005) Exact trend test.
[d]Statistically significant (p<0.0001) Exact trend test.
[e]Statistically significant (p<0.05) Fisher's exact test.
[f]Statistically significant (p<0.0001) Fisher's exact test.

Historically, tumors of the duodenum or small intestine are very rare in $B6C3F_1$ mice in NTP studies. The following discussion of historical tumor occurrence in NTP studies addresses both tumors only of the duodenal section of the small intestine (where most of the tumors were detected in the mouse) and tumors of all sections of the small intestine.

In control male mice, NTP reported detecting nine adenomas and three carcinomas of the duodenum in 1,549 animals examined (in studies involving all exposure routes). Ten adenomas and 30 carcinomas (39 adenomas or carcinomas) of the small intestine were detected out of 1549 animals examined (all routes). In control female mice, three adenomas and one carcinoma was detected in the duodenum of 1,648 examined and three adenomas and eight carcinomas (eleven adenomas or carcinomas) were detected in the small intestine out of 1,648 mice examined (all routes, data as of March 2, 2007). Thus, historical data from NTP on the low incidence of small intestinal tumors observed in control $B6C3F_1$ mice is consistent with the concurrent control data from the NTP's studies of Cr VI in mice (NTP, 2008).

No statistically significant increases in tumors of the oral cavity were observed at any dose, unlike what was observed in the studies in the rat. No statistically significant increases in tumors were observed in the forestomach, unlike what was observed in mice in the Borneff *et al.* (1968) study. The statistically significant increase in stomach tumors observed in humans exposed to Cr VI in drinking water in China (Zhang and Li, 1987) may or may not be consistent with what was observed in the duodenum of mice as the precise site of the tumors in the human study is unclear.

Maximum Tolerated Dose - Rats

No differences in survival were evident in male or female rats treated with Cr VI compared to controls. Decreases in body weight were observed in the high dose groups of both sexes which NTP attributed, in part, to a decrease in water intake. NTP stated, "No clinical findings were attributed to sodium dichromate dihydrate exposure." NTP reported, "Non-neoplastic lesions were not observed in the oral mucosa."

Changes in hematology were noted by NTP: "An exposure concentration-related erythrocyte microcytosis, evidenced by decreased mean cell volumes, occurred on day 4 and persisted throughout the study in the 172 and 516 mg/L groups..." "The severity of the microcytosis ameliorated with time." Exposure-related anemia was also observed in the 57.3, 172 and 516 mg/L groups. NTP noted "the anemia was most severe on day 22 (an approximate 30 percent decrease in the 516 mg/L group), but resolved with time." "In fact, at 3 months, erythrocyte counts were increased, in contrast to the lower hematocrit and hemoglobin values in the 516 mg/L group.…" NTP concluded: "Taken together, it appears that the erythropoietic tissues were able to respond to the anemia.…"

Statistically significant increases in chronic inflammation were observed in the livers of all female rats administered Cr VI. Fatty changes were also observed. The inflammation was described as minimal to mild in severity except in the high dose females, where it was described as mild to moderate in severity. Chronic inflammation was also observed in male rats administered 172 mg/L of Cr VI.

There was very little evidence of toxicity in rats treated with Cr VI. These findings do not indicate that the maximum tolerated dose was exceeded.

Maximum Tolerated Dose - Mice

No difference in survival was evident in male or female mice receiving Cr VI, indicating the animals tolerated the chemical reasonably well. A decrease in body weight was

observed from roughly twenty to seventy weeks in the high dose group of female mice and the high dose group of male mice. By the end of the study, the mean body weight of male mice (high dose) was not substantially different from control, while the mean body weight of female mice (high dose) still appeared to be below the mean body weight of the control group. Previous studies in animals (Borneff *et al*., 1968) and humans (Zhang and Li, 1987) revealed that at high levels of Cr VI, drinking water becomes unpalatable. In the NTP 2008 studies, reduced water consumption (normalized to body weight) was observed in male and female mice receiving the high dose of Cr VI (Figures 13 and 14).

The initial marked reduction in drinking water consumption in male and female mice appeared to be consistent with and likely responsible for much of the reduced weight gain in these animals. With time, water consumption in higher dose females returned to control levels, indicating the animals tolerated Cr VI in their drinking water with time. However, bodyweights remained approximately 20 percent lower than controls. It has been suggested that this was due to an exceedance of the MTD in the high dose female mice (NJDEP, 2009). Although water consumption in high dose males did not return to levels observed in the control group, the recovery of body weight to levels observed in the control group indicated that high dose males tolerated Cr VI better with time.

No notable non-neoplastic pathology was reported in rats or mice. The NTP reported, "no clinical findings were attributed to sodium dichromate dihydrate exposure." Exposure-related microcytosis as evidenced by decreased mean red blood cell volume was seen in the mice, although NTP indicated "the mice were less affected than the rats."

Figure 13. Effect of hexavalent chromium on water intake in male mice, by week.

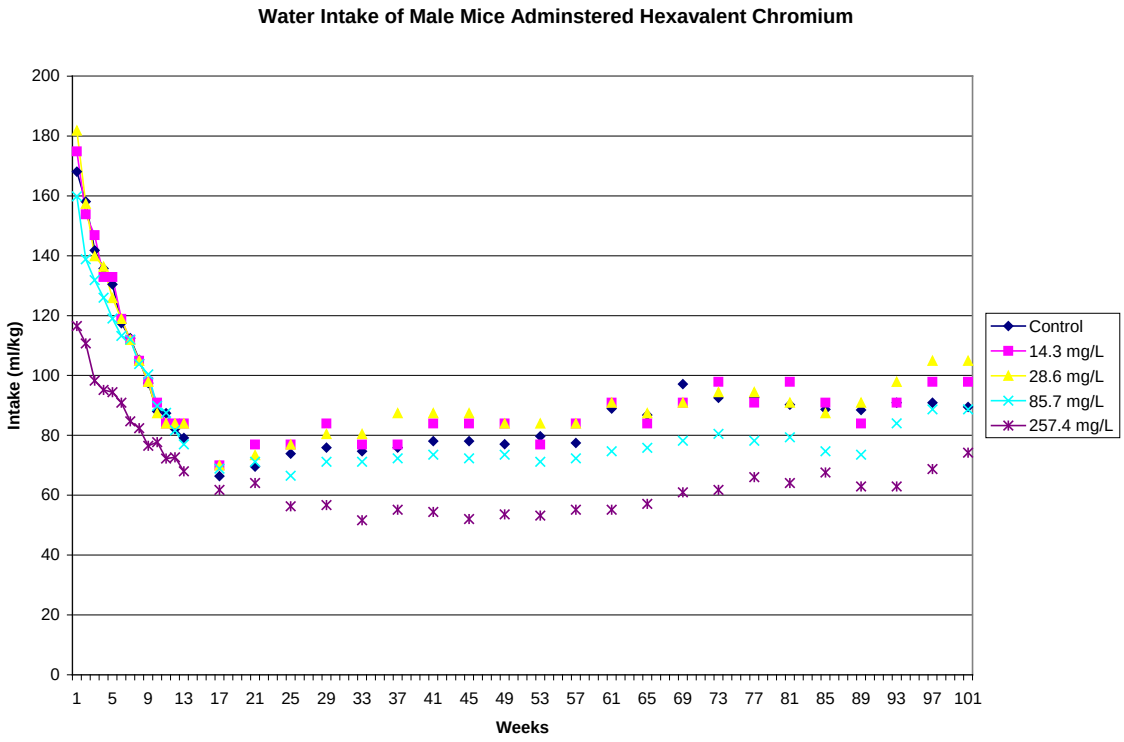
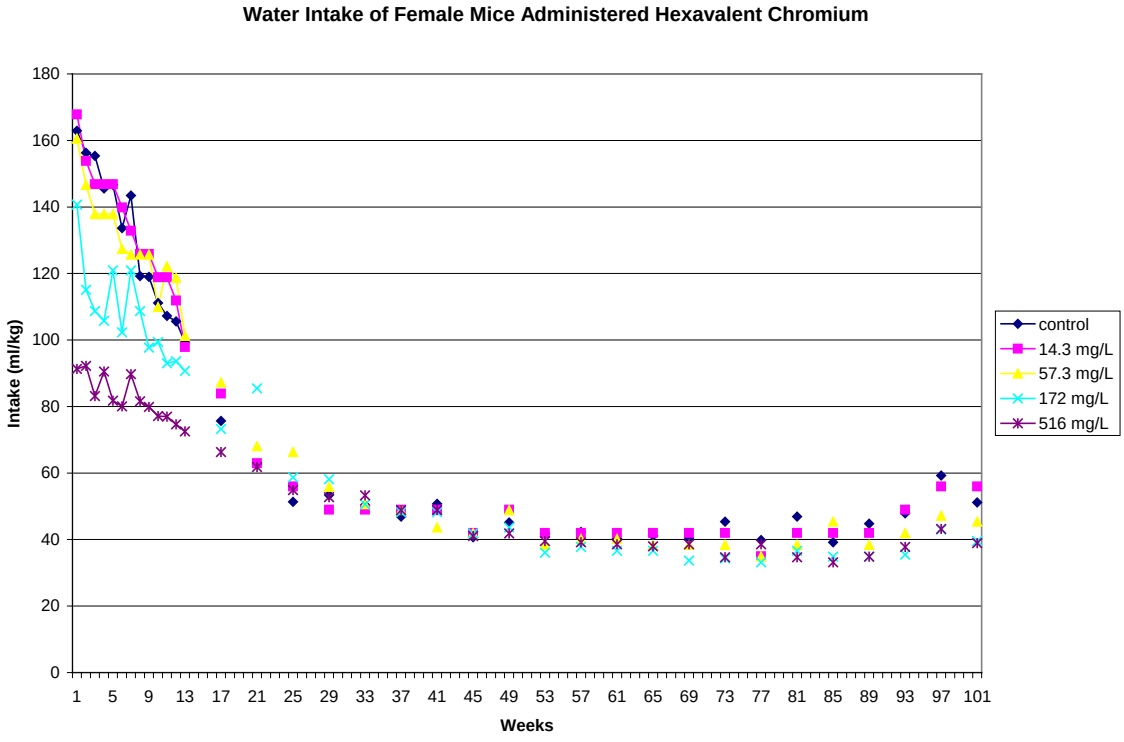


Figure 14. Effect of hexavalent chromium on water intake in female mice, by week.



A statistically significant and dose related increase in diffuse hyperplasia in the duodenum was observed in mice. NTP indicated "that collectively, these lesions are considered consistent with regenerative hyperplasia secondary to previous epithelial cell injury." Note however, that tissue damage and cell injury were ***not*** observed (see below).

In conclusion, very little evidence of toxicity was observed in mice treated with Cr VI. These findings do not indicate that the maximum tolerated dose was exceeded.

On May 16, 2007, the NTP Technical Reports Review Subcommittee reviewed the draft NTP Technical Report (NTP TR 546) on the Toxicity and Carcinogenesis studies of Sodium Dichromate Dihydrate and reported: "The Subcommittee accepted unanimously (6 yes, 0 no) the conclusions as written, *clear evidence of carcinogenic activity* of sodium dichromate dihydrate in male and female F344/N rats and *clear evidence of carcinogenic activity* in male and female B6C3F1 mice" (NTP, 2007a).

Non-neoplastic findings - Possible relationships between tissue damage, inflammation, hyperplasia and tumors in rats and mice

In describing non-neoplastic lesions in tissues with significant increases in tumors, NTP (2008) reported, "The incidences of diffuse epithelial hyperplasia were significantly increased in the duodenum of all exposed groups of male and female mice (Tables 13, C4, and D4). In the jejunum, the incidence of diffuse epithelial hyperplasia was significantly increased in 516 mg/L females. Diffuse epithelial hyperplasia generally involved the entire mucosa. Compared to the controls, the duodenal villi of exposed mice were short, broad, blunt, and lined by densely packed, tall columnar epithelial cells that were more basophilic than the shorter epithelial cells lining the duodenum villi of the controls (Plates 19 to 22). The epithelial cells and cell nuclei were often piled up in multiple layers along the long axis of the villi. Intestinal crypts were often elongate and generally appeared to contain increased numbers of epithelial cells with increased numbers of mitotic figures. Collectively, these lesions are considered consistent with regenerative hyperplasia secondary to previous epithelial cell injury."

OEHHA examined the findings of NTP (2008) (Pages 42-46, 57-61 and Table A4, B4, C4 and D4) for evidence of damage to the epithelium, inflammation and hyperplasia in the small intestine (duodenum), oral cavity and liver of male and female rats and mice (Table 7 below). No Cr VI-related tissue damage or inflammation was evident in the small intestine of male or female mice (or rats) while hyperplasia (and tumors) was observed in the duodenum of the mouse (but not the rat). A subsequent report by NTP scientists stated, "We observed no increases in non-neoplastic histopathology lesions in either species suggestive of overt tissue damage due to the oxidant properties of Cr (VI)" (Stout *et al*., 2009).

These findings of duodenal hyperplasia in the absence of tissue damage are supported by the results of the subchronic rodent study (NTP, 2007). Male and female mice exposed to Cr VI in drinking water exhibited duodenal hyperplasia at all dose levels (62.5 to 1,000 mg/L sodium dichromate dihydrate), while duodenal tissue damage was only observed at the highest dose level. Thus, after an exposure lasting three months, hyperplasia of mouse duodenal tissue occurred that was not regenerative. One possible explanation is

that Cr VI is mitogenic. This is not unexpected, since a number of carcinogens (some of them mutagenic) are also mitogens.

Table 7. Summary of NTP (2008) Findings of Neoplastic and Non-neoplastic Effects or Lesions in Male and Female Mice and Rats

Species	Site	Gender	Histocyte Infiltration	Inflammation	Hyperplasia	Tumors
Rat	Duodenum	M	Yes	No	No	No
		F	Yes	No	No	No
	Liver	M	Yes	Chronic	No	No
		F	Yes	Chronic	No	No
	Oral Cavity	M	No	No	No	Yes
		F	No	No	No	Yes
Mouse	Duodenum	M	Yes	No	Yes	Yes
		F	Yes	No	Yes	Yes
	Liver	M	No	No	No	No
		F	Yes	Chronic[a]	No	No
	Oral Cavity	M	No	No	No	No
		F	No	No	No	No

[a]At a concentration of 172 mg/L.

Chronic inflammation was observed in the male and female rat liver and female (but not male) mouse liver. Hyperplasia and increases in tumors were not evident in the rat or mouse liver. Neither chronic inflammation nor hyperplasia was reported in the oral cavity of female or male rats or mice.

Histiocytic infiltration (but no increase in tumors) was observed in the duodenum and liver of male and female rats while tumors were observed in the oral cavity without histiocytic infiltration. Histiocytic infiltration was reported in the duodenum of male and female mice and in the female (but not male) mouse liver.

OEHHA examined the patterns of these non-neoplastic effects and their possible association with tumors in the small intestine, oral cavity and liver. Damage to tissue (epithelial damage) was not reported in any of the tissues. Chronic inflammation was reported in tissues where significant increases in tumors were not observed (male and female rat liver, female mouse liver) and was **not** observed in tissues where significant increases in tumors were observed (female and male mouse duodenum; oral cavity of male and female rats). Hyperplasia was observed in the duodenum of male and female

mice, which are tissues that had significant increases in tumors. However, no hyperplasia was reported in the oral cavity of male and female rats.

With regard to histiocyte infiltration, NTP (2008) stated: "The biological significance of the histiocytic cellular infiltrates is unknown but may suggest phagocytosis of some insoluble chemical precipitate." Histiocyte infiltration was reported in tissues with no inflammation and no hyperplasia and no significant increase in tumors (male and female rat duodenum). Histiocytic infiltration was observed in tissues with reported chronic inflammation, but no hyperplasia and no increase in tumors (male and female rat liver). Histiocyte infiltration was reported in the male and female mouse duodenum, along with hyperplasia and increases in tumors but no inflammation.

In the male mouse liver, no histiocytic infiltration, inflammation, hyperplasia or tumors were reported. In the female mouse liver, histiocytic infiltration along with inflammation but no hyperplasia or tumors were reported. OEHHA could not discern a consistent pattern of histiocytic infiltration, inflammation, hyperplasia and the occurrence of tumors in the mouse or rat duodenum, oral cavity or liver in the NTP (2008) study. Therefore, an MOA other than that of genotoxicity or mutagenicity is not supported by these findings. The standard approach for carcinogens operating via a genotoxic or mutagenic MOA is to apply a linearized multistage model to calculate the cancer potency (U.S. EPA, 2005; OEHHA, 2009a).

Non-Oral routes/ Unorthodox Protocol

Cancer bioassays of animals exposed to Cr VI by non-oral routes have been thoroughly reviewed by others (unlike the Borneff *et al*., 1968 study) and another review is not needed (ATSDR, 2000; IARC, 1990). To summarize, four cancer inhalation studies were identified that evaluated Cr VI compounds in mice, and one in rats. In one study of mice exposed to chromium trioxide mist by inhalation (Adachi, 1987), statistically significant increases in nasal papillomas were observed. In other studies in mice, non-significant increases in lung adenomas and adenocarcinomas were observed following inhalation of calcium chromate dust (Nettesheim *et al.*, 1971) or chromium trioxide mist (Adachi *et al.*, 1986). In the rat study, inhalation of sodium dichromate mist resulted in non-significant increases in lung tumors and a single carcinoma of the pharynx (Glaser *et al.*, 1986). Although the data are rather sparse, it appears that rodents are relatively insensitive to Cr VI-induced cancer when it is administered by the inhalation route.

In a short-term cancer study conducted by Davidson and associates, groups of 6-week old hairless SK1-hrBR mice (20 animals per group) were exposed to potassium chromate in their drinking water and/or UV light and observed for skin tumor formation (Davidson *et al.*, 2004). The exposure groups were as follows: controls (Group 1), UV radiation only (Group 2), 2.5 ppm K_2CrO_4 (Group 3), 5.0 ppm K_2CrO_4 (Group 4), UV + 0.5 ppm K_2CrO_4 (Group 5), UV + 2.5 ppm K_2CrO_4 (Group 6), and UV + 5.0 ppm K_2CrO_4 (Group 7). The Cr VI was administered in the drinking water for 182 days. UV light exposures (1.18 kJ/m^2) were begun after the first month of chromate treatment at a frequency of 3 days per week and continued for three months. After a 1-week break, UV treatments resumed for 3 additional months on 2 days/week. Animals were sacrificed at approximately 224 days of age. No skin tumors were observed among controls or mice treated only with the chromate (Groups 1, 3 and 4). However, co-exposure to UV and

chromate resulted in increased skin tumors that demonstrated a clear dose-response with increasing chromate concentration in drinking water (Groups 2, 5, 6, and 7). Since many humans are exposed to both UV radiation from sunlight and Cr VI in drinking water, the authors concluded that the findings support concern over the potential carcinogenic hazards posed by Cr VI in drinking water.

Multiple studies have also been conducted in which Cr VI compounds have been directly placed in the pulmonary tract or pleural space by intratracheal instillation or intrabronchial or intrapleural administration. Hexavalent chromium induced lung tumors in mice (basic potassium zinc chromate, Steffee and Baetjer, 1965) and rats (sodium dichromate and calcium chromate, Steinhoff *et al.*, 1986), but not guinea pigs, rabbits (basic potassium zinc chromate and lead chromate, Steffee and Baetjer, 1965), or hamsters (calcium chromate, Reuzel *et al.*, 1986), following intratracheal instillation. Intrabronchial implantation in rats of stainless-steel mesh pellets containing calcium chromate, zinc potassium chromate, or strontium chromate, but not chromium trioxide, sodium dichromate, sodium chromate, or lead chromate resulted in increased incidences of bronchial carcinoma and squamous cell carcinoma of the lung (Laskin *et al.*, 1970; Levy and Venitt, 1986; Levy *et al.*, 1986). Intrapleural implantation in rats of a variety of Cr VI compounds, namely strontium chromate, lead chromate, basic zinc chromate, and calcium chromate induced implantation site tumors (Hueper, 1961; Hueper and Payne, 1962).

Additional routes of exposure include subcutaneous and intramuscular administration. Treatment-related injection site sarcomas were reported in rats following subcutaneous administration of lead chromate, basic lead chromate, basic zinc chromate and mixtures containing lead chromate, sulfate and molybdate (Maltoni, 1974, 1976; Maltoni *et al.*, 1982). The one subcutaneous injection study conducted in mice reported a single tumor at the site of injection of calcium chromate (Payne, 1960). Intramuscular administration of Cr VI compounds resulted in a treatment-related increase in injection site sarcomas in the mouse with calcium chromate (Payne, 1960), but not lead chromate (Furst *et al.*, 1976). In the rat, treatment-related increases in injection site sarcomas were observed following intramuscular injection of calcium chromate, sintered chromium trioxide, basic zinc chromate, strontium chromate, and lead chromate, but not sodium dichromate or barium chromate (Hueper and Payne, 1959, 1962; Hueper, 1961; Roe and Carter, 1969; Furst *et al.*, 1976). In the studies of Furst *et al*. (1976), intramuscular injection of lead chromate to the rat was also associated with induction of renal carcinomas; however, as noted by IARC, 1990, it is likely that the renal tumor response was due to the known carcinogenic action of lead in the rodent kidney.

Toxicological Effects in Humans

Acute Toxicity

A 14-year old boy died in the hospital eight days after ingesting 7.5 mg Cr VI/kg as potassium dichromate. Death resulted from gastrointestinal ulceration and severe liver and kidney damage (Kaufman *et al*., 1970). The autopsy revealed an enlarged brain and cerebral edema. However, this effect may be secondary to kidney failure rather than a direct effect on the nervous system (Kaufman *et al*., 1970). A 22-month-old boy died of

cardiopulmonary arrest after ingesting an unknown amount of sodium dichromate (Ellis *et al*., 1982). In another case report, a 17-year-old male died of cardiac arrest after ingesting potassium dichromate at 29 mg Cr VI/kg (Clochesy, 1984). Effects on the cardiovascular, respiratory, gastrointestinal, hematological, hepatic and renal systems have been observed in humans who ingested large amounts of Cr VI (ATSDR, 2000).

Developmental and Reproductive Toxicity

The status of spermatogenesis was evaluated in workers in an electroplating factory in China (Li *et al*., 2001). Workers exposed to harmful chemicals including Cr VI were compared to workers that were not exposed. Sperm counts and motility were significantly reduced in workers exposed to harmful chemicals. No information regarding amount of chromium exposure was reported. No differences in serum and semen chromium levels were observed. It is unclear whether these measures indicate no difference in exposure. Other factors including exposure to other hazardous chemicals (e.g., lead) and high workplace temperatures could also be responsible for the reported effects in the workers.

Chromium (hexavalent compounds) was considered by the Developmental and Reproductive Toxicant Identification Committee (DARTIC) of the Office of Environmental Health Hazard Assessment's (OEHHA) Science Advisory Board at a public meeting held on November 20, 2008 (OEHHA 2009c, 2010). At this meeting, the DARTIC determined that chromium (hexavalent compounds) was clearly shown through scientifically valid testing according to generally accepted principles to cause reproductive toxicity (developmental toxicity, male reproductive toxicity and female reproductive toxicity). The DARTIC's action added chromium (hexavalent compounds) to California's list of chemicals known to cause reproductive toxicity pursuant to the Safe Drinking Water and Toxic Enforcement Act of 1986, more commonly known as Proposition 65.

OEHHA has developed a Proposition 65 maximum allowable dose level (MADL) for chromium (hexavalent compounds) (OEHHA, 2010). For purposes of Proposition 65, a MADL is the No Observable Effect Level (NOEL, generally considered equivalent to a NOAEL) divided by 1,000, based on the most sensitive study of sufficient quality. The MADL for chromium (hexavalent compounds) is based on Murthy *et al.* (1996). In this study of female reproductive toxicity, two sets of adult female Swiss albino mice were exposed to potassium dichromate as a source of hexavalent chromium in drinking water. For the oral route of exposure, the following calculations were performed to derive the $MADL_{oral}$ for chromium (hexavalent compounds), based upon the Murthy et al. (1996) study in mice that provided a NOAEL of 0.142 mg/kg-day for female reproductive toxicity:

Calculation of the NOAEL for a 58 kg woman:
0.142 mg/kg-day X 58 kg = 8.236 mg/day

The NOAEL for a woman was divided by 1,000 to obtain the MADL:

$MADL_{oral}$ = 8.236 mg/day ÷ 1,000 = 0. 008236 mg/day or 8.2 μg/day after rounding. This MADL applies to exposure to chromium (hexavalent compounds) by the oral route.

The concentration of Cr VI in drinking water resulting in this level of exposure to a 58 kg woman will depend on the drinking water ingestion rate. As an example, the adult drinking water ingestion rate of 0.039 L/kg-day (see footnote to Table 17) can be used to estimate the Cr VI concentration of drinking water yielding exposure at the MADL:

0.039 L/kg-day X 58 kg = 2.3 L/day

8.2 μg/day ÷ 2.3 L/day = 3.6 μg/L or ppb.

This value is slightly higher than the health-protective concentration based on non-cancer effects (2 ppb) and greater than 100-fold higher than the PHG based on cancer effects (0.02 ppb).

Immunotoxicity

Dermal exposure to Cr VI has been linked to allergic contact dermatitis (ATSDR, 2000). The North American Contact Dermatitis Group Patch-Test Results, 1996-1998 revealed that 2.8 percent of 3440 patients tested by 12 North American dermatologists exhibited a positive allergenic reaction to 0.25 percent potassium dichromate solution (Marks *et al.*, 2000). The test methods typically completely occlude the skin for around 48 hours; less response would be expected during the shorter duration of a shower or during bathing or swimming. The cumulative percent of responders in sensitive individuals (those that tested positive) at various concentrations in various studies was summarized by Felter and Dourson (1997). Virtually no response was detected at concentrations below 4 to 5 ppm of Cr VI. However, this 4-5 ppm cut-off has several associated uncertainties including individual susceptibility and the use of different compounds for testing.

Chronic Toxicity

A village in the People's Republic of China had a drinking water well contaminated from a nearby alloy plant with 20 mg Cr VI/L. A cross sectional study of people living in this village revealed that they suffered from leukocytosis and immature neutrophils (Zhang and Li, 1987). Villagers who drank this water experienced oral ulcer, diarrhea, abdominal pain, indigestion, and vomiting. The dose was estimated to be 0.57 mg chromium VI/kg-day (Zhang and Li, 1987). The alloy plant began operation in 1961, and the study was conducted in 1965. No data are available on the chromium concentration in the water before the plant began to operate.

Carcinogenicity

Selected recent human epidemiological studies of Cr VI exposure and cancer risk were reviewed. The major focus of many of these studies was the increase in cancer associated with inhalation exposure. We used the data from these studies to estimate a cancer potency for inhalation exposure to Cr VI. We also evaluated the data on tumors at

multiple sites in these studies to address the extent to which secondary ingestion of particles cleared from the lungs might provide evidence on oral carcinogenicity of Cr VI.

Inhalation studies

In 1998, the U.S. EPA reviewed the available human epidemiological evidence on Cr VI and respiratory cancer risk (U.S. EPA, 1998) and concluded that Cr VI is a strong carcinogen for the respiratory system. The U.S. EPA report also contained a risk quantification (potency estimate) based upon the best data available at the time, from Mancuso (1975). The following discussion focuses on selected studies and reports published since the U.S. EPA review.

