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**Public Health Goal for
Hexavalent Chromium
in Drinking Water**

Prepared by

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PREFACE

**Drinking Water Public Health Goals
Pesticide and Environmental Toxicology Branch
Office of Environmental Health Hazard Assessment
California Environmental Protection Agency**

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 proposing a Public Health Goal (PHG) for hexavalent chromium of 0.06 parts per billion (ppb) or micrograms per liter ($\mu\text{g/L}$) in drinking water. OEHHA has reviewed the available data on the toxicity of hexavalent chromium and has identified the proposed 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 concern 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, 2007b). To calculate the proposed PHG, OEHHA derived both an oral cancer slope factor of $0.6 \text{ (mg/kg-day)}^{-1}$, based on a dose-related increase of tumors of the small intestine in male mice (NTP, 2007b), and an inhalation cancer slope factor of $510 \text{ (mg/kg-day)}^{-1}$, based on occupational studies. OEHHA also used an exposure assessment (Keating and McKone, 1993) to estimate inhalation of waterborne hexavalent chromium during showering. 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 resulting proposed PHG is quite low, based on the linear extrapolation to a one in one million lifetime cancer risk from the high incidence of tumors observed in the mice.

A health-protective level of 2 ppb for non-carcinogenic effects is also proposed based on liver toxicity (mild chronic inflammation, fatty changes) in female rats in the NTP study (2007b). 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 chromium in whole blood and plasma were observed, while little change was observed following trivalent chromium administration. Increases in

blood/plasma 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 hexavalent chromium is completely converted to trivalent chromium in the acidic environment of the stomach, and therefore 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, as mentioned above, differences in blood/plasma levels have been observed. In addition, studies in animals and humans have revealed that orally administered hexavalent chromium results in elevated chromium tissue levels and increased urinary half-life compared to administered 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 indicates 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 route of exposure. 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 a statistically significant increase in papillomas or carcinomas of the oral cavity in the high dose group (NTP, 2007b). Administration of hexavalent chromium in drinking water to male and female B6C3F₁ mice resulted in a statistically significant and dose-related increase in adenomas or carcinomas of the small intestine (NTP 2007b).

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). Review of occupational studies in which humans were exposed to hexavalent chromium primarily by the inhalation route revealed an increase in stomach cancer, which suggests that cells in the stomach are being exposed to hexavalent chromium, although the primary exposure route was inhalation. 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 are 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.

The proposed 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 chromium VI, is more water soluble, more easily enters living cells, and is much more toxic than the trivalent ionic form, known as chromium III. Chromium III is an essential trace element in the human diet. Trivalent chromium is thought to potentiate the action of insulin, acting in combination with the glucose tolerance factor (ATSDR, 2000). Chromium VI 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 chromium 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 chromium 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 chromium 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 $1,875^{\circ}\text{C}$, and boils at $2,680^{\circ}\text{C}$; 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 $<0.01 \mu\text{g}/\text{m}^3$ in rural areas, and in the range of 0.01 to $0.03 \mu\text{g}/\text{m}^3$ 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 $30 \mu\text{g}/\text{L}$ of chromium. U.S. lakes usually have $<5 \mu\text{g}/\text{L}$ 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 $8.0 \mu\text{g}/\text{L}$, with a mean of $1.8 \mu\text{g}/\text{L}$ (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 $10 \mu\text{g}/\text{L}$. The range of total chromium levels in the samples where chromium was detected was from $10 \mu\text{g}/\text{L}$ up to a maximum of $1,100 \mu\text{g}/\text{L}$, with a mean of $23 \mu\text{g}/\text{L}$ and a median of $17 \mu\text{g}/\text{L}$. The chromium was not speciated, so we do not know how many of these sources would have had detectable amounts of chromium VI.

In January 2001 the California Department of Health Services (CDHS) adopted a regulation adding hexavalent chromium 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 hexavalent chromium levels between 1 and 5 ppb, and 13 percent of the sources detected hexavalent chromium concentrations

from 6 to 10 ppb. Six percent of the sources had hexavalent chromium levels between 11 and 20 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).

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 ranging 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).

Other Exposure Sources

Workers in chromium production, stainless steel production and welding, chromium plating, ferrochrome and chromium pigment industries may have occupational exposures to chromium III and chromium 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 hexavalent chromium 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 hexavalent chromium, the assessment of exposure to chromium 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 Teledye 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.

$$\begin{aligned} V_L &= V_d \times \text{number of droplets} \\ &= 187.4 \mu\text{m}^3 / \text{droplet} \times 1022 \text{ droplets}/\text{cm}^3 \text{ air} \\ &= 191,500 \mu\text{m}^3 \text{ liquid}/\text{cm}^3 \text{ air} \end{aligned}$$

In this equation V_d 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. V_d 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} $\text{mg}/\mu\text{m}^3$ water.

$$\begin{aligned} \text{ML} &= 191,500 \mu\text{m}^3 \text{ liquid}/\text{cm}^3 \text{ air} \times (10^{-9} \text{ mg}/\mu\text{m}^3 \text{ water}) \\ &= 1.92 \times 10^{-4} \text{ mg liquid}/\text{cm}^3 \text{ air} \end{aligned}$$

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

$$\begin{aligned} V_{ra} &= (20 \text{ m}^3/\text{day}) \times (1 \text{ day}/24 \text{ hrs}) \times (1 \text{ hr}/60 \text{ min}) \times (10 \text{ min}/\text{shower}) \\ &= 0.14 \text{ m}^3/\text{shower} \end{aligned}$$

The 20 m^3/day is the standard respiratory rate for an adult that was used in calculating the inhalation cancer potency for chromium 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 (M_{rl}) that an individual would inhale during a 10 minute shower is calculated as:

$$\begin{aligned} M_{rl} &= \text{ML} \times V_{ra} \\ &= 1.92 \times 10^{-4} \text{ mg liquid}/\text{cm}^3 \text{ air} \times 0.14 \text{ m}^3 \text{ air} \times 10^6 \text{ cm}^3/\text{m}^3 \\ &= 27 \text{ mg of water that is inhaled, or } 27 \times 10^{-6} \text{ L} \end{aligned}$$

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. 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 hexavalent chromium levels in air in the breathing-zone height ranging from 87 to 324 ng Cr VI+/m³ 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 proposed PHG of hexavalent chromium will address both the inhalation and ingestion routes of exposure to water using the estimate of 27 mg/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 hexavalent chromium 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 hexavalent chromium in humans. Four subjects were submersed in water containing 22 parts per million (ppm) of hexavalent chromium for three hours (Corbett *et al.*, 1997). Following exposure, a total of 6.1 µg of chromium (average value) 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 cm² of skin surface area had been exposed to water containing hexavalent chromium.

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

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

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

Comparison of the dermal to ingestion dose of hexavalent chromium

Drinking water ingestion rate = 2 L/day.

Surface area of whole body in shower = 20,000 cm²

Time in shower = 10 minutes or 1/6 hr/day

Assume a concentration of hexavalent chromium 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).

$$\begin{aligned}\text{Absorbed dose (dermal)} &= K_p \times \text{concentration} \times \text{surface area} \times 1/6 \text{ hr} \\ &= 7 \times 10^{-6} \text{ cm/hr} \times 0.01 \text{ ug/cm}^3 \times 20,000 \text{ cm}^2 \times 1/6 \text{ hr} = 1.5 \times 10^{-4} \text{ ug/day}\end{aligned}$$

$$\begin{aligned}\text{Absorbed dose (ingestion)} &= \text{concentration} \times \text{ingestion rate} \times 0.03 \text{ (absorbed)} \\ &= 10 \text{ ug/L} \times 2 \text{ L/day} \times 0.01 = 0.60 \text{ ug/day}\end{aligned}$$

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 hexavalent chromium. Most of the toxicokinetic research that was conducted to address inhalation exposure to hexavalent chromium is relevant to the evaluation of exposure to hexavalent chromium via the oral route. The findings of these studies are very useful in gaining an understanding of whether, or under what conditions, exposure to hexavalent chromium 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 trivalent chromium. In biological environments, little trivalent chromium is converted to the hexavalent form of the metal. Once inside the cell, highly reactive hexavalent chromium is thought to directly damage macromolecules or generate reactive metabolites that damage macromolecules, thereby producing toxicity. The rapid uptake of hexavalent chromium into cells may also play a role in its toxicity. While administered trivalent chromium does not result in toxicity comparable to that of hexavalent chromium, once hexavalent chromium has penetrated the cell, it is possible that trivalent chromium produced by intracellular reduction is also a proximal toxicant. The evidence that hexavalent chromium gets into tissues following oral exposure is a concern regardless of whether the toxicity is due to the reaction of hexavalent chromium 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 hexavalent chromium 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 hexavalent and not trivalent chromium 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 trivalent chromium 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 hexavalent chromium reduction to trivalent chromium by saliva and stomach fluids because this reduction would markedly reduce or eliminate chromium absorption into the body. Complete conversion of hexavalent chromium to trivalent chromium 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 hexavalent chromium 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 hexavalent chromium per day and that gastric juices have the ability to reduce at least 80.3 to 84 mg of hexavalent chromium 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 stimulated 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 hexavalent chromium. 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 hexavalent chromium within minutes. Neither dilution nor the addition of an antacid markedly altered the reducing properties of the simulated stomach fluid.

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 hexavalent chromium in water (roughly 5 mg/L or greater), which is not likely to be ingested due to organoleptic considerations. Based on this level, hexavalent chromium 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 hexavalent chromium 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 hexavalent chromium added to orange juice, coffee, tea or lemonade was reduced to trivalent chromium.

Given that the maximum plausible levels of hexavalent chromium in water that would likely be ingested by humans has been estimated to be less than 5 mg/L, exhaustion of the capacity of saliva and gastric fluids to reduce hexavalent chromium appears unlikely. Moreover, evidence of hexavalent chromium 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 hexavalent chromium to trivalent chromium does not necessarily mean that complete reduction always occurs. If complete reduction were to occur, then hexavalent chromium administration would be expected to behave as if trivalent chromium had been administered. Evidence summarized below indicates that this is frequently untrue.

Absorption

Most studies that have investigated oral absorption of hexavalent or trivalent chromium 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 trivalent chromium 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 trivalent chromium 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 trivalent chromium 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 trivalent chromium 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 hexavalent chromium 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 hexavalent chromium to trivalent chromium in the stomach. While the absorption of hexavalent chromium was low, these studies do indicate significantly greater oral

absorption of hexavalent chromium than trivalent chromium (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) administered 20 ng of radiolabeled Cr VI, Kerger *et al.* (1996a) administered 5 mg of Cr VI, Finley *et al.* (1996) administered 0.005 mg/kg-day of Cr VI for three days and Finley *et al.* (1997) administered 0.1, 0.5, 1.0, 5.0 or 10 mg/day of Cr VI for four days. In the study of Finley *et al.* (1997), the percent of the administered dose of hexavalent chromium recovered in the urine did not increase with dose. Therefore, the results of these studies do not indicate that oral absorption of administered hexavalent chromium begins to occur when the reducing capacity of the stomach is exhausted.

Infusion of hexavalent chromium 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 hexavalent chromium in human urine when the metal was infused into the intestine (only one to two percent of the dose of trivalent chromium was absorbed). Fifty seven percent of the dose of hexavalent chromium administered into the ligated jejunum of rats was recovered in the jejunum after 60 minutes while approximately 98 percent of the dose of trivalent chromium was recovered in the jejunum under the same experimental conditions (Febel *et al.*, 2001). Following the oral administration of hexavalent chromium 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 and associates administered hexavalent chromium 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 (Kerger *et al.*, 1996a). The addition of hexavalent chromium to orange juice prior to its ingestion was a *de facto* reductive pretreatment of hexavalent chromium. In spite of this, the fraction of the administered dose of chromium recovered in the urine appeared to be greater for hexavalent chromium than when trivalent chromium was administered (0.6 percent versus 0.13 percent). However, the absorbed fraction was considerably less than when hexavalent chromium was administered in water (6.9 percent).

Blood/plasma and tissue levels of chromium

Finley and associates observed marked increases in plasma chromium levels in some individuals (but not in others) that ingested three daily doses of hexavalent chromium, at total doses as low as 0.1 mg/day (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 hexavalent chromium. 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 hexavalent chromium 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 hexavalent chromium 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 2007b). A more detailed analysis of the findings of this study is found in Appendix A. Thus, the difference in absorption of hexavalent versus trivalent chromium 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 hexavalent chromium (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 hexavalent chromium is incubated with washed isolated RBCs, almost the entire dose is taken up by the cells. It is reduced inside the cells to trivalent chromium, essentially trapping it inside the RBC. In contrast, little trivalent chromium appears to be taken up by RBCs in *in vitro* incubations (Gray and Sterling, 1950; Bentley, 1977; Donaldson and Barreras, 1966; Aaseth *et al.*, 1982). When hexavalent chromium is incubated with whole blood or RBCs plus plasma, only a fraction (depending on conditions) of the hexavalent chromium 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 hexavalent chromium to trivalent chromium outside of the RBC (Capellmann and Bolt, 1992; Korallus *et al.*, 1984; Richelmi *et al.*, 1984). The converted trivalent component of chromium is then largely excluded from the RBC.

Negligible amounts of trivalent chromium were associated with RBC in many *in vivo* studies (Minoia and Cavalleri, 1988; Doisy *et al.*, 1971; Wiegand *et al.*, 1984; Sayato *et al.*, 1980; Coogan *et al.*, 1991b; Suzuki *et al.*, 1984; Onkelinx, 1977). However, in other studies there is some evidence that trivalent chromium 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 trivalent chromium uptake by the RBC appears to be substantially less than that of hexavalent chromium, it could be noticeable when a large dose of trivalent chromium is administered or when hexavalent chromium is mostly absent. Some of the trivalent chromium 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 hexavalent chromium 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 hexavalent chromium was inhaled or administered intratracheally, intraperitoneally, or intravenously, much of the chromium in the blood (25 to 70 percent) was taken up by RBCs (Gao *et al.*, 1993; Minoia and Cavalleri, 1988; Wiegand *et al.*, 1984; Weber, 1983; Sayato *et al.*, 1980; Edel and Sabbioni, 1985). At the same time, a sizable portion of the amount in blood remained in the plasma fraction (30 to 75 percent). The reduction of hexavalent chromium to trivalent chromium at the site of administration as well as in the plasma probably accounts for this result (Korallus *et al.*, 1984; Suzuki, 1988; Cavalleri *et al.*, 1985; Richelmi and Baldi, 1984; Lewalter *et al.*, 1985). 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 administered 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 (Yamamoto *et al.*, 1981; Aaseth *et al.*, 1982; Aisen *et al.*, 1969). There is little evidence that hexavalent chromium binds to the various proteins and peptides that bind trivalent chromium. In the plasma, trivalent chromium preferentially binds to transferrin but as transferrin binding sites become saturated, a greater fraction of trivalent chromium begins to bind to other molecules such as albumin (Frankendal and Stigbrand, 1973; Ani and Moshtaghie, 1992; Moshtaghie *et al.*, 1992; Aisen *et al.*, 1969; Yang and Black, 1994; Lim *et al.*, 1983). At higher levels, more chromium also occurs in the ultrafiltrate of plasma, also indicating transferrin-binding sites have become saturated (Onkelinx, 1977; Frankendal and Stigbrand, 1973). 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 trivalent chromium in the blood was associated with RBCs 24 hours post-administration. Up to 35 percent of the trivalent chromium in the blood was associated with RBCs in the study of Gao *et al.*, 1993. Increased blood levels of chromium following oral administration of trivalent chromium 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 hexavalent chromium 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 hexavalent chromium into RBCs are present in other cells. Therefore, other cells would be expected to readily take up hexavalent chromium while little trivalent chromium would be expected to be taken up by most cells. Indeed, oral, intratracheal, intravenous,

or intraperitoneal administration of hexavalent chromium results in increased chromium levels in a number of tissues, while little uptake occurs following the administration of trivalent chromium (Baetjer *et al.*, 1959; Yamaguchi *et al.*, 1983; MacKenzie *et al.*, 1958; Edel and Sabbioni, 1985; Wiegand *et al.*, 1984; NTP, 2007b). The uptake of hexavalent chromium was very rapid in the isolated perfused rat liver (Wiegand *et al.*, 1986). Relative to hexavalent chromium, little uptake of trivalent chromium occurred even when it was administered intravenously, which ensured that the metal was immediately available for tissue and cellular uptake (Visek *et al.*, 1953; Sayato *et al.*, 1980; Baetjer *et al.*, 1959).