Gibb *et al.*, 2000 - Gibb *et al*. (2000) examined mortality rates from lung cancer, prostate cancer, and all cancers combined among 2,357 male chromate production workers first employed between 1950 and 1974. This report was an update of a cohort in Baltimore, Maryland, that was first described by Hayes *et al*. in 1979. The cohort definition used by Hayes *et al*. (1979) was altered by Gibb *et al*. (2000) by including all lengths of employment (instead of a 90-day minimum) and by excluding workers first employed before 1950 (because of less complete exposure information prior to 1950). Observation of the cohort's mortality experience was updated to cover the period 1950 through 1992, and comparison was made to United States and state of Maryland general population cancer rates. Analyses controlled the potentially confounding effects of age, calendar year, gender (males only), and race. The investigators found a statistically significant increased risk of mortality from lung cancer compared to U.S. rates (SMR=1.80, 95 percent CI 1.49-2.14 based on 122 deaths). In contrast, risk of mortality from prostate cancer was only slightly elevated and was statistically consistent with no increased risk (SMR= 1.22, 95 percent CI 0.70-1.98, based on 16 deaths) (note the lower 95 percent CI in the publication is in error; the correct number is given here).

Dose-response for lung cancer was assessed using two methods. The first method was comparison of lung cancer rates for four cumulative exposure categories to Maryland rates using stratification for age, calendar year, gender, and race. A significant monotonic trend was found, with standardized mortality ratios (SMRs) of 0.96, 1.42, 1.57, and 2.24 for "mean cumulative Cr VI" exposures of 0.00045, 0.0042, 0.03, and 0.45 mg Cr_2O_3/m^3-years, respectively. The second method was internal comparison (no external reference population) of lung cancer rates for the same four cumulative dose categories using a proportional hazards regression model to control for age, calendar year, gender, race, and smoking. The regression model showed cumulative dose to be significantly predictive, and the best fit was obtained with log transformation of the four cumulative exposure values. When average rather than cumulative exposure was assessed, poorer model fits resulted, even with log transformation of exposure.

Major strengths of the Gibb *et al.* (2000) study included relatively precise exposure information, a relatively large number of lung cancer deaths, and control of smoking in some analyses. The strengths of the Gibb *et al*. (2000) study make it a better candidate for potency estimation than the 1975 Mancuso study that has been the basis of previous risk quantifications (U.S. EPA, 1998; California Air Resources Board, 1985).

Limitations of the Gibb *et al.* (2000) study included: 1) coding of observed deaths by a single revision of the International Classification of Diseases (ICD 8) when the observation period covered four revisions (ICD 6-9), 2) lack of stratification by, or control of, time-since-first-exposure (TSFE) in the dose-response analyses, 3) unclear calculation of "mean" cumulative exposures (the unit of observation when calculating the mean was not known), and 4) publication of results for just lung and prostate cancers.

Sorahan and Harrington, 2000 - Sorahan and Harrington (2000) updated a cohort of 1,087 chromium platers exposed to chromic acid mist in the United Kingdom that was previously analyzed by Royle in 1975 (Sorahan and Harrington, 2000; Royle, 1975). Mortality rates were calculated for the period 1972-1997 and were compared to rates for England and Wales after adjustment for age, calendar year, and gender.

The investigators found a statistically significant increased risk of lung cancer in men (SMR=1.85, 95 percent CI 1.41-2.38, based on 60 observed deaths) and small, nonsignificant increased risks for several other cancer sites (stomach, large intestine, rectum, nose and sinuses, and prostate). The only measure of exposure was duration of employment, thus the study was not useful for potency estimation.

Luippold *et al.*, 2003; Crump *et al.*, 2003 – Luippold and coworkers evaluated a cohort of 482 worker exposed to Cr VI in a chromate production facility in Painesville, Ohio. The cohort in this study started work after 1940 and was different from the cohort evaluated in the Mancuso (1975, 1997) studies. Fifty-one of the 304 deaths in the cohort were due to lung cancer. The increases in overall and lung cancer (SMR of 239; 95 percent CI 179-313) were statistically significant. A test for trend revealed a strong relationship between lung cancer mortality and cumulative exposure to Cr VI.

Cole and Rodu, 2005 – Cole and Rodu conducted meta-analyses of cancer rate ratios reported in studies of humans ostensibly exposed to Cr VI. The authors included 48 occupational studies with inhalation exposures and one community study with drinking water exposure. The meta-analyses were conducted for lung cancer, stomach cancer, prostate cancer, kidney cancer, central nervous system cancer, leukemia, Hodgkin's disease, and other hematological cancers. Based on the results of the meta-analyses, the authors concluded that Cr VI is a weak cause of lung cancer and not a cause of the other cancers evaluated. OEHHA has concluded, however, that the Cole and Rodu paper is of limited usefulness because it included studies in which there was no exposure to Cr VI (e.g., steel polishers in Jarvholm, 1982), did not include studies in which there was Cr VI exposure (e.g., chromate spray painters in Boice, 1999), and included a study that has since been retracted by the journal that published it (Zhang, 1997; Brandt-Rauf, 2006).

Cancers of ingestion- and digestion-related organs reported in occupational studies

While inhalation is the primary method of exposure to Cr VI in occupational populations, much of what is inhaled is ingested after it is cleared by the mucociliary motion of the upper respiratory tract. Thus there is the potential for digestive and other non-respiratory cancers to be elevated in populations with respiratory exposure. OEHHA conducted a literature search (methods described below) for occupational studies that have reported results for cancers of ingestion and digestion-related organs in order to determine if any

have reported an association with Cr VI exposure. Only published papers and published results within those papers were included in this PHG document.

Identification and Selection of Studies. To identify relevant epidemiology studies, OEHHA searched the automated citation files of PubMed, a service of the National Library of Medicine that includes over 15 million citations for biomedical articles dating back to the 1950s. The citations in PubMed are from the MEDLINE biomedical database and from additional life science journals. The PubMed database was searched using the following string of terms: *(chrome OR chromium OR chromate* OR bichromate* OR dichromate* OR "chromic acid") OR (stainless AND weld*) OR (cement OR concrete OR mason* OR brickmason* OR bricklayer*) OR (chromeplat* OR electroplat* OR "chrome plating" OR "chrome platers") AND (cancer* OR tumor OR tumors OR tumour* OR malignan* OR carcinoma* OR sarcoma*) NOT ("asbestos cement" OR "bone cement" OR trial* OR therap* OR treat* OR vertebroplasty OR implant* OR replace* OR reconstruct*),* with limitation to human studies, journal articles, and titles and abstracts. (Note - the "*" symbol is a PubMed wildcard search feature that includes all endings of words.) The articles were screened to identify epidemiologic studies of occupational populations potentially exposed to hexavalent chromium that reported results for non-respiratory cancers. OEHHA also reviewed the reference lists of major reports from health agencies and of articles that discussed the carcinogenicity of Cr VI within the last 10 years to ensure that no publications were missed [OSHA 2006; IARC 1990; EPA 1998; NTP 2005; ATSDR 2000].

The articles incorporated in the review met the following inclusion criteria: 1) the study focused on occupations or industries that included potential airborne Cr VI exposure (manufacturing of chromates, chromate paint pigments, or ferrochromium; spraying of chromate pigmented paints; chrome plating; stainless steel welding; and manufacturing or use of dry portland cement except asbestos-containing portland cement); 2) the epidemiologic design was cohort-based cancer incidence or mortality rates or proportions; 3) employment was documented by employer, labor organization, or government records; 4) our professional judgment that it was likely that at least half of the employees in the cohort or a subcohort were likely to have been exposed to Cr VI; 5) the article contained results for organ-site-specific categories of non-respiratory cancers; 6) there was no obvious reporting bias of organ site-specific results (e.g., presentation of positive associations only); 7) the statistical analyses controlled for the potentially confounding variables age, calendar time, race, and gender; 8) the data were presented in a complete article or report (as opposed to an abstract only); and 9) the article was the most recent update if more than one article regarding a study population was published.

We used several rules for abstracting data from the articles. If results were presented only for specific categories of sex, race or factory, and no distinction was made in the exposure levels, we combined the observed and expected values for the races, genders, and factories to make a single rate ratio and confidence interval. If results were presented for categories of time since first exposure (TSFE) and for all TSFE, we used the results for all TSFE because few studies presented results for categories of TSFE. Similarly, if results were presented for categories of duration of employment (DOE) and for all DOE, we used the results for all DOE because few studies presented results for categories of DOE. If results were available for a subcohort with substantially higher Cr VI exposure

than the remainder of the cohort, the results for the higher-exposed subcohort were abstracted. For example, in the Axelsson *et al.*(1980) study of ferrochromium manufacturing, arc furnace workers were exposed to higher levels than other workers (0.25 mg/m^3 Cr VI versus a maximum of 0.05 mg/m^3 in other subcohorts); thus the results for the arc furnace workers were abstracted. In the Sorahan *et al.* (1987) study of metal platers, chrome bath workers were said to be "more heavily exposed;" thus the results for the subcohort of workers whose first employment was "chrome bath" were abstracted. If results were presented separately for "hard" and "bright" chrome electroplating processes, the results for "hard" chrome plating were abstracted because Cr VI exposures are known to be higher in hard chrome plating (Guillemin, 1978; Franchini, 1983).

For studies that presented rate ratio estimates and observed numbers of cancers but not expected numbers, we calculated the expected numbers by dividing the observed numbers by the rate ratios. For the rate ratio estimates in the individual studies we calculated 95 percent confidence intervals using the mid-P method for the expectation of a Poisson distribution with the WINPEPI DESCRIBE version 1.36 computer program in the Computer Programs for Epidemiologic Analyses (PEPI) statistical package (Kulkarni, 1998; Abramson, 2004). We calculated mid-P confidence intervals for the studies instead of using the exact intervals presented in the papers because traditional exact Poisson intervals are conservative for hypothesis testing due to the discreteness of the Poisson distribution (Berry, 1995). The mid-P method is recommended for assessing the strength of evidence against the null hypothesis when a distribution is discrete, because the coverage probability for nominally 95 percent confidence intervals averages around 0.95 rather than having 0.95 as a lower bound (Barnard, 1989; Cohen, 1994).

The 30 occupational studies that were examined are listed in Table 8, organized alphabetically by last name of the first author. Seven studies were of chromate chemical manufacturing, six of chrome plating, six of Portland cement manufacturing or concrete mixing, four of chromate pigment production, three of ferrochromium manufacturing, three of stainless steel welding, and two of chromate pigment spray painting. One study (Boice *et al*. 1999) is counted in two manufacturing categories because it reported only combined results for chrome plating and chromate pigment spray painting.

For stomach cancer, three studies reported statistically significant associations. The occupations in the significant studies were chromate production (RR=1.7, 95% CI 1.2-2.3), Portland cement manufacturing (RR=1.8, 95% CI 1.1-2.6), and concrete mixing (RR=1.4, 95% CI 1.2-1.6) (Rosenman *et al.*, 1996, McDowall *et al*. 1984; Kuntsson *et al.*, 2000). In the Rosenman (1996) and Knutsson (2000) studies, lung cancer was also significantly increased, indicating that Cr VI respiratory exposures may have been substantial in those populations. These results are consistent with an association between occupational exposure to Cr VI (via inhalation) and stomach cancer. For cancers of the oral cavity and pharynx, none of nine studies exhibited significant increases in exposed workers. A common limitation of the studies was lack of data on socioeconomic status, which may be associated with stomach cancer as noted by Cole and Radu (2005).

Table 8. Summary of Results for Selected Cancers and Nonmalignant Respiratory Diseases Reported in Studies of Occupational Populations Potentially Exposed to Hexavalent Chromium.

First Author, Year Published, and Country	Industry/ Occupation (minimum duration)	Rate Ratio Method (Control)[1]	Rate Ratio (95 percent Confidence Interval) (Observed/Expected)										
			Non-Respiratory Cancers								Indicator Diseases[@]		
			Oral Cav. & Pharynx (ICD 140-149)[*]	All Digestive (ICD 150-159)	Esophagus (ICD 150)	Stomach (ICD 151)	Small Intestine (ICD 152)	Colon (ICD 153)	Rectum (ICD 154)	Liver and Gall Bladder (ICD 155-156)	Pancreas (ICD 157)	Lung Cancer (ICD 162)	Nonmalig. Resp. Dis. (ICD 460-519)
Amandus 1986 United States	Portland cement manufacturing	SMR (United States)				1.35 (0.90-1.93) (27/20.1)							
Axelsson 1980 Sweden	Ferrochromium manufacturing arc furnaces (1 year)	SMR (county of factory)				0.78 (0.25-1.89) (4/5.1)		0.83[4] (0.14-2.75) (2/2.4)	0.00 (0.00-2.00) (0/1.5)			0.42 (0.02-2.05) (1/2.4)	0.60 (0.22-1.32) (5/8.4)
Becker 1999 Germany	Stainless steel welding coated electrodes (6 months)	SMR (Germany)	1.07 (0.06-5.48) (1/0.9)	0.66 (0.29-1.31) (7/10.6)	1.21 (0.06-6.16) (1/0.8)	0.59 (0.08-1.54) (2/4.3)		0.00 (0.00-1.30) (0/2.3)	1.51 (0.26-5.08) (2/1.3)	0.00 (0.00-3.33) (0/0.9)	1.38 (0.24-4.72) (2/1.4)	1.22 (0.64-2.12) (11/9.0)	1.09 (0.51-2.08) (8/7.3)
Birk 2006 Germany[10]	Chromate production	SMR (Germany)	0.49 (0.03-2.43) 1/2.03	0.62 (0.32-1.11) 10/16.06		0.50 (0.08-1.64) 2/4.04		1.08 (0.34-2.60) 4/3.71"	1.02 (0.17-3.39) 2/1.95		0.41 (0.02-2.00) 1/2..46	1.48 (0.95-2.21) 22/14.83	0.22 (0.04-0.72) 2/9.14
Boice 1999 United States	Chrome plating and chromate painting aircraft manufacturing (1 day)[19]	SMR (California white and US nonwhite)	0.14 (0.01-0.69) (1/7.14)		1.04 (0.48-1.98) (8/7.69)	1.03 (0.54-1.79) (11/10.7)		1.02 (0.66-1.50) (23/22.6)	1.08 (0.44-2.24) (6/5.56)	1.07 (0.47-2.12) (7/6.54)	1.00 (0.57-1.64) (14/14.0)	1.02 (0.82-1.26) (87/85.3)	0.98 (0.79-1.21) (88/89.8)
Dalager 1980 United States	Zinc chromate spray painting of aircraft (3 months)	PMR (United States)	2.50 (0.64-6.80) (3/1.2)	1.00 (0.53-1.74) (11/11.0)								1.84[12] (1.17-2.77) (21/11.4)	0.68 (0.42-1.57) (9/10.5)

First Author, Year Published, and Country	Industry/ Occupation (minimum duration)	Rate Ratio Method (Control)[1]	Rate Ratio (95 percent Confidence Interval) (Observed/Expected)										
			Non-Respiratory Cancers								Indicator Diseases[@]		
			Oral Cav. & Pharynx (ICD 140-149)[*]	All Digestive (ICD 150-159)	Esophagus (ICD 150)	Stomach (ICD 151)	Small Intestine (ICD 152)	Colon (ICD 153)	Rectum (ICD 154)	Liver and Gall Bladder (ICD 155-156)	Pancreas (ICD 157)	Lung Cancer (ICD 162)	Nonmalig. Resp. Dis. (ICD 460-519)
Danielsen 1996 Norway	Stainless steel boiler welders (ever)	SIR (Norway)				1.03 (0.26-2.82) (3/2.9)		1.21 (0.39-2.92) (4/3.3)	1.82 (0.58-4.39) (4/2.2)	0.00[3] (0.00-9.99) (0/0.3)		1.03 (0.42-2.15) (6/5.8)	
Davies 1991 England & Scotland	Chromate production (1 year)	SMR (England, Wales, & Scotland)[11]	2.17 (0.88-4.51) (6/2.77)		1.62 (0.79-2.97) (9/5.56)	0.73 (0.45-1.12) (19/26.11)		0.62 (0.27-1.23) (7/11.33)	1.02 (0.47-1.94) (8/7.85)	1.56 (0.57-3.46) (5/3.20)	1.71 (0.90-2.97) (11/6.44)	1.97 (1.69-2.27) (175/89.0)	1.21[13] (0.95-1.61) (55/44.13)
Deschamps 1995 France	Lead and zinc chromate pigment production (6 months)	SMR (northern France)	0.52 (0.03-2.54) (1/1.94)	1.30 (0.64-2.39) (9/6.91)	1.48 (0.38-4.02) (3/2.03)	1.52 (0.26-5.04) (2/1.31)		3.08[4] (0.98-7.42) (4/1.30)	0.00 (0.00-1.73) (0/1.73)	0.00 (0/0.31) (0.00-9.66)	0.00 (0.00-4.34) (0/0.69)	3.60 (2.20-5.58) (18/5.0)	0.43 (0.11-1.18) (3/6.94)
Enterline 1974 United States	Chromate production (not stated)	SMR (United States)		1.53 (0.91-2.45) (16/10.4)								9.43 (7.41-11.89) (69/7.3)	1.45 (0.81-2.43) (13/8.9)
Franchini 1983 Italy	"Hard" chrome plating[18] (1 year)	SMR (Italy)				3.33 (0.69-9.74) (3/0.9)						4.29 (1.09-11.66) (3/0.7)	
Hayes 1979[7] United States	Chromate production (90 days)	SMR (Baltimore)		0.60[5] (0.33-1.00) (13/21.7)								2.02 (1.55-2.59) (59/29.2)	0.67 (0.40-1.05) (17/25.33)
Hayes 1989 United States	Chromium pigment production (1 month)	SMR (United States)		1.11 (0.72-1.82) (18/15.3)		1.79 (0.73-3.73) (6/3.35)						1.84 (1.11-2.89) (17/9.23)	0.53[6] (0.32-0.83) (17/32.1)

First Author, Year Published, and Country	Industry/ Occupation (minimum duration)	Rate Ratio Method (Control)[1]	Rate Ratio (95 percent Confidence Interval) (Observed/Expected)										
			Non-Respiratory Cancers								Indicator Diseases[@]		
			Oral Cav. & Pharynx (ICD 140-149)[*]	All Digestive (ICD 150-159)	Esophagus (ICD 150)	Stomach (ICD 151)	Small Intestine (ICD 152)	Colon (ICD 153)	Rectum (ICD 154)	Liver and Gall Bladder (ICD 155-156)	Pancreas (ICD 157)	Lung Cancer (ICD 162)	Nonmalig. Resp. Dis. (ICD 460-519)
Horiguchi 1990 Japan	Chrome plating, hard or bright not stated (1 day)	SMR (Osaka Prefecture)				1.30 (0.22-4.29) (2/1.54)						1.74 (0.09-8.65) (1/0.57)	
Itoh 1996 Japan	Chrome plating, hard or bright not stated	SMR (Japan)			0.49 (0.02-2.35) (1/2.1)	0.79 (0.39-1.45) (9/11.4)				0.99[3] (0.40-2.05) (6/6.1)	0.00 (0.00-1.30) (0/2.3)	1.81 (1.03-2.98) (14/7.7)	
Jakobsson 1993 Sweden	Portland cement manufacturing	SIR (Sweden)		1.16 (0.88-1.49) (56/48.4)		1.01 (0.57-1.65) (14/13.9)		1.55 (0.96-2.37) (19/12.3)	1.26 (0.70-2.10) (13/10.3)		1.13 (0.46-2.34) (6/5.33)	1.07 (0.58-1.82) (12/11.2)	
Kano 1993 Japan	Chromium pigment production (1 year)	SMR (Japan)			2.20 (0.37-7.26) (2/0.91)	1.20 (0.56-2.30) (8/6.66)		2.33 (0.39-7.68) (2/0.86)			1.00 (0.05-4.93) (1/1.00)	1.02 (0.26-2.77) (3/2.95)	1.12 (0.36-2.71) (4/3.56)
Knutsson 2000 Sweden	Concrete mixing	SIR	1.21 (0.99-1.46) (103/84.9)		1.03 (0.77-1.37) (46/44.5)	1.39 (1.22-1.57) (243/174.6)		0.80 (0.69-0.93) (187/232.7)	1.01 (0.86-1.17) (167/165.5)	0.98[3] (0.78-1.21) (81/82.9)	0.98 (0.80-1.18) (108/110.1)	1.25 (1.14-1.37) (473/378.0)	
Korallus 1993 Germany[10]	Chromate production (1 year)	SMR (North Rhine-Westphalia)				1.27 (0.75-2.02) (16/12.6)						2.07 (1.63- 2.59) (75/36.3)	0.47 (0.29-0.74) (17/35.9)
Langard 1979 (digestive) & 1983 (lung) Norway	Chromium pigment production (3 years)	SIR[14] (Norway)		6.38 (1.63-17.37) (3/0.47)								44.44 (18.01-92.44) (6/0.135)	

First Author, Year Published, and Country	Industry/ Occupation (minimum duration)	Rate Ratio Method (Control)[1]	Rate Ratio (95 percent Confidence Interval) (Observed/Expected)										
			Non-Respiratory Cancers								Indicator Diseases[@]		
			Oral Cav. & Pharynx (ICD 140-149)[*]	All Digestive (ICD 150-159)	Esophagus (ICD 150)	Stomach (ICD 151)	Small Intestine (ICD 152)	Colon (ICD 153)	Rectum (ICD 154)	Liver and Gall Bladder (ICD 155-156)	Pancreas (ICD 157)	Lung Cancer (ICD 162)	Nonmalig. Resp. Dis. (ICD 460-519)
Langard 1990 Norway	Ferrochromium manufacturing (1 year)	SIR (Norway)				1.40 (0.61-2.77) (7/5.0)			0.80 (0.13-2.64) (2/2.5)		1.58 (0.40-4.30) (3/1.9)	1.54 (0.78-2.74) (10/6.5)	
McDowall 1984 United Kingdom	Portland cement manufacturing	SMR (England and Wales)			1.16 (0.30-3.15) (3/2.59)	1.75 (1.12-2.61) (22/12.57)		0.89 (0.36-1.85) (6/6.74)	1.86 (0.94-3.31) (10/5.38)			0.85 (0.58-1.21) (28/32.94)	0.86 (0.66-1.10) (60/69.77)
Moulin 1990 France	Ferrochromium & stainless steel manufacturing (some workers exposed to PAHs) (1 year)	SMR (France)	0.58[9] (0.15-1.59) (3/5.14)		0.00 (0.00-1.36) (0/2.20)	2.75 (0.87-6.61) (4/1.46)		0.00[4] (0.00-2.52) (0/1.19)	0.00 (0.00-4.61) (0/0.65)		0.00 (0.00-3.70) (0/0.81)	2.04 (1.07-3.54) (11/5.40)	0.00 (0.00-0.79) (0/3.80)
Rafnsson 1997 Iceland	Concrete mixing and spraying	SIR (Iceland)			1.15 (0.23-3.35) 3/2.62	1.08 (0.43-2.58) (21/19,47)	4.23 (0.85-12.35) (3/0.71)	0.56 (0.18-12.35) (5/8.91)	1.06 (0.28-2.70) (4/3.79)		1.49 (0.60-3.06) (7/4.71)	1.69 (1.09-2.49) (25/14.81)	
Rosenman 1996 United States	Chromate production[15] (1 day)	PMR (United States)		1.25 (1.05-1.48) (130/103.7)	1.15 (0.74-2.05) (15/11.8)	1.66 (1.17-2.29) (34/20.5)		1.15[16] (0.86-1.51) (48/41.7)		1.43[17] (0.75-2.48) (11/7.7)		1.93 (1.69-2.19) (224/115.9)	1.01 (0.83-1.20) (113/112.1)
Satoh 1981 Japan	Chromate production (1 year)	SMR (Japan)				0.95 (0.50-1.65) (11/11.58)				1.22[2] (0.31-3.33) (3/2.45)		9.46 (6.31-13.66) (26/2.75)	

First Author, Year Published, and Country	Industry/ Occupation (minimum duration)	Rate Ratio Method (Control)[1]	Rate Ratio (95 percent Confidence Interval) (Observed/Expected)										
			Non-Respiratory Cancers								Indicator Diseases[@]		
			Oral Cav. & Pharynx (ICD 140-149)[*]	All Digestive (ICD 150-159)	Esophagus (ICD 150)	Stomach (ICD 151)	Small Intestine (ICD 152)	Colon (ICD 153)	Rectum (ICD 154)	Liver and Gall Bladder (ICD 155-156)	Pancreas (ICD 157)	Lung Cancer (ICD 162)	Nonmalig. Resp. Dis. (ICD 460-519)
Sjogren 1987 Sweden	Stainless steel welding mostly coated electrodes (5 years)	SMR (Sweden)							4.31 (0.72-14.24) (2/0.46)			2.49 (0.91-5.51) (5/2.01)	
Smailyte 2004 Lithuania	Portland cement manufacturing	SIR (Lithuania)	1.30 (0.60-2.47) (8/6.15)			0.77 (0.25-1.86) (4/5.18)		0.77 (0.28-1.70) (5/6.51)	1.25 (0.61-2.29) (9/7.22)		0.90 (0.29-2.17) (4/4.44)	1.51 (1.12-1.99) (47/31.1)	0.83[20] (0.51-1.27) (19/23.0)
Sorahan 1987 United Kingdom (Midlands)	"Bright" chrome plating baths (6 months)	SMR (England & Wales)				1.49 (0.84-2.44) (14/9.4)				5.00 (0.84-16.52) (2/0.4)		1.85 (1.40-2.41) (52/28.1)	1.10 (0.81-1.44) (47/42.9)
Sorahan 2000 United Kingdom (Yorkshire)	Chrome plating, hard or bright not stated (3 months)	SMR (England & Wales)				1.56 (0.84-2.65) (12/7.7)		1.14[4] (0.53-2.17) (8/7.0)	1.40 (0.57-2.90) (6/4.3)			1.79 (1.39-2.28) (62/34.6)	

[*] Code ranges for the 9th ICD Revision are presented for illustration; the actual ICD revisions used in the studies ranged from 5th through 9th.

[@] Lung cancer is an indicator of Cr VI inhalation exposure and nonmalignant respiratory disease is an indicator of heavy cigarette smoking.

[1] SMR = standardized mortality ratio, SIR = standardized incidence ratio, PMR = proportionate mortality ratio, PCMR = proportionate cancer mortality ratio. "Control" is the comparison population.

[2] The liver cancer category in Satoh *et al.* 1981 was labeled "Liver" with no mention of gall bladder and an incompatible ICD code (157, which was pancreatic cancer in the 8th Rev. ICD used by the study).

[3] Liver cancer only (gallbladder excluded).

[4] Included cancer of the small intestine.

[5] Hayes *et al*. 1979 used a nonstandard ICD code grouping for all digestive cancer (ICD codes 140-154, instead of 150-159) which included buccal cavity and pharynx, and excluded biliary passages, liver, gall bladder, pancreas, and peritoneum.

[6] In Hayes *et al*., 1989 the nonmalignant respiratory diseases SMR was for all factory employees (not limited to workers with 1 month or more Cr+6 exposure).

[7] The Baltimore cohort studied by Hayes *et al.* (1979) was updated by Gibb *et al*. (2000), but only Hayes *et al*. reported findings for cancers of the buccal cavity, pharynx, or digestive system.

[9] Included laryngeal cancer.