The widespread distribution of chromium into tissues following hexavalent chromium 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 hexavalent chromium from reaching and being taken up by tissues. While chromium was detected in high levels in the kidney, spleen, RBCs, and liver when hexavalent chromium was administered, little chromium was detected in these tissues following the administration of trivalent chromium except at the site of its excretion, the kidney (and at much lower levels than when hexavalent chromium was administered) (Weber, 1983; Costa, 1997; Yamaguchi *et al.*, 1983; Yamamoto *et al.*, 1981; Suzuki *et al.*, 1984). Substantial uptake of hexavalent chromium by the liver is indicated by elevated levels of chromium in the bile following intravenous administration of hexavalent chromium, compared to trivalent chromium administration (Cikrt and Bencko, 1979; Manzo *et al.*, 1983; Cavalleri *et al.*, 1985). One particularly notable finding was the detection of hexavalent chromium 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 hexavalent chromium in their drinking water (Trivedi *et al.*, 1989; Junaid *et al.*, 1996a; 1996b).

Oral administration of hexavalent chromium 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 (Sutherland *et al.*, 2000; Thomann *et al.*, 1994; Witmer *et al.*, 1989; NTP 2007b). 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 trivalent chromium. If hexavalent chromium were rapidly and completely reduced to trivalent chromium it should have been distributed in a manner that is virtually identical to that observed following trivalent chromium 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 hexavalent and trivalent chromium to humans, with an average half-life of 10 hours following trivalent chromium administration versus an average half-life of 39 hours following administration of hexavalent chromium (Kerger *et al.*, 1996a). The prolonged urinary half-life following hexavalent chromium 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 hexavalent chromium 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 hexavalent chromium 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 hexavalent chromium in the liver following intravenous administration by Sayato *et al.* (1980) also suggests that liver is a site of hexavalent chromium uptake. The half-life of chromium in various tissues (other than plasma) of rats administered hexavalent chromium 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 hexavalent chromium would be consistent with the behavior of hexavalent but not trivalent chromium.

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

For some of the subjects in the human studies, changes in chromium levels in RBC following hexavalent chromium behaved as if trivalent chromium 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 hexavalent chromium, unlike the pattern observed following trivalent chromium administration. In other individuals, RBC and plasma chromium levels remained essentially unchanged following hexavalent chromium administration.

That changes in the RBC chromium level following hexavalent chromium administration appeared as if trivalent chromium had been administered is not surprising if most of the chromium in the blood was trivalent chromium. The pattern of rapid increase and decrease in RBC chromium levels does not exclude the presence of hexavalent chromium, but only indicates that the trivalent chromium predominates. At the high doses administered in these studies trivalent chromium may have sorbed onto the RBC surface proteins. Thus, a sizable portion of the increase of chromium levels in the plasma and RBC following oral administration of hexavalent chromium to humans is probably trivalent chromium. This is due to: 1) extensive reduction of absorbed hexavalent chromium 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 trivalent chromium 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 trivalent chromium had cleared from the blood.

Elimination

Administered trivalent chromium 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 hexavalent chromium, RBC chromium levels or the ratio of RBC to plasma chromium either did not decline as rapidly or remained elevated for quite some time (Gao *et al.*, 1993; Weber, 1983; Sayato *et al.*, 1980; Coogan *et al.*, 1991b; Langard *et al.*, 1978; Suzuki *et al.*, 1984). Some of the initial decline in RBC chromium levels following hexavalent chromium administration probably reflects the portion of the dose that was immediately converted to trivalent chromium.

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

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 hexavalent chromium in drinking water, the saliva, stomach and blood have abundant and essentially inexhaustible ability to rapidly convert hexavalent chromium to trivalent chromium. Based on this belief that orally administered hexavalent chromium is completely converted to trivalent chromium in the stomach and saliva, no differences in absorption, distribution, or elimination should be apparent for hexavalent versus trivalent chromium. 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) or animals (MacKenzie *et al.*, 1958; Costa, 1997) do not support the conviction that hexavalent chromium is completely converted to trivalent chromium. Orally administered hexavalent chromium does not behave as if trivalent chromium had been administered in humans or experimental animals.

OEHHA proposes two models that account for the differences in behavior of hexavalent and trivalent chromium 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 hexavalent chromium (Sutherland *et al.*, 2000; Witmer *et al.*, 1989; Thomann, *et al.*, 1994; Costa, 1997; NTP, 2007b). The prolonged plasma and urinary half-life appear to result from chromium being taken up and then eluted from cells. The behavior of administered trivalent chromium - 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 hexavalent and trivalent chromium 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 hexavalent chromium exposure. If the absorbed hexavalent chromium was rapidly reduced to trivalent chromium 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 hexavalent chromium administration, the findings of a prolonged plasma and urinary half-life and its distribution to the liver and other tissues (relative to trivalent chromium) indicate that the hexavalent chromium form of the metal is moving into cells prior to its reduction to trivalent chromium.

One finding that at first glance appears to contradict the aforementioned models is that following the administration of hexavalent chromium, the half-life of chromium in the erythrocyte, while prolonged compared to when trivalent chromium 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 hexavalent chromium is distributed more to the liver and other tissues and less to erythrocytes. Minimal amounts of the absorbed hexavalent chromium 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 trivalent chromium. The chromium associated with the erythrocytes probably was bound to macromolecules on the outside of the cell. Non-specific binding of trivalent chromium 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 trivalent chromium 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 hexavalent chromium 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 hexavalent chromium to trivalent chromium 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

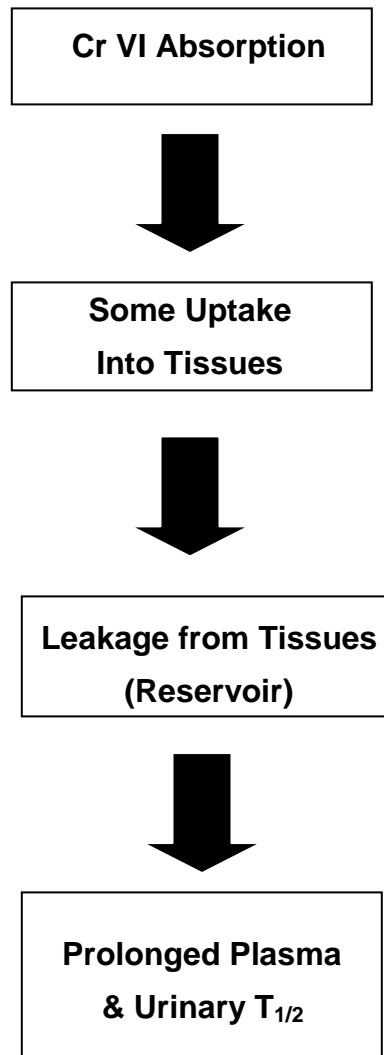
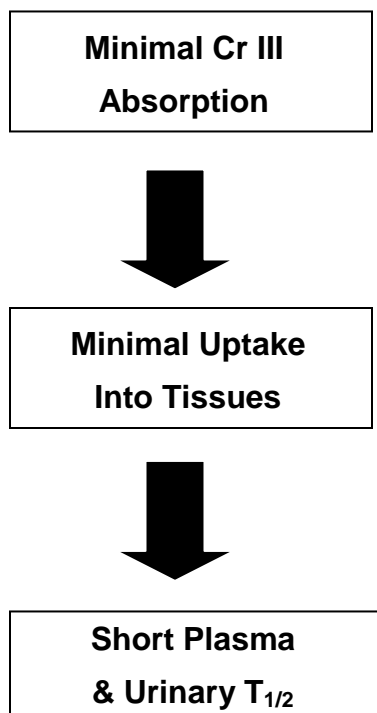


Figure 2. Toxicokinetic Model - Trivalent Chromium



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 hexavalent chromium into cells, while trivalent chromium is largely excluded from cells. The difference in the amount of absorption from the gut may also reflect the uptake of hexavalent chromium but not trivalent chromium by cells.

Quantitative differences in the propensity of hexavalent and trivalent chromium to associate with RBCs and differences in other characteristics such as the rate of decline of chromium in RBC following uptake of trivalent chromium (rapid) and hexavalent chromium (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 hexavalent chromium, 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 hexavalent chromium. 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 hexavalent chromium in the plasma would also be expected to substantially affect the levels of hexavalent chromium 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 hexavalent chromium into tissues as well as the amount of non-specific binding of trivalent chromium to the outside of the RBC.

Physiological/Nutritional Role

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

TOXICOLOGY

Toxicological Effects in Animals and Plants

Acute Toxicity

Oral LD₅₀s for chromium 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 chromium VI salts had greater acute toxicity than Cr III salts, and female rats appeared to be more sensitive than males to chromium VI salts (ATSDR, 2000).

Developmental and Reproductive Toxicity

BALB/c mice were administered hexavalent chromium (potassium dichromate) or trivalent chromium (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 trivalent or hexavalent chromium. The decrease in sperm counts appeared to be related to dose. Other effects were also reported including marked decreases in spermatogonia, increases in resting spermatocytes, and alteration in the proportions of germ cells in different mitotic phases (decreases in leptotene and zygotene and marked increases in pachytene). These findings appeared to be 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 hexavalent chromium 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 hexavalent chromium 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 hexavalent chromium 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 hexavalent chromium for three, six or nine weeks.

Male and female BALB/c mice were exposed to hexavalent chromium for 13 weeks, one week of pre-cohabitation exposure followed by 12 weeks of cohabitation exposure in a continuous breeding study that yielded several litters (F₁ generation) (NTP, 1997b). The F₁ 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 litter in the F₁ generation 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 F₀ generation, week 1 doses of hexavalent chromium 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 F₁ 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 F₀ and F₁ 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 F₀ 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 F₁ 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 (F₂ generation) born to the F₁ 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 F₁ generation. The body weights of F₁ male and female mice administered 400 ppm of potassium dichromate in the diet were decreased by about 9 percent on 74 day. The body weights of F₁ 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 CrO₃ (5, 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 hexavalent chromium (as potassium dichromate) in drinking water on day 0 through 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 level indicating maternal toxicity. At 1000 ppm of potassium dichromate, no implantations were observed. Increases in gross and skeletal abnormalities were notable in fetuses of animals administered 500 ppm of potassium dichromate. 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 hexavalent chromium (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 hexavalent chromium) 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 hexavalent chromium 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 hexavalent chromium)) 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 hexavalent chromium 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 hexavalent chromium pre- and postnatally.

Female rats were administered hexavalent chromium in drinking water (250, 500 or 750 ppm (31, 60, 75 mg/kg-day) Cr VI+ 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) Cr VI+ 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 hexavalent chromium in drinking water (250, 500 or 750 ppm (52, 98, 169 mg/kg-day) Cr VI+ 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 hexavalent chromium 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 hexavalent chromium 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 hexavalent chromium 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 hexavalent chromium 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 K₂CrO₄, 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 F₀ 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 K₂CrO₄) was administered to the F₁ 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 F₂ generation occurred in the twelfth month of the study, indicating that the breeding of the F₁ generation commenced around the eleventh month. Only 364 offspring resulted from breeding 220 F₁ females, indicating reproductive toxicity in the F₁ generation.

This finding is consistent with the reproductive toxicity observed by other investigators. The difference in the number of offspring between the F₀ and F₁ generations may be related to a substantial difference in the length of exposure of the mice (six weeks exposure prior to breeding in the F₀ generation as opposed to 11 months of exposure in the F₁ generation). In other studies, rats exposed to hexavalent chromium for 90 days were much more severely impacted by exposure to hexavalent chromium 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 hexavalent chromium, 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 K₂CrO₄ (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 K₂CrO₄ in their drinking water.

Exposure of male Wistar rats to a chromium VI (Na₂Cr₂O₇) aerosol (25, 50, 100 or 200 µg/m³ 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 µg/m³ 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 hexavalent chromium in air.

Exposure of male Wistar rats to a chromium VI (Na₂Cr₂O₇) aerosol (50, 100, 200 or 400 µg/m³ chromium) for 30 or 90 days, (22 hr/day) resulted in significant increases in lung weight the 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 as well as increased macrophage levels (at 200 and 400 µg/m³) were observed in bronchioalveolar lavage fluid.

Subchronic Toxicity

Eight subchronic animal studies were identified (NTP, 1996, 1997a,b; NTP, 2002; Chopra *et al.*, 1996; Acharya *et al.*, 2001; Kumar *et al.*, 1985; Kumar and Rana, 1982, 1984; Vyskocil *et al.*, 1993) in which hexavalent chromium 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 hexavalent chromium 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 hexavalent chromium administration).

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) in this study. 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, 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 week prior to sacrifice. It also should be noted that the microscopic pathology examination was limited to a few tissues. 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 hexavalent chromium.

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 five or six female Wistar rats per group for 22 weeks. The control group received untreated water. A third group was administered 10 percent ethanol in their drinking water and a fourth group was administered both ethanol and hexavalent chromium. 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 animals 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 animals receiving hexavalent chromium revealed “degeneration with reticular arrangement of hepatocytes, widened sinusoidal spaces, vacuolation and necrosis,” which was more pronounced in the periportal region. Similar significant changes in the histology 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, confirming 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 regarding the number of animals examined or the number of animals displaying histopathology was provided. Serum cholesterol levels were reduced while serum triglyceride and glucose levels were significantly increased above control in rats administered hexavalent chromium. 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 five to six male Wistar rats/group 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 hexavalent chromium. 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 animals 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 hexavalent chromium 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 regarding the number of animals examined or the number of animals displaying histopathology was provided. Decreased levels of triglycerides and glycogen, and increased levels of cholesterol compared to control, were observed in the livers of animals administered hexavalent chromium.

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 F₀ generation, week 1 doses of hexavalent chromium 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 F₁ 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 mice in the F₁ generation 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 observed in the 100 ppm F₁ female mice.

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

Potassium chromate was given by gavage to male rats (10 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 hexavalent chromium 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 animals administered chromium (Kumar and Rana, 1984).

Vyskocil et al., 1993

Male and female Wistar rats were given hexavalent chromium (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 observed 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, 2007a

The NTP reported findings of a 3 month-study in which F344 rats and B6C3F₁ mice were administered sodium dichromate in their drinking water (NTP, 2007a). Sodium dichromate dihydrate (0, 62.5, 125, 250, 500 or 1000 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 chromium for male rats and 0, 1.8, 3.5, 6.2, 11.5 or 21.4 mg/kg-day of chromium 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 (in all but the lowest dose group) 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 of hexavalent chromium to the mice was 0, 3.1, 9.1, 15.7 or 30.0 mg/kg-day of chromium for males and 0, 3.1, 9.4, 15.4 or 26.2 mg/kg-day of chromium 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 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 hexavalent chromium in the test article (other investigators had found that hexavalent chromium 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 had been administered to rats. Consequently, the methods used in the hexavalent chromium 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 regarding 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 reported that no differences in weight gain or food consumption were found 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 hexavalent chromium on these animals.

NTP, 2007b

Groups of 50 male and female rats (F-344) and mice (B6C3F₁) 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, 2007b). 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 6.0 mg/kg-day of chromium VI+, while female rats received 0.2, 0.9, 2.6 or 7.0 mg/kg-day of chromium VI+ (OEHHA calculations). Based on measured amounts of water consumption, male mice received an average dose of 0.45, 0.9, 2.4, or 5.7 mg/kg-day of chromium VI+, while female mice received 0.3, 1.2, 3.2 or 8.8 mg/kg-day of chromium VI+ (OEHHA calculations).

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 chromium VI+ probably accounts, in part, for the decreases in weight gain in the high dose groups (NTP, 2007b).

Similar to what has been observed in other studies (NTP 1996; 2007a), 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 groups as evidenced by decreased hematocrit, hemoglobin and erythrocyte counts. The animals appeared to recover from the anemia by 12 months.

Treatment related non-neoplastic lesions were not 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 animals after 2 years of exposure. However, the high dose that was administered in the two-year study (516 mg/L) was substantially lower than the high dose in the three month study (1000 mg/L).

Administration of chromium VI+ to female rats resulted in a dose-related increase in liver toxicity as evidenced by increased fatty changes and chronic inflammation. Statistically significant increases in the number of animals exhibiting fatty change plus chronic inflammation were observed in female rats administered 57.3 mg/L or more of Cr VI+, and chronic inflammation alone in animals administered 14.3 mg/L. 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 mice (both male and female) was good. There was no evidence of reduced survival in animals receiving hexavalent chromium. Body weight gains were largely unaffected by chromium VI+ administration in the mouse except in the high dose groups. As in the rat, water consumption was reduced in mice in the high dose groups. As with the rat, 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 hexavalent chromium. No hematology was performed in the male mouse.

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 doses that were actually administered. 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 hexavalent chromium. 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 hexavalent chromium in drinking water.

Table 1. Strengths and Weaknesses of Available Hexavalent Chromium Bioassays

Study	Strengths	Weaknesses
MacKenzie <i>et al.</i> , 1958	<ul style="list-style-type: none"> -5 dose levels in one study, 1 dose level in second study -Food and water intake monitored -Body weights monitored -Drinking water vehicle 	<ul style="list-style-type: none"> -Little information on animal husbandry 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 <i>et al.</i> , 1968	<ul style="list-style-type: none"> -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 	<ul style="list-style-type: none"> -Little information on animal husbandry 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

Study	Strengths	Weaknesses
National Toxicology Program, 1996; 1997a	<ul style="list-style-type: none"> -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 	<ul style="list-style-type: none"> -Cr VI+ in feed -Small number of animals group (6 males and 12 females per group) -Length of study only 9 weeks -Histopathology examination limited to a few tissues
National Toxicology Program, 1997b	<ul style="list-style-type: none"> -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 	<ul style="list-style-type: none"> -Cr VI+ in feed -Small number of animals/treatment group (20 animals/group) -Limited histopathology examination -Length of study only 90 days
National Toxicology Program, 2007a	<ul style="list-style-type: none"> 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 	<ul style="list-style-type: none"> -Small number of animals/treatment group (10 animals/group) -Length of study only 90 days -Limited histopathology
Chopra <i>et al.</i> , 1996	<ul style="list-style-type: none"> -Drinking water vehicle -Animal husbandry and QA/QC -Food and water intake monitored 	<ul style="list-style-type: none"> -Small number of animals/treatment group (five or six animals) -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 Program, 2007b	<ul style="list-style-type: none"> -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 -Chronic two year study 	<ul style="list-style-type: none"> -Cancer bioassays that employed relatively high doses. -Limited data on clinical chemistry (male rats) and hematology (male rats, female mice).
Acharya <i>et al.</i> , 2001	<ul style="list-style-type: none"> -Drinking water vehicle -Animal husbandry and QA/QC -Food and water intake monitored 	<ul style="list-style-type: none"> -Small number of animals/treatment group (5 or six animals) -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 <i>et al.</i> , 1985 Kumar and Rana, 1982; 1984	<ul style="list-style-type: none"> -Water vehicle 	<ul style="list-style-type: none"> -Small number of animals/treatment group (10 rats /group) -dose administered by gavage -Length of study only 20 days -Limited histopathology -Unclear if Cr VI++ levels in administered solution were analyzed

Study	Strengths	Weaknesses
		-Only one Cr VI++ dose administered
Vyskocil <i>et al.</i> , 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 hexavalent chromium 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 (reviewed in De Flora *et al.*, 1990; IARC, 1990; ATSDR, 2000). Hexavalent chromium compounds induced gene mutations in multiple species and strains of bacteria, and gene mutations, DNA-protein crosslinks, DNA strand breaks, chromosomal aberrations, sister chromatid exchanges, unscheduled DNA synthesis, and other forms of DNA damage in mammalian cells *in vitro*.

While the genotoxicity of hexavalent chromium compounds associated with *in vivo* exposures of humans and animals has been reviewed elsewhere (De Flora *et al.*, 1990; IARC, 1990; ATSDR, 2000), several new studies have recently been published. The following summarizes the evidence of genotoxicity of hexavalent chromium, emphasizing studies by the oral route because of the importance of this route in assessing the potential risk associated with hexavalent chromium in drinking water. Studies of exposure via other routes are also described.

Inhalation, intratracheal, intraperitoneal and intravenous exposures - IARC (1990) reviewed the studies of DNA damage in peripheral blood lymphocytes of workers exposed to hexavalent chromium. IARC noted that "Elevated levels of sister chromatid exchange were observed in workers exposed to chromium VI compounds in electroplating factories in four out of six studies. Chromosomal aberrations were found in all three studies of exposed workers." Relatively few *in vivo* genotoxicity studies of hexavalent chromium following exposures to the respiratory system were located (Bigaliev *et al.*, 1997; Izzotti *et al.*, 1998; Cheng *et al.*, 2000). 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) dosed Sprague-Dawley rats with intratracheal installations of 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. Some have argued that DNA damage in the lung and distant tissues will only occur above some threshold dose (Izzotti *et al.*, 1998; De Flora, 2000).

Over 15 genotoxicity studies in which rodents were administered soluble hexavalent chromium 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), and ATSDR (2000). 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 hexavalent chromium 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 (as reviewed by De Flora *et al.*, 1990; IARC, 1990; ATSDR, 2000). In rodents administered hexavalent chromium 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 – Nine primary studies of the potential genotoxic effects following ingestion of hexavalent chromium by humans or other mammalian species were located (Bigaliev *et al.*, 1977; Shindo *et al.*, 1989; Coogan *et al.*, 1991a; Sarkar *et al.*, 1993; Bagchi *et al.*, 1995a, Bagchi *et al.*, 1995b, Bagchi *et al.*, 1997; Kuykendall *et al.*, 1996; Mirsalis *et al.*, 1996). A summary of these studies is provided in Table 2. Six of the nine studies reported positive genotoxicity findings in various tissues. Chromosomal aberrations of the bone marrow, DNA single strand breaks of the liver and brain, or DNA-protein crosslinks of the liver were observed following exposure of rodents via drinking water or via chronic dosing by gavage. Surprisingly, no study to date has looked for DNA damage in the oral cavity or gastrointestinal tract following oral administration of hexavalent chromium. The data are consistent with the idea that, following low to moderate bolus doses (gavage) or higher concentrations in drinking water, hexavalent chromium is absorbed by the intestines and is transported to distant tissues where it damages DNA. Studies of genotoxicity of the oral cavity and gastrointestinal tract following oral ingestion of hexavalent chromium are needed.

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 ¹
Bigaliev, et al., 1997	white rats	gavage ²	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
Shindo <i>et al.</i> , 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 <i>et al.</i> , 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 <i>et al.</i> , 1993	Swiss mice	gavage	20 mg/kg, chromium VI oxide, single dose, measured 24 hr after dosing	+	chromosomal aberrations in bone marrow
Bagchi <i>et al.</i> , 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
Bagchi <i>et al.</i> , 1995a	Sprague-Dawley rats	gavage	25 mg/kg, sodium dichromate, single dose, measured 48 hr after dosing	+	DNA single strand breaks in liver

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 ¹
Bagchi <i>et al.</i> , 1997	Sprague-Dawley rats	gavage	2.5 mg/kg-d, sodium dichromate, 120 days	+	DNA single strand breaks in liver and brain
Kuykendall <i>et al.</i> , 1996	humans	drinking water	5 mg (~0.007 mg/kg), potassium dichromate, in 0.5 L water	-	DNA-protein crosslinks in leukocytes
Mirsalis <i>et al.</i> , 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
	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

¹ It is important to note that no group has looked for genotoxicity of the oral cavity or gastrointestinal tract following oral administration of hexavalent chromium.

² 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 K₂Cr₂O₇." 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.

The three oral genotoxicity studies which reported negative findings each employed short-term exposures: Shindo *et al.* (1989) administered a single dose by gavage; Kuykendall *et al.* (1996) administered a single dose in 0.5 L of drinking water; and Mirsalis *et al.* (1996) administered either two doses by gavage or dosed the animals via drinking water over a two-day period. One study assayed for DNA-protein crosslinks in leukocytes (Kuykendall *et al.*, 1996), a biomarker of Cr(VI) exposure whose usefulness has been called into question (Coogan *et al.*, 1991a; Paustenbach and Finley, 1999).

There is some concern that high doses of hexavalent chromium, 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 hexavalent chromium 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 hexavalent chromium-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 hexavalent chromium by gavage at doses (1.0 mg/kg-d, Bigaliev *et al.*, 1977; 2.5 mg/kg-d, Bagchi *et al.*, 1997) not likely to overwhelm the reductive capacities of the stomach, intestines and blood.

Summary

Hexavalent chromium has been shown to be genotoxic by all routes of administration in rodents treated with high doses of hexavalent chromium. 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 hexavalent chromium.

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 hexavalent chromium compounds via inhalation, ip, 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 hexavalent chromium, 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 hexavalent chromium is whether it causes DNA damage to the

oral cavity or gastrointestinal tract following oral ingestion. Unfortunately, none of the genotoxicity studies employing oral exposures examined DNA damage at the site of administration.

In summary, hexavalent chromium is reduced to trivalent chromium 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 hexavalent chromium can evade reduction in the oral cavity, gastrointestinal tract and blood. Currently, it is unknown whether significant portions of lower oral doses of hexavalent chromium evade *in situ* reduction and cause DNA damage in the oral cavity and gastrointestinal tract.

Mechanism of Genotoxicity and Carcinogenicity

Although hexavalent chromium 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). 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, hexavalent chromium 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 hexavalent chromium (IARC, 1990). It is during the reduction of hexavalent chromium 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 thiol 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). Additionally, the newly formed Cr III may build up to high concentrations within the cell, and may be itself an important mediator of hexavalent chromium 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 hexavalent chromium as a compound that "functions as a sort of Trojan horse" (De Flora, 2000). It is widely believed that DNA damage from hexavalent chromium 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 hexavalent chromium and DNA damage is not well understood (Sugden and Stearns, 2000).

The postulated mechanisms of hexavalent chromium-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 hexavalent chromium (reviewed in De Flora and Wetterhahn, 1989; Cohen *et al.*, 1993; Sugden and Stearns, 2000). This evidence includes the measurement of reactive oxygen species in *in vitro* tests of hexavalent chromium 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 hexavalent chromium treatment *in vitro* and *in vivo*, and observations that hexavalent chromium toxicity is reduced in the presence of free radical scavengers (reviewed in ATSDR, 2000; Sugden and Stearns, 2000).

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

In support of the third mechanism, researchers have observed direct binding of chromium with DNA and other cellular macromolecules (reviewed in ATSDR, 2000). 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).

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 hexavalent chromium compounds. IARC 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, 1980b)." IARC (1990) stated "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).”

Borneff *et al.*, 1968

Until the recent publication of the results of the NTP bioassay for sodium dichromate (NTP 2007b), only one long-term animal cancer bioassay where hexavalent chromium 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 K₂CrO₄ 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, and this effectively ended the epidemic and the study continued. Two carcinomas of the stomach were observed in female mice exposed to K₂CrO₄. No malignant stomach tumors were found in control mice. Nine benign stomach tumors were observed in female mice exposed to K₂CrO₄. 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 stomach tumors (11/66) in K₂CrO₄-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 2007b

Groups of 50 male or female F-344 rats and B6C3F₁ mice 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, 2007b). Based on measured water consumption rates and body weight, male rats received a time-weighted average dose of 0.2, 0.8, 2.1, or 6.0 mg/kg-day of chromium VI+, while female rats received 0.2, 0.9, 2.6 or 7.0 mg/kg-day of chromium VI+ (OEHHA calculations). Based on measured amounts of water consumption, male mice received an average dose of 0.45, 0.9, 2.4, or 5.7 mg/kg-day of chromium VI+, while female mice received 0.3, 1.2, 3.2 or 8.8 mg/kg-day of chromium VI+ (OEHHA calculations).

Rat

The survival of rats (both male and female) was good. Survival in rats receiving hexavalent chromium was similar to that in the control group (Figures 3 and 4). Body weight gains were largely not affected by chromium VI+ administration in the rat except in high dose male and female rats (Figure 5 and 6).