[10] There was some overlap between the Birk et al 2006 studies and the Korallus et al. 1993 studies. Many of the cohort members in Birk (2006) were part the study by Korallus (1993), but Birk et al. excluded employees working before completing a changeover to a no-lime process and added more recently hired employees.
[11] Expected deaths adjusted for geographic area and social class.
[12] Included all respiratory cancer (7th ICD 160-164).
[13] Chronic obstructive airways disease portion adjusted for geographic area and social class.
[14] Follow-up through 1980 for lung cancer and through 1975 for digestive system cancers.
[15] Rate ratio abstracted for men only because the investigators said women were likely to have had office jobs not directly involved with production.
[16] Included rectal cancer.
[17] Described as "liver" cancer in the article, thus may not have included gall bladder.
[18] Franchini 1983 gave results for "hard" and "bright" plating. The hard plating results were abstracted because the investigators said Cr+6 exposures were much higher in hard plating.
[19] Minimum of one year of employment at the facility, of which as little as one day could have involved exposure to chromate.
[20] In Smailyte 2004, the nonmalignant respiratory disease finding was for mortality rather than incidence.

Gatto *et al.* (2010) published a meta-analysis of 32 studies of cancer in populations with potential occupational exposures to Cr VI. The investigators focused on results of the studies for ingestion- and digestion-related organs, citing possible redistribution of inhaled Cr VI to the GI tract as one reason for their interest. They found that none of the summary rate ratios (RR) were statistically significantly elevated, as follows: oral cavity RR=1.0 (95% CI = 0.8–1.3); esophagus RR=1.2 (95% CI = 0.9–1.5); stomach RR=1.1 (95% CI = 0.9–1.3); colon RR=0.9 (95% CI = 0.7–1.1); and rectum RR=1.2 (95% CI = 0.98–1.4). Analyses of more highly exposed subgroups based on geographic region, industry, and occupational exposure categories did not result in significantly elevated summary RRs, except that esophageal cancer was significantly elevated among U.S. occupational populations (RR = 1.5, 95% CI = 1.1–2.1) (the investigators noted that the result was based on only four studies, one of which was a PMR study).

The Gatto *et al.* (2010) summary results may have been affected by inclusion of occupational populations with little or no exposure to Cr VI. For example, three populations of leather tanning workers were included, but there was potential for Cr VI exposure only if an older two-bath tanning process that had chromate in the first vat was used. Even the two-bath process may have entailed little Cr VI exposure, as animal skins were generally stirred with paddles, and OEHHA has been unable to find reports of misting, bubbling, or other aerosol generation. Respiratory exposures may have occurred only to workers who poured dry sodium or potassium dichromate into the first vat. According to Constantini *et al*. (1989), in Italy the two-bath tanning process has not been generally used since the Second World War. According to Stern *et al*. (1987), in the U.S., exposure to Cr VI has been minimal since 1940. Of the leather tanning populations included in the meta-analysis by Gatto *et al.*, only the tanners studied by Pippard *et al.* (1985) appear to have been potentially significantly exposed to Cr VI, as employment in the year 1939 was required. In the included Iaia *et al.* (2006) study of leather tanners followed through 1998, only 21% of the tanners were observed more than 20 years, only 7% were deceased at the end of the study, and the rate ratio for lung cancer was not increased (RR=0.4, 95% CI 0.2-0.9), all of which suggest that few tanners in the cohort were employed in tanning prior to WW II when the two-bath process was being used (Iaia *et al.* did not provide data on calendar years of employment). In the included Montanaro *et al.* (1997) study of leather tanners followed through 1994, the authors stated that cancer risk "among workers employed in earlier periods was hard to evaluate due to the few person-years."

Conversely, the Gatto *et al.* (2010) summary results may have been affected by exclusion of occupational populations with significant exposure to Cr VI. Notably, occupations exposed to Portland cement were generally excluded (the one exception was a study of masons by Rafnsson *al.*, 1997). Portland cement has been known to contain Cr VI since at least 1950, when Jaeger *et al.* (1950) demonstrated the presence of water extractable chromate in Swiss cements, and reported that 30 of 32 men with cement dermatitis were allergic to chromates in patch testing. Hexavalent chromium is created inadvertently during the cement manufacturing process from Cr III in raw materials and chrome steel grinders. According to Kjuus *et al.* (2003), the Cr III is transformed into Cr VI because of alkaline conditions, high kiln temperature, and presence of air in the kiln. Many investigators have reported levels of Cr VI in bulk samples of Portland cement. For

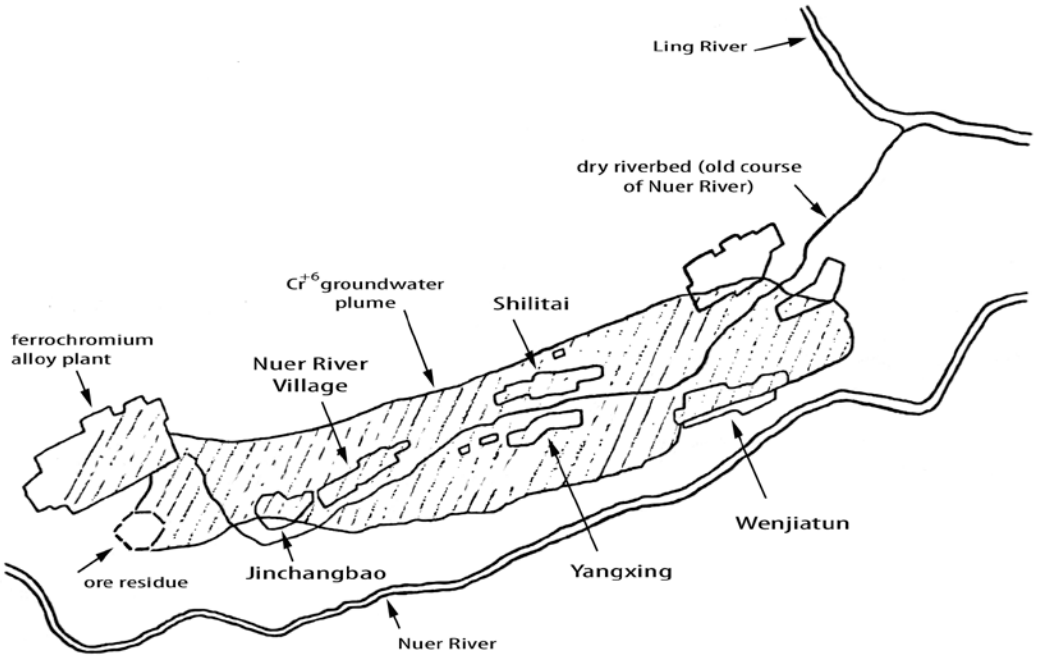
example, Rafnsson *et al.* (1986) reported Cr VI concentrations in the range of 5.8 – 9.4 mg/kg in cement used by masons in Iceland in 1983. A few studies have reported breathing-zone concentrations. Vestbo *et al.* (1991) in Denmark reported mean Cr VI concentrations (in dust by weight) of 9 mg/kg in personal air samples in 1975 among Portland cement manufacturing workers, and said that after 1981 all cements were "*chromate-reduced*," resulting in mean Cr VI concentrations lower than 2 mg /kg. Further evidence of Cr VI in Portland cement comes from the fact that many countries now limit the amount of Cr VI in Portland cement in order to prevent chromate-sensitive dermatitis from handling wet cement and concrete (a chemical reducing agent such as ferrous sulfate is commonly added during the manufacturing process to lower the Cr VI content). Amandus *et al.* (1986), who studied cancer rates at 23 Portland cement manufacturing plants in the U.S., mentioned that Cr VI had been found in dust samples and that the study's purpose was "to evaluate the hypothesis that exposure to cement dust is associated with an increased risk of death from stomach cancer." Thus it seems reasonable to include studies of Portland cement-exposed workers in a meta-analysis of Cr VI-exposed workers. It also would have been helpful to include the Danielsen *et al.* (1996) study of stainless steel welders and the results of studies for "all digestive" cancers, such as the studies by Enterline *et al.* (1974) and Langard *et al.* (1979) of chromate pigment production workers.

Ingestion studies

Two human studies were identified that reported organ-specific cancer rates in a population demonstrably exposed to Cr VI in drinking water. The first, by Zhang and Li (1987), investigated the occurrence of cancer in rural villages (Figure 15) near JinZhou, China and reported an increase in stomach cancer. A reevaluation of these data concluded the increase in cancer was unrelated to the exposure to chromium in drinking water (Zhang and Li, 1997, later retracted by Brandt-Rauf, 2006, and replaced with Kerger *et al*., 2009). OEHHA's reevaluation (Beaumont *et al*, 2008), based on the findings in the original report (Zhang and Li, 1987) as well as other reports (JHAS, 1979; Zhang and Li, 1986; Zhang and Li, 1980), is summarized below.

The source of the contamination was a chromium ore processing facility. Releases of Cr VI began around 1960 and full-scale production, which began in 1965, was associated with dramatic increased releases of production wastes. The releases were reportedly not fully controlled until 1980–1982. Groundwater from wells in two villages near the plant began to appear yellow (contaminated) in 1964. The movement of groundwater contamination appeared to be rapid and by the end of 1965, groundwater contamination had expanded to approximately half (41 percent) of the wells in the nearest village and 96 percent of the wells in the second nearest village. The detection of high levels of Cr VI in groundwater samples does not necessarily indicate that all of the population in the area was exposed to high chromium levels.

Figure 15. Approximate locations in 1979 of the ferrochromium alloy plant, ore residue pile, Cr VI groundwater contamination plume (shaded area), and five villages included in a study of cancer mortality 1970-1978. Adapted from Figure 3 of the report *Chromium Contamination in the City of Jinzhou* (Zhang and Li, 1986).



Kerger and coworkers and the retracted 1997 report noted that the villages closest to the plant with higher levels of Cr VI in drinking water in 1965 had lower cancer rates over the period 1970-1978 than villages with lower levels of Cr VI, and concluded that the risk of cancer was probably unrelated to exposure to Cr VI (Zhang and Li, 1997; Kerger *et al*., 2009). However, based on the recently available reports from China, this conclusion does not appear to be credible. First, it did not address the actual pattern of exposures to Cr VI during the entire period. In villages nearest to the contamination source, the water from some of the wells became essentially unpalatable in 1965 and was not necessarily consumed, while populations down-gradient may have continued to drink the well water. Second, the proportion of wells contaminated in each village (and the proportion of people exposed) is likely to have increased as the plume spread out down-gradient. Third, the reduction of contamination at the source may have resulted in a peak of the contaminant moving down-gradient over the study period. This pattern would be consistent with elevated levels of cancer in the more distant villages.

The Kerger *et al*. (2009) analysis is principally based on the findings of 1965 sampling (samples collected right after high levels of contamination were first detected in wells near the plant) to characterize exposure that occurred over the entire duration of the study

(over a decade). Wells in the more distant villages which would have been responsible for oral exposure to Cr VI in those villages were not contaminated in 1965. By using data collected in 1965, it is assumed that the distant wells never became contaminated over the entire study period. In essence, the Kerger *et al*. analysis assumed that after 1965, when contaminated groundwater had rapidly moved from the plant to the nearest villages, the movement of contamination stopped.

Beaumont *et al.* (2008) did not find that assumption to be plausible. While the precise date that contamination was first released from the plant is unknown, it is clear that the contamination moved with the flow of groundwater, and its movement was rapid, impacting nearby villages by 1965. It is unlikely that once the contamination reached the nearest villages, it stayed there, and did not impact wells in the more distant villages, while groundwater continued to flow.

Given that almost no sampling of the wells occurred after 1965, Beaumont and coworkers did not ascribe exposure in the individual villages based on the results of the 1965 sampling. Their analysis was based on comparing cancer rates in the impacted villages with nearby unimpacted reference populations. There is good evidence that the residents of the villages were exposed to Cr VI but the magnitude of the exposure is very unclear. Given the uncertainties regarding the levels of Cr VI in groundwater after 1965, no conclusions are warranted concerning whether certain villages were exposed to more Cr VI than other villages.

OEHHA combined the population and cancer data for the five villages with documented Cr VI drinking water contamination to form a single exposed population. Rates for mortality from all cancer, or stomach cancer in the combined exposed villages, were compared to the rates in Liaoning Province (in which the villages were located) by calculating rate ratios (rate in combined exposed villages/rate in province). Rates for the province adjusted to the 1964 age distribution of China were obtained from the *Atlas of Cancer Mortality in the People's Republic of China, rates for 1973–75* (Editorial Committee for the Atlas of Cancer Mortality, 1979). Exact mid-P 95 percent confidence intervals and 2-sided hypothesis test probabilities were calculated for 70 or fewer deaths, and approximate Fisher's confidence intervals and probabilities for more than 70 deaths, using the PEPI Describe program for the Poisson distribution (Abramson, 2004).

The rate ratio (RR) for all cancers combined (1.23; 95 percent CI=0.97-1.53) was slightly elevated when compared to the rate in the whole province and not statistically significant ($p = 0.078$). The rate ratio for stomach cancer compared to the province (1.69; 1.12-2.44), was higher and statistically significant ($p = 0.013$).

The Zhang and Li (1987) findings have several important limitations. The study employed an ecological epidemiological design, in which cancer rates in geographic areas were compared without data on exposure to individual residents. It is likely that not all persons in the villages classified as exposed were actually exposed to contaminated drinking water (not all wells were contaminated). Another limitation was the study's relatively short observation time (14 years) after residents first noticed the yellow color of the water, which would limit the study's ability to detect increases in cancer. Other limitations of the study included lack of data on factors that could be related to the risk of

mortality from stomach cancer, including cigarette smoking, diet, *Helicobacter pylori* infection of the stomach, and socioeconomic status.

While the study had substantial limitations, it is clear that Cr VI was released from the alloy plant, that underground water became contaminated, and that the contaminated water was used as a source of drinking water in villages adjacent to the plant. Additional information resulting from a thorough groundwater hydrological investigation, information whether certain villages were provided alternative sources of drinking water, and information on the effectiveness of remedial measures could be employed to yield a more complete exposure analysis.

The relationship between the levels of chromium (total) and other metals in drinking water throughout Nebraska in 1986-1987 and mortality from cancer and other diseases was investigated using records from Nebraska counties (Bednar and Kies, 1991). No correlation between cancer and the level of chromium in drinking water was found. An important limitation of this study was that the sampling in the study occurred for only two years. Unlike the Zhang and Li (1987) study, no specific source of Cr VI exposure was identified. Also, while overall cancer mortality rates were evaluated, tumors at specific sites were not.

Armienta-Hernandez and Rodriguez-Castillo (1995) measured chromium in groundwater, soil, and human urine from releases from a chromate compounds factory and tanneries in the Leon valley in central Mexico. Unfortunately the investigators did not conduct an epidemiologic study of cancer. The study was helpful, however, with respect to the possibility of self-limited exposure at high concentrations. The investigators reported that residents did not want to consume water with Cr VI concentrations above 0.5 mg/L because of its yellowish color.

A possible relationship between Cr VI exposure and overall and lung cancer mortality was investigated in communities adjacent to cooling towers that used Cr VI (Fryzek *et al.*, 2001). Rates of cancer in the nearby communities were not significantly elevated when compared to more distant communities. The Public Health Assessment for the Pacific Gas and Electric (a/k/a Hinkley site) power plant (DHHS, 2000) found that "Twelve families consisting of 46 individuals were determined to have used eight contaminated drinking water wells." At least nine of the 46 individuals were less than 10 years old. How much exposure this population received and for how long is unclear. Given that a very small population was exposed to Cr VI from the facility, it is not clear whether increases in tumors could have been detected.

The rate of incident cancer of all types combined over the years 1996-2008 in the census tract that included Hinkley, California, were the subject of a preliminary surveillance report from the California Cancer Registry in March, 2011 (Morgan, 2011). Historically, some drinking water in Hinkley has been contaminated with Cr VI from cooling water released from a natural gas compression facility. A total of 196 cancer cases occurred during the study period, when 224.2 were expected based on state-wide rates (rate ratio = 0.87). Unfortunately, results were not presented for specific types of cancer, so it is possible that there was an excess of a specific type of cancer.

An epidemiologic study performed in Greece is the second study examining the relationship between Cr VI in drinking water and organ-specific mortality from cancer. Linos *et al.* (2011) conducted a historical cohort study of mortality rates among selected residents of the Oinofita municipality. Cr VI contamination from industrial wastes had been detected previously in some drinking water samples.

A chronology of events related to the Cr VI contamination (including concentrations found in sampling) and the mortality study is shown in Table 9 and briefly described here. Starting in approximately the early 1970s, the Oinofita region of Greece transformed from a rural region into a major industrial region and became a municipality containing four villages. For a period of time liquid industrial wastes were discharged without restriction into the Asopos River that runs through the region. Aquifers that supplied wells in the region may have been contaminated with Cr VI from either the Asopos River and/or direct injection of industrial wastes containing Cr VI (Economou-Eliopoulos *et al.*, 2011). Cr VI concentrations in drinking-water samples taken previously in the municipality ranged widely, with the five highest concentrations reported in the Linos *et al.* (2011) article being 44, 48, 51, 53, 54, and 156 μg/l. The article did not present details of the water sampling methods in the previous studies, such as whether the water sampling was meant to be representative of the supply system or was worst-case sampling. In 2007, fines were imposed on 20 businesses in the municipality for disposing waste with "high levels" (not defined in article) of Cr VI into the Asopos river.

The exposed cohort was defined as all people who were ever legally registered as a citizen and also as a permanent resident in Oinofita municipality during the period 1/1/1999 – 12/31/2009 (11 years). The municipality's electronic records were searched to find all persons meeting this cohort definition. The resulting cohort was comprised of 5842 individuals. The mortality observation period was also 1999-2009 (same as the cohort eligibility period). Person-time at risk in the Oinofita cohort was calculated starting from either 1/1/1999 for individuals registered in the municipality before this date, or the date of registration for those who registered after 1/1/1999. Person-time accumulation ended on the date of death, the date of deletion from the records because of registering in another municipality, or the end of the mortality observation period (December 31, 2009).

The Linos *et al.* (2011) article did not describe how deaths among cohort members were identified, other than to say that "the municipality's vital statistics department maintained death certificates" and "death certificates of each individual … were obtained from the local vital statistics registry and from burial records of the local church." Cause of death was coded using the four-digit ICD-9 classification system by one of the investigators.

For statistical analysis, the person-time and deaths in the cohort were stratified by sex, age (five-year categories), and calendar year (one-year categories). Expected numbers of deaths were calculated by multiplying corresponding rates in surrounding Voiotia prefecture (described as similar in population density, socioeconomic level, and ethnicity) times the person-time in the strata. The article presented a table showing the distribution of the cohort's person-time by age, gender, and year, and it was notable that the age

distribution was rather young, with over 50 percent of the person-time occurring under the age of 40.

Table 9. Chronology of events related to Cr VI contamination of drinking water and study of cancer mortality in the Oinofita municipality of Greece, paraphrased from the article by Linos *et al.* (2011).

Year of Event	Location of Event	Description of Event
Prior to 1970s	Oinofita	Area was rural and had four villages.
Early 1970s	Oinofita	The villages transformed into industrial areas.
1969-early 1970s	Asopos River	Liquid industrial wastes began to be discharged into the river.
1984+	Oinofita	Industrial growth in municipality accelerated due to new restrictions on types of industries allowed in a neighboring prefecture.
1990s or earlier	Oinofita drinking water	Citizens began to complain about the color and turbidity of the drinking water.
1996	Oinofita drinking water	A Cr VI concentration of 54 µg/l was found in one water sample by the Oinofita municipality.
1/11999	*Oinofita study cohort*	*Start of cohort eligibility (resided in Oinofita municipality at any time in 1999-2009)* *Start of mortality observation period (1999-2009).*
2007	Asopos River	20 industries were fined "for disposing waste with high levels of CrVI into the Asopos river."
2007-2008	Oinofita wells	Cr VI concentrations were 10 µg/l or greater in 35 water samples (out of 87) "taken from different wells, with a maximum value 156 µg/l," in a study by the Institute of Geology and Mineral Exploration. The total number of wells was not stated.
2007-2008	Oinofita drinking water	Cr VI concentrations were above 8 µg/l in all 16 samples collected in 2007 and 2008 by the Oinofita municipality.
2007-2010	Oinofita drinking water	Cr VI levels were above 10µg/l in13 samples of water, with a maximum value of 51 µg/l (study by the Oinofita municipality). The total number of samples was not stated.

2008	Oinofita drinking water	Cr VI levels ranged from 41 to 53 μg/l in three samples of public drinking water in a study by the University of Athens. The total number of samples was not stated.
12/31/2009	*Oinofita study cohort*	*End of cohort eligibility period.* *End of mortality observation period.*
2009	Oinofita drinking water	Oinofita begins to receive water from a lake (that also supplies Athens) as part of its water supply.

The investigators reported results for 15 specific cancer categories and five residual ("other") cancer categories. The most striking finding was a statistically significant rate ratio for primary liver cancer mortality (RR=11.0, $p<0.001$, based on 6 deaths). When the liver cancer data were stratified by sex, significant associations were found separately for males (RR=8.1, $p=0.003$, based on 4 deaths) and females (RR=39.5, $p=0.002$, based on 2 deaths).

It was notable that no significant associations were found for stomach cancer and other cancers that might be hypothesized to be at increased risk because of previous Cr VI epidemiology and animal cancer bioassay studies.

An unfortunate but non-serious limitation of the Linos *et al.* (2011) article was confusion about the study design. The article's title, abstract, and introduction implied that the design was ecological, but the methods section clearly describes a cohort study. The use of the word ecological was unfortunate because only people who read the full article will understand that it was a cohort study. The authors may have been confused by the fact that, while their exposure variable was ecological (residence in the municipality), other variables were at the individual level (e.g., sex, age, calendar year).

One validity issue in the study appeared to be accuracy of person-time calculation. The investigators noted that in Greece, one can move into a new place of residence without registering in the new municipality for some period of time. When this occurs, apparently the old municipality doesn't know the person has moved out.

Another validity issue was that the article did not describe how deaths among cohort members were identified outside of the Oinofita municipality. There may have been no method of identifying deaths that occurred among cohort members who moved out of the municipality after they moved out. If so, and person-time continued to accumulate for those members, a bias in mortality rates could occur. The bias would affect all categories of death, however, not just liver cancer.

Sensitive Subpopulations

Toxicokinetic studies suggest that absorption of Cr VI following oral exposure is substantially reduced by acidic stomach juices that facilitate the conversion of Cr VI to Cr III (Donaldson and Barreras, 1966). Little Cr III is absorbed from the gut (Donaldson and Barreras, 1966). Therefore, human populations that are characterized by elevated pH

in the stomach are likely to experience increased absorption of Cr VI, and this factor is likely to be responsible for much of the observed variability in gastrointestinal absorption of Cr VI.

Early life exposures to carcinogens may result in greater lifetime risk of cancer compared to exposures later in life (OEHHA, 2009b). Infants' stomachs are near neutral pH during the first days to weeks after birth, and stomach pH levels generally remain higher than adults during the first three months of life (OEHHA, 2001).

There are a variety of human conditions in which gastric acid production is dramatically decreased, including pernicious anemia (10-20 cases/100,000 people of Celtic and Scandinavian descent), pancreatic tumors, infection with *Helicobacter pylori*, mucolipidosis type IV, and some autoimmune diseases (Isselbacher *et al.*, 1994). Increased absorption of Cr VI was observed in humans with pernicious anemia (Donaldson and Barreras, 1966).

A considerable fraction of the population consumes medications which raise gastric pH, either by reducing production of gastric acid or by neutralizing acid. Common disorders treated with these agents include gastroesophageal reflux disease, peptic ulcer disease, and chronic gastritis (Isselbacher *et al.*, 1994). Recent statistics from the U.S. Department of Health and Human Services indicate that about seven million people in the U.S. suffer from gastroesophageal reflux disease (GERD) (National Digestive Diseases Data Working Group, 1984).

The goal in treatment of these disorders is to maintain gastric pH above 4 for the maximal number of hours daily. Recommended therapeutic regimens result in a pH>4 for between 4 and 20 hours daily. The newer agents, proton pump inhibitors (PPIs), can achieve a pH>4 for 20 hours with a single daily dose (Hunt, 1999). Prolonged treatment by physician prescription is common for acid suppression, as is long term self medication in the absence of clear symptoms (Morales Suarez-Varela *et al.*, 1998).

A 1999 survey of office-based physician medication recommendations revealed over 11 million prescriptions for omeprazole (a single PPI) in the U.S. for that year (Cherry *et al.*, 2001). Other recommendations in 1999 for medications that affect the pH of the stomach include: famotidine, over 4 million; cimetidine, nearly 3 million; and over the counter antacids, 2.6 million (Cherry *et al.*, 2001). A survey of 1202 adults in America conducted by Princeton Survey Research Associates in 1997 for Prevention Magazine and the American Pharmaceutical Association reported that 57 percent used an over-the-counter antacid (Princeton Survey Research Associates, 1997).

In summary, infants are a sensitive group, with increased susceptibility to carcinogen exposures, and increased pH levels in the stomach. In addition, there is substantial evidence that a sizable portion of the population is consuming medications that are aimed at increasing the pH of the stomach. The targeted pH of 4 or higher is in the range of pH of the forestomach in rodents (Browning *et al.*, 1983; Browning *et al.*, 1984; Kunstyr *et al.*, 1976; Ward *et al.*, 1986) where Cr VI administration resulted in a statistically significant increase in tumors in female mice (Borneff *et al.*, 1968). For this population, oral intake of Cr VI would be expected to result in a higher effective dose in the stomach compared to individuals with a more acidic stomach environment.

Examination of Evidence for Chromium Carcinogenicity

Human studies - Human occupational exposure to Cr VI has been linked to increased rates of lung cancer. A number of retrospective studies have associated significant increases in respiratory cancer to Cr VI exposure in workers engaged in chromate production and chromate pigment production (IARC, 1990). Increased incidence of lung cancer has also been observed in workers employed in the chromium plating industries. A summary of the findings of multiple studies where workers were exposed to Cr VI by the inhalation route (conducted by OEHHA) found several reports of associations between inhalation exposure to Cr VI and cancer of the digestive organs (Table 8). These data are consistent with an association between occupational exposure to Cr VI via inhalation and stomach cancer. In the only two studies of human exposure to Cr VI in drinking water that specifically measured organ-specific cancer, statistically significant increases in stomach cancer mortality (Zhang and Li, 1987; statistical analysis conducted by OEHHA) and primary liver cancer mortality (Linos *et al.*, 2011) were detected in the exposed populations.

Animal studies - The administration of Cr VI to rats or mice by inhalation, intratracheal instillation, intrabronchial or intrapleural implantation, subcutaneous, and intramuscular injection resulted in statistically significant increases in tumors compared to controls. Five rodent cancer bioassays, three in mice and two in rats, were identified in which Cr VI was given orally for the lifetime of the animal (NTP, 2008; Borneff *et al.*, 1968). The administration of Cr VI to male and female mice resulted in statistically significant and dose-related increases in tumors (adenomas; adenomas or carcinomas) of the duodenum and tumors of the small intestine (duodenum, jejunum and ileum) in male and female mice compared to controls (NTP, 2008). The administration of Cr VI to male or female rats resulted in statistically significant increases in tumors of the oral cavity in both sexes (NTP, 2008).