Figure 3. Survival curves for female rats

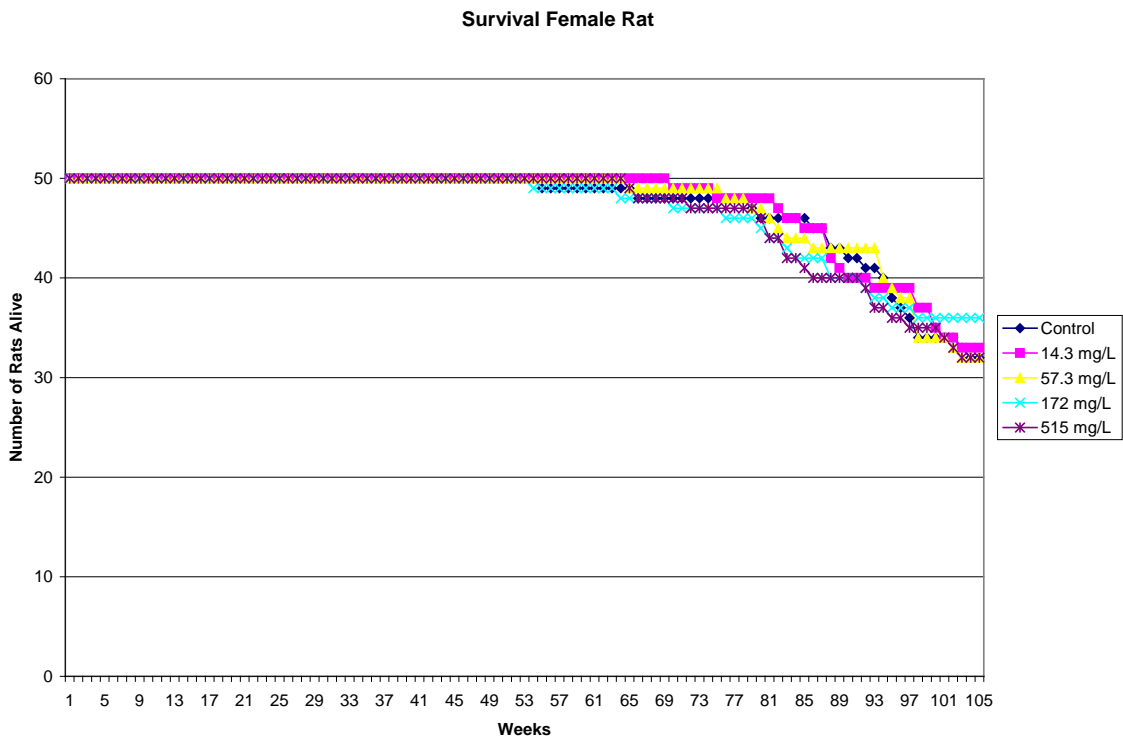


Figure 4. Survival curves for male rats

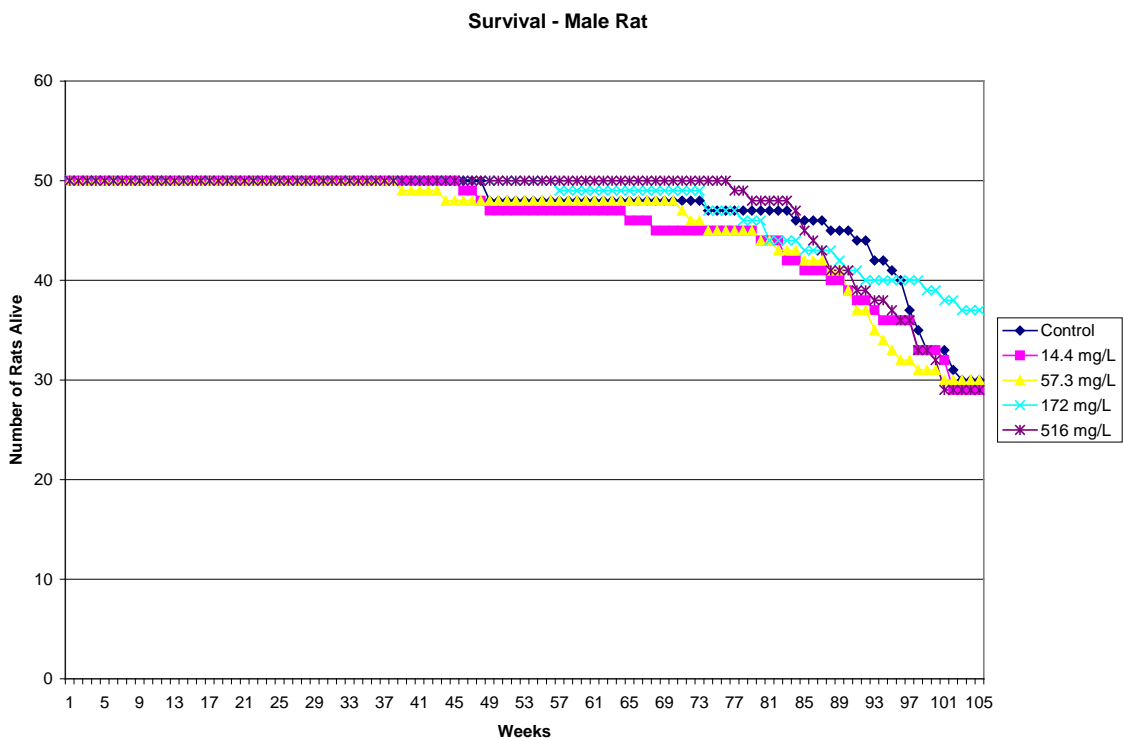


Figure 5. Female rats body weights, by week

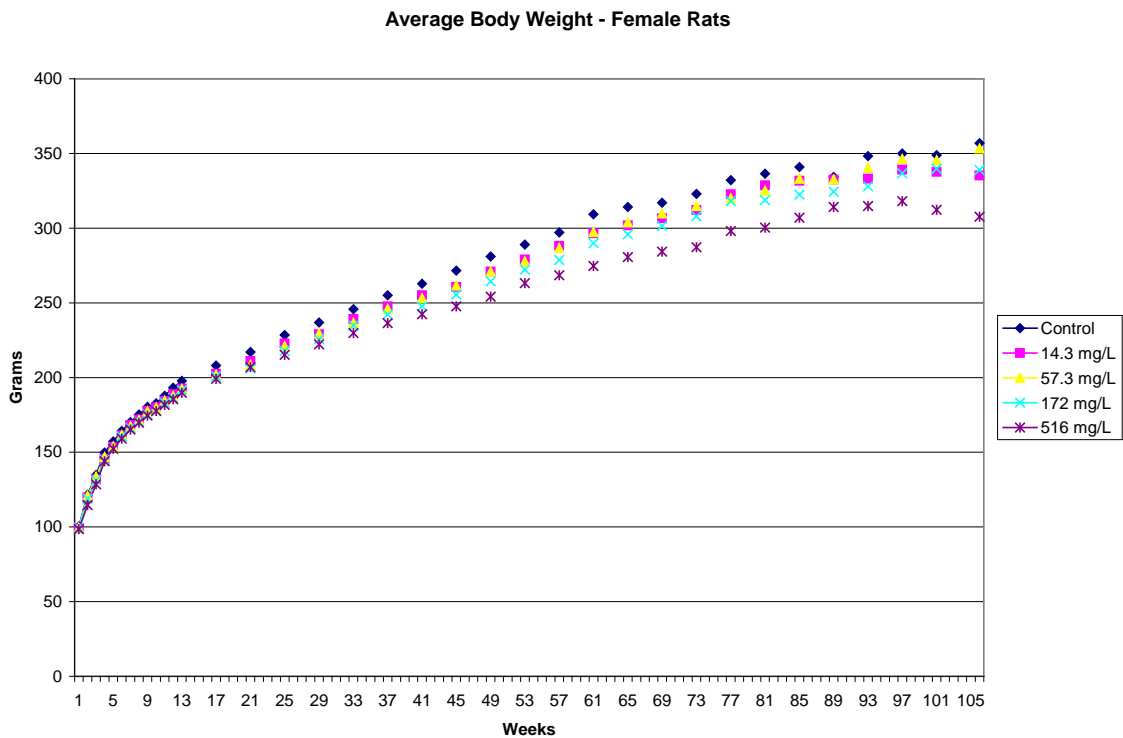
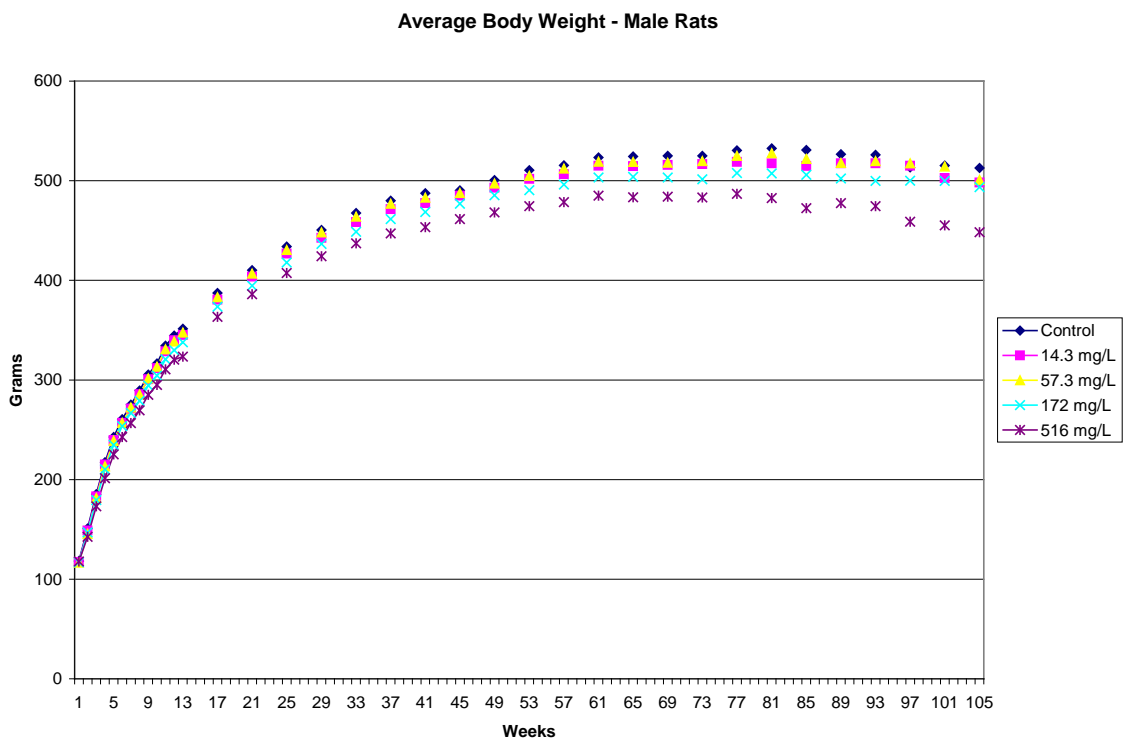


Figure 6. Male rat body weights, by week



Neoplasms

The administration of hexavalent chromium resulted in a statistically significant increase 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 increase was observed for squamous cell carcinomas alone and combined carcinomas or squamous cell papillomas. The tests for trend were positive, but this is not all that meaningful given that tumors were only increased at the highest dose level. NTP reported that squamous cell carcinomas of the oral mucosa of the rat were rarely observed in historical controls.

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.

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 13.4 mg/L group. The tests for trend were not positive at this site.

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

Tumor	0 mg/L	14.3 mg/L	57.3 mg/L	172 mg/L	516 mg/L
Papillomas	0/48 ^a	0/46	0/47	0/49	1/49
Carcinomas	0/48 ^b	1/46	0/47	0/49	6/49 ^c
Papillomas or Carcinomas	0/48 ^b	1/46	0/47	0/49	7/49 ^c

^aNumber of tumors/number of animals at risk. Animals were considered to be at risk of developing a tumor if the animal lived beyond when the first tumor was observed (within 45 days of the appearance of the first tumor) and if tissues were examined (tissues not lost or autolysed).

^bStatistically significant ($p < 0.05$) Mantel-Haenszel trend test.

^cStatistically significant ($p < 0.05$) 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. Occurrence of Tumors in the Oral Mucosa and Tongue in Female Rats Administered Hexavalent Chromium.

Tumor	0 mg/L	14.3 mg/L	57.3 mg/L	172 mg/L	516 mg/L
Papillomas	1/50 ^a	1/48	0/49	0/49	0/50
Carcinomas	0/50 ^b	0/48	0/49	2/49	11/50 ^c
Papillomas or Carcinomas	1/50 ^b	1/48	0/49	2/49	11/50 ^c

^aNumber of tumors/number of animals at risk. Animals were considered to be at risk of developing a tumor if the animal lived beyond when the first tumor was observed (within 45 days of the appearance of the first tumor) and if tissues were examined (tissues not lost or autolysed).

^bStatistically significant ($p < 0.05$) Mantel-Haenszel trend test.

^cStatistically significant ($p < 0.05$) Fisher's Exact test.

Mouse

The survival of mice (both male and female) was good. Survival in animals receiving chromium VI+ was similar to that in the control group (Figures 7 and 8). Body weight gains were largely unaffected by chromium VI+ administration in the mouse 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 in the high dose groups. The reduced body weight was partly attributed by the NTP to the reduced water consumption.

Figure 7. Survival curves for female mice

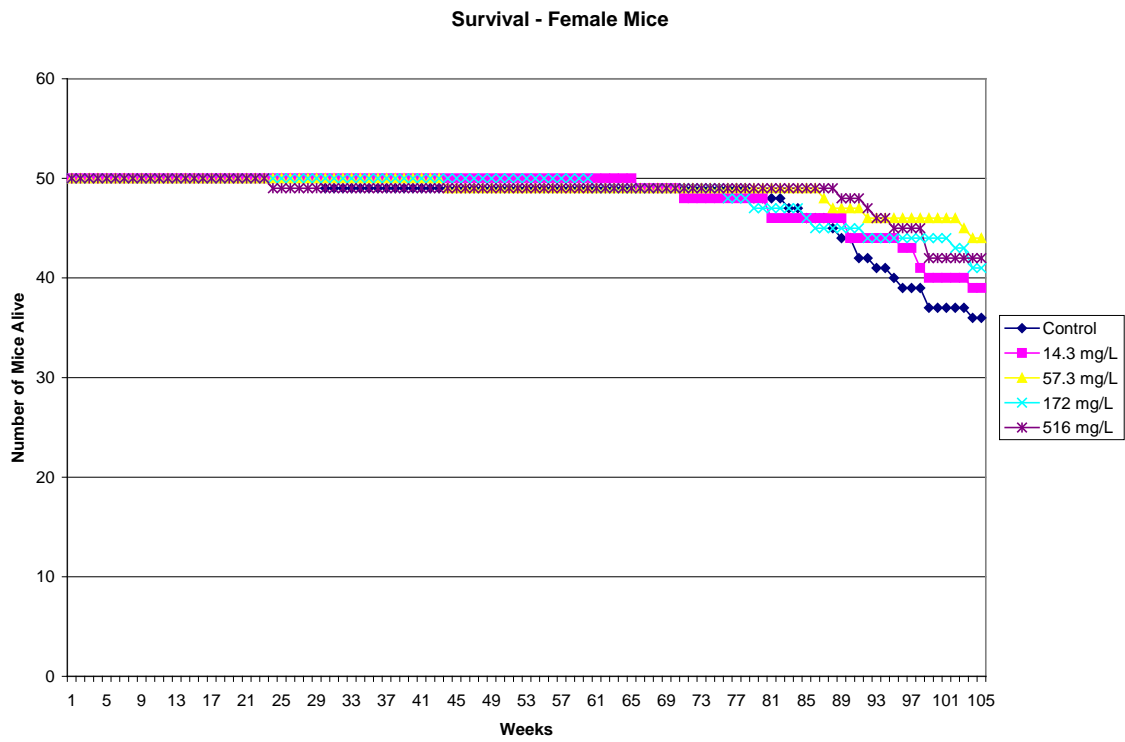


Figure 8. Survival curves for male mice

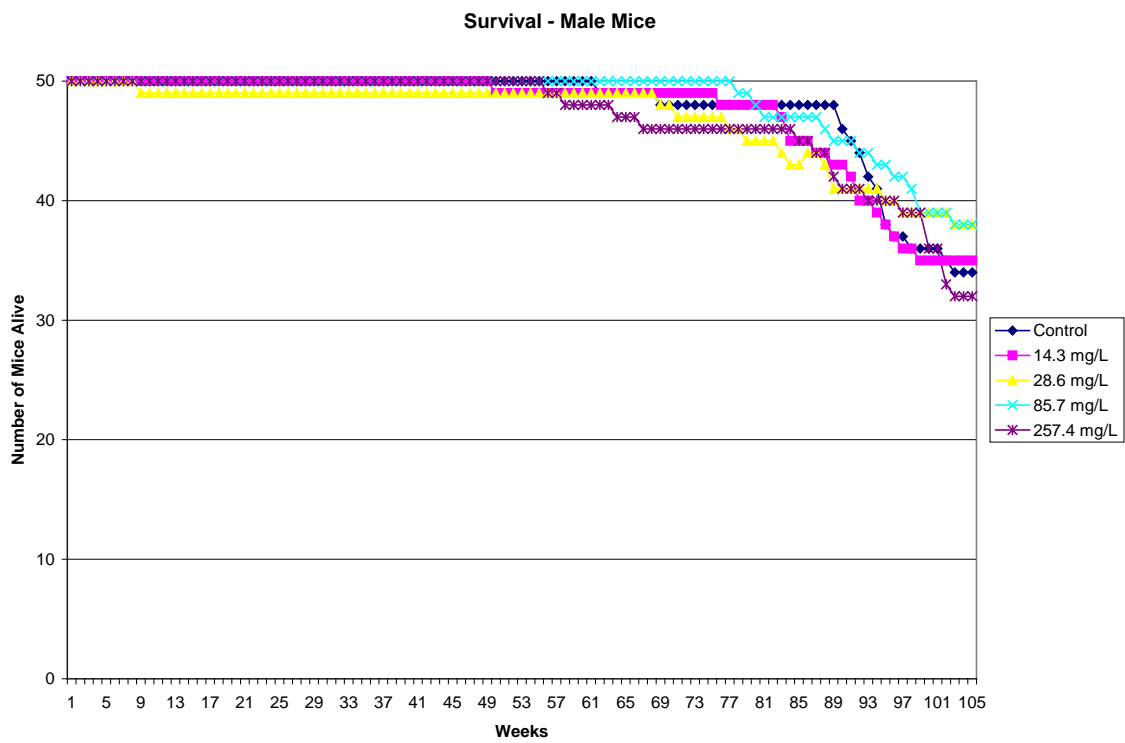


Figure 9. Male mouse body weights, by week

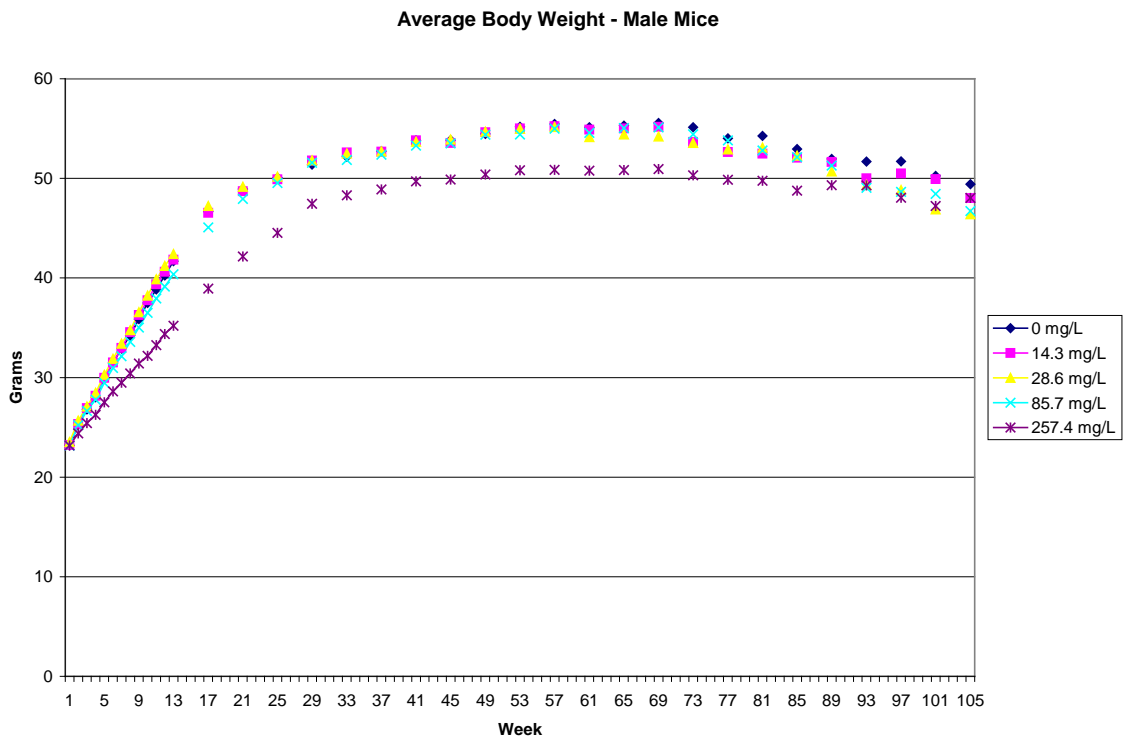


Figure 10. Female mouse body weights, by week

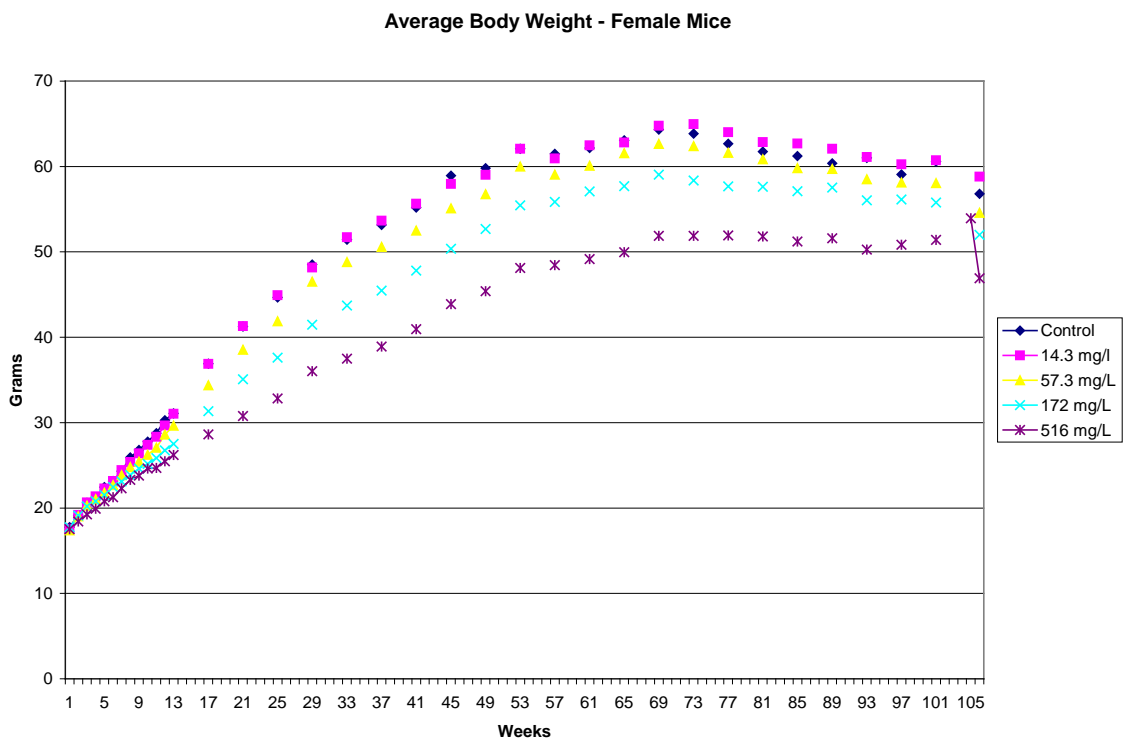


Figure 11. Body weights of male mice, compared to control

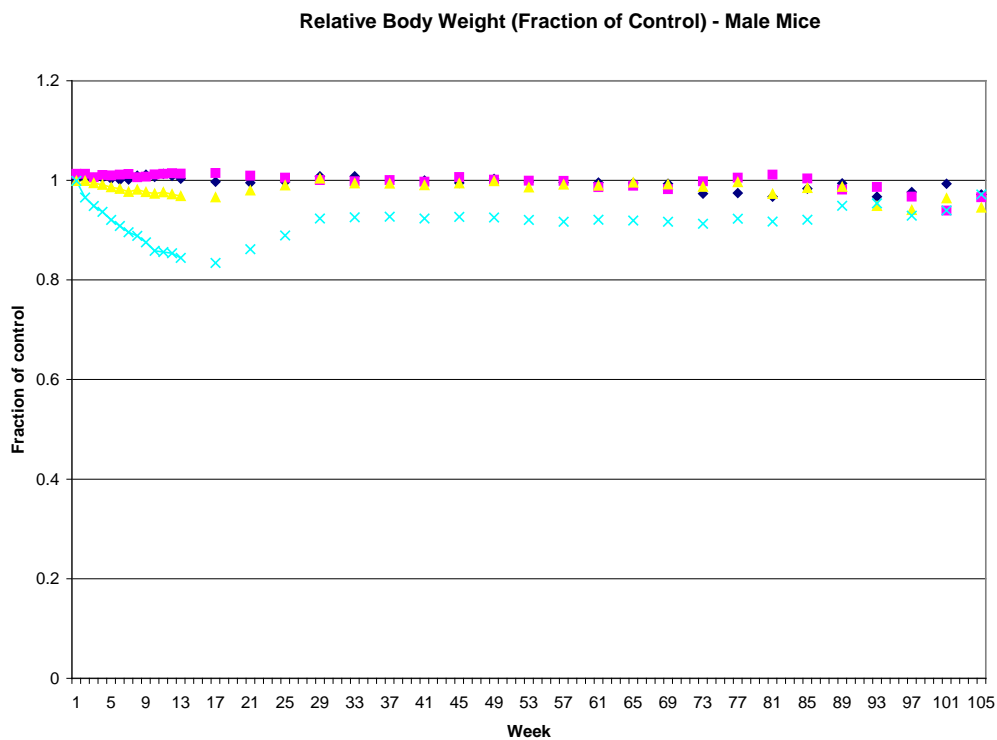
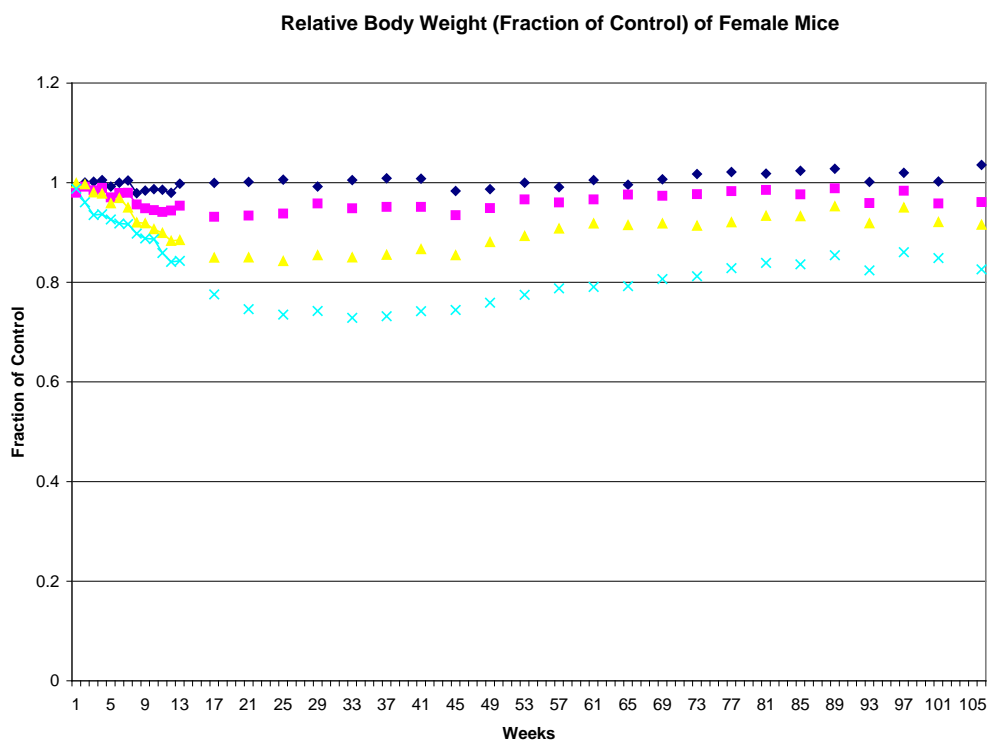


Figure 12. Body weights of female mice, compared to control



Neoplasms

The administration of hexavalent chromium to male and female mice resulted in a statistically significant and dose-related increase in adenomas or carcinomas in the duodenum and the entire small intestine (duodenum, jejunum and ileum) (Tables 5 and 6, Figure 13). The dose-response relationship between chromium VI+ and tumors of the small intestine appeared to be quite similar in male and female mice (Figure 13).

The effective number of mice in Tables 5 and 6 (the denominator) reflects animals whose duodenum (where most of the tumors occurred) was examined and excludes animals whose duodenum was not examined due to autolysis or where the tissue was missing. Animals were also excluded if they died more than 40 days prior to the appearance of the first tumor in the small intestine. Statistical analysis in which the effective number of animals was based on animals where the duodenum **or** jejunum were examined (slightly increasing the denominator) resulted in essentially the same findings (data not shown).

The intestinal tumors occurred late, 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.

Table 5. Occurrence of Duodenum or Small Intestine Tumors in Male Mice Administered Hexavalent Chromium.

Organ	Tumor Type	Concentration of Cr VI+ in Drinking Water				
		0 mg/L	14.3 mg/L	28.6 mg/L	85.7 mg/L	257.4 mg/L
Duodenum	Adenomas	1/39 ^{a,b}	0/43	1/44	5/48	15/39 ^c
	Carcinomas	0/39 ^b	0/43	0/44	2/48	3/39
	Adenomas or Carcinomas	1/39 ^b	0/43	1/44	6/48	16/39 ^c
Small Intestine ^d	Adenomas	1/39 ^b	1/43	1/44	5/48	17/39 ^c
	Carcinomas	0/39 ^b	2/43	1/44	3/48	5/39 ^c
	Adenomas or Carcinomas	1/39 ^b	3/43	2/44	7/48 ^c	20/39 ^c

^aNumber of tumors/number of animals at risk. Animals were considered to be at risk of developing a tumor if the animal lived beyond when the first tumor was observed (within 40 days of the appearance of the first tumor) and if tissue was examined (tissue was not lost or autolysed).

^bStatistically significant ($p < 0.05$) Mantel-Haenszel trend test.

^cStatistically significant ($p < 0.05$) Fisher's exact test.

^dIncludes duodenum, ileum and jejunum.

Table 6. Occurrence of Duodenum or Small Intestine Tumors in Female Mice Administered Hexavalent Chromium.

Organ	Tumor Type	Concentration of Cr VI+ in Drinking Water				
		0 mg/L	14.3 mg/L	57. mg/L	172 mg/L	516 mg/L
Duodenum	Adenomas ^{a,b}	0/40 ^{a,b}	0/40	2/47	13/41 ^c	12/47 ^c
	Carcinomas ^b	0/40 ^b	0/40	0/47	1/41	6/47 ^c
	Adenomas or Carcinomas ^b	0/40 ^b	0/40	2/47	14/41 ^c	17/47 ^c
Small Intestine ^d	Adenomas	0/40 ^b	1/40	2/47	15/41 ^c	16/47 ^c
	Carcinomas	1/40 ^b	0/40	2/47	3/41	7/47 ^c
	Adenomas or Carcinomas	1/40 ^b	1/40	4/47	17/41 ^c	22/47 ^c

^aNumber of tumors/number of animals at risk. Animals were considered to be at risk of developing a tumor if the animal lived beyond when the first tumor was observed (within 40 days of the appearance of the first tumor) and if tissue was examined (tissue was not lost or autolysed).

^bStatistically significant ($p < 0.05$) Mantel-Haenszel trend test.