Hexavalent chromium administration yielded a statistically significant increase in tumors of the forestomach in female mice compared to control (OEHHA's statistical analysis) (Borneff, *et al*., 1968). The findings in the Borneff *et al* (1968) study were diminished for several reasons: the occurrence of viral infection that caused substantial intercurrent mortality; the use of only one dose group; differences in the length of survival and total dose received in different generations in this study; and animals within each treatment group were related to one another. However, the statistically significant increase in forestomach tumors was found despite these study limitations, none of which should have led to such results in the absence of a true effect.

Genotoxicity - Hexavalent chromium displayed genotoxic activity in both *in vitro* and *in vivo* bioassays. Exposure to Cr VI by the inhalation route or intratracheal instillation yielded elevated levels of sister chromatid exchange, chromosomal aberrations, mutations, DNA fragmentation, DNA-protein crosslinks, or oxidized DNA bases in the lung, kidney or liver (Bigaliev *et al*., 1977; Izzotti *et al.*, 1998; Cheng *et al.*, 2000). Oral administration of Cr VI resulted in chromosomal aberrations, DNA single strand breaks or DNA-protein crosslinks in the liver, brain, or bone marrow (Bigaliev *et al*., 1977; Coogan *et al.*, 1991a; Sarkar *et al.*, 1993; Bagchi *et al.*, 1995a,b, 1997).

Toxicokinetics - The toxicokinetics of Cr VI has been studied in animals and humans. Inhalation or oral exposure to Cr VI resulted in detectable chromium increases in the erythrocyte, plasma, and other tissues of humans and experimental animals. Because of the rapid conversion of Cr VI to Cr III in the stomach, only a small fraction of the oral dose of Cr VI appears to be absorbed. The amount of absorption is highly variable, although it generally is much greater than the gastrointestinal absorption when Cr III is administered. The oral absorption of Cr VI does not appear to be a consequence of exhaustion of the reducing capacity of gastric fluids and saliva, because the doses administered in toxicokinetic studies did not exceed the ability of the stomach to reduce Cr VI to Cr III.

There is evidence that a portion of an orally administered dose of Cr VI is distributed to tissues as Cr VI, based on the distribution and elimination pattern *in vivo*. Experimental evidence also suggests that an increased amount of orally administered Cr VI is absorbed when the pH of the stomach is elevated. Infants and individuals who regularly take medications that increase the pH of the stomach would appear to be sensitive populations because decreased reduction of Cr VI in their stomachs would be expected to result in an increased gastrointestinal absorption of Cr VI. Infants and children are also considered to be more susceptible to carcinogen exposures than adults.

Toxicity - Hexavalent chromium appears to be more toxic than Cr III when administered by the oral route. These differences in toxicity of Cr VI and Cr III are consistent with toxicokinetic findings that a portion of the Cr VI is orally absorbed and enters cells, rather than being fully converted to Cr III in the acidic environment of the stomach. The GI tumors (NTP, 2008) may result from the "site-of-contact" transfer of Cr VI into the cells of the GI tract that ultimately form tumors. Alternatively, Cr VI might first enter the systemic circulation and then return to the GI tract. The available data cannot distinguish between these two possibilities.

Mechanism - Hexavalent chromium rapidly enters the cell via the anion transport system. Hexavalent chromium is then reduced to Cr III and "trapped" inside the cell. Trivalent chromium itself has been linked to DNA damage and therefore its buildup inside the cell should not be considered innocuous (Costa, 1997; Cohen *et al.*, 1993).

More importantly, there is evidence for generation of the reactive intermediates Cr V and Cr IV as well as the formation of reactive species such as hydroxyl free radicals and singlet oxygen during the reduction process (De Flora and Wetterhan, 1989; Cohen *et al.*, 1993; Sugden and Stearns, 2000). These highly reactive species have been associated with oxidative DNA damage.

Conclusion - Exposure to Cr VI has been linked to increased incidences of tumors in humans and experimental animals. Increased tumor incidences were observed not only following occupational inhalation exposures but also were observed in humans and animals exposed to Cr VI in the drinking water. Hexavalent chromium displayed genotoxic activity *in vitro* and *in vivo* in animals and humans following oral or inhalation exposure. In humans and animals, there is substantial evidence of oral uptake of Cr VI and that Cr VI penetrates into cells following oral exposure. There is substantial evidence of DNA damage following oral exposure to Cr VI; however, it is not known if this would occur at environmental exposure levels. There is evidence that Cr VI may

damage DNA by the generation of free radicals during its metabolism, due to direct metal-mediated oxidation and by directly binding to DNA.

The findings of available human, animal, genotoxic, and toxicokinetic studies all indicate that Cr VI is a possible human carcinogen by the oral route. Given these observations and until more human and/or animal studies become available that clearly indicate otherwise, it is prudent to consider this hazard in the development of a PHG for Cr VI.

DOSE-RESPONSE ASSESSMENT

Noncarcinogenic Effects

Six studies were identified which allowed an assessment of non-carcinogenic effects of Cr VI. The strengths and weakness of each study are summarized in Table 1. The results are summarized here:

NTP, 1997a - Doses of Cr VI ranging from 1.1 to 29.3 mg/kg-day were administered orally to mice as potassium chromate in their diet for nine weeks in this subchronic study in mice. The NOAEL for Cr VI of 1.1 mg/kg-day was identified by the NTP. At doses of 3.6 mg/kg-day and above, vacuoles were detected in hepatocytes.

Mackenzie *et al.*, 1958 - Doses of Cr VI ranging from 0.0045 to 2.5 mg/kg-day were administered orally to rats as potassium chromate in their drinking water in a one year study. No toxicity was reported in these animals, resulting in identification of a NOAEL of 2.5 mg/kg-day.

Chopra *et al.*, 1996 – In this 22-week study in female rats, 25 ppm of potassium dichromate was administered in the drinking water. Cellular necrosis in the liver and kidney was reported in these animals. A LOAEL for Cr VI of 1.40 mg/kg-day was estimated based on standard drinking water consumption rates and body weights.

Acharya *et al.*, 2001 – In this 22-week study in male rats, 25 ppm of potassium dichromate was administered in the drinking water. Cellular necrosis in the liver and kidney was reported in these animals. A LOAEL for Cr VI of 1.1 mg/kg-day was estimated based on standard drinking water consumption rates and body weights.

NTP, 2007 - In a 90 day study in rats and mice, sodium dichromate dihydrate (0, 62.5, 125, 250, 500 or 1000 ppm) was administered in drinking water to male and female rats. Based on average water consumption, the mean effective doses were 0, 1.6, 3.1, 5.8, 11.0 or 21.1 mg/kg-day of chromium for male rats and 0, 1.8, 3.5, 6.2, 11.5 or 21.4 mg/kg-day of chromium for females. A LOAEL of 1.6 mg/kg-day was identified based on effects on blood forming tissues (decreased erythrocyte levels, mean cell volume, mean cell hemoglobin (total and concentration) and platelet concentrations) in male rats.

NTP, 2008 - Groups of 50 male or female rats (F-344) and mice ($B6C3F_1$) were administered sodium dichromate in drinking water (male and female rats and female mice: 14.3, 57.3, 172 or 516 mg/L; male mice: 14.3, 28.6, 85.7 or 257.4 mg/L) for two-years (NTP, 2008). Based on measured water consumption rates and body weights, male rats received a time weighted average dose of 0.2, 0.8, 2.1, or 5.9 mg/kg-day of Cr VI,

while female rats received 0.2, 0.9, 2.4 or 7.0 mg/kg-day of Cr VI. Male mice received an average dose of 0.38, 0.9, 2.4, or 5.9 mg/kg-day of Cr VI, while female mice received 0.38, 1.4, 3.1 or 8.7 mg/kg-day of Cr VI. Indications of mild hepatotoxicity (chronic inflammation, fatty changes) were detected in female rats at the lowest doses administered (0.2, 0.9 mg/kg-day). A LOAEL of 0.2 mg/kg-day was identified.

The critical noncarcinogenic endpoint for risk assessment of Cr VI by the oral route is considered to be liver damage (mild chronic inflammation, fatty changes). A LOAEL of 0.2 mg/kg-day is the lowest dose where toxicity was detected. No NOAEL below the LOAELs can be identified from these studies.

Carcinogenic Effects

The derivation of the oral potency of Cr VI is based on the results of animal studies, given the limitation of the available human studies. Cancer potency could not be reliably calculated for the stomach tumor data reported by Zhang and Li (1987) because of inadequate exposure information. OEHHA's analysis of stomach tumors associated with occupational exposure to Cr VI (see earlier section) was judged to be unsuitable for deriving a dose-response relationship for Cr VI. Estimates of the amount of Cr VI that was inhaled and then swallowed, judged likely to be responsible for the increase in stomach cancer in the analysis of occupational studies, are highly uncertain.

Treatment-related increases in tumors have been observed in five studies of laboratory animals exposed to Cr VI in drinking water (Borneff *et al.*, 1968; NTP, 2008). The recent NTP (2008) studies provide the most suitable Cr VI oral carcinogenicity data for dose-response assessment and cancer potency derivation.

Oral Potency Estimates Based on Animal Studies

Standard methods for estimation of lifetime theoretical cancer risks (OEHHA, 1999c; U.S. EPA, 2000, 2005b) were employed in the development of the oral cancer potency estimates. Further, McCarroll *et al.* (2010) reported that the weight of evidence supports the plausibility that Cr VI may act through a mutagenic mode of action (MOA). Based on U.S. EPA Cancer Guidelines (2005) and a mutagenic MOA for Cr VI, a linear extrapolation and the application of age sensitivity factors are recommended. Thus, this procedure is consistent with OEHHA's cancer guidelines (2009a) as well as the U.S. EPA Cancer Guidelines (2005). Four cancer bioassays, conducted in male rats, female rats, male mice, and female mice, were identified in which animals given Cr VI in drinking water displayed statistically significant increases in tumors (NTP, 2008). Treatment-related increases in tumors were greater and were observed at lower Cr VI doses in the NTP mouse studies, as compared to the NTP rat studies, indicating the mouse was the more sensitive species. Cancer potency estimates that utilize animal data are typically derived using the findings from the most sensitive sex and species. This health-protective assumption is intended to ensure that the cancer risk in humans is not underestimated. Therefore the findings in the mouse studies were judged to be most appropriate for deriving an oral cancer slope factor for Cr VI.

Dose-Response Modeling

Dose-response relationships were derived using U.S. EPA (1995b, 2000a) BMDS (Version 2.1.1). The lifetime time-weighted average dose was employed as the dose metric (see NTP, 2008 above for ingested doses in mg/kg-day). The multistage model in the U.S. EPA BMDS program was fitted to combined incidence data of adenomas and carcinomas of the small intestine for male $B6C3F_1$ mice and for female $B6C3F_1$ mice (NTP, 2008; Tables 5 and 6 of this PHG document). The multistage model was used to model the tumor incidence data because this is the model preferred by OEHHA (2009a) and U.S. EPA (2010) for conducting cancer dose-response assessments. The model generated both the mean and lower-bound estimates of the dose (ED_{10} and LED_{10}) associated with a ten percent increase in tumors.

Male Mice

The U.S. EPA BMDS multistage model yielded an acceptable fit for tumors of the small intestine, and estimated the dose associated with a 10 percent incidence of tumors (LED_{10}) as 1.2 mg/kg-day (Table 10). This mouse dose associated with a 10 percent increase in the incidence in tumors was then scaled to a human equivalent dose based on the ratio of mouse to human body weight to the ¾ power (U.S. EPA, 2005b). For this purpose, the time-averaged weight of a male $B6C3F_1$ mouse in the NTP (2008) study of 0.050 kg is used, and a 70 kg adult human body weight. This yields a human equivalent dose of:

$1.2\ \text{mg/kg-day}_{\text{mouse}} * (0.050\ \text{kg}/70\ \text{kg})^{1/4} = 0.196\ \text{mg/kg-day}_{\text{human}}$

Table 10. Cancer Potency Calculations for Combined Incidence of Adenomas and Carcinomas in the Small Intestine of Male $B6C3F_1$ Mice (NTP, 2008)

Model	Chi-square	P value	ED_{10}[a] (mg/kg-day)	LED_{10}[b] (mg/kg-day)
Multistage	1.03	0.60	2.2	1.2

[a] ED_{10} = maximum likelihood estimate of the dose producing a 10 percent extra risk of adenomas and adenocarcinomas in the small intestine of male mice
[b] LED_{10} = lower 95 percent confidence interval on the ED_{10}

Thus, 0.196 mg/kg-day is the lower bound estimate of dose in humans associated with a ten percent increase in tumors. The oral cancer slope factor, a measure of potency, is calculated from the dose associated with a ten percent increase in tumors, as:

Slope factor = tumor response / dose associated with that response, or

Slope factor = 0.1 / 0.196 mg/kg-day = $0.5\ (\text{mg/kg-day})^{-1}$

This value is essentially the same as that used by the U.S. EPA (2010) and by Stern (2010) for the state of New Jersey.

Female Mice

Using all dose levels, the multistage model in the BMDS did not yield an acceptable fit ($p>0.1$) for combined incidence of adenomas and carcinomas of the intestine in female mice. When the high dose group was excluded, the model yielded an acceptable fit, as shown below in Table 11.

Table 11. Cancer Potency Calculations for Combined Incidence of Adenomas and Carcinomas in the Small Intestine of Female B6C3F$_1$ Mice (excluding the high dose, 8.7 mg/kg-day)

Model	Chi-square	P value	ED_{10}[a] (mg/kg-day)	LED_{10}[b] (mg/kg-day)
Multistage	0.23	0.89	1.53	1.03

[a] ED_{10} = maximum likelihood estimate of the dose producing a 10 percent extra risk of adenomas and adenocarcinomas in small intestine of female mice
[b] LED_{10} = lower 95 percent confidence interval on the ED_{10}

Using the LED_{10} based on the standard multistage model (1.03 mg/kg-day) and the time-averaged weight of the female B6C3F$_1$ mice reported in NTP (2008) of 0.052 kg yields a human equivalent dose of:

$$1.03 \text{ mg/kg-day}_{mouse} * (0.052 \text{ kg}/70 \text{ kg})^{1/4} = 0.17 \text{ mg/kg-day}_{human}$$

Thus, 0.17 mg/kg-day is the lower bound estimate of dose in humans associated with a ten percent increase in tumors. The oral cancer slope factor, a measure of potency, is calculated from the dose associated with a ten percent increase in tumors as:

Slope factor = tumor response / dose associated with that response, or

$$\text{Slope factor} = 0.1 / 0.17 \text{ mg/kg-day} = 0.59 \text{ (mg/kg-day)}^{-1}$$

Dose-response relationships were successfully derived for tumors of the small intestine in male mice (all dose groups included) and in female mice when the high dose group was excluded. The modeling yielded similar results in male and female mice. Because of the better fit of the male mice data (no discarded data points), OEHHA selected the cancer slope factor for male mice of 0.5 $(\text{mg/kg-day})^{-1}$ to calculate the PHG.

Cancer Potency for the Inhalation Route

A cancer potency value was developed for the inhalation route of exposure associated with contaminants in drinking water as mandated by the The California Safe Drinking Water Act of 1996 ("OEHHA shall consider additive effects of exposure to contaminants in media other than drinking water, including food and air, and the resulting body burden"). Previous estimates of the carcinogenic potency of airborne chromium have relied largely on data furnished in an occupational cohort study of lung cancer by Mancuso (1975). Using those data, the U.S. EPA (1984b) pioneered methods to determine potency of Cr VI, and other agencies followed, including the California

Department of Health Services (CDHS, 1985) and National Health and Welfare Canada (1993). Crump (1995) performed an extensive re-analysis of the Mancuso (1975) data for the U.S. Occupational Health and Safety Administration. In those analyses the potency values depended proportionally on different scale factors assumed for the ratio of Cr VI to the total chromium values given in the study.

Since the California DHS (CDHS, 1985) analysis, Mancuso (1997) obtained follow-up data on the same cohort and also reported airborne Cr VI exposure measurements. Gibb *et al*. (2000) performed a new lung cancer study using these new data that is appropriate for determining a dose response relationship for airborne Cr VI. The authors present logarithmic dose-response relationships not well suited for low dose extrapolation, because they are likely to overpredict risk at low doses. The present work is intended to update the DHS (1985) analysis. The findings of Gibb *et al*. (2000) are employed to develop linear dose-response relationships suitable for estimating the low dose carcinogenic potency of Cr VI. The results of these analyses were then compared with existing potency estimates to determine the best value to use in this assessment.

The Gibb *et al*. (2000) study data are (1) observed and expected lung cancer deaths, (2) person years at risk, and (3) cumulative exposure to Cr VI for each age category and occupational exposure level in the chromium production workers. Cumulative exposures were lagged 5 years. Reported Cr_2O_3 exposure values were multiplied by the ratio of formula weights, 52/100, to obtain Cr VI exposures. Initially, Gibb *et al*. (2000) reduced their data to four exposure categories and seven age categories (cut-points at 10 year increments from 20 to more than 80 years of age) and then pooled over the age categories to yield results in person-year weighted data for four exposure categories. We used the first reduction for statistical analysis and the second reduction for a simpler statistical analysis with graphical insights.

Methods for Analysis I: Simple Dose-Response Analysis

The lung cancer dose-response data presented in Table 12 were used to derive cancer potency estimates for ambient Cr VI exposure. A relative risk (RR) model that adjusts for estimated uncontrolled confounding bias (Arrighi and Hertz-Picciotto, 1994; Arrighi and Hertz-Picciotto, 1996; Robins, 1987) was fit to the cohort data. The model may be described as:

$$RR = \beta_0(1+\beta_1 d) + \varepsilon \qquad \text{[Equation (1)]}$$

where β_0 is a parameter representing the ratio of the background rate of cancer in the population studied to the rate in the general population, β_1 is a parameter characterizing the potency in units of $(mg/m^3\text{-years})^{-1}$, d represents the cumulative (mg/m^3-years) Cr VI exposure level (i.e., dose), and ε is the error.

To determine parameter estimates for β_0 and β_1, Equation (1) is reformulated such that the dependent variable is the observed number of lung cancer cases. The number of observed lung cancer cases was assumed to be a Poisson random variable and hence ε will follow a known distribution. The resulting model fit to the cohort data is:

$$obs = \beta_0 EXP(1 + \beta_1 d) \quad \text{[Equation (2)]}$$

where *obs* is observed number of lung cancer cases and *EXP* is the expected number of lung cancer cases (i.e., using the relationship *EXP = obs/RR*).

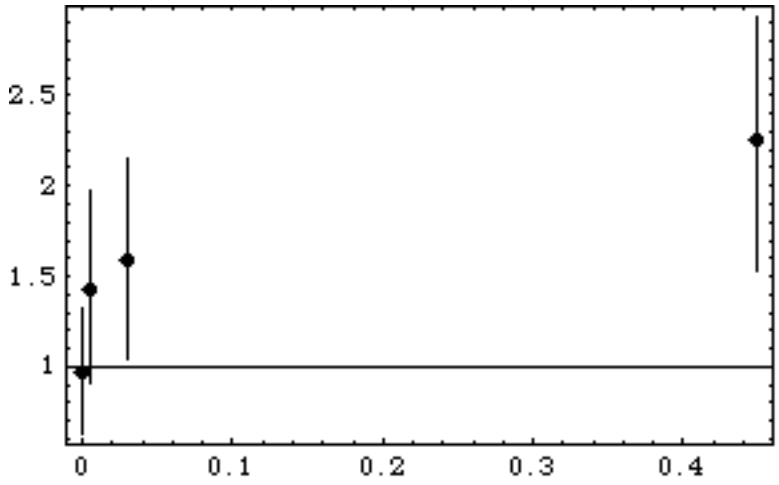
Table 12. Gibb *et al*. (2000) Lung Cancer Occupational Cohort Data; Quartiles of Exposure Without Categories for Age

Mean cumulative $Cr0_3$ exposure (mg/m^3-years)	Observed lung cancer cases (obs)	Expected number of lung cancer cases for unexposed persons (background)	Relative Risk
0.00045	26	27.1	0.96
0.0042	28	19.8	1.41
0.030	30	19.1	1.57
0.449	38	17.0	2.24

Maximum likelihood estimation techniques were used to determine the values of the parameter estimates in the equation above and their corresponding 90 percent profile likelihood-based confidence intervals.

Figure 16 displays the observed dose-response data reported by Gibb *et al*. (2000). Clearly, the dose-response does not conform to a linear model. Rather, a supralinear curve describes the relationship more accurately, as the greatest per-dose effects occur at the very lowest levels of exposure. Keeping this point in mind, the analyses presented next estimate linear dose-response relationships not only using all four exposure categories but also determine linear relationships by using the lowest three categories and the lowest two categories. Attention was paid to the fits of these models to the data selected. The low dose range is of particular importance in potency estimation since low levels often represent exposure to the general population.

Figure 16. Gibb *et al*. (2000) Observed Dose-response Data



Methods for Analysis II: Stratified Person-Years Dose-Response Analysis.

For the analyses stratified by age and exposure classifications, the number of lung cancer deaths in each stratum of Table 13 was assumed to be a Poisson distributed random variable with expectation

$$PY_{a,d}[h(a,d)]$$

$PY_{a,d}$ is the number of person-years at risk in the stratum corresponding to age (a) and cumulative exposure (d). *h(a,d)* is the incidence function (hazard), which was defined as:

$$h(a,d) = \alpha h_0(a)[1+\beta d]$$

h_0(a) represents the background rate of lung cancer deaths as a function of age category, while α represents a parameter that adjusts the background for each analysis. The background hazard for a given age category was derived from general population lung cancer deaths divided by the person years for that age category. Gibb *et al*. (2000) used the age-, calendar-, and race-specific mortality rates for Maryland to determine the background rates of lung cancer deaths. If α is statistically equivalent to 1 (at the 5 percent significance level), then no adjustment to the background rate is required. Otherwise it may be considered a correction for bias in matching of the target population to the reference population. β represents the slope parameter for cumulative exposure. Parameter estimation was accomplished via maximum likelihood estimation (Breslow and Day, 1987), as applied in Dawson and Alexeeff (2001).

Table 13. Gibb *et al*. (2000) Lung Cancer Occupational Cohort Data; Quartiles of Cumulative Exposure with Categories for Age

Person years	Observed lung cancer deaths	Cr_2O_3 exposure (mg/m^3-yr)	Midpoint age (yr)	Expected lung cancer deaths	Expected lung cancer rate (yr^{-1})
5003	0	0.21	25	0.02	3.60E-06
7684	1	0.41	35	0.39	5.08E-05
6509	0	0.51	45	2.50	3.84E-04
5184	14	0.53	55	7.56	1.46E-03
3104	8	0.5	65	10.79	3.48E-03
865	2	0.46	75	5.00	5.78E-03
163	1	0.4	85	0.88	5.40E-03
349	0	4.2	25	0.00	2.87E-06
3139	0	4.3	35	0.18	5.73E-05
4643	2	4.3	45	1.97	4.24E-04
3928	10	4.2	55	6.09	1.55E-03
2183	10	4.2	65	7.85	3.60E-03
558	4	3.9	75	3.25	5.82E-03
79	2	3.7	85	0.44	5.57E-03
457	0	31	25	0.00	4.38E-06
3520	0	31	35	0.19	5.40E-05
4732	3	30	45	1.93	4.08E-04
3720	10	30	55	5.70	1.53E-03
2128	11	28	65	7.66	3.60E-03
559	4	29	75	3.26	5.83E-03
78	2	27	85	0.38	4.87E-03
200	0	210	25	0.00	5.00E-06
2874	0	330	35	0.17	5.92E-05
4294	8	410	45	1.82	4.24E-04
3663	8	520	55	5.63	1.54E-03
1926	18	630	65	6.71	3.48E-03
423	3	780	75	2.48	5.86E-03
29	1	860	85	0.18	6.21E-03

Conversion Factors

In order to express the estimated model slope parameter (in units of concentration-year) as a potency value (in units of $(\mu g/m^3)^{-1}$), the following conversion factors were applied. The estimated slope parameter was multiplied by 70 years of age for a nominal lifetime at risk. This product was then multiplied by the background risk of lung cancer in the target population, 0.0247 for California (OEHHA, 1998). To account for the occupational nature of the exposure, i.e., the proportion of air breathed at work compared to the total breathed in a day and the proportion of the year spent at work, an intermittency factor

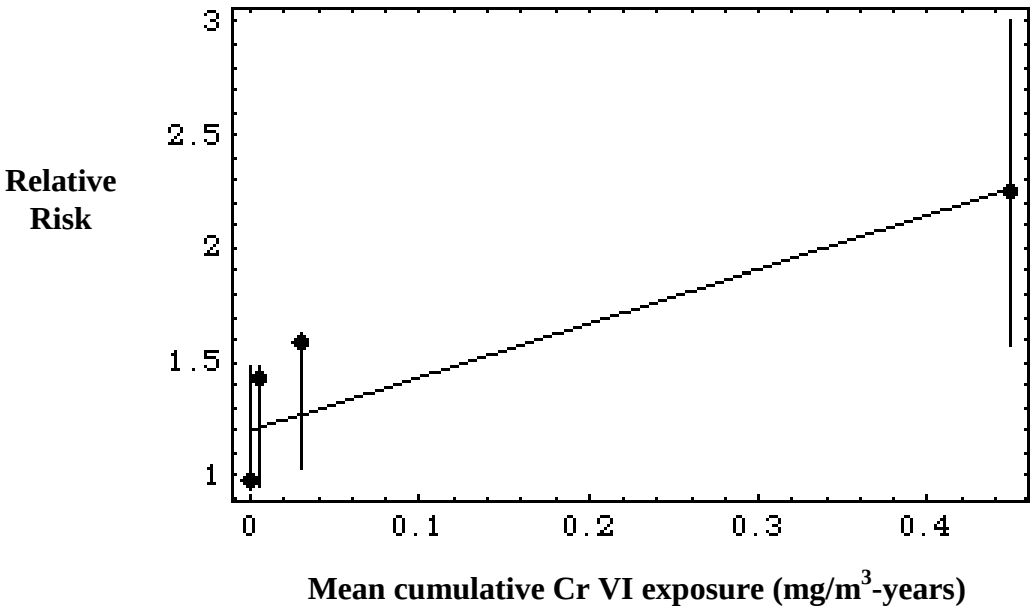
was then applied, described by the equation (10 m^3 / 20 m^3) * (240 day / 360 day) = 0.33, which was then divided into the aforementioned product.