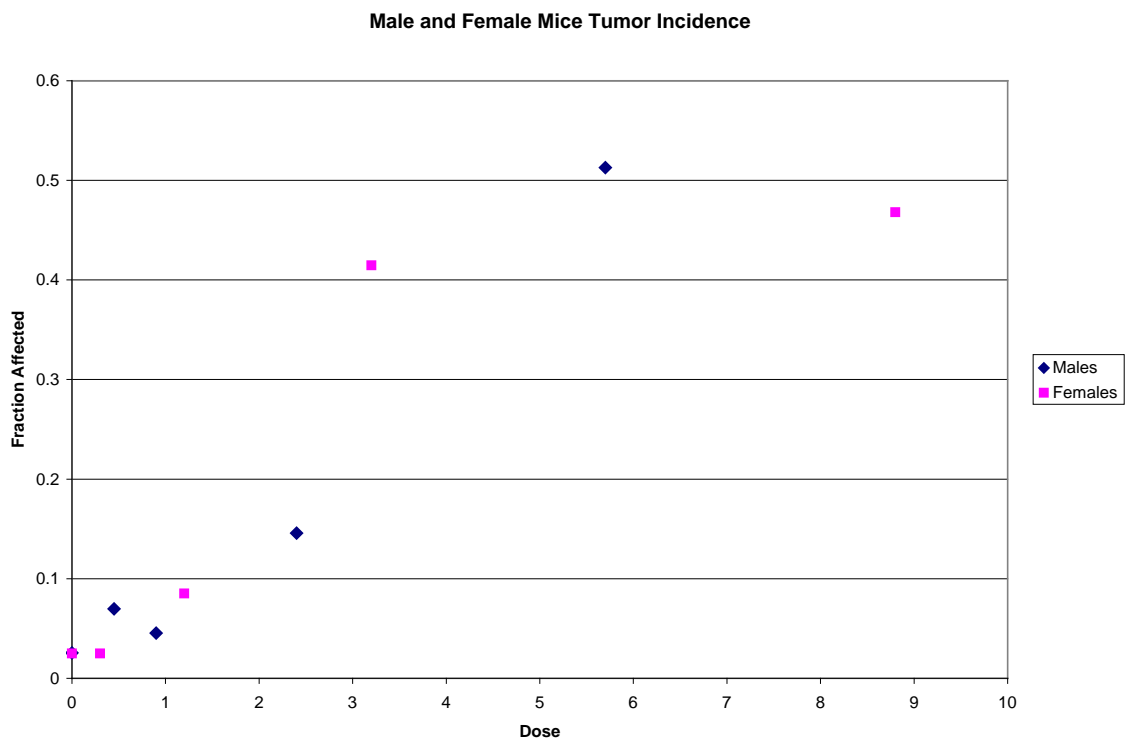
^cStatistically significant ($p < 0.05$) Fisher's exact test.

^dIncludes duodenum, ileum and jejunum.

Historically, tumors of the duodenum or small intestine are very rare in B6C3F₁ mice in NTP studies. In control male mice, NTP reported detecting nine adenomas and three carcinomas of the duodenum of 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, the appearance of small intestinal tumors in mice administered chromium VI+ does not appear to be due to chance

No statistically significant increases in tumors of the oral cavity were observed at any dose, unlike what was observed 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 chromium 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.

Figure 13. Dose-response for combined intestinal tumors in male and female mice.



Maximum Tolerated Dose - Rats

No difference in survival was evident in male or female rats treated with chromium VI+ compared to control. A decrease in body weight was observed in the high dose group 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: “A concentration-related erythrocyte microcytosis, evidenced by decreased mean cell volume, occurred on day 4 and persisted throughout the study in the 172 and 516 mg/L group.” “The severity of the microcytosis ameliorated with time.” Exposure-related anemia was also observed in the 57.2, 172 and 516 mg/L groups. NTP noted “the anemia was most severe on day 22 (approximately 30 percent decrease in the 516 mg/L group), but resolved with time.” “In fact, at 3 months, erythrocyte counts were increased and contradictory 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 liver of female rats administered 57.3 mg/L or greater of hexavalent chromium. 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 hexavalent chromium.

There was very little evidence of toxicity in rats treated with hexavalent chromium. 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 hexavalent chromium, 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 chromium VI+, drinking water becomes unpalatable. In the NTP 2007b study, reduced water consumption (normalized to body weight) was observed at the high dose of chromium VI+ (Figures 14 and 15).

Figure 14. Effect of chromium VI+ on water intake in male mice, by week.

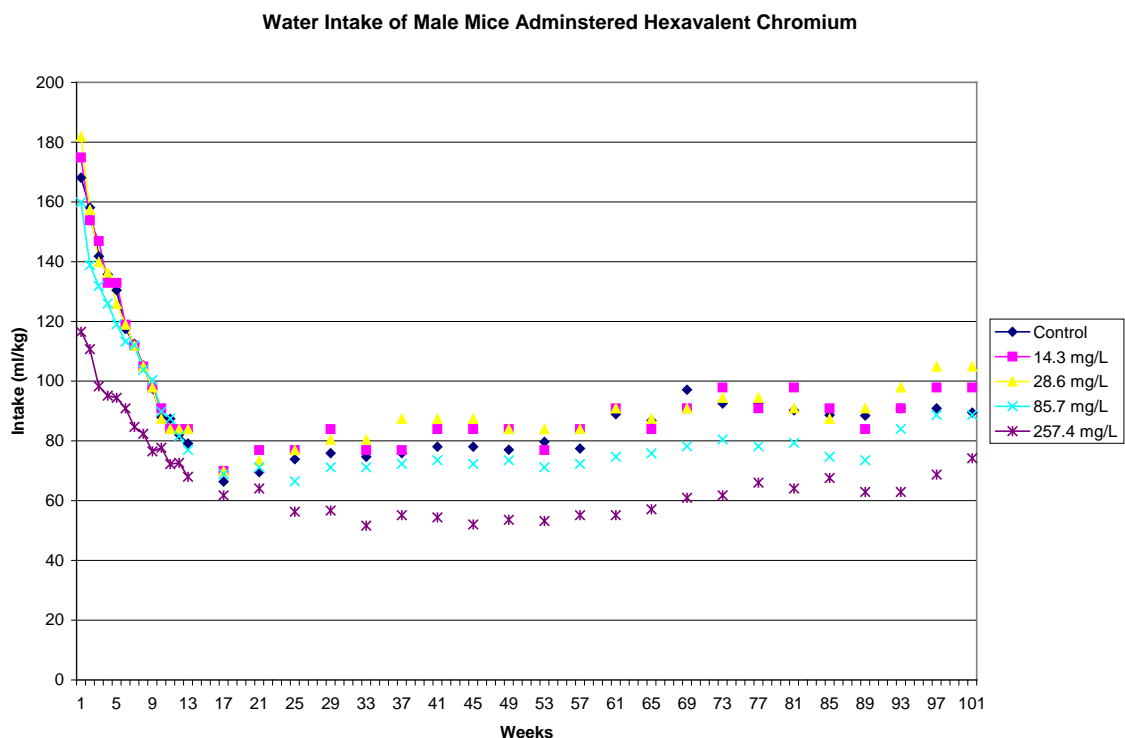
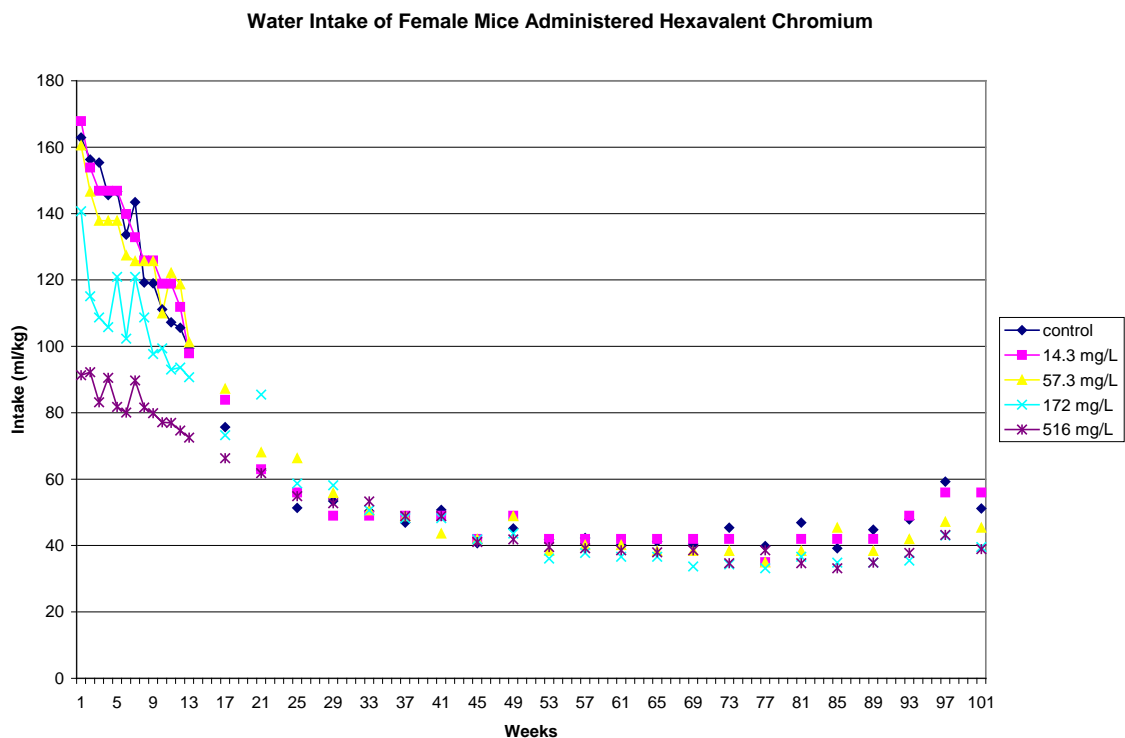


Figure 15. Effect of chromium VI+ on water intake in female mice, by week.



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 hexavalent chromium in their drinking water with time. 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 also tolerated chromium 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.”

A statistically significant and dose related increase in diffuse hyperplasia in the duodenum was observed in mice. This finding was not unexpected given that hyperplasia may be a precursor to the observed tumors in the duodenum. While no injury was reported, NTP indicated “that collectively, these lesions are considered consistent with regenerative hyperplasia secondary to previous epithelial cell injury.”

In conclusion, very little evidence of toxicity was observed in mice treated with hexavalent chromium. 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, 2007c).

Non-Oral Routes

Cancer bioassays of animals exposed to hexavalent chromium by non-oral routes have been thoroughly reviewed by others (unlike the Borneff *et al.*, 1968 study) and another review is not needed (ATSDR, 2002; IARC, 1990). To summarize, four cancer inhalation studies were identified that evaluated hexavalent chromium 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 hexavalent chromium when it is administered by inhalation.

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

Multiple studies have also been conducted in which hexavalent chromium 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 hexavalent chromium 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 hexavalent chromium 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 CrVI/kg as potassium dichromate. Death resulted from gastrointestinal ulceration and severe damage to the liver and kidneys (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 chromium 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 chromium 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 hexavalent chromium were compared to workers that were not exposed. 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. Sperm counts and motility were significantly reduced in workers exposed to harmful chemicals. 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.

Immunotoxicity

Dermal exposure to hexavalent chromium 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 hexavalent chromium. .

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

Recent human epidemiological studies of hexavalent chromium exposure and cancer risk are reviewed. The major focus of many of these studies is the increase in cancer associated with inhalation exposure. We used the data from these studies to estimate a cancer potency for inhalation exposure to hexavalent chromium. 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 hexavalent chromium.

Inhalation studies

In 1998, the U.S. EPA reviewed the available human epidemiological evidence on hexavalent chromium and respiratory cancer risk (U.S. EPA, 1998) and concluded, as did IARC in 1990, that hexavalent chromium 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 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 hexavalent chromium" exposures of 0.00045, 0.0042, 0.03, and 0.45 mg CrO₃/m³-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 hexavalent chromium 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 hexavalent chromium

Cole and Rodu, 2005 – Cole and Rodu conducted meta-analyses of cancer rate ratios reported in studies of humans ostensibly exposed to hexavalent chromium. 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 hexavalent chromium 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 hexavalent chromium (e.g., steel polishers in Jarvholm, 1982), did not include studies in which there was hexavalent chromium 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 hexavalent chromium 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 exposed populations. OEHHA systematically searched for occupational studies that reported results for ingestion and digestion-related organs to determine if there may be a link between occupational exposure to hexavalent chromium and cancers of the digestive organs.

Identification and Selection of Studies. Over a thousand articles related to human exposure to hexavalent chromium and cancer were identified. These articles were screened to identify epidemiologic studies of occupational populations exposed to hexavalent chromium that reported any results for the buccal (oral) cavity, pharynx, or the digestive system. The articles incorporated into the summary met the following inclusion criteria: 1) employment in an occupation or industry with potential Cr⁶⁺ exposure was documented by employer, labor organization, or government records; 2) the article stated that exposure to a Cr⁺⁶-containing substance occurred; 3) at least half of the study population was likely exposed to Cr⁺⁶; 4) the statistical analysis controlled for the potentially confounding variables age, calendar time, race, and gender; 5) the data

were presented in a complete article or report (as opposed to an abstract only); and 6) the article was the most recent update of cancer findings if more than one article was published regarding the study population.

Data Abstraction. The following rules were followed in abstracting the rate ratios and numbers of cancers from the articles. If results were presented only for specific gender or race categories or factories, 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. For studies of chrome platers, if results were presented separately for “hard” and “bright” chrome electroplating processes, the results for “hard” chrome plating were abstracted because hexavalent chromium exposures are known to be higher in hard chrome plating (Guillemain, 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).

Results. The 30 studies that met the inclusion criteria are listed in Table 7, 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, 18 of 25 (72 percent) estimated a rate ratio above 1, while in 7 out of 25 studies, the rate ratio was below 1 (suggesting a reduction in stomach cancer) (Table 7 and 8). The rate ratios were above 1 in 18 of 26 studies for cancer in all digestive organs, 8 out of 11 studies for cancer of the esophagus and 12 out of 16 studies for cancer of the rectum. Interestingly, for stomach cancer, only in 3 out of 25 studies did the lower confidence interval of the rate ratio exceed 1 (Table 7).

Table 7. 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)	Colon (ICD 153)	Rectum (ICD 154)	Liver and Gall Bladder (ICD 155-156)	Pancreas (ICD 157)	Lung Cancer (ICD 162)	Nonmalignant 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	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 ¹¹	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)
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)	

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)	Colon (ICD 153)	Rectum (ICD 154)	Liver and Gall Bladder (ICD 155-156)	Pancreas (ICD 157)	Lung Cancer (ICD 162)	Nonmalignant Resp. Dis. (ICD 460-519)
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 (0.00-9.66)	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)
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)	

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)	Colon (ICD 153)	Rectum (ICD 154)	Liver and Gall Bladder (ICD 155-156)	Pancreas (ICD 157)	Lung Cancer (ICD 162)	Nonmalignant Resp. Dis. (ICD 460-519)
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	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)	
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)

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)	Colon (ICD 153)	Rectum (ICD 154)	Liver and Gall Bladder (ICD 155-156)	Pancreas (ICD 157)	Lung Cancer (ICD 162)	Nonmalignant Resp. Dis. (ICD 460-519)
Moulin 1990 France	Ferrocromium & 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 1984 Iceland	Concrete mixing	SMR (Iceland)				1.16 (0.43-2.58) (5/4.30)	0.93 ¹⁰ (0.05-4.61) (1/1.07)	1.85 (0.09-9.13) (1/0.54)			3.14 (1.53-5.75) (9/2.87)	0.64 (0.11-2.10) (2/3.14)
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)	
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)	
Smalyte 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)

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)	Colon (ICD 153)	Rectum (ICD 154)	Liver and Gall Bladder (ICD 155-156)	Pancreas (ICD 157)	Lung Cancer (ICD 162)	Nonmalignant Resp. Dis. (ICD 460-519)	
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 hexavalent chromium 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 The Rafnsson results for large intestine may have included small intestine. While they were labeled “large intestine,” the 7th Revision ICD codes were said to be 152 and 153 (152 is small intestine).

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.

Table 8. Occupational Studies That Reported Results for Stomach Cancer, Sorted by Rate Ratio, in Descending Order.

Row No.	First Author, Year Published, and Country	Manufacturing Process	Stomach Cancer Rate Ratio (95% CI) (obs/exp)
1	Franchini 1983 Italy	Hard chrome plating	5.00 (0.84-16.52) (2/0.4)
2	Moulin 1990 France	Ferrochromium and stainless steel manufacturing	2.75 (0.87-6.61) (4/1.46)
3	Hayes 1989 United States	Chromium pigment production	1.79 (0.73-3.73) (6/3.35)
4	McDowall 1984 United Kingdom	Portland cement manufacturing	1.75 (1.12-2.61) (22/12.57)
5	Rosenman 1996 United States	Chromate production	1.66 (1.17-2.29) (34/20.5)
6	Sorahan 2000 United Kingdom	Chrome plating, hard or bright not stated	1.56 (0.84-2.65) (12/7.7)
7	Deschamps 1995 France	Lead and zinc chromate pigment production	1.52 (0.26-5.04) (2/1.31)
8	Sorahan 1987 United Kingdom	“Bright” chrome plating	1.49 (0.84-2.44) (14/9.4)
9	Langard 1990 Norway	Ferrochromium manufacturing	1.40 (0.61-2.77) (7/5.0)
10	Knutsson 2000 Sweden	Concrete mixing	1.39 (1.22-1.57) (243/174.6)
11	Amandus 1986 United States	Portland cement manufacturing	1.35 (0.90-1.93) (27/20.1)
12	Horiguchi 1990 Japan	Chrome plating, hard or bright not stated	1.30 (0.22-4.29) (2/1.54)
13	Korallus 1993 Germany	Chromate production	1.27 (0.75-2.02) (16/12.6)
14	Kano 1993 Japan	Chromium pigment production	1.20 (0.56-2.30) (8/6.66)
15	Rafnsson 1984 Iceland	Concrete mixing	1.16 (0.43-2.58) (5/4.30)
16	Boice 1999 United States	Chrome plating and chromate painting aircraft manufacturing	1.03 (0.54-1.79) (11/10.7)
17	Danielsen 1996 Norway	Stainless steel boiler welders	1.03 (0.26-2.82) (3/2.9)
18	Jakobsson 1993 Sweden	Portland cement manufacturing	1.01 (0.57-1.65) (14/13.9)
19	Satoh 1981 Japan	Chromate production	0.95 (0.50-1.65) (11/11.58)

Row No.	First Author, Year Published, and Country	Manufacturing Process	Stomach Cancer Rate Ratio (95% CI) (obs/exp)
20	Itoh 1996 Japan	Chrome plating, hard or bright not stated	0.79 (0.39-1.45) (9/11.4)
21	Axelsson 1980 Sweden	Ferrochromium manufacturing (arc furnaces)	0.78 (0.25-1.89) (4/5.1)
22	Smailyte 2004 Lithuania	Portland cement manufacturing	0.77 (0.25-1.86) (4/5.18)
23	Davies 1991 England & Scotland	Chromate production	0.73 (0.45-1.12) (19/26.11)
24	Becker 1999 Germany	Stainless steel welding with coated electrodes	0.59 (0.08-1.54) (2/4.3)
25	Birk 2006 Germany	Chromate production	0.50 (0.08-1.64) (2/4.04)

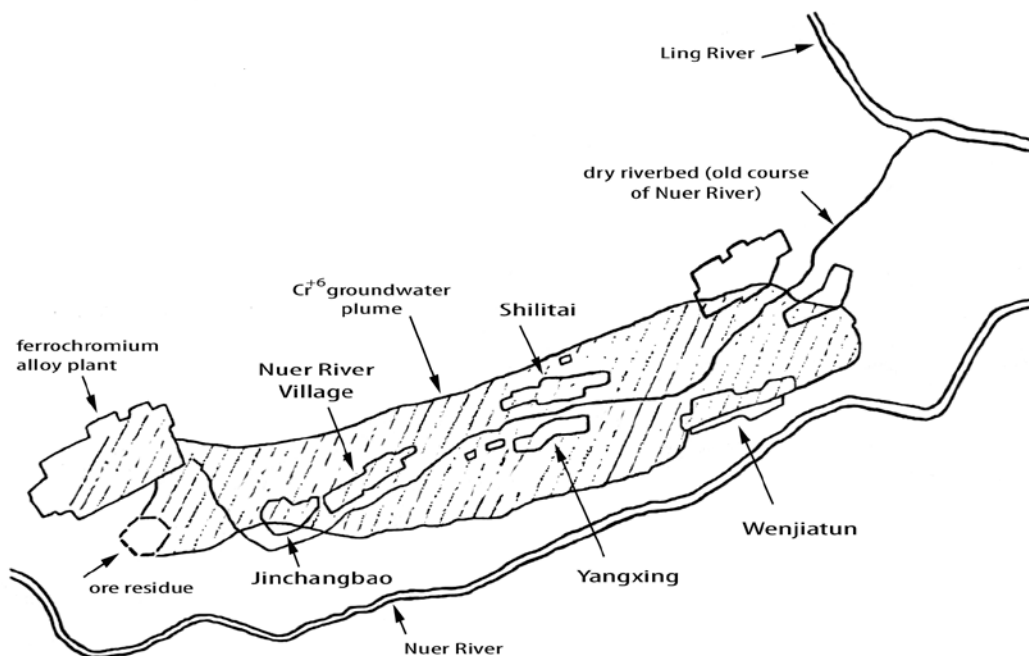
Ingestion studies

Only one study was identified in which cancer risk was investigated in a population demonstrably exposed to hexavalent chromium in drinking water. Zhang and Li (1987) investigated the occurrence of cancer in rural villages (Figure 16) near JinZhou, China and reported an increase in stomach cancer. Another report concluded the increase in cancer was unrelated to the exposure to chromium in drinking water (Zhang and Li, 1997) although this paper was later retracted (Brandt-Rauf, 2006). 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), are summarized below.

The source of the contamination was a chromium ore processing facility. Releases of hexavalent chromium 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 hexavalent chromium in groundwater samples does not necessarily indicate that all of the population in the area was exposed to high chromium levels.

Health surveys found evidence of exposure in the villages. A survey of residents in Nuer River Village in 1965 revealed mouth ulcer, diarrhea, stomach pains, indigestion and vomiting. In 1971, a survey of subjects in the second farthest village from the plant revealed 92 percent developed oral ulcers, 48 percent had diarrhea, and 36 percent had abdominal pains. These symptoms were observed in 1974 in the most remote of the five villages near the alloy factory.

Figure 16. 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).



The retracted 1997 report noted that the villages closest to the plant with higher levels of hexavalent chromium in drinking water in 1965 had lower cancer rates over the period 1970-1978 than villages with lower levels of hexavalent chromium, and concluded that the risk of cancer was probably unrelated to exposure to hexavalent chromium (Zhang and Li, 1997). 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 hexavalent chromium 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. Given the uncertainties regarding the levels of hexavalent chromium in groundwater after 1965, no conclusions are warranted concerning whether certain villages were exposed to more hexavalent chromium than other villages.

Because it is unclear which of the villages were exposed to the higher levels of hexavalent chromium in their drinking water, OEHHA combined the population and cancer data for the five villages with documented hexavalent chromium 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 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. However, increases in stomach and lung cancers were detected in spite of this limitation.

While the study had substantial limitations, it is clear that hexavalent chromium 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.

Sensitive Subpopulations

Toxicokinetic studies suggest that absorption of hexavalent chromium following oral exposure is substantially reduced by acidic stomach juices that facilitate the conversion of hexavalent chromium to trivalent chromium. Little trivalent chromium 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 hexavalent chromium, and this factor is likely to be responsible for much of the observed variability in gastrointestinal absorption of hexavalent chromium.

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*,

mucopolysaccharidosis type IV, and some autoimmune diseases (Isselbacher *et al.*, 1994). Increased absorption of hexavalent chromium 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, 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 hexavalent chromium administration resulted in a statistically significant increase in tumors in female mice (Borneff *et al.*, 1968). For this population, oral intake of hexavalent chromium 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 hexavalent chromium has been linked to increased rates of cancer. A number of retrospective studies have associated significant increases in respiratory cancer to hexavalent chromium 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 hexavalent chromium by the inhalation route (conducted by OEHHA) was suggestive of a link between inhalation exposure to hexavalent chromium and cancer of the digestive organs.

In the single study of human exposure to hexavalent chromium in drinking water identified, a statistically significant increase in stomach cancer mortality (statistical analysis conducted by OEHHA) was detected in the exposed population (Zhang and Li, 1987).

Animal studies - The administration of hexavalent chromium 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. Three cancer bioassays were identified in which hexavalent chromium was administered orally for the lifetime of the animal (NTP, 2007b; Borneff *et al.*, 1968). The administration of hexavalent chromium to mice resulted in statistically significant and dose related increase in tumors (adenomas; adenomas or carcinomas) of the duodenum or tumors of the small intestine (duodenum, jejunum and ileum) in male and female mice compared to control (NTP, 2007b). The administration of hexavalent chromium to male or female rats resulted in statistically significant increases in tumors of the oral cavity (NTP, 2007b).

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 stomach 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 hexavalent chromium 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, Turebaev, and Elemesova, 1977; Izzotti *et al.*, 1998; Cheng *et al.*, 2000). Oral administration of hexavalent chromium 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, 1977).

Toxicokinetics - The toxicokinetics of hexavalent chromium has been studied in animals and humans. Inhalation or oral exposure to hexavalent chromium resulted in detectable chromium increases in the erythrocyte, plasma, and other tissues of humans and experimental animals. Because of the rapid conversion of hexavalent chromium to trivalent chromium in the stomach, only a small fraction of the oral dose of hexavalent chromium appears to be absorbed. The amount of absorption is highly variable, although it generally is much greater than the gastrointestinal absorption when trivalent chromium is administered. The oral absorption of hexavalent chromium 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 hexavalent chromium to trivalent chromium.

There is evidence that a portion of an orally administered dose of hexavalent chromium is distributed to tissues as hexavalent chromium, based on the distribution and elimination pattern *in vivo*. Experimental evidence also suggests that an increased amount of orally administered hexavalent chromium is absorbed when the pH of the stomach is elevated. Individuals who regularly take medications that increase the pH of the stomach would appear to be a sensitive population because decreased reduction of hexavalent chromium in their stomachs would be expected to result in an increased gastrointestinal absorption of hexavalent chromium.

Toxicity - Hexavalent chromium appears to be more toxic than trivalent chromium when administered by the oral route. These differences in toxicity of hexavalent and trivalent chromium are consistent with toxicokinetic findings that a portion of the hexavalent chromium is orally absorbed and enters cells, rather than being fully converted to trivalent chromium in the acidic environment of the stomach.

Mechanism - Hexavalent chromium rapidly enters the cell via the anion transport system. Hexavalent chromium is then reduced to trivalent chromium 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 hexavalent chromium 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 in the only available oral studies. 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 hexavalent chromium and that hexavalent chromium penetrates into cells following oral exposure. There is substantial evidence of DNA damage following oral exposure to hexavalent chromium; however, it is not known if this would occur at environmental exposure levels. There is evidence that hexavalent chromium 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 hexavalent chromium is a possible human carcinogen by the oral route. Given these observations and until more human and/or animals studies become available, it is prudent to consider this hazard in the development of a proposed PHG.

DOSE-RESPONSE ASSESSMENT

Noncarcinogenic Effects

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

NTP 1997a - Doses of hexavalent chromium 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 chromium 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 hexavalent chromium 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 chromium 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 chromium VI of 1.1 mg/kg-day was estimated based on standard drinking water consumption rates and body weights.

NTP, 2007a - 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, 2007b - Groups of 50 male or female rats (F-344) and mice (B6C3F₁) 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, 2007b). 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 6.0 mg/kg-day of chromium VI+, while female rats received 0.2, 0.9, 2.6 or 7.0 mg/kg-day of chromium VI+. Based on measured amounts of water consumption, male mice received an average dose of 0.45, 0.9, 2.4, or 5.7 mg/kg-day of chromium VI+, while female mice received 0.3, 1.2, 3.2 or 8.8 mg/kg-day of chromium 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 hexavalent chromium 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 aforementioned LOAELs can be identified from these studies.

Carcinogenic Effects

In the studies of Borneff *et al.* (1968) and Zhang and Li (1987), exposures to hexavalent chromium in drinking water resulted in a statistically significant increase in tumors. However, estimation of a health-protective level for hexavalent chromium that necessitates consideration of dose-response relationships is problematic with the available data in these studies.

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. Two cancer bioassays, one in rats and one in mice, were identified in which animals administered hexavalent chromium in drinking water displayed statistically significant increases in tumors (NTP, 2007b). The increased incidence of intestinal tumors in mice was statistically significant compared to control in the two highest dose groups while statistically significant increases in oral tumors in the rat were observed only in the high dose group. Also, the incidence of intestinal tumors in the mouse was higher, indicating the mouse was the more sensitive species. Therefore the findings in the mouse were judged to be most appropriate for deriving an oral cancer slope factor for hexavalent chromium.

Dose-Response Modeling

Dose-response relationships were derived using U.S. EPA (1995b, 2000a) BMDS (Version 1.4.1). The lifetime time-weighted average dose was employed as the dose metric. Available models in the U.S. EPA BMDS program were fitted to combined incidence of adenoma and carcinomas of the duodenum or small intestine of male B6C3F₁ mice (NTP, 2007b). Similar analyses were performed using the combined incidence of adenomas and carcinomas of the small intestine of female B6C3F₁ mice. The models generated both the mean and lower-bound estimates of the dose (ED₁₀ and LED₁₀) associated with a ten percent increase in tumors. Acceptable fits ($p > 0.1$) are shown in Tables 9-11.

Male Mice

All eight models in the U.S. EPA BMDS yielded acceptable fits for duodenum tumors (Table 9) and tumors of the entire small intestine (Table 10). The models yielded a narrow range of lower confidence intervals of the dose associated with a 10 percent incidence of tumors (LED₁₀), ranging from 2.5 to 1.2 mg/kg-day for tumors in the duodenum and 2.0 to 0.8 mg/kg-day for tumors in the entire small intestine. As expected the LED₁₀ values were lower (higher potency) when tumors of the jejunum and ileum were combined with tumors in the duodenum.