Results

Analysis I: Simple Dose-Response Analysis

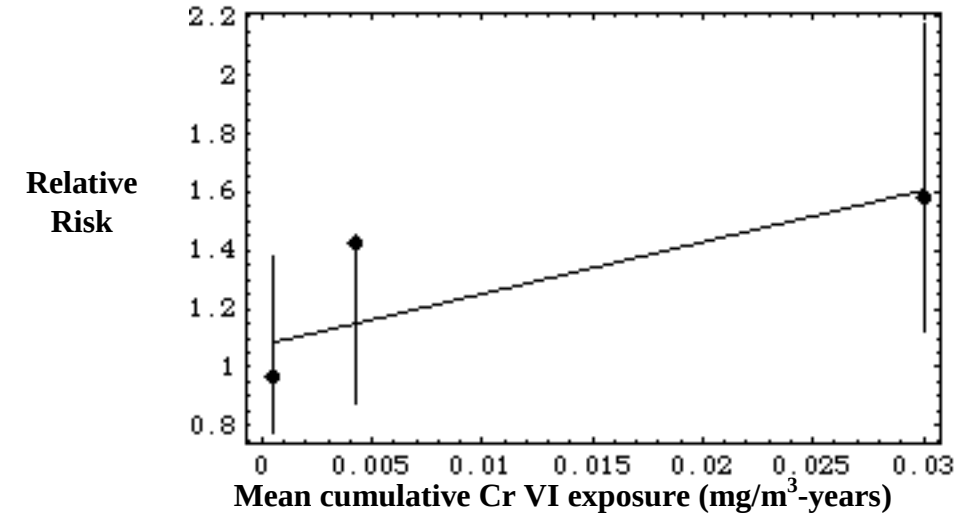
The dose-response data presented in Table 12 were fit to the model represented by Equation (2). Figures 17, 18, and 19 display the fits of the model to the observed data. Table 14 presents model parameter estimates (in terms of cumulative exposure), the 90 percent profile likelihood-based confidence intervals for the model parameters, and the goodness-of-fit statistics associated with the model fits to the observed data. Because of the supralinear nature of the observed dose-response data, the model when fit to all of the data points (Figure 17) may over-predict the number of lung cancer cases in the lowest dose category and underestimate lung cancer cases for the middle two dose categories. The model appears to accurately predict the cases observed for the highest dose category.

Figure 17. Complete Observed Dose-response Data with the Model-predicted Line and 95 Percent Pointwise Confidence Intervals



In view of the model deficiencies at the lowest levels of exposure, the highest dose category is eliminated and the data are refit. A similar pattern of over-estimation of lung cancer cases at the lowest exposure level and under-prediction at the middle exposure level occurs in this scenario (Figure 18). A comparison of the potency parameters from the model fit to all of the data versus the data fit excluding the highest dose category shows approximately an order of magnitude difference among the estimates (Table 15).

Figure 18. Observed Dose-response Data Excluding the Highest Dose Category with the Model-predicted Line and 95 Percent Pointwise Confidence Intervals



Because the low dose range represents exposure to the general population, an analysis of the lowest two levels of exposure was conducted. In this situation, the observed data conform to a linear dose-response. A perfect fit is achieved in this instance (Figure 19) since the model consists of two parameters and two data points are being fit, i.e., saturated model. The potency estimate from this fit is approximately two orders of magnitude greater than the potency estimate from fitting the entire data set.

Figure 19. Dose-response Data Excluding the Two Highest Dose Categories with the Model-predicted Line and 95 Percent Pointwise Confidence Intervals

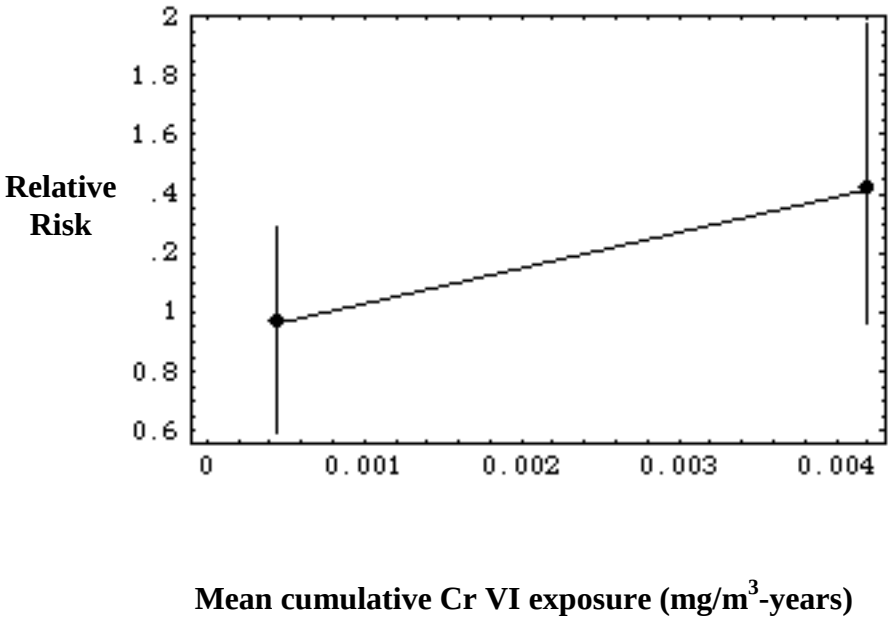


Table 14. Estimated Model Parameters for Gibb *et al*. (2000) Four Points, Analysis I

Dose categories included in the model	Estimate of β_0 (90% CI)	Estimate of β_1[1] (90% CI) ([mg/m^3-yr]$^{-1}$)	χ^2 Goodness-of-Fit p-value	Potency[2] ([μg/m^3]$^{-1}$)	
				MLE	95% UCL
All doses included in the model	1.20 (1.18, 1.23)	3.77E-03 (3.54E-03, 4.02E-03)	0.19	1.98 E-02	2.11E-02
Drop the highest dose category	1.07 (1.05, 1.10)	3.17E-02 (2.81E-02, 3.53E-02)	0.21	1.66 E-01	1.85E-01
Drop the highest two dose categories	0.90 (0.88, 0.93)	2.58E-01 (2.28E-01, 2.87E-01)	1.00 (saturated model)	1.35E+00	1.50E+00

[1]Estimates of β_1 were multiplied by 100/52 to obtain slope for Cr VI exposures
[2]Conversion factors applied to β_1 to obtain potency.

Analysis II: Stratified Person-Years Dose-Response Analysis

This analysis is based on the age-dose stratified person-years data displayed in Table 14; the results are presented in Table 15. The slope based upon excluding the highest two exposure categories is 38 percent above that in Analysis I. The upper bound slope based upon excluding the highest dose category is 88 percent above, and the upper bound slope based upon all four dose categories is 33 percent above that in Analysis I. As indicated by the narrow confidence intervals, the slopes are all statistically significantly different from 0. When all exposure categories are included in the model, the confidence interval for α is sufficiently narrow such that the estimate (1.26) is statistically significantly greater than 1. The intercepts in the analyses with the upper exposure categories removed are not statistically significantly different than 1. There is no statistical indication of a lack of fit for any of these data selections, but the greatly increased slope with decreased exposures suggests a departure from linearity in the overall relationship.

Table 15. Model Results for Gibb *et al*. (2000) Using Hexavalent Chromium Data with Age Categories, Analysis II

Dose categories included in the model	Estimate of α	Estimate of β (90% CI) ([mg/m^3-yr]$^{-1}$)	Deviance p-value	Potency ([μg/m^3]$^{-1}$)	
				MLE	95% UCL
All doses included	1.26	2.45E-03 (4.18E-04, 5.33E-03)	0.19	1.28 E-02	2.79E-02
Drop the highest dose category	1.10	3.10E-02 (7.24E-03, 6.64E-02)	0.34	1.62 E-01	3.48E-01
Drop the highest two doses	0.90	2.66E-01 (0.00E+00, 3.96E-01)	0.18	1.40E+00	2.08E+00

Discussion

In evaluating the results for Gibb *et al*. (2000) we first note that there is considerable consistency between the results of Analysis I and Analysis II above. We also outline previous potency calculations based on the Mancuso (1975) data and find a degree of consistency between the above results for Gibb *et al*. (2000) and those for Mancuso (1975), which made similar exposure assumptions. Finally we discuss some of the advantages of using the Gibb *et al*. (2000) data.

Comparison with previous results

The U.S. EPA (1984b) calculated Cr VI potency by two methods, both of which used total chromium to represent Cr VI. First, a method taking into account competing risk used the age dependence of cancer rates as developed in a multistage model. Second, a crude model was based upon collapsing all the 18 Mancuso data points to a single point of relative risk and exposure to determine the slope of the exposure response. Details are provided in U.S. EPA (1984b), and, with modification of the dose scale, in the California Department of Health Services report (CDHS, 1985). The slopes obtained were converted to potency for an equivalent continuous lifetime exposure by the equation on page 85, and the average relative risk was obtained by the equation on page 90 in the CDHS report. Their intermittency factor for yearly exposure is 0.22. The background risk (called a "rate" in CDHS, 1985) of lung cancer for the 1964 U.S. population is 0.036.

Although previous potency estimates in Table 16 primarily used the Mancuso (1975) data, some results differed considerably from one another, depending mostly on the assumptions about how to scale the exposure measurements. The CDHS analyses assumed that Cr VI is only 1/7 of total chromium and produced 7-fold higher risks than those of U.S. EPA, which used total chromium to represent Cr VI. The Crump (1995) analyses assumed that 43 percent of total chromium is Cr VI. OEHHA used the above intermittency, background mortality rate and seventy-year lifetime exposure to convert the (occupational) potencies in Crump (1995), which are actually slopes in our terminology, to obtain continuous 70-year potencies. Crump (1995) presented critical justifications for an alternative analysis to that of U.S. EPA (1984b).

Table 16. Comparison of Potency Estimates $(\mu g/m^3)^{-1}$ for Hexavalent Chromium Based on Mancuso (1975)

Data/analysis	**Potencies**	
	MLE	**95% UCL**
Competing risk (U.S. EPA, 1984b) for total Cr	0.012	--
Crude (U.S. EPA, 1984b) for total Cr	0.014	--
Competing risk (CDHS, 1985) for Cr VI = 0.14 x total Cr	0.081	--
Crude (CDHS, 1985) for Cr VI = 0.14 x total Cr	0.101	0.146
Crump (1995) for Cr VI = 0.4 x total Cr	0.019	0.026

Usefulness of the new data

Both Mancuso (1997) and Gibb *et al*. (2000) provided new data on workers exposed to airborne chromium. The Gibb *et al*. (2000) study was well conducted, and it contains a comparison documenting superiority to Mancuso (1997) in several ways. Some of the most important are the concurrent measurements of exposure, 7-fold larger cohort, 5-fold large number of person years, and 2-fold larger number of cancer deaths. Most importantly, Gibb *et al*. (2000) provided data on expected cancer cases by calendar year, whereas Mancuso (1975, 1997) did not give information allowing assured reconstruction of expected cancer deaths in that regard. The background rate of lung cancer was increasing annually over the course of the study, as pointed out by Crump (1995). This increase is likely to bias risk slopes upwards with no referent population in the modeling. Although Crump (1995) did make estimates of the calendar-year effect for Mancuso (1975), those estimates are quite uncertain. The uncertainty increases with the longer follow-up of Mancuso (1997), which was therefore not used in this assessment.

However, the Gibb *et al*. (2000) study also has limitations. One is the lack of accounting for time since first exposure, which if accounted for might prevent possible bias due to lag in the effect of exposure. Also, without the individual work histories, the present analysis is limited in exploring different modeling approaches, such as the use of time-dependent multistage models.

At the Painesville, Ohio plant, where the Mancuso studies took place, Luippold *et al*. (2003) studied former employees who started work after 1940, whereas the employees in the Mancuso studies started in the decade before that. Luippold *et al*. (2003) found that their data were consistent with a linear threshold or non-threshold relationship of relative risk to cumulative exposure to Cr VI. Using the least squares method and a non-threshold model to ascribe a slope to the results of Luippold *et al*. (2003), we derived a slope of 0.0018 (yr-μg Cr VI/m^3)$^{-1}$, yielding an MLE potency of 0.01 (μg Cr VI/m^3)$^{-1}$. This is the same value obtained based on the four-point slope calculated using Gibb *et al*. (2000). The Luippold *et al*. (2003) exposures were mostly higher than those of Gibb *et al*. (2000). The Gibb *et al*. (2000) unit risk of 0.16 (μg Cr VI/m^3)$^{-1}$ with a 95 percent UCL potency of 0.35 (μg/m^3)$^{-1}$ was based on the lowest three exposure levels. We judge that the unit risk from Gibbs *et al.* (2000) provides a sounder value because it is based on lower exposures, which are nearer and therefore more relevant to environmental levels.

Conclusions regarding inhalation potency - The uncertainties in the Mancuso (1975) exposure data were much less than in other studies analyzed as alternatives in the earlier reports (U.S. EPA, 1984b; CDHS, 1985; Crump, 1995). The measured values of Cr VI in Mancuso (1997) apparently reduce some of the uncertainty about the Mancuso (1975) exposure to Cr VI, but especially because it does not have a referent population, Mancuso (1997) is subject to too much bias to be useful by the present approaches. The earlier CDHS (1985) discussion of uncertainty in the Mancuso (1975) study applies to Mancuso (1997), especially reliance on sampling after the major exposures occurred. OEHHA concentrated on the Gibb *et al*. (2000) data because it provided superior exposure measurements, which were generally much lower.

The slope of the line modeled with the 3 lowest exposure categories in Gibb *et al*. (2000) provided a 95 percent UCL potency of 0.35 (μg/m^3)$^{-1}$. The line using the 2 lowest

exposure categories is much steeper, and the line using all 4 points is much shallower. Using rounded values, the steeper slope with a 95 percent UCL potency of 2 $(\mu g/m^3)^{-1}$ provides the top of the range of potencies and the shallower slope furnishes the bottom of the range, 0.01 $(\mu g/m^3)^{-1}$. The various slope estimates obtained for both Mancuso (1975; 1997) studies are in the lower half of this range. This range also includes the estimate used by OEHHA (1999c) to designate the 95 percent UCL value of potency for Cr VI obtained by the crude model, 0.15 $(\mu g/m^3)^{-1}$ or 510 $(mg/kg\text{-}day)^{-1}$ to be used for lifetime risk assessments (OEHHA, 1999c).

Correction for Early-in-Life Exposures

Cancer potency is corrected by an Age Sensitivity Factor (ASF), as defined earlier (OEHHA, 2009b). The procedure for application of cancer potency factors has been revised to take into account information which suggests that children can be especially susceptible to carcinogens. Weighting factors are utilized to calculate cancer risks from exposures of infants, children and adolescents, to reflect their anticipated special sensitivity to carcinogens. Cancer risk is weighted by a factor of 10 for exposures that occur from the third trimester of pregnancy to <2 years of age, and by a factor of 3 for exposures that occur from ≥2 years through <16 years of age. This approach applies to all carcinogens, regardless of purported mechanism of action, unless chemical-specific data exist that could be used to make more specific adjustments to risk.

Calculations using the age sensitivity factors are made by applying the age-specific adjustment factors and durations to age-specific consumption rates, where:

R	=	Total risk;
C	=	Concentration in water;
p_o	=	Oral cancer potency;
p_i	=	Inhalation cancer potency;
ASF_1	=	Age sensitivity factor for 3rd trimester + infancy, value 10;
ASF_2	=	Age sensitivity factor for childhood (ages 2-16), value 3;
ASF_3	=	Age sensitivity factor for adult (ages 16-70), value 1.

For this calculation, the duration of sensitive periods is expressed as fractions of the standard lifetime of 70 years, as follows:

d_0	=	3rd trimester, value 0.25/70;
d_1	=	infancy, value 2/70;
d_2	=	childhood, value 14/70;
d_3	=	adult, value 54/70.

The equivalent water exposure values (L/kg-day) for each age range are expressed as follows:

$cons^o_1$	=	Oral route, infancy;
$cons^o_2$	=	Oral route, childhood;
$cons^o_3$	=	Oral route, adult;

$cons^i_2$ = Inhalation route, childhood;

$cons^i_3$ = Inhalation route, adult.

For the risk equation, the overall lifetime risk is the sum of all partial risk components for each age bin and route. Note that for the third trimester of pregnancy, the ASF_1 for early-in-life exposures is applicable, but the consumption rate is assumed to be that of an adult (i.e., maternal consumption). Also, infants are assumed not to take showers (but mothers do). Then,

$$\begin{aligned} R = {} & p_o \times ASF_1 \times d_0 \times cons^o_3 \times C + \\ & p_o \times ASF_1 \times d_1 \times cons^o_1 \times C + \\ & p_o \times ASF_2 \times d_2 \times cons^o_2 \times C + \\ & p_o \times ASF_3 \times d_3 \times cons^o_3 \times C + \\ & p_i \times ASF_1 \times d_0 \times cons^i_3 \times C + \\ & p_i \times ASF_2 \times d_2 \times cons^i_2 \times C + \\ & p_i \times ASF_3 \times d_3 \times cons^i_3 \times C \end{aligned} \qquad \text{Equation 1}$$

This can be simplified by taking the common factor C outside a top-level bracket, and the common factors p_o and p_i can be taken outside second-level brackets:

$$\begin{aligned} R = C \times \Big[& p_o \times \big(ASF_1 \times d_0 \times cons^o_3 + \\ & \quad ASF_1 \times d_1 \times cons^o_1 + \\ & \quad ASF_2 \times d_2 \times cons^o_2 + \\ & \quad ASF_3 \times d_3 \times cons^o_3 \big) + \\ & p_i \times \big(ASF_1 \times d_0 \times cons^i_3 + \\ & \quad ASF_2 \times d_2 \times cons^i_2 + \\ & \quad ASF_3 \times d_3 \times cons^i_3 \big) \Big] \end{aligned} \qquad \text{Equation 2}$$

It is important to note that the calculation cannot be simplified further to any important degree, since there are no other persistent common factors inside the second-level brackets. In other words, you cannot achieve an accurate result by summing consumption values and adjustment factors separately and then multiplying the results together.

Rearranging Equation 2:

$$C = \frac{R}{\begin{aligned} & \Big[p_o \times \big(ASF_1 \times d_0 \times cons^o_3 + \\ & \quad ASF_1 \times d_1 \times cons^o_1 + \\ & \quad ASF_2 \times d_2 \times cons^o_2 + \\ & \quad ASF_3 \times d_3 \times cons^o_3 \big) + \\ & p_i \times \big(ASF_1 \times d_0 \times cons^i_3 + \\ & \quad ASF_2 \times d_2 \times cons^i_2 + \\ & \quad ASF_3 \times d_3 \times cons^i_3 \big) \Big] \end{aligned}} \qquad \text{Equation 3}$$

The PHG is determined by solving Equation 3 for $R = 10^{-6}$.

CALCULATION OF THE PHG

Calculations of concentrations of chemical contaminants in drinking water associated with negligible risks for carcinogenic or noncancer endpoints must take into account the toxicity of the chemical and the potential exposure of individuals using the water. Tap water is used directly for drinking, and for preparing foods and beverages. It is also used for bathing or showering, and in washing, flushing toilets, and other household uses resulting in potential dermal and inhalation exposures. Therefore, three routes of exposure, ingestion, inhalation and dermal contact with domestic water are addressed in developing the PHG.

Noncarcinogenic Effects

The results from six studies in which Cr VI was administered to animals (NTP, 1997a; NTP, 2002; MacKenzie *et al.*, 1958; Chopra *et al.*, 1996; Acharya *et al.*, 2001; NTP, 2007, 2008) will be employed to develop a health-protective level based on non-cancer health-based criteria for Cr VI. For this purpose, acceptable daily doses (ADD) will first be calculated from the NOAELs and LOAELs of these studies to illustrate the range of potential choices based on the study limitations and the application of appropriate uncertainty factors. From these data, a health-protective concentration in drinking water will then be calculated.

Choosing Appropriate Uncertainty Factors

Uncertainty associated with use of the NOAEL from an animal study - Concern that humans may develop toxic effects at levels below those in experimental animals (interspecies sensitivity) is typically addressed by using an uncertainty factor of ten in deriving a health-based criterion. Heightened sensitivity could be due to differences in absorption, metabolism, or tissue responses to the chemical. Chromium levels in different tissue of rats and mice ingesting Cr VI in their drinking water for two years differed by five-fold or less between the two species on day 182 (Collins *et al.*, 2010). Similar data are not available for estimating the toxicokinetic differences in chromium accumulation by humans compared to rodents. Also, the toxicodynamic interspecies differences have not been adequately investigated. In addition, there is uncertainty associated with the protocols employed in the NTP (1997), MacKenzie *et al.* (1958), Chopra *et al.* (1996), and Acharya *et al*. (2001) studies and their ability to detect toxic effects (see Table 1). The limited scope of the evaluations may not have been adequate to detect all likely toxic effects. The U.S. EPA has addressed deficiencies in the available toxicology studies by employing an additional uncertainty factor or modifying factor in deriving a RfD for certain toxicants (U.S. EPA, 1993). These two sources of uncertainty suggest that an uncertainty factor of ten may not be sufficient.

Uncertainty associated with the use of a less than lifetime study to establish a NOAEL for chronic exposure - The assessment of risks associated with exposure to low levels of Cr VI in water focused on the most sensitive toxicological endpoint, which in the NTP (1997a), Chopra *et al.* (1996), and Acharya *et al*. (2001) studies was hepatotoxicity. Animals in the NTP (1997) study were exposed to Cr VI for only nine weeks while

animals in the Chopra *et al.* (1996) and Acharya *et al.* (2001) studies were exposed for 22 weeks. Concern that toxic effects observed in 90-day subchronic studies (NTP, 2002) may occur at lower doses with lifetime exposures is typically addressed by the addition of a ten-fold uncertainty factor when subchronic studies are used. The NTP (1997a) study was conducted for only nine weeks, notably shorter than a typical 90-day subchronic study. This introduces additional uncertainty, from which we infer that an uncertainty factor of ten may not be sufficient for this study.

For the subchronic NTP (2007) study conducted for 90 days, an uncertainty factor of 10 is employed to address the uncertainty associated with the short duration of the study. For the chronic NTP (2008) study, no uncertainty factor is needed to address uncertainty associated with a less than lifetime exposure duration.

Uncertainty associated with extrapolating a NOAEL from a LOAEL - Ideally, the NOAEL associated with the most sensitive toxic effect is identified and employed to develop a health-based criterion. However, toxic effect(s) are sometimes observed at the lowest dose administered in the study. Under these circumstances, an uncertainty factor of ten is often employed to extrapolate a NOAEL from the LOAEL in the study.

Uncertainty associated with human variability – Genetic, life-stage, and lifestyle variations among humans is generally accounted for with an uncertainty factor of ten. This variability can occur because of differences in absorption and metabolism of a chemical, or in the toxicological response. However, there is concern that certain human populations (such as infants) may have extra sensitivity not encompassed by the default factor of ten. In the case of Cr VI there is also a question as to whether antacid consumption or gastrointestinal disease may result in marked increases in the absorption of Cr VI from drinking water. Also individuals with liver disease may be particularly sensitive to the hepatotoxic effects of Cr VI, given that their livers are already compromised.

An aggregate uncertainty factor of 3,000 is generally considered the maximum, based on recommendations of California's Risk Assessment Advisory Committee (1996) and the U.S. EPA (2002b).

NTP, 1997a - In a limited study with a small number of animals aimed at investigating the reproductive toxicity of Cr VI, doses of 1.1 to 29.3 mg/kg-day of Cr VI were administered to mice for nine weeks. A NOAEL of 1.1 mg/kg-day was identified by the NTP based on hepatic cytoplasmic vacuolization at doses of 3.6 mg/kg-day and above. Because of the study's limitations (short duration, small number of animals per dose and limited toxicological evaluation), uncertainty factors appropriate to provide an adequate margin of safety for human exposure to Cr VI in drinking water from this study include 10 for extrapolating from a subchronic study, 10 to extrapolate between species, and 10 to protect potentially sensitive human subpopulations (including antacid users). An additional factor of 3 or 10 could be considered for very limited data (small number of animals/group, short study, only a few tissues examined). A limited-data factor of 3 is chosen to restrict the aggregate uncertainty factor to the maximum of 3,000.

ADD = 1.1 mg/kg-day / 3,000 = 0.00037 mg/kg-day

MacKenzie *et al.*, 1958 - In a study focused on investigating tissue levels of chromium following oral exposure to Cr VI, doses of 0.0045 to 2.5 mg/kg-day of Cr VI were administered to rats for one year. A NOAEL of 2.5 mg/kg-day was identified based on a lack of observed toxicity at any dose. While no toxicity was reported in any of the dose groups, the thoroughness of the toxicological investigation is unclear (given the study was focused on investigating chromium update into tissues). Only two sentences in the published account of the study addressed toxicity: "neither gross changes in appearance nor pathological changes in blood or other tissues were observed" and "No toxic symptoms were observed in any of the groups fed low concentration of chromium over a period of one year, although quite high concentrations were found in the tissues." Almost no details of the protocol were provided by the authors. However, a companion study of cadmium toxicity (that used the same protocol) failed to observe toxicity at doses where it would be expected. Other problems with this study include limited number of animal and reports of respiratory infections and deaths occurring in the animals without specifying the extent of this problem. Because of the study's limitations (small number of animals per dose and limited toxicological evaluation), uncertainty factors appropriate to provide an adequate margin of safety for human exposure to Cr VI include 10 for animal to human extrapolation, 10 to protect sensitive populations and 10 due to limitation of the study's protocol (small number of animals per treatment group, early mortality, limited data reporting, no monitoring of Cr VI in the water). Thus the aggregate uncertainty factor is 1,000.

ADD = 2.5 mg/kg-day / 1,000 = 0.0025 mg/kg-day

Chopra *et al.*, 1996 - Only one dose level was used in this 22-week study of Cr VI administered to female rats in their drinking water. The LOAEL for Cr VI is estimated to be 1.4 mg/kg-day, based on cellular necrosis in the liver and kidney. Because of the study's limitation (short duration, small number of animals per dose, only a few tissues examined), uncertainty factors appropriate to provide an adequate margin of safety for human exposure to Cr VI in drinking water from this study include 10 for LOAEL to NOAEL extrapolation, 10 for animal to human extrapolation, and 10 to protect sensitive subpopulations. Additional factors could be applied for the less than lifetime study (10) and other limitations of the study protocol (3-10) (small number of treated animals, few tissues examined, unclear if Cr VI levels in the water were monitored). The aggregate factor is limited to 3,000.

ADD = 1.4 mg/kg-day / 3,000 = 0.00046 mg/kg-day

Acharya *et al.*, 2001 – Only one dose level was used in this 22-week study of Cr VI administered to male rats in their drinking water. The LOAEL for Cr VI is estimated to be 1.1 mg/kg-day, based on cellular necrosis in the liver and kidney of these animals. Because of the study's limitation (short duration, small number of animals per dose, only a few tissues examined), uncertainty factors appropriate to provide an adequate margin of safety for human exposure to Cr VI in drinking water from this study include 10 for LOAEL to NOAEL extrapolation, 10 for animal to human extrapolation, and 10 to

protect sensitive subpopulations. Additional factors could be applied for the less than lifetime study (10) and other limitations of the study protocol (10) (small number of treated animals, few tissues examined, unclear if Cr VI levels in the water were monitored). The aggregate factor is limited to 3,000.

ADD = 1.1 mg/kg-day / 3,000 = 0.00037 mg/kg-day

NTP, 2007 - Doses of 1.6 to 21.4 mg/kg-day of Cr VI were administered to male rats for thirteen weeks in this study. A LOAEL of 1.6 mg/kg-day was identified based on effects on blood forming tissues (decreased erythrocyte levels, mean cell volume, mean cell hemoglobin (total and concentration) and platelet concentrations). The uncertainty factors appropriate to provide an adequate margin of safety for human exposure to Cr VI in drinking water include 10 for extrapolating from a subchronic study, 10 to extrapolate between species, and 10 to protect potentially sensitive human subpopulations (including antacid users). An additional factor of 3 or 10 could be considered for limited data (small number of animals/group, short study, only a few tissues examined). The aggregate factor is limited to 3,000.

ADD = 1.6 mg/kg-day / 3,000 = 0.00053 mg/kg-day

NTP, 2008 - Female rats received 0.2, 0.9, 2.4 or 7.0 mg/kg-day of Cr VI administered in drinking water. A LOAEL of 0.2 mg/kg-day was identified based on effects in the female rat liver: mild chronic inflammation and fatty changes (note that while the incidence of fatty changes was increased relative to controls at all dose levels, the increase was only statistically significant at the three highest dose levels). The uncertainty factors appropriate to provide an adequate margin of safety for human exposure to Cr VI in drinking water include 10 for using a LOAEL, 10 to extrapolate between species, and 10 to protect potentially sensitive human subpopulations (including antacid users). The aggregate uncertainty factor is 1000.

ADD = 0.2 mg/kg-day / 1,000 = 0.0002 mg/kg-day

A public health-protective concentration (C, in mg/L) for Cr VI in drinking water for noncarcinogenic endpoints is calculated from the ADD as follows:

$$C = \frac{\text{ADD (mg/kg-day)} \times \text{RSC}}{\text{water intake (L/kg-day)}}$$

where,

RSC = relative source contribution (usually in the range of 20 to 80 percent);

Water intake = values for drinking water intake, calculated on a body weight basis, are derived from values in U.S. EPA (2008) and Kahn and Stralka (2009) as described in the footnote to Table 17 below.

Drinking water intake refers to tap water consumed as a beverage or tap water used in the home or local establishments to prepare food or drink (Kahn and Stralka, 2009).

The maximum default relative source contribution of 0.8 is used in this case, based upon the assumption that the major source of Cr VI is likely to be from drinking water. Little or no Cr VI exposure is expected from air, food, incidental inhalation, dermal and oral exposure to soil and dust. For drinking water intake, either 90 or 95 percentile water consumption values may be considered to be health-protective.

The results of six studies were evaluated for derivation of a health protective concentration for Cr VI based on non-cancer toxic endpoints (Table 17). In five of the studies, toxic effects were detected (NTP 1997a; Chopra *et al*., 1966; Acharya *et al*., 2001; NTP, 2007, 2008) although a NOAEL was not identified in four of these studies (Chopra *et al*., 1966; Acharya *et al*., 2001; NTP, 2007, 2008). The MacKenzie *et al*. (1958) study did not identify toxic effects, but this was not its purpose.

When several toxicity studies are available, it is advisable to employ studies that are clearly superior to identify sensitive toxicological endpoint(s). The most sensitive endpoint is then identified and employed to derive a health-protective concentration. The 2008 NTP study is clearly the best of the available studies for deriving a health-protective concentration. Therefore the health-protective level for non-carcinogenic effects is based on the LOAEL from this study.

Table 17. Health Protective Concentrations for Hexavalent Chromium based on Non-cancer Endpoints

Study	ADD (mg/kg-day)	Health Protective Concentration (mg/L)[a]	
		Child	Adult
NTP, 2007	0.00053	0.0063	0.011
NTP, 2008	0.0002	0.0024	0.0041
NTP, 1997a	0.00037	0.0044	0.0076
MacKenzie *et al*., 1958	0.0025	0.030	0.051
Chopra *et al.*, 1966	0.0005	0.0055	0.010
Acharya *et al.*, 2001	0.00037	0.0044	0.0076

[a]Upper 95th percentile water intakes for a child (0 to <11 years) and adults (16 to 70 years) are 0.067 and 0.039 L/kg-day, respectively, calculated using the "Consumers only" data in U.S. EPA (2008) and Kahn and Stralka (2009). The child value is a time weighted average of the values for birth<1 month, 1<3 months, 3<6 months, 6<12 months, 1<2 years, 2<3 years, 3<6 years and 6<11 years. The adult value is a time weighted average of the values for 16<18, 18<21 and >21 years.

Alternatively, a composite value (usually a median or mean value) of the various health protective concentrations can be employed to derive the health-protective concentration, when the studies are of a similar quality. The matrix of potential health protective concentrations based exclusively on non-cancer effects in the six studies described above, as shown in Table 17, presents health protective concentrations for children and adults

based on their body weight and their water consumption rates. Five of the studies yielded similar health protective concentrations for children (the most sensitive receptor) that ranged from 0.002 to 0.006 mg/L with a median value of 0.004 mg/L (NTP, 1997a; NTP, 2007; Chopra *et al*., 1966; Acharya *et al*., 2001). The health protective concentration of 0.002 mg/L based on the 2008 NTP study is similar to the values derived from the other studies.

Carcinogenic Effects

Calculation of a health-protective concentration to protect against carcinogenic effects of Cr VI considered three routes of exposure: water ingestion (tap water consumed as a beverage or tap water used in the home or local establishments to prepare food or drink), inhalation of water droplets generated during showering, and dermal exposure during showering. All three of these routes could be relevant because of the concern that Cr VI may be carcinogenic by each of these exposure routes. However, as explained earlier in this document, the dermal contribution to exposure is very small, and is expected to add little compared to the risk posed by other exposure routes. A health-protective concentration (C) that addresses the inhalation and oral routes of exposure for carcinogenic effects is derived using the following general equation, which collapses the separate calculations for each exposure period (shown above in the Dose Response section) into a single bracket for convenience of expression:

$$C = \frac{R}{P_o \times (\sum_j [ASF_j \times d_j \times cons^o_j]) + P_i \times (\sum_j [ASF_j \times d_j \times cons^i_j])}$$

where:

R	=	a default risk level of one in one million, or 10^{-6};
P_o	=	oral cancer potency, in mg/kg-day;
P_i	=	inhalation cancer potency, in mg/kg-day;
$\sum_j$	=	sum of contributions at each age range;
ASF_j	=	age sensitivity factors for the 3rd trimester + infants, children and adults;
d_j	=	duration of exposure factors for the 3rd trimester + infants, children and adult life stages;
$cons^{i/o}_j$	=	equivalent water exposure values for each age range.

Estimates of the oral potency of Cr VI were obtained from the results of an animal study (NTP, 2008) because epidemiology studies of human exposure to Cr VI were judged to be unsuitable for deriving a dose-response relationship for Cr VI. Cancer potency values could not be reliably calculated for the stomach tumor data reported by Zhang and Li (1987) because of inadequate exposure information. Similarly, estimates of the amount of Cr VI that was inhaled and then swallowed in occupational studies are highly uncertain.

Statistically significant increases in tumors (adenoma or carcinoma) were observed in the oral cavity of male and female F344 rats and the small intestine of male and female $B6C3F_1$ mice following Cr VI administration in drinking water (NTP, 2008). The findings in male mice were judged to yield the best dose-response relationship for oral exposure to Cr VI and therefore are the basis of the oral slope factor of 0.5 $(mg/kg\text{-}day)^{-1}$. For the inhalation route, the human cancer potency value of 510 $(mg/kg\text{-}day)^{-1}$ as derived above in the dose response assessment section was used.

Drinking water exposure is estimated for this calculation for the age ranges used above in the Dose Response section. The drinking water consumption values utilized are upper 95^{th} percentile values calculated by OEHHA of 0.125, 0.047, and 0.039 L/kg-day for infancy, childhood, and adult life stages using the "Consumers only" values from U.S. EPA (2008) and Kahn and Stralka (2009). The infant value is the time weighted average of the values for 0<2 months, 1<3 months, 3<6 months, 6<12 months, and 1<2 years. The childhood value is the time weighted average of the values for 2<3, 3<6, 6<11 and 11<16 years. The adult value is a time weighted average of the values for 16<18, 18<21 and >21 years. The adult (maternal) value was used for the 3^{rd} trimester calculation. The value for exposure to water droplets in showering is $3.86x10^{-7}$ L/kg-day (Keating and McKone, 1993), which is applied to children and adults only, since infants are presumed not to take showers.

Estimation of the drinking water exposures x age sensitivity factors and duration adjustments in the equation above for each life stage (ASF_j x d_j x $cons^o_j$) provides values in the units of equivalent L_{ingest}/kg-day as in the standard risk calculation (C = R / (potency x dose)), as shown in Table 18.

Table 18. Calculation of Adjusted Exposures by Life-stage (ASF_j x d_j x $cons_j$) for Hexavalent Chromium

Life Stages	**ASF_j x d_j x $cons_j$**	
	Oral	**Inhalation**
3^{rd} Trimester	0.0014	$0.138x10^{-7}$
Infant (0-2)	0.0357	--
Child (2-16)	0.0282	$2.316x10^{-7}$
Adult (16-70)	0.0300	$2.978x10^{-7}$
Exposure Totals (L/kg-day)	0.0953	$5.432x10^{-7}$

Inserting the exposure values in the equation above,

$$C = \frac{R}{0.5\ (mg/kg\text{-}day)^{-1} \times (0.0953)(L/kg\text{-}day) + 510\ (mg/kg\text{-}day)^{-1} \times (5.432x10^{-7})}\ (L/kg\text{-}day)$$

$$C = \frac{10^{-6}}{0.04765 + 0.00028} = 2.09 \times 10^{-5}\ mg/L = 0.02\ \mu g/L \text{ or ppb (rounded)}$$

As shown above, the proportion of the total cancer risk contributed by inhalation is very small (~0.6%), despite the high cancer potency by the inhalation route. The PHG for Cr VI is therefore set at 0.02 µg/L or 0.02 ppb, representing a lifetime cancer risk of 1 in 1 million. Other toxic effects associated with Cr VI were observed at higher exposure levels. The PHG for carcinogenic effects is protective against these other toxic effects.

RISK CHARACTERIZATION

The PHG for Cr VI of 0.02 µg/L is based on risk associated with the ingestion of drinking water, with a very small contribution from the inhalation of aerosol droplets generated during showering. Various sources of uncertainty regarding the development of health-protective criteria for the oral and inhalation route are discussed.

Hazard Identification - While there is considerable evidence that occupational inhalation exposures of humans to Cr VI have resulted in increased incidences of lung cancer, studies in humans characterizing the carcinogenicity of oral exposures to CrVI are more limited. Only two epidemiological studies were identified that measured organ-specific cancer in humans exposed to Cr VI in drinking water (Zhang and Li, 1987; Linos *et al.*, 2011). Five long-term cancer bioassays, three in mice and two in rats, have been conducted in which Cr VI was administered in the drinking water (Borneff *et al.*, 1968; NTP, 2008). OEHHA's analysis of findings of Borneff and coworkers found a statistically significant increase in tumors of the forestomach in the female mouse. There is uncertainty associated with this finding because of a viral infection that caused substantial intercurrent mortality, a single dose level, differences in the length of survival in different generations, and other factors. Although there is no evidence that the increase in tumors was due to the viral infection, or that other factors limiting this study would have led to these findings, the results have been judged inappropriate for quantitative risk assessment.

The recent NTP cancer bioassays in rats and mice of both sexes (NTP, 2008) revealed statistically significant dose related increases in tumors in the oral cavity in male and female rats and tumors of the small intestine in male and female mice. The data in mice were judged to be suitable for quantitative risk assessment.

Once inside cells, Cr VI has been shown to damage DNA. The finding of genotoxicity in the liver following oral administration of Cr VI is consistent with both the toxicokinetic findings and the proposed DNA-damaging mechanism of action. Taken together, the toxicity and cancer studies in humans and animals, plus the mechanistic, toxicokinetic and genotoxicity studies, provide sufficient evidence for the carcinogenicity of Cr VI in humans.

The NTP studies in which Cr VI was administered to rodents in the feed suggest that liver and blood-forming tissues may also be affected by Cr VI (NTP, 1996, 1997a,b, 2007). Three studies in male and female rats given Cr VI orally for 22 weeks or two years suggest that the liver is a target organ (Acharya *et al.*, 2001; Chopra *et al.*, 1996; NTP 2008). These studies appear to indicate that Cr VI is entering liver cells, which is consistent with the findings of toxicokinetic studies in which increased chromium levels were observed in liver following oral administration of Cr VI. However, in one early

study, no toxicity was reported in rats administered Cr VI for one year (MacKenzie *et al.*, 1958).

Dose Response – cancer endpoint

Oral exposure - The available human studies provided limited information on the dose-response relationship for Cr VI by the oral route. Cancer potency values based on a dose response relationship could not be reliably calculated from the findings of Zhang and Li (1987). The Borneff *et al*. (1968) study in mice provided limited data regarding increases in tumors in mice and was judged unsuitable for deriving a dose-response relationship for Cr VI. The findings of the NTP (2008) studies in rats and mice of both sexes provided sufficient information for developing dose-response relationships for Cr VI. Dose-response data for tumors of the small intestine seen in male and female mice were analyzed. An acceptable fit to the multistage model in the BMDS was obtained using all dose groups in the male mouse study; for the female mouse study the high dose group was dropped. Thus the findings in male mice were judged to be the most suitable for developing a dose-response relationship for Cr VI.

Inhalation exposure - A dose-response relationship was derived from an occupational exposure to Cr VI, based on lung cancer in workers in a plant in Painesville, Ohio. A linear model was applied to correlate cumulative exposure to chromium with relative risk. Exposure estimates are relatively uncertain, but were judged adequate to develop a cancer potency factor.

Dose response – non-cancer endpoint

The recent NTP (2008) study was judged to be the best study for identifying the lowest dose associated with an adverse effect. The health-protective level for non-carcinogenic effects was developed from the LOAEL by applying appropriate uncertainty factors. Health-protective values derived from other animal studies for the same endpoint (liver toxicity) were at similar levels (see Table 17).

Exposure Assessment - The non-cancer health-based criterion reflects a relative source contribution of 80 percent of the total exposure coming from drinking water. While these are typical conventions employed to estimate exposure, there is uncertainty attendant with their use.

The estimate of exposure to water inhaled during showering relies on the results of a study by Keating and McKone (1993), and assumes a daily 10-minute shower. Different shower conditions including the average duration, type of showerhead, water temperature and pressure, and size and ventilation of the shower and bathroom would result in varying exposure by this route. The early-in-life exposure factor correction was not applied to infants for the inhalation route, since they generally do not take showers. We recognize that average shower duration may change markedly over the age range from two to 16, but data are not available to more precisely estimate the varying exposure. This route of exposure contributed very little to the total exposure to Cr VI in drinking water.

Cancer risk from exposure to drinking water was estimated based on the upper 95% confidence limits of exposure to tap water, by life stage, as described by U.S. EPA (2008) and Kahn and Stralka (2009). The values used were derived from a study by U.S. EPA

(2004) of intake measured in USDA's 1994-1996 and 1998 continuing survey of food intakes by individuals, and represent values for tap water consumers only. These drinking water exposure values are significantly larger than the default value of 2 L/day that OEHHA has used in many previous cancer risk assessments. The use of the 95% upper confidence limit drinking water consumption value provides extra assurance that the risk to the entire population, including sensitive subpopulations, is being considered.

Risk Characterization – There are many sources of uncertainty in the calculation of the PHG. The NTP carcinogenicity studies provide robust data for the assessment of oral cancer risk attributed to Cr IV. Protection of public health requires that health-based criteria be developed in a manner to ensure that risk is not markedly underestimated.

OTHER REGULATORY STANDARDS

The U.S. EPA MCLG and MCL for total chromium are set at 0.1 mg/L, or 100 ppb (U.S. EPA, 2005a). The U.S. EPA stated: "There was inadequate data to demonstrate that Cr VI has oncogenic potential via ingestion" (U.S. EPA, 1989). The RfD for Cr VI is $3x10^{-3}$ mg/kg-day (U.S. EPA, 1998, 2002a). The MCLG and RfD were based on the absence of observed toxic effects in the study of MacKenzie *et al*. (1958). U.S. EPA does not have separate drinking water standards for Cr III and Cr VI. The California MCL for total chromium is 0.05 mg/L, or 50 ppb (22 CCR, section 64431, Table 64431-A-Inorganic Chemicals), is also based on a non-cancer risk estimate.

The U.S. EPA also has 1 day and 10 day health advisories of 1 mg/L (1,000 ppb) for total chromium.

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APPENDIX A

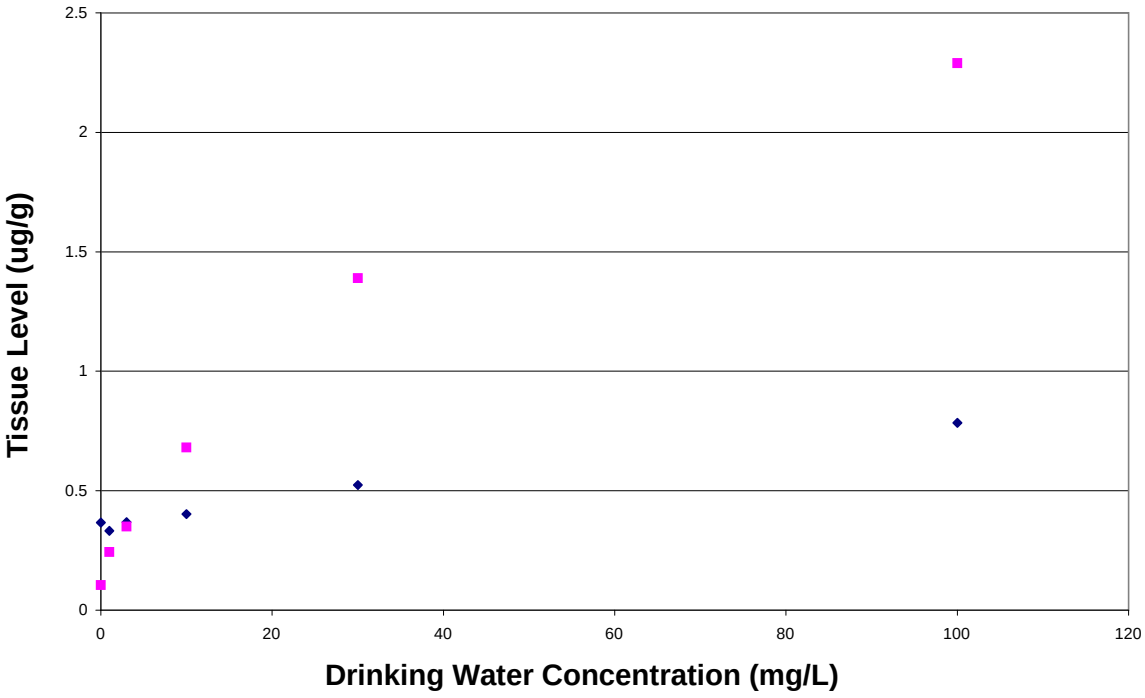
Carcinogenic Threshold: Was the reductive capacity of the rodent GI tract exceeded in the NTP (2008) two-year bioassay?

Because hexavalent chromium is rapidly converted to the trivalent form in the GI tract, several investigators have asserted that negligible amounts of Cr VI are orally absorbed (De Flora and Wetterhahn, 1989; De Flora *et al*., 1997; De Flora, 2000; Proctor *et al.*, 2002b). DeFlora and associates estimate a reducing potential of the human GI tract in excess of 80 mg/day. Consistent with the estimates of DeFlora and associates, studies by Proctor and coworkers also showed that stomach fluids rapidly reduced Cr VI to Cr III at levels that ranged from 0.3 to 1 mg/L (Proctor *et al.*, 2002a). These investigators also reported that initial Cr VI levels from 100 to 400 μg/L did not alter the rate of reduction.

In studies in humans where the oral administration of Cr VI resulted in increased blood chromium levels and an increase in urinary half-life, the metal was administered at levels that would not exhaust the reducing capacity of stomach fluids (based on the findings of DeFlora and coworkers 1989, 2000 and Proctor and associates, 2002b). Increased absorption and a prolonged urinary half-life of chromium, compared to what would be expected using Cr III, were also observed in a study where Cr VI was administered in an acidic vehicle (orange juice) (Kerger, 1996). Other studies by Kerger and associates indicated a rapid and essentially complete reduction of Cr VI to Cr III (*in vitro*) when added to orange juice (Kerger, 1996). Thus while considerable amounts of chromium are reduced to Cr III in the GI tract, toxicokinetic studies in humans that were conducted at relatively high doses (necessary to detect Cr absorption), but at doses well below the reducing potential of the GI tract, indicate a portion of the dose is absorbed. The absorption at the doses that were tested does not appear to be due to the exhaustion of the reducing capacity of the GI tract.

Studies in animals also do not indicate that the absorption of Cr VI was a consequence of the exhaustion of the capacity of the GI tract to reduce Cr VI to Cr III. Chromium blood and kidney levels were determined in male B6C3F$_1$ mice administered Cr VI in drinking water at 1 to 300 mg/L for 21 days (NTP, 2007). Blood and kidney chromium levels increased with the concentration of chromium in water with no threshold evident (Figures A1 and A2). Figure A2 is the same data as Figure A1 with the addition of the highest dose group.

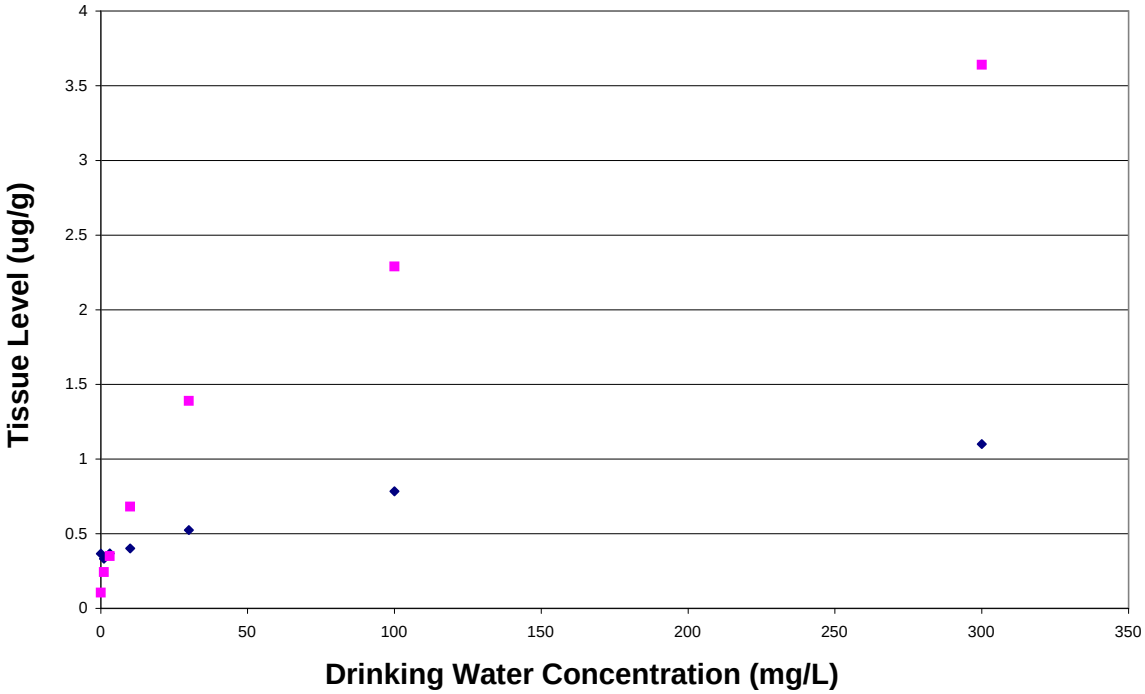
Figure A1. Blood and Kidney Chromium Levels in Male Mice



Square: Kidney chromium levels Diamond: Blood chromium levels

In another study, Cr VI was administered in drinking water at 5 to 180 mg/L to female $B6C3F_1$ mice for 6 to 371 days (NTP, 2008). Chromium levels in erythrocytes, plasma, liver and kidney were measured (Figures A3-A6). Notable increases in chromium levels were observed in the liver (probably due to blood flow via the portal circulation) and kidney (the site of elimination), while little increase was observed in the red blood cell and the plasma, an observation consistent with previous studies (Witmer *et al.*, 1989; Thomann *et al.*, 1994; Costa, 1997). At the four times when measurements were performed, chromium levels in the liver and kidney were markedly increased with dose (perhaps beginning to plateau at the highest doses). The plots of chromium accumulation in tissue versus Cr VI concentration in drinking water were linear or supralinear, suggesting that there was no saturation of reductive capacity of the GI tract over this dose range (Collins *et al.*, 2010). The findings of this study are consistent with the aforementioned 21 day study. The findings of both of these studies are not consistent with the assertion that Cr VI absorption occurs only when the reducing capacity of the GI tract is exhausted.

Figure A2. Blood and Kidney Chromium Levels in Male Mice



Square: Kidney chromium levels. Diamond: Blood chromium levels

Figure A3. Chromium Tissue Levels on Day 6 in Female Mouse

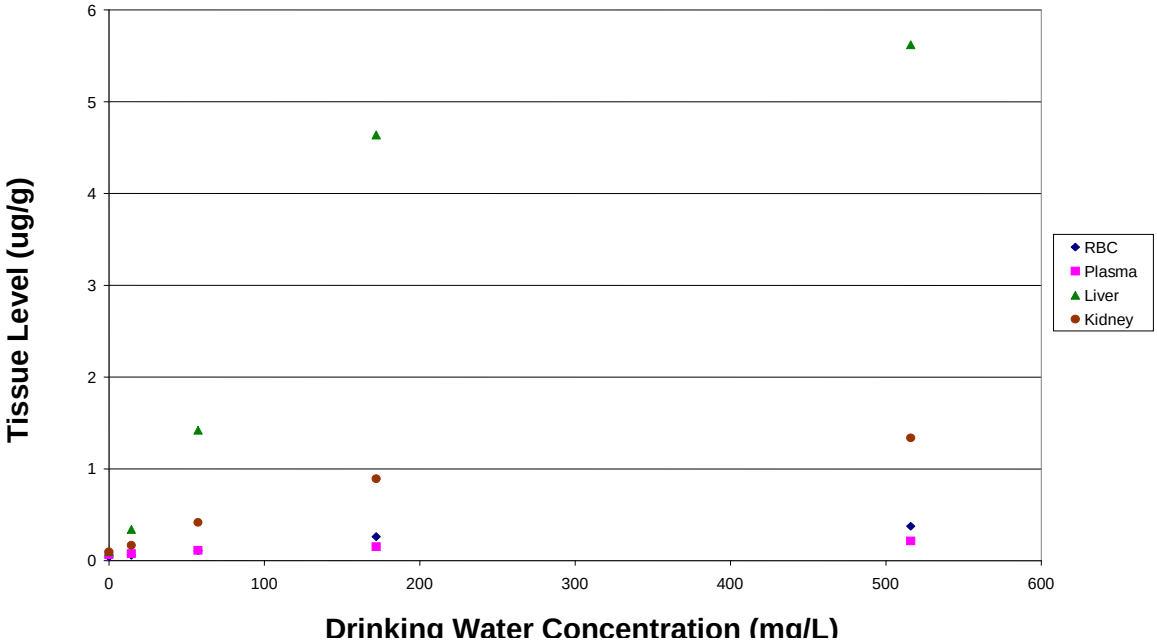


Figure A4. Chromium Tissue Levels on Day 13 in Female Mouse

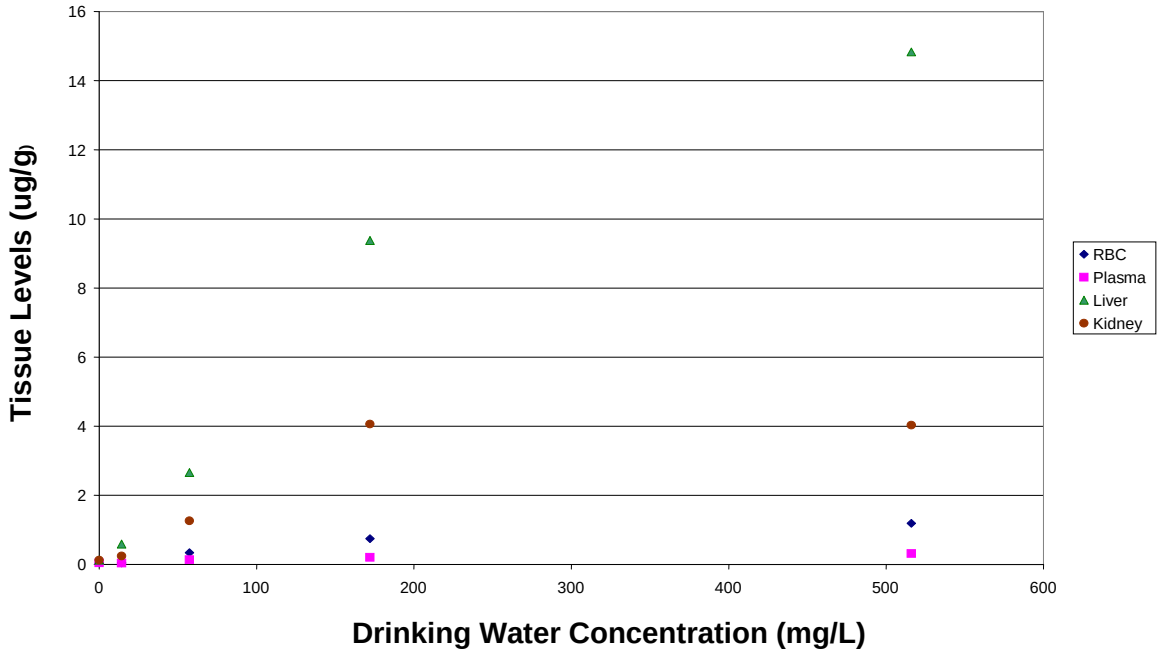


Figure A5. Chromium Tissue Levels on Day 18

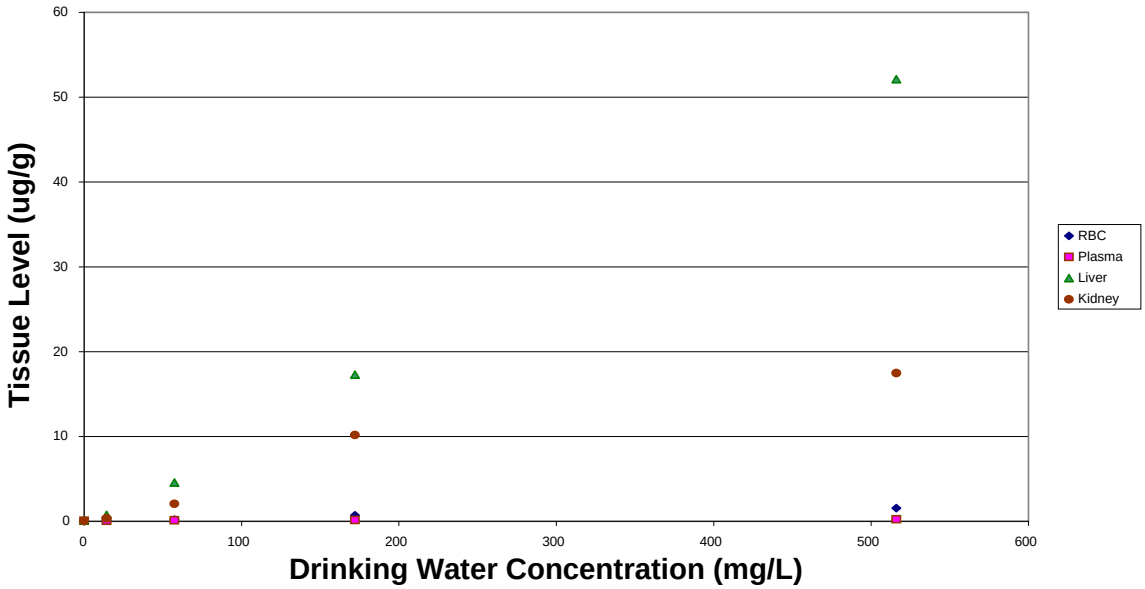
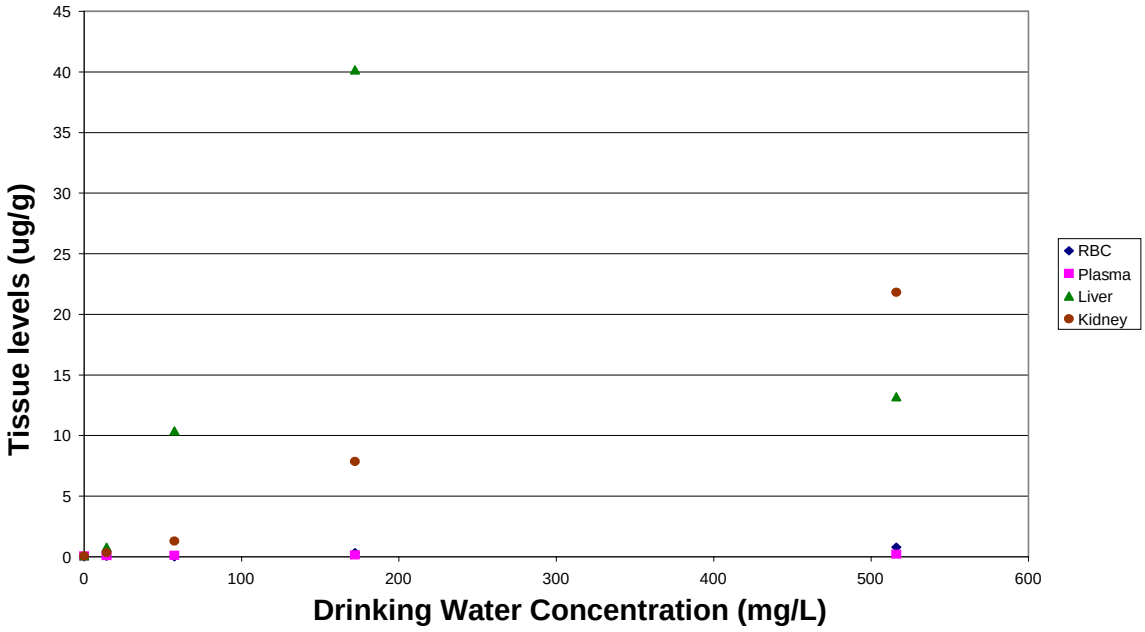


Figure A6. Chromium Tissue Levels on Day 371



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APPENDIX B

Mouse Cancer Study of Borneff et al. (1968)

Using a three-generation study design, Borneff *et al.* (1968) treated 120 female and 10 male NMRI mice with 1 mg K_2CrO_4 per day (500 ppm) in drinking water (containing 3 percent household detergent). An equal number of animals received drinking water (3 percent detergent) only. In addition, two groups of 120 females and 10 males which received either benzo[a]pyrene alone or benzo[a]pyrene + 500 ppm K_2CrO_4 in drinking water were included in the study. The Cr VI drinking water concentration (135 mg/L) was approximately twice the lowest drinking water concentration giving intestinal tumors in the two-year bioassay (NTP, 2008). Animals were mated six weeks after the start of treatment. Two mice from each litter were selected as the first generation (F_1) mice.

Three weeks after birth these mice were separated by sex and received the same food and concentration of test substance [0 or 500 ppm K_2CrO_4, or benzo[*a*]pyrene or benzo[*a*]pyrene + 500 ppm K_2CrO_4] in their drinking water as did the parent (F_0) generation. An outbreak of mousepox (ectromelia) virus occurred during the eighth month of the experiment, and within three months the majority (512) of the animals died. All animals received a mousepox vaccination two months after the outbreak, and this effectively ended the epidemic.

First generation (F_1) mice were mated after the mousepox epidemic had ended. The numbers of offspring from the mating of F_1 mice were much less than after the breeding of the F_0 animals. The F_2 generation mice received the same food and concentration of test substance [0 or 500 ppm K_2CrO_4, or benzo[*a*]pyrene or benzo[*a*]pyrene + 500 ppm K_2CrO_4] in their drinking water as did the F_0 and F_1 generations. The F_2 mice received the pox vaccine at two months of age, and all animals received a second dose of the vaccine three months later. These studies were terminated after 880 days. At the time of termination, F_2 mice had been exposed for approximately 17 months (510 days). Necropsies were performed on all mice killed on the 880th day plus those that died during the course of the studies, with the exception of those that had died of ectromelia.

Two carcinomas of the forestomach were observed in female mice exposed to K_2CrO_4. No malignant stomach tumors were found in control mice. Nine benign forestomach tumors were observed in female mice exposed to K_2CrO_4. These tumors were identified as papillomas and described histologically as having a more or less branched structure. Nine tumors (combined carcinomas and papillomas) were observed in the F_0 generation, 1 tumor in the F_1 generation and 2 tumors in the F_2 generation. The authors indicated in their discussion that the carcinomas and benign tumors occurred in different animals.

Benign and malignant neoplasms were combined for the statistical analysis (McConnell *et al.*, 1986; U.S. EPA, 2005). The combined incidence of malignant and benign forestomach tumors (11/66) in K_2CrO_4-exposed-female mice (all three generations combined) was significantly different than the combined incidence of tumors in control female mice (2/79) [Fisher's Exact test, $p<0.05$, (OEHHA analysis)]. Analysis of tumor incidence by generation finds that in F_0 animals, 22 percent of K_2CrO_4-exposed mice had

forestomach tumors compared to 3.6 percent of controls. In the F_1 and F_2 animals, tumor incidence was similar to controls.

Borneff and coworkers suggest that the mousepox epidemic may have delayed tumor growth in the F_1 generation (as suggested by other studies and as evidenced by the five month delay in the appearance of benzo[*a*]pyrene-induced tumors in this study). Borneff and coworkers also cite a study in which growth of melanoma was inhibited after massive pox vaccination. In contrast to the F_1 generation, tumor growth had already begun in F_0 mice at the time that the mousepox epidemic occurred (experimental month eight). The F_2 generation was not exposed to mousepox virus; however, they were vaccinated and this could have affected tumor development.

Borneff and coworkers calculated that the K_2CrO_4 –exposed mice who developed forestomach carcinomas were exposed to more than 700 mg of chromate, and postulated that a minimum dosage of 700 mg was needed for expression of chromate's carcinogenic effect. Based on this, Borneff and coworkers suggested that the dose received by the F_2 generation (corresponding to a total dose of about 510 mg of chromate over a 17 month lifetime), was not sufficient for the induction of tumors in these animals.

Issues related to experimental design and adequacy of the animal model

Certain aspects of the three-generation drinking water studies reported by Borneff *et al*. (1968) henceforth referred to as "the study," should be considered in a positive light. A large number of female mice per treatment group was used in the study. The study contained a vehicle and positive control that are critical for interpreting the results of the study. The animals were exposed to Cr VI in drinking water for their lifetime and the drinking water solution containing K_2CrO_4 was analyzed at regular intervals to confirm its stability.

Because the study contained a vehicle control group and a positive control group, the statistically significant increase in forestomach tumors that occurred in female animals administered Cr VI compared to the vehicle/negative control group would appear to be due to the administration of Cr VI. However, certain aspects of the study complicate and may compromise the findings of the study. (1) The animals were housed in groups. There has been some suggestion that this may have influenced the results of the study. (2) A major outbreak of mousepox virus caused significant mortality in the F_0 generation. (3) Only one dose level of Cr VI was employed in the study. There has been some suggestion that the dose was excessive. (4) Tumors observed in the forestomach of mice are not representative of what may occur in the stomach of humans. (5) There were no reported preneoplastic lesions in the forestomachs of mice in this study. (6) There was no increase in tumors in animals exposed in utero. (7) The multigenerational design raises certain issues about how to interpret the increased incidence of tumors in the study.

The importance of each aspect on the overall study findings is discussed below:

1) Group housing. The tumors occurred in female mice. Group housing of female mice is standard NTP practice, and is not associated with differences in forestomach tumors (Haseman *et al.*, 1994).

2) Mousepox Virus. While there was significant mortality in the F_0 generation due to the outbreak of the mousepox (ectromelia) virus, there is no evidence that the increase in tumors observed in female mice were due to the virus. There is no evidence that the forestomach of the mouse is a site where mousepox lesions occur (Dick *et al.*, 1996). Borneff and coworkers characterized the forestomach papillomas histologically as displaying a branched structure, which is typical of papillomas. If these lesions were instead a result of the mousepox infection, then an equal increase in papillomas should have been observed in "surfactant only" vehicle control animals, which did not occur. The high early mortality in the F_1 generation resulting from the mousepox epidemic and the shorter lifespans of the F_1 and F_2 generations are a concern because the high mortality could have compromised the ability of the study to detect a carcinogenic response. Fortunately, because the study began with rather large numbers of animals, enough of the animals survived to allow sufficient sensitivity to detect a carcinogenic response.

3) Dose of Cr VI in the study. Only one dose level of Cr VI was administered to the mice. The dose administered did not appear to be excessive such that the study could be considered compromised. Borneff *et al.* (1968) stated that the dose chosen was "close to the maximum concentration that is tolerated by mice without developing any damage." The paper did not report any toxicity, excess mortality, or weight loss associated with K_2CrO_4 treatment.

The level of Cr VI employed did not appear to have achieved the maximum tolerated dose (MTD) that is normally targeted in cancer bioassays. As defined in the 1976 Guidelines for Carcinogen Bioassay in Small Rodents (Sontag *et al.*, 1976, the MTD is the "highest dose of the test agent during the chronic study that can be predicted not to alter the animals' longevity from effects other than carcinogenicity." It was also defined as a dose that caused "no more than a 10 percent weight decrement" (compared to controls) and "does not produce mortality, clinical signs of toxicity or pathologic lesions (other than those that may be related to a neoplastic response) that would be predicted [in the chronic study] to shorten an animal's natural life span." Over time, histopathological appearance became more important in design of the chronic NTP studies, with effects on weight gain of secondary importance (McConnell, 1989).

In evaluating study design, McConnell has stated "if significant toxicity was not achieved at the highest dose, one can say that the MTD was not achieved." He also stated that "overall, probably the best design for choosing doses in cancer bioassays …is to use an MTD for the high dose." In particular, the MTD is "clearly justified when one is designing studies of chemicals found in drinking water, food, air and the work environment" (McConnell, 1989).

In its *Report of the Ad hoc Panel on Chemical Carcinogenesis Testing and Evaluation of the NTP Board of Scientific Counselors* (NTP, 1984), the National Toxicology Program stated that the MTD should be used in animal bioassays for carcinogenic agents as the highest level administered. The International Agency for Research on Cancer (IARC, 1980) stated that the high dose is one that produces some toxicity during the course of the study. Regarding lower doses, IARC (1980) stated: "The chief purpose of the lower dose is to ensure that at least one group of animals can be compared meaningfully with the controls, even if a misjudgment occurred in the selection of the high dose (i.e., if the high

dose group suffers such severe mortality that few animals live long enough for tumours to arise or suffer such severe toxic effects that the relevance of the findings in the high dose group is doubtful)."

In the Guideline for Carcinogen Risk Assessment (2005), the U.S. EPA stated that "an adequate high dose would generally be one that produces some toxic effects without unduly affecting mortality from effects other than cancer or producting significant adverse effects on the nutrition and health of the test animals." It further stated that "The high dose would generally be considered inadequate if neither toxicity nor change in weight gain is observed (U.S. EPA, 2005)." Based on these guidelines, there is no evidence that a dose in excess of the MTD was administered, since there were no signs of excess mortality, toxicity or weight loss in the study.

4) pH of the forestomach. Because a major portion of the dose of orally ingested Cr VI appears to be reduced to Cr III in the acidic environment of the human stomach (De Flora, 2000), the occurrence of tumors in the mouse forestomach may not be representative of what would occur in humans if the mouse forestomach is a neutral environment with a pH of seven. However, there is no evidence that the pH of the mouse forestomach is neutral. No studies in the scientific literature were identified in which mouse forestomach pH has been measured. However, studies measuring the pH of the rat forestomach consistently found that the forestomach is acidic (Kunstyr *et al.*, 1976; Browning *et al.*, 1983; Browning *et al.*, 1984; Ward *et al.*, 1986). Kunstyr *et al.* (1976) reported that pH values were dependent on the degree of filling of the forestomach and varied between pH 3 and 5. Browning *et al.* (1984) reported that forestomach pH in male rats was 4.3$\pm$0.1 except in starving animals where it was much more acidic (pH 2.3$\pm$0.5).

While the stomach in humans is typically acidic, there is a sizable population with near neutral pH in their stomach due to disease (e.g., pernicious anemia, *Helicobactor pylori* infection) and due to medications (e.g., proton pump inhibitors, histamine receptor blockers). Infant's stomachs are also near neutral pH during the first days to weeks after birth. A more detailed discussion can be found in the sensitive subpopulation section of the PHG.

5) Lack of preneoplastic lesions. If oral exposure to Cr VI in drinking water induced tumors in female mice, preneoplastic forestomach lesions might also be expected, but none were reported by the investigators. The Borneff *et al.* (1968) study also included a positive control, benzo[*a*]pyrene, which caused significant increases in forestomach tumors in this study and in previous studies of Borneff (1963) and others **(**Rigdon and Neal, 1966). In the Borneff *et al.* (1968) study, preneoplastic lesions were not reported in mice administered benzo[*a*]pyrene. The reason for this is unknown, but is likely due to the same factor in mice exposed to chromium and those exposed to benzo[*a*]pyrene). Thus, the significance of the lack of reported preneoplastic lesions in mice receiving Cr VI in this study is unclear.

6) In utero exposure. The Borneff study used a multigenerational protocol, which resulted in two generations exposed *in utero* and during weaning (F_1 and F_2) and one generation that was not (F_0). Under certain circumstances this additional exposure might be expected to result in an increased response. With an increased focus on assessing impacts of toxicants on children (U.S. Congress, 1996), the U.S. EPA explored the use of

protocols similar to that employed by Borneff *et al.*, which included perinatal exposure of animals (U.S. EPA, 1996). They concluded, "quantitatively, perinatal carcinogenicity dosing may or may not result in higher tumor incidence than standard dosing."

It has not been demonstrated that the perinatal period is a period of increased susceptibility to Cr VI. The reducing ability of the dam's stomach, blood and placenta may protect the fetus. In addition, pups feed on dam's milk and and do not directly consume drinking water, so pups may not receive much exposure to Cr VI until after weaning. However, in light of chromium accumulation in various tissues of adult animals ingesting Cr VI, chromium accumulation in the fetus and adverse fetal effects cannot be ruled out.

7) Multigenerational design. While there are certain advantages to bioassays that evaluate exposure to toxicants for several generations, this design may complicate the evaluation of findings of the study. The animals in the Borneff study were related to one another across generations and therefore each generation cannot be considered to be independent from a statistical standpoint. No information was provided as to which specific animals had tumors.

The animals in each generation were administered the identical test articles, received the same food, housing, and housekeeping, and were monitored in the same way (at the same time and in the same cages for much of their lifetime). The F_0 generation that survived the mousepox virus received a greater cumulative dose of Cr VI because they lived the longest, which perhaps explains the occurrence of tumors primarily in the F_0 generation.

In any event, each generation of mice in this study should not be considered to be a separate (independent) study, and representing them as such would not be advisable. The decision to combine tumors across the three generations of female mice for statistical analysis seems the most appropriate thing to do for these limited data.

Potential Influence of Helicobacter Infections on Stomach Tumors

Statistically significant increases in stomach tumors were observed in the Borneff study in the F_0 generation, while no significant increases were observed in the F_1 and F_2 generations. Why the increase was only detected in the F_0 generation is unclear. OEHHA hypothesizes that this effect may have occurred because of the presence of *helicobacter* in the stomach of the F_0 generation mice. Since the time of the Borneff study, *helicobacter* species have been closely related to stomach ulcers and stomach tumors in humans (Correa, 1988, 1992; Centers for Disease Control, 2002). Studies in animals exposed to carcinogens have also revealed stomach tumors when the animals were infected with *helicobacter* and no increases in uninfected animals. The mice in the Borneff study were exposed to infectious agents but it is unknown if they were infected with *helicobacter* (the agent was unknown at the time of the study). NTP has detected *helicobacter* infection in animals in past NTP studies (Hailey *et al.*, 1998).

The location of the tumors in the forestomach in the Borneff study is consistent with *helicobacter* thriving in the less acidic environment. Recent studies in animals with other carcinogens showed that neither *helicobactor* nor the carcinogen alone yielded increases in stomach tumors whereas the combination of both agents resulted in an increase in stomach tumors. The treatment of mice with Cr VI may have prevented the transmission

of this agent to the F_1 and F_2 generations, thereby accounting for the lack of tumors in the F_1 and F_2 generation (the newborn stomach is characterized by lower acidity which may have substantially reduced the conversion of Cr VI to Cr III, precipitating the eradication of the *helicobacter* infection in the newborn). A more thorough review of the research associated with this hypothesis follows.

The Helicobacter Hypothesis

Helicobacter pylori, a bacterium that commonly occurs in the human stomach, has been linked to various stomach maladies including gastritis, gastric and duodenal ulcers, and cancer. Stomach cancer in humans associated with *H. pylori* infection appears to occur when and where the local environment in the stomach favors the organism. While the incidence of gastritis is quite high in people with *H. pylori* infections, most people with these infections do not develop stomach cancer.

In humans, *H. pylori* growth occurs in condition of moderate acidity. Similarly, *Helicobacter* infections in the stomach of animals tend to occur in less acidic environments. This suggests that the organism should thrive in the less acidic environment of the rodent forestomach, the site of most chemically-induced stomach tumors in rodent bioassays.

Recently, a model of *H. pylori* infection that more closely mirrored what is observed in humans was developed in the Mongolian gerbil. Chemically induced tumors in the stomach of Mongolian gerbil occurred mostly when the chemical agent was administered in combination with *Helicobacter* and not when the potent chemical agent or *Helicobacter* was administered alone. The occurrence of stomach tumors in the rodent bioassays, primarily in the forestomach, may be due to the bacterium preferentially colonizing this portion of the stomach and the combined actions of the bacterium and the chemical agent. An interaction of *Helicobacter* species with chemical carcinogens may help explain some of the variability in animal bioassay results as well as the localization of tumors.

Only certain human populations with a high prevalence of *H. pylori* infections develop stomach cancer, while others do not. Only a small fraction of individuals who are infected by *H. pylori* develop stomach cancer. Given the results of studies in the Mongolian gerbil, other factors such as exposure to chemical agents combined with the bacterial infection may be involved. Correspondingly, current bioassays may not be optimal for detecting chemicals that induce stomach cancer.

Helicobacter infections in people are transmissible, and incidence increases with age. The same pattern is likely in rodent colonies. The possible role of *Helicobacter* infection is discussed in relation to studies on Cr VI, a chemical linked to stomach tumors in humans and rodents. Research is proposed to evaluate if colonization by *Helicobacter* could have an important role in the development of tumors in animals exposed to Cr VI (and other agents). Such studies could provide valuable information related to the mechanisms of stomach cancer induction in humans as well as in the standard rodent bioassays.

Helicobacter Pylori

Helicobacter pylori is a gram-negative spiral-shaped bacterium that colonizes the stomach of humans. Other species of *Helicobacter* occur in the stomachs of cats, dogs, ferrets and rodents. Large portions of the world's population are infected with *H. pylori*. Since 1982 when the bacterium was "discovered," *H. pylori* has been linked to gastritis, gastric and duodenal ulcers, and gastric cancer (Isselbacher *et al.*, 1994; IARC, 1994; Hansson *et al.*, 1996). While much has been learned since the discovery of *H. pylori*, remarkably little is known about the pathophysiology of *H. pylori* infection, particularly how the infection is acquired and how infection results in disease.

H. pylori occurs in all human populations but is much more prevalent in developing countries. Seventy to ninety percent of adults harbor *H. pylori* in China, Africa and India (Lee *et al.*, 1996). The prevalence of *H. pylori* infection is low in young children but then rapidly increases with age (IARC, 1994; Lynch, 2002). Infection rates are higher in 55-64 years-old males and females compared to 25-34 years old (IARC, 1994). Within the United States, *H. pylori* infections are more common among Mexican-Americans (62 percent) and non-Hispanic blacks (53 percent) compared to non-Hispanic whites (26 percent) (National Institute of Diabetes and Digestive and Kidney Diseases, 2002). The prevalence of *H. pylori* infection appears to be declining among non-Hispanic whites but not in minority groups (National Institute of Diabetes and Digestive and Kidney Diseases, 2002).

Early reports that bacteria occur in the human stomach were dismissed because it was believed that no organism could survive in the highly acidic environment of the stomach (Lynch, 2002). Any bacterium observed in tissue samples from the stomach was considered to have resulted from contamination of the sample. Investigators in Australia, after observing spiral-shaped bacteria in the stomach epithelium of a number of patients with gastritis, resolved that the pathology was likely from these bacteria (Marshall and Warren, 1984; Marshall, 1983; Warren, 1983). The investigators were able to culture the bacterium and then reproduce symptoms after inoculating themselves with the bacterium. Since these pioneering studies, a number of epidemiological studies have linked *H. pylori* infections with various stomach pathologies (IARC, 1994).

While *H. pylori* occurs in the stomach, it is only acid-tolerant; it is not impervious to the low stomach pH. The organism employs ingenious strategies to survive in a highly acidic stomach environment. *H. pylori* tends to colonize portions of the human stomach that are normally less acidic (e.g., the antrum) (Lee *et al.*, 1996). It resides between the mucus layer and stomach epithelium in the human stomach (Isselbacher *et al.*, 1994). The mucus layer is believed to contribute to protecting the stomach's epithelial lining from the harsh acidic luminal environment. The organism uses multiple flagella and perhaps secretes enzymes to move through the mucus layer. *H. pylori* then attaches to the epithelial lining, probably by binding to cellular membrane proteins on the epithelial cells. The organism produces large amounts of the enzyme urease that converts urea to ammonia and carbon dioxide. This reaction provides a localized less-acidic environment that protects the organism from the effects of gastric acid.

H. pylori survival is tenuous at neutral pHs. This may be due to the loss of its transmembrane potential in alkaline environments. The effect of pH on transmembrane

potential which is needed to generate ATP was investigated in *H. pylori in vitro* (Sachs *et al.*, 1996; Meyer-Rosberg *et al.*, 1996). The organism was able to maintain transmembrane potential differences over a pH range of 3.5 to 8.5. When the pH was greater than 8.5, the transmembrane potential collapsed. Thus when the pH is greater than 8.5, ATP would not be synthesized, which is not compatible with the survival of the organism. When little acid is present in the stomach, the organism would appear to self-destruct as it continues to produce ammonia from urea, raising the pH of its microenvironment. Effective treatment of *H. pylori* infections in humans involves the combination of antibiotics with acid suppressing medications (Centers for Disease Control, 2002). The combined therapy, which is much more effective than administrating antibiotics alone, probably is due to a much less hospitable environment in the stomach for *H. pylori* (although a modestly elevated pH may stimulate the growth of *H. pylori*, thereby making the organism more vulnerable to antibiotics).

The influence of local acid production on *H. pylori* colonization in the human stomach has been reviewed by Van Zanten and coworkers (Van Zanten *et al.*, 1999; Lee *et al.*, 1996). While *H. pylori* survives between pH 4 and 8, it tends to flourish (multiply) in a less acidic environment (above a pH of 5) and therefore normally occurs in the antrum, the less acidic portion of the human stomach (Van Zanten *et al.*, 1999). When the pH is increased due to acid suppression by proton pump inhibitors, vagotomy, or gastric atrophy caused by *H. pylori* itself (gastritis leading to atrophic gastritis) colonization begins to occur in the body of the stomach, which is normally characterized by a lower pH (Lee *et al.*, 1996). Less colonization occurs in the antrum, as a higher pH is less hospitable to the organism (Lee *et al.*, 1996; Van Zanten *et al.*, 1999).

Raising the pH of the stomach by administering proton pump inhibitors has been linked to increased atrophic gastritis (Kuipers *et al.*, 1996). Gastric atrophy is characterized by an increase in luminal pH because of the loss of secretory glands. Pernicious anemia is characterized by an almost total lack of secretory glands in the stomach. *H. pylori* is absent in the stomach of patients with pernicious anemia, becomes absent in areas of the stomach characterized by gastric atrophy, and does not normally colonize the small intestine. This is probably due to the organism's need for a minimally acidic environment to survive. There have been suggestions that duodenal ulcers occur as acidic conditions begin to occur in the small intestine, which would favor *H. pylori* colonization (Van Zanten *et al.*, 1999).

The influence of acid on *H. pylori* colonization in the human stomach is mirrored in the stomach of animals (Lee *et al.*, 1996). Danon and coworkers inoculated female BALB/c mice with *Helicobacter felis* and then examined various portions of the glandular stomach 2, 6, 23 and 26 months post-inoculation (Danon *et al.*, 1995). *H. felis* colonization occurred in the antrum and cardia at various times post-infection, while colonization was not observed in the body of the stomach, the acid secreting portion of the mouse glandular stomach. Colonies occurred throughout the glandular stomach when mice received omeprazole, an inhibitor of acid secretion.

Colonization and Transmission

It is not known how *H. pylori* infection is acquired (Centers for Disease Control, 2002). The prevalence of infection is much lower in infants and children than adults, suggesting

that transmission occurs postnatally. Transmission is likely through oral-oral or fecal to oral routes (Centers for Disease Control, 2002). Transmission of the disease has been documented through the use of contaminated endoscopes (Centers for Disease Control, 2002). Humans probably remain infected with *H. pylori* for life unless a therapeutic intervention occurs, although there is some evidence of reversion to uninfected status (Xia and Talley, 1997).

Mutant strains of *H. pylori* with limited urease activity or deficient flagellin genes were compromised in their ability to colonize the stomachs of gnotobiotic pig (IARC, 1994; Eaton *et al*., 1991, 1996; Tsuda *et al.*, 1994). However, once an infection was established, the inhibition of urease activity did not eradicate the bacteria. This suggests possible vulnerability of the organism before it becomes established in the stomach.

Gastritis and Ulcers

Helicobacter pylori causes gastritis in virtually all infected individuals (Isselbacher *et al.*, 1994). However, many individuals are asymptomatic to the gastritis that results from the *H. pylori* infection (Lynch, 2002). Chronic gastritis may lead to atrophic gastritis, which is characterized by a loss of the normal architecture of the mucosa including the loss of acid secreting glands. The loss of a portion of the acid-secreting glands results in an increase in stomach pH, which leads to the growth of *Helicobacter* in a more hospitable stomach environment. While most duodenal ulcers (up to 90 percent) and gastric ulcers (up to 80 percent) are linked to H. pylori infections, fewer than 20 percent of individuals that test positive for *H. pylori* have ulcers (Centers for Disease Control, 2002).

Cancer

In 2000, cancer of the stomach resulted in the third (females) or second (males) highest rates of mortality of all tumor sites worldwide (IARC, 2000). Mortality from stomach cancer is highest in developing countries (e.g., China) (Centers for Disease Control, 2002). The high incidence of stomach cancer in developing countries has been attributed to dietary factors, nutritional status, and the lack of refrigeration. These countries are also characterized by a widespread occurrence of *H. pylori* in the population (Lynch, 2002). Greater than 80 percent of the population in China is believed to be infected with *H. pylori*.

Individuals infected with *H. pylori* have a 2- to 6-fold increased risk of developing gastric cancer and mucosal-associated, lymphoid-type lymphoma compared to uninfected individuals (Centers for Disease Control, 2002). IARC determined that there was sufficient evidence that "infection with *Helicobacter pylori* is carcinogenic to humans (Group 1)" (IARC, 1994). The high incidence of stomach cancer in China cannot be attributed only to the high prevalence of *H. pylori* infection, given that other populations with high incidence of *H. pylori* (such as in Africa and India) do not display a comparable high incidence of stomach cancer (Miwa *et al.*, 2002). Most people infected with *H. pylori* do not develop stomach cancer so *H. pylori* infection does not appear to be the sole causative agent (Crespi and Citarda, 1998).

The occurrence of adenocarcinoma of the stomach is believed to be the culmination of a sequence of events. Adenocarcinoma is preceded by gastritis, chronic atrophic gastritis, intestinal metaplasia, dysplasia, and then cancer (Correa, 1988, 1992). These events are

associated with *H. pylori* infections. The sequence suggests that the loss of the glandular features, particularly the acid secreting character of the stomach, precedes changes that ultimately lead to stomach cancer.

The mechanisms by which *H. pylori* infection produces gastritis, ulcers and gastric cancer are still largely unknown. The organism secretes lipases, cytotoxic proteins and urease, which generates toxic ammonium. All these agents may contribute to the pathogenesis that leads to gastritis and ultimately to the occurrence of gastric cancer. In addition the organism causes an immune response characterized by the attraction of neutrophils and monocytes which generate reactive oxygen species (ROS). The immune cells are not able to eliminate the bacterium from the stomach. The chronic gastritis associated with *H. pylori* infection is consistent with release of reactive metabolites such as ROS during an immunological response. Evidence of oxidative DNA damage has been detected in samples of stomach epithelium from areas of chronic gastritis associated with *H. pylori* infection in humans (Farinati *et al.,* 1998; Hahm *et al.,* 1997).

Animal Models.

The link between *H. pylori* infection and gastritis, ulcers and cancer in humans triggered the search for animal models to aid in understanding the pathophysiology of the infection. Various species of *Helicobacter* have been detected in rodents, dogs, cats and ferrets. While animals can be inoculated with *H. pylori*, the organism does not thrive in most animal models. Related *Helicobacter* species such as *H. felis* more closely mimic the disease in rodents. However, rodents infected with *H. felis* or *H. pylori* generally do not precisely mimic what is observed in human infections (Dubois, 1998; Lee, 2000). Even when mice are successfully infected with *H. pylori*, much lower levels of inflammation occur and mononuclear but not polymorphonuclear lymphocytes characterize the infiltrating inflammatory cells (Dubois, 1988; Nedrud, 1999). Gastritis is rarely seen in *H. felis* or *H. pylori* infections in mice (Lee, 2000). *H. felis* does not appear to attach itself to the stomach epithelium in rodents, but appears to remain "free floating" within or below the mucus layer (Dubois, 1998). Recently, an animal model of *H. pylori* infection was developed in the Mongolian gerbil that yields pathophysiology that is reasonably close to what is observed in humans (Lee, 2000).

The Forestomach

The rodent stomach is composed of two distinct parts, the forestomach and the glandular stomach, separated by the limiting ridge. The forestomach is believed to function as a temporary storage depot for ingested food (Nagayo, 1973). Studies have shown it is not essential for the survival of the animal (Kunstyr *et al.*, 1976).

The two portions of the rodent stomach are connected, and mixing of their content does occur. Acid is secreted in the rodent's glandular stomach, particularly during the time of feeding. Food mixes with stomach secretions then is stored in the forestomach. Measurements of the pH of the rat forestomach ranged from 3 to 5, with an average measurement of about 4 (Browning *et al.*, 1983, 1984; Kunstyr *et al.*, 1976**;** Ward *et al.*, 1986). This is considerably higher than the pH levels measured in the glandular stomach (Ward *et al.*, 1986).

The higher pH of the rodent forestomach would appear to be more hospitable to

Helicobacter than the glandular stomach. The apparent lack of attachment to epithelial cells (at a specific site) suggests *Helicobacter* (*H. felis*) would not be limited to a specific segment of the rodent stomach, and it might be expected to occur in greater numbers in the rodent forestomach. Unfortunately, measurements of the distribution of *Helicobacter* in the rodent forestomach are lacking. Investigators that study the pathophysiology of *Helicobacter* in rodent models generally ignore the forestomach because the well-defined anatomical division does not occur in humans.

The common presumption that forestomach tumors in gavage studies result from a selective direct contact of the gavage solution with the forestomach (rather than the glandular portion of the stomach) appears to be inconsistent with the anatomy. The esophagus empties in the area of the limiting ridge at the junction of the two portions of the rodent stomach (analogous to the human stomach). Gavage administration would appear to deposit solutions into this area of the rodent stomach, similar to normal food delivery.

Because of the idiosyncratic growth characteristics of *Helicobacter*, this organism may not be detected in standard bacterial cultures. In their pioneering study, Marshall and Warren nearly failed to grow it in culture because of its growth requirements and long incubation period (Lynch, 2002). *Helicobacter* infections in the rodent stomach are not characterized by the inflammation (gastritis) observed in the human stomach (Lee, 2000). *Helicobacter* is not usually observed on routine histological examination of H&E stained sections (at least in liver sections) (Hailey *et al.*, 1998). Thus its occurrence in the glandular stomach or forestomach would not necessarily have been detected in past rodent bioassays.

In 1993, liver lesions were identified in treated and control male mice in two completed NTP bioassays (Nyska *et al.*, 1997). These lesions (hepatitis, oval cell hyperplasia and karyomegaly, and chronic inflammation) were consistent with infection with *Helicobacter hepaticus*, an organism closely related to *H. pylori*. Further investigation detected *H. hepaticus* in 9 long-term completed NTP cancer bioassays where hepatitis was reported (Hailey *et al.*, 1998). The presence of this organism may be confounding the findings of hepatic tumors in these bioassays associated with exposure to chemical agents (Nyska *et al.*, 1997).

Chemical Carcinogens

In long term animal bioassays conducted by the NCI and NTP, neoplasms of the forestomach were much more common than neoplasms of the glandular stomach (fifth most common tumor versus the 32nd most common tumor, respectively) (Huff, 1999). Nineteen chemicals in male and 13 chemicals in female rats, and 20 chemicals in male and 21 chemical in female mice were positive for forestomach tumors. Two chemicals were positive for tumors of the glandular stomach and only in the female rat (Huff, 1999). Given the association of *Helicobacter* infection with stomach tumors, the occurrence of tumors in the portion of the rodent stomach with elevated pH could be related to a more hospitable environment for the growth of *Helicobacter*.

Although tumors of the forestomach are much more common than tumors of the glandular stomach in rodent cancer bioassays, the relevance of these tumors is somewhat

problematic given the lack of a comparable structure in the human stomach. The pathophysiology of *Helicobacter* infection in human stomach cancer involves a progression that results in the loss of glandular structure. Describing the sequence of events in the human stomach preceding carcinoma, IARC states "They follow a sequential presentation of chronic nonatrophic gastritis, atrophic gastritis, intestinal metaplasia and dysplasia. Atrophy (loss of gastric glands) is a pivotal change in the precancerous process" (IARC, 1994). A "de-glandular process" appears to occur in the stomach before cancer occurs in the humans infected with *Helicobacter pylori*. Cancer in the aglandular portion of the rodent stomach, the forestomach, may be very relevant to what is occurring in the human stomach. Colonization of *Helicobacter* tends to occur in the portion(s) of the human stomach (e.g., antrum) with no acid-secreting glands.

While *Helicobacter* infections have been detected in the stomach of a number of species, it is unclear what role they play in the carcinogenesis process in animals. Recent studies (discussed below) suggest that *Helicobacter* may have a role in carcinogenesis in animals, particularly in combination with chemical carcinogens.

The inoculation of Mongolian gerbils with *H. pylori* prior to or following the administration of N-methyl-N-nitrosourea (MNU) in drinking water resulted in a statistically significant increase in adenocarcinoma of the glandular stomach after 40 weeks (Sugiyama *et al.*, 1998). No tumors were observed in animals exposed to *H. pylori* or MNU alone.

In a study that lasted for 50 weeks, Mongolian gerbils were administered N-methyl-N-nitroso guanidine (MNNG), *H. pylori*, or a combination of the two agents (Shimizu *et al.*, 1999). No tumors were observed in a control group infected with *H. pylori* alone although almost of the animals in this control group exhibited inflammation, edema, hemorrhagic spots and erosions, and hyperplasia of the stomach. These effects were not observed in an uninfected control group.

Statistically significant increases in tumors of the glandular stomach were observed when 60 or 300 ppm of MNNG was administered in drinking water for 10 weeks, followed by an infective dose of *H. pylori* (after one week), when compared to MNNG alone. In a separate experiment, animals infected with *H. pylori* and then administered 100 or 20 ppm of MNNG for 30 weeks (one week after the *H. pylori* was administered) showed statistically significant increases in stomach tumors compared to MNNG alone, but only in the low dose group. Fewer tumors in the high dose group may be related to *H. pylori* being eradicated from the stomachs of many of the animals in the high dose group (possible due to a direct toxic effect of MNNG on the bacteria).

Mongolian gerbils were first inoculated with *H. pylori* and after four weeks MNNG (50 μg/ml) was administered in drinking water for an additional 20 weeks (Tokieda *et al.*, 1999). Eighteen weeks later, four of six animals exposed to *H. pylori* and MNNG displayed adenocarcinomas in the glandular stomach, while only 3 of 17 animals displayed tumors in animals receiving MNNG alone. No tumors were observed in animals exposed to *H. pylori* alone. Histopathological examination of the forestomach revealed hyperkeratotic changes and hypertrophy in animals exposed to MNNG but not in animals exposed to just *H. pylori* alone. Forestomach tumors occurred in one animal exposed to MNNG and *H. pylori* and one animal exposed to MNNG alone. Ninety-three

percent of animals exposed to *H. pylori* alone remained infected at the end of the study but only 40 percent in animals exposed to MNNG and *H. pylori*, indicating that the chemical may have had bactericidal activity.

N-methyl-N-nitrosourea was administered in drinking water to Mongolian gerbils (10 ppm for 20 weeks or 30 ppm for six of 10 weeks), which were sacrificed after 41 weeks (Maruta *et al.*, 2001). The gerbils were inoculated with *H. pylori* one week prior to (10 ppm) or one week subsequent to (30 ppm) MNU treatment. Control groups consisted of animals inoculated with *H. pylori* alone or animals treated with MNU and not inoculated with H. pylori. Fourteen of 39 animals developed carcinomas of the stomach in animals inoculated with *H. pylori* and then treated with 10 ppm MNU. Six of 18 animals treated with 30 ppm of MNU and then inoculated with *H. pylori* developed carcinomas. No carcinomas were observed in the stomach of animals treated with 10 ppm or 30 ppm of MNU alone, or animals inoculated with *H. pylori* alone.

The administration of 10 ppm of MNU in drinking water to Mongolian gerbils for 20 weeks, with sacrifice after an additional 20 weeks, yielded seven adenocarcinomas of the stomach in 20 animals exposed to *H. pylori* one week prior to treatment but no tumors in animals inoculated with *H. pylori* 24 weeks prior to treatment with MNU (Maruta *et al.*, 2000). Animals treated with MNU alone did not develop stomach tumors.

Vagotomy

Vagotomy, a procedure involving the resection of the vagus nerve, has been used to reduce the secretion of acid into the stomach (stimulation of the vagus nerve results in the release of gastrin and increased section of acid into the stomach). Vagotomy has been linked to increases in gastric tumors in humans and animals (Capper and Johnson, 1964; Haukland and Johnson, 1981; Morgenstern, 1968). While changes in acid secretion (hypochlorhydria) and duodenal reflux have been suggested as being involved in the increase in cancer, the mechanism remains unknown. Increases in gastric tumors have also been observed in vagotomized animals administered 20-methylcholanthrene (Vilchez and Echeve-Llanos, 1964; Morgenstern, 1968) or MNNG (Fujita *et al.*, 1979; Tatsuta *et al.*, 1985) when compared to sham-operated animals. In vagotomized rats administered MNNG, Tatsuta *et al.* (1985) observed increased stomach pH and atypical glandular hyperplasia. In addition, there were increased numbers of rats with gastric cancer and an increase in the number of gastric cancers per rat compared to animals treated with MNNG alone**.**

An increase in stomach pH that is associated with vagotomy in these studies is consistent with conditions that are more hospitable to *Helicobacter* infections. The increases in stomach tumors and glandular hyperplasia are consistent with effects associated with *Helicobacter* infection.

Stomach Irritation and Cancer

Helicobacter pylori infection results in gastritis in humans and has also been linked to stomach cancer. However, most individuals infected with the organism do not develop stomach cancer and certain populations with high prevalence of *Helicobacter* infection have a high incidence of stomach cancer while other populations do not. Other factors appear to be involved.

The Mongolian gerbil, when infected with *H. pylori*, develops gastric symptoms that mimic what is observed in humans. Tumors of the stomach were observed in animals exposed to MNU or MNNG in combination with *H. pylori*. Stomach tumors were not observed following exposure to *H. pylori* alone in the Mongolian gerbil. Exposure to chemical agents may be one of the "other factors" involved in the pathophysiology of stomach cancer associated with *H. pylori* infection in humans.

Little inflammation of the stomach is observed when mice are infected by *Helicobacter*. However, irritation is detected in the stomach of mice exposed to some agents that produce stomach cancer (Wilkinson and Killeen, 1996; Frederick *et al.*, 1990; Boorman *et al.*, 1986). The irritation (and cancer) has been attributed to the agent alone (particularly since there is no evidence that something else could be causing the irritation). However, the irritation could be evidence of the presence of *Helicobacter* infection and perhaps the combined actions of *Helicobacter* and the carcinogenic agent, given that *Helicobacter* infection and its associated gastritis or irritation precedes stomach cancer in humans and the Mongolian gerbil. While a role for *Helicobacter* infection in the pathophysiology of chemicals linked to stomach cancer in rodents is intriguing, little information regarding the possible occurrence of the organism in the stomach or forestomach of rodents in past bioassays is available.

Hexavalent Chromium - Toxicity Studies

Three studies have linked exposure to Cr VI in drinking water with statistically significant increases in cancer of the GI tract (NTP, 2007; Zhang and Li, 1987; Borneff *et al.*, 1968). Zhang and Li (1987) was an ecological epidemiology study that revealed statistically significant increases in the incidence of both stomach cancer and overall cancer rates in rural villagers exposed to what appears to be high concentrations of Cr VI in drinking water. The NTP (2007) study revealed a statistically significant and dose-related increase in duodenum tumors in both male and female mice. Borneff *et al.* (1968) was an animal study that revealed a statistically significant increase in the incidence of tumors of the forestomach in female mice exposed to 500 ppm of potassium dichromate in drinking water.

Zhang and Li, 1987. A statistically significant increase in the incidence of stomach tumors was detected in rural villagers in China exposed to a relatively high level of Cr VI in their drinking water. Most notable about this increase was that it occurred after a rather short duration of exposure and latency period, 12 to 17 years. The villagers in this study were likely to have been infected by *Helicobacter pylori*, given its very high prevalence in the Chinese population. The brief exposure duration and latency period before stomach cancer was detected is reminiscent of the short exposure and latency period for stomach tumors in the Mongolian gerbil following the administration of MNNG and MNU.

Borneff *et al.*, 1968. The forestomach tumors in this study (for the protocol see Appendix B) were found almost exclusively in the F_0 generation. This generation was characterized by a slightly later onset of exposure, a slightly longer duration of exposure

than the F_1 generation, and a significantly longer duration of exposure than the F_2 generation. While tumor incidence was markedly increased only in the F_0 generation, exposure duration was markedly shorter only in the F_2 generation and not in the F_1 generation. Thus, differences in the duration of exposure do not appear to explain why tumors occurred primarily in the F_0 generation.

We postulate that an earlier exposure of mice to Cr VI in the F_1 and F_2 generations (which occurred following weaning) may have "prevented" tumors in these generations. This finding could have resulted from a combined exposure to Cr VI and a *Helicobacter* infection, analogous to the studies in which MNNG or MNU was administered to Mongolian gerbils.

Mice in the F_0 generation infected with *Helicobacter* and exposed to Cr VI developed forestomach tumors. The lack of tumors in subsequent generations in the Borneff study may simply reflect the elimination of *Helicobacter* from the forestomach at an early age by the high concentration of Cr VI in their drinking water. Mutagenicity tests have revealed that Cr VI is cytotoxic to *E. coli* at concentrations of 10 to 15 ppm (Lantzsch and Gebel, 1997) or 100 to 150 ppm (Olivier and Marzin, 1987). In the newborn mouse essentially no acid is secreted into the stomach (Helander, 1970). At ten days of age (the last time period in the Helander study), stomach pH level in fasted mice was around 4, well above levels measured in adult animals (Helander, 1970). If rates of acid secretion were still reduced at 21 days of age, the rate of chromium reduction to Cr III in the stomach and forestomach at the time of weaning in the Borneff *et al.* (1968) study may have been reduced. Higher Cr VI levels in the stomach and forestomach may have prevented colonization or eliminated *Helicobacter* from the forestomachs of the mice in the F_1 and F_2 generations. The elimination of a *Helicobacter* infection from the forestomach in the Borneff *et al.* (1968) study would be analogous to apparent bactericidal effects of MNNG on *Helicobacter* in the Mongolian gerbil (Tokieda *et al.*, 1999; Shimizu *et al*., 1999).

Once established, *Helicobacter* is difficult to eliminate from the stomach. In humans, one or more antibiotics are administered in combination with a drug that acts as a proton pump inhibitor. An established infection with *Helicobacter* in the F_0 generation may have been refractory to the bactericidal effects of Cr VI in drinking water, particularly at the pH levels in the adult stomach. However, the organism may have been more vulnerable in the young pups. The high concentration of chromium in drinking water may have prevented the transmission of *Helicobacter* to the F_1 and F_2 generation because of the antibiotic properties of a high chromium concentration.

An ectromelia epidemic occurred in the eighth month of the Borneff *et al*. (1968) study, which resulted in significant mortality in the F_0 and F_1 generations. The epidemic was ended by vaccination of the entire colony. Thus, the mouse colony was obviously not free of infective agents. Mouse infection with ectromelia is not associated with stomach tumors (Dick *et al.*, 1996), in contrast to the occurrence of certain species of *Helicobacter* in the stomach of mice and their association with stomach tumors.

Any role that *Helicobacter* infection may have played in the increase in forestomach tumors observed in Borneff *et al.* (1968) and stomach tumors in Zhang and Li (1987) will remain unresolved. There are no data or possibility of obtaining data from these studies

to support or refute a possible role of *Helicobacter* infection in the occurrence of stomach cancer. These studies were conducted prior to the discovery of the role of *Helicobacter* in the etiology of stomach cancer.

NTP, 2007. The NTP study was conducted in mice free of *Helicobacter* infection. Interestingly, the tumors occurred in the duodenum and not the stomach (Zhang and Li, 1987) or forestomach (Borneff *et al*., 1968). *Helicobacter* infection is characterized by the occurrence of intestinal metaplasia, a transformation of the stomach into a tissue that resembles the intestine.

Toxicity Mechanisms

Hexavalent chromium rapidly enters the cell via the anion transport system and then is rapidly reduced to Cr III inside the cell. There is evidence of the generation of reactive intermediates Cr V and Cr IV as well as the formation of reactive species such as hydroxyl free radicals and singlet oxygen during the reduction process (De Flora and Wetterhan, 1989; Cohen *et al.*, 1993; Sugden and Stearns, 2000). These highly reactive species have been associated with oxidative DNA damage.

Similar mechanisms of action have been attributed to *Helicobacter* effects on the stomach epithelium, namely the generation of reactive intermediates such as reactive oxygen species by infiltrating neutrophils and monocytes. As mentioned earlier, oxidative DNA damage has been detected in samples of stomach epithelium from areas of chronic gastritis associated with *H. pylori* infection in humans (Farinati *et al.*, 1998).

Future Studies

Stomach Cancer

While the stomach is one of the most common sites of neoplasms in humans, cancer bioassays in animals have yielded almost no tumors in the glandular stomach. Tumors in the rodent forestomach are much more common. But given that this portion of the stomach does not occur in humans, it is unclear if tumors of the rodent forestomach are representative of what occurs in the human stomach (Nagayo, 1973).

The lack of tumors in the glandular stomach in cancer bioassays is problematic. It seems unlikely that tumors of the human stomach are not caused by exposure to chemical agents, considering the large variation in rates among different populations, apparently associated with environmental causes. Alternatively, it could be postulated that the tumors that are occurring in the human stomach may be due to exposure to agents not yet tested in animal cancer bioassays.

Many potent carcinogens have been tested in animal bioassays and they have typically been administered by the oral route, allowing direct contact with the stomach epithelium. Under these circumstances, tumors in the glandular portion of the rodent stomach probably should have been observed. The lack of tumors in the glandular stomach in cancer bioassays suggests that the current animal bioassays are not an appropriate model

for detecting agents that cause stomach cancer in humans (particularly if tumors of the forestomach are considered to be irrelevant to humans).

Recent studies have linked exposure to chemical carcinogens to tumors in the glandular stomach in the Mongolian gerbil, for the most part only when *Helicobacter* infection was present. In the Mongolian gerbil model, potent carcinogens were inactive or much less active unless *Helicobacter* infection was present. This finding suggests a role for *Helicobacter* infection in the etiology of stomach cancer associated with chemical agents.

Tumors in previous cancer bioassays in rodents may have occurred because the animals were infected by *Helicobacter*. Accordingly, *Helicobacter* infection may be necessary or appropriate for an animal model of human stomach carcinogenesis.

Helicobacter infections produce changes in the human stomach including atrophic gastritis and intestinal metaplasia prior to the appearance of stomach tumors. *Helicobacter* infections are producing a "de facto" aglandular epithelium (reminiscent of the rodent forestomach) prior to the occurrence of gastric cancer in humans. Thus, the rodent forestomach may be an appropriate model for tumors of the human stomach.

Given the emerging understanding of the possible involvement of *Helicobacter* in various pathologies of the stomach, future bioassays should at a minimum account for its presence. Other research should investigate the possible role that it may play in fostering carcinogenic response to various chemical agents in animals and humans.

Specific Areas of Investigation

1) The higher pH of the rodent forestomach suggests that this organ is a more hospitable environment for *Helicobacter* than the glandular stomach. This may be the reason that tumors occur in the forestomach and not glandular stomach in rodent bioassays. It ought to be determined if *Helicobacter* occurs in the rodent forestomach, and if the organism preferentially colonizes this portion of the rodent stomach.

2) Future bioassays ought to determine if *Helicobacter* is occurring in the stomach of rodents used in the bioassay.

3) Evidence of *Helicobacter* colonization in archived samples from past rodent bioassays would be useful in investigating if there is role of this organism in stomach cancer. This type of investigation is equivalent to previous efforts that demonstrated the occurrence of *Helicobacter hepaticus* in the liver of rodents in past NTP studies (Hailey *et al.*, 1998).

4) Given that a large portion of the human population is infected by *Helicobacter pylori*, the hypothesis that chemical agents are acting in combination with *Helicobacter* to cause stomach cancer ought to be investigated.

5) If there is strong evidence that *Helicobacter* infection has a role in carcinogenic response to chemicals in the stomach, it may be advisable to use rodents that are infected with *Helicobacter* in cancer bioassays.

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