Table 9. Cancer Potency Calculations for Combined Incidence of Adenomas and Carcinomas in the Duodenum of Male B6C3F₁ Mice (NTP, 2007b)

Model	Chi-square	P value	ED ₁₀ ^a (mg/kg-day)	LED ₁₀ ^b (mg/kg-day)
Gamma	1.35	0.51	2.4	1.7
Logistic	2.52	0.47	3.0	2.5
Log logistic	1.38	0.50	2.4	1.7
Multistage	1.52	0.68	2.5	1.8
Probit	2.09	0.55	2.8	2.3
Log probit	1.13	0.57	2.3	1.7
Quantal linear	6.02	0.11	1.6	1.2
Weibull	1.51	0.47	2.4	1.7
^a ED ₁₀ = maximum likelihood estimate of the dose producing a 10 percent extra risk of adenomas and adenocarcinomas in duodenum of male mice ^b LED ₁₀ = lower 95 percent confidence interval on the ED ₁₀				

Table 10. Cancer Potency Calculations for Combined Incidence of Adenomas and Carcinomas in the Small Intestine of Male B6C3F₁ Mice (NTP, 2007b)

Model	Chi-square	P value	ED ₁₀ ^a (mg/kg-day)	LED ₁₀ ^b (mg/kg-day)
Gamma	0.95	0.62	2.3	1.2
Logistic	0.83	0.84	2.4	2.0
Log logistic	0.94	0.62	2.3	1.3
Multistage	0.78	0.68	2.2	1.1
Probit	0.84	0.84	2.2	1.8
Log probit	0.92	0.63	2.4	1.5
Quantal linear	4.26	0.23	1.2	0.8
Weibull	0.95	0.62	2.3	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				

The mouse doses associated with a 10 percent increase in the incidence in tumors that were generated by the various models (Tables 9 and 10) are then scaled to a human equivalent dose based on the ratio of mouse to human body weight to the ³/₄ power (U.S. EPA, 2005b).

Using the LED₁₀ based on the standard multistage model (1.1 mg/kg-day) yields a human equivalent dose of:

$$1.1 \text{ mg/kg-day}_{\text{mouse}} * (0.035 \text{ kg}/70 \text{ kg})^{1/4} = 0.16 \text{ mg/kg-day}_{\text{human}}$$

Thus, 0.16 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

$$\text{Slope factor} = 0.1 / 0.16 \text{ mg/kg-day} = 0.6 \text{ (mg/kg-day)}^{-1}$$

Female Mice

Using all dose levels, none of the models in the BMDS yielded an acceptable fit (p>0.1) for combined incidence of adenomas and carcinomas of the intestine in female mice. There was no evidence of saturation, given the incidences of intestinal tumors in the two highest dose groups were well below 100 percent. When the high dose group was excluded, all but one of the models yielded acceptable fits (Table 11).

Table 11. Cancer Potency Calculations for Combined Incidence of Adenomas and Carcinomas in the Small Intestine of Female B6C3F₁ Mice (excluding the high dose, 8.8 mg/kg-day).

Model	Chi-square	P value	ED ₁₀ ^a (mg/kg-day)	LED ₁₀ ^b (mg/kg-day)
Gamma	0.00	0.95	1.50	0.87
Logistic	0.10	0.95	1.67	1.36
Log logistic	0.01	0.94	1.50	0.87
Multistage	0.01	0.91	1.52	0.86
Probit	0.08	0.96	1.53	1.24
Log probit	0.0	0.99	1.47	0.99
Quantal linear	3.33	0.19	0.87	0.61
Weibull	0.01	0.92	1.52	0.87
^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 LED₁₀ based on the standard multistage model (0.86 mg/kg-day) yields a human equivalent dose of:

$$0.86 \text{ mg/kg-day}_{\text{mouse}} * (0.035 \text{ kg}/70 \text{ kg})^{1/4} = 0.13 \text{ mg/kg-day}_{\text{human}}$$

Thus, 0.13 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

$$\text{Slope factor} = 0.1 / 0.13 \text{ mg/kg-day} = 0.8 \text{ (mg/kg-day)}^{-1}$$

Dose-response relationships were successfully derived for tumors of the small intestine in male mice and in female mice when the high dose group was excluded. The modeling yielded similar results in male and female mice. The results from male mice will be employed in the derivation of the PHG as the data used in the modeling was more robust (based on more data points).

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 hexavalent chromium, 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 hexavalent chromium 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 hexavalent chromium 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 hexavalent chromium. 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 hexavalent chromium. 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 hexavalent chromium for each age category and occupational exposure level in the chromium production workers. Cumulative exposures were lagged 5 years. Reported CrO₃ exposure values were multiplied by the ratio of formula weights, 52/100, to obtain hexavalent chromium 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 was used to derive cancer potency estimates for ambient hexavalent chromium 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:

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

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 $(\text{mg}/\text{m}^3\text{-years})^{-1}$, d represents the cumulative $(\text{mg}/\text{m}^3\text{-years})$ hexavalent chromium 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:

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

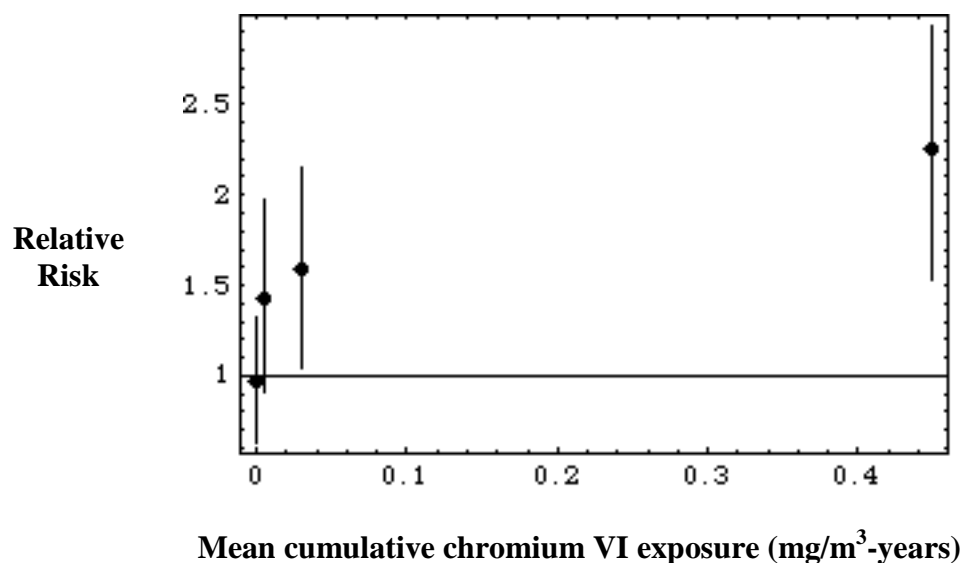
where *obs* is observed number of lung cancer cases and *EXP* is the expected number of lung cancer cases (i.e., using the relationship $EXP = obs/RR$).

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

Mean cumulative CrO 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 17 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 17. 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	CrO 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 (μg/m³)⁻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

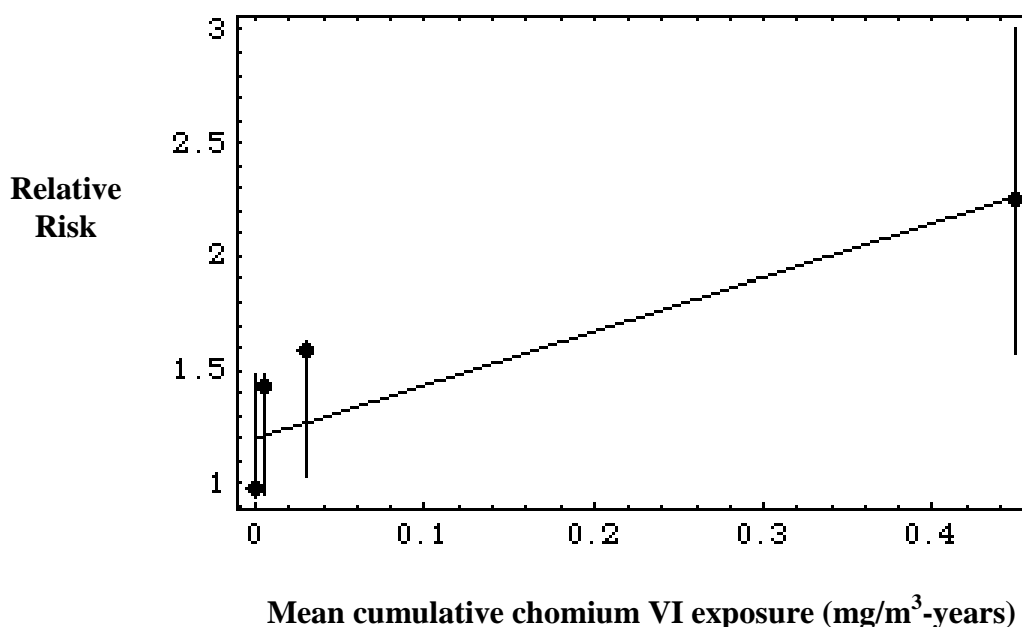
breathed in a day and the proportion of the year spent at work, an intermittency factor was then applied, described by the equation $(10 \text{ m}^3 / 20 \text{ m}^3) * (240 \text{ day} / 360 \text{ day}) = 0.33$, which was then divided into the aforementioned product.

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 18, 19, and 20 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 18) 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.

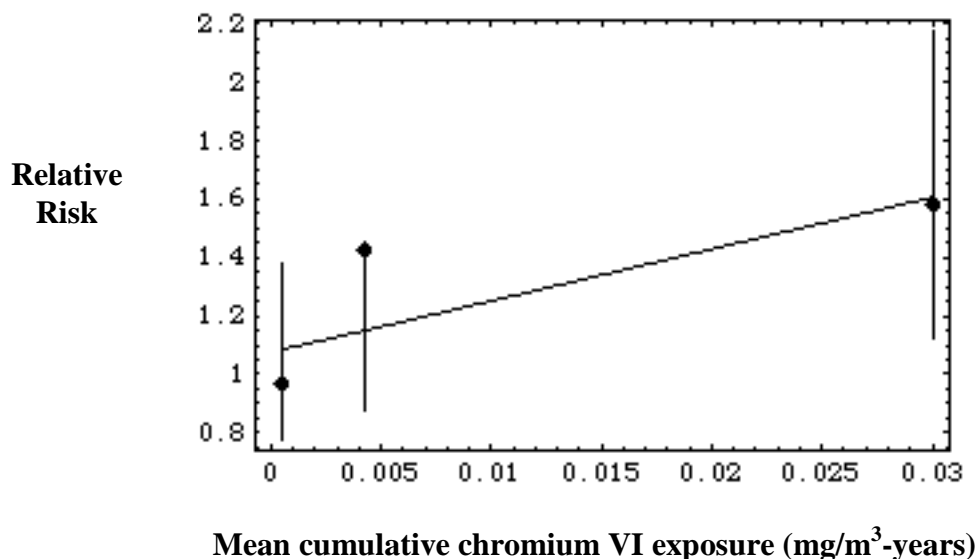
Figure 18. Complete Observed Dose-response Data with the Model-predicted Line and 95 Percent Pointwise Confidence Intervals



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 19). 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 19. 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 20) 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 20. Dose-response Data Excluding the Two Highest Dose Categories with the Model-predicted Line and 95 Percent Pointwise Confidence Intervals

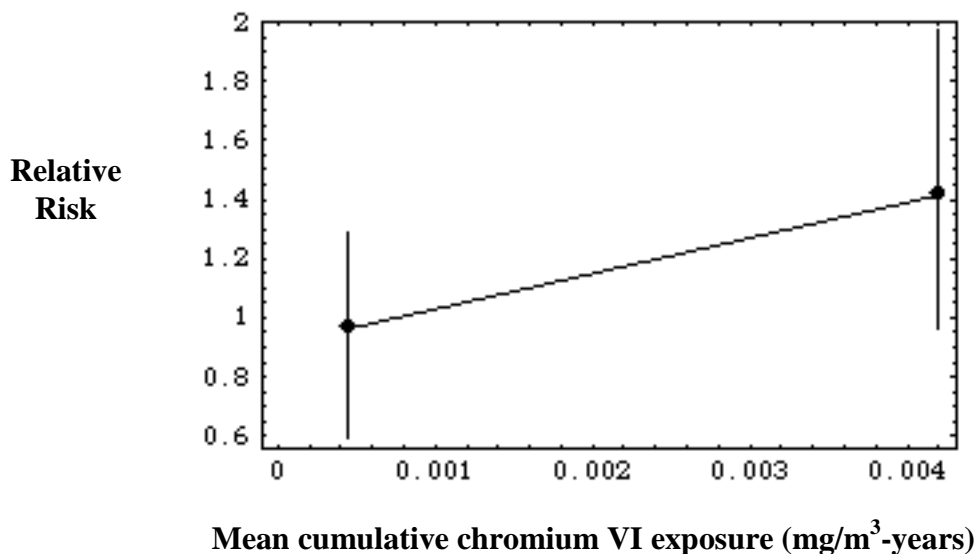


Table 14. Estimated Model Parameters for Gibb *et al.* (2000) Four Points, Analysis I

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 ([μ g/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

¹Estimates of β_1 were multiplied by 100/52 to obtain slope for hexavalent chromium exposures

²Conversion 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) ($[\text{mg}/\text{m}^3\text{-yr}]^{-1}$)	Deviance p-value	Potency ($[\mu\text{g}/\text{m}^3]^{-1}$)	
				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 the potency of hexavalent chromium by two methods, both of which used total chromium to represent hexavalent chromium. 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 for determining the slope of the exposure response. Details are provided in the U.S. EPA (1984b) report, 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 U.S. population in 1964 is 0.036.

Although previous potency estimates in Table 16 primarily used the data of Mancuso (1975), some results differed considerably from one another, depending mostly on the assumptions about how to scale the exposure measurements. The CDHS analyses assumed that hexavalent chromium is only 1/7 of total chromium. Hence those results produced 7-fold higher risks than those of U.S. EPA, which used total chromium to represent hexavalent chromium. The Crump (1995) analyses assumed that 43 percent of total chromium is hexavalent. 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 ($\mu\text{g}/\text{m}^3$)⁻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 hexavalent chromium. 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 $0.0018 \text{ (yr-}\mu\text{g Cr VI+}/\text{m}^3)^{-1}$, which translates to an MLE potency of $0.01 \text{ (}\mu\text{g Cr VI+}/\text{m}^3)^{-1}$. 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 $0.16 \text{ (}\mu\text{g Cr VI+}/\text{m}^3)^{-1}$ with a 95 percent UCL potency of $0.35 \text{ (}\mu\text{g}/\text{m}^3)^{-1}$ was based on the lowest three exposure levels. We judge that the unit risk from Gibb *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 hexavalent chromium in Mancuso (1997) apparently reduce some of the uncertainty about the Mancuso (1975) exposure to hexavalent chromium, 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 $0.35 \text{ (}\mu\text{g}/\text{m}^3)^{-1}$. 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 $2 \text{ (}\mu\text{g}/\text{m}^3)^{-1}$ provides the top of the range of potencies and the shallower slope furnishes the bottom of the range, $0.01 \text{ (}\mu\text{g}/\text{m}^3)^{-1}$. 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 hexavalent chromium obtained by the crude model, $0.15 \text{ (}\mu\text{g}/\text{m}^3)^{-1}$ or $510 \text{ (mg/kg-day)}^{-1}$ to be used for lifetime risk assessments (OEHHA, 1999c).

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 hexavalent chromium was administered to animals (NTP, 1997a; NTP, 2002; MacKenzie *et al.*, 1958; Chopra *et al.*, 1996; Acharya *et al.*, 2001, NTP, 2007a,b) will be employed to develop a health-protective level based on non-cancer health-based criteria for hexavalent chromium. For this purpose, health-protective doses (HPD) 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. 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 hexavalent chromium 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 hexavalent chromium 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 (2007a) study conducted for 90 days which is less than lifetime exposure, an uncertainty factor of 10 is employed to address the uncertainty associated

with the short duration of the study. For the chronic NTP (2007b) 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 hexavalent chromium there is also a question as to whether antacid consumption or gastrointestinal disease may result in marked increases in the absorption of hexavalent chromium from drinking water. Also individuals with liver disease may be particularly sensitive to the hepatotoxic effects of hexavalent chromium, 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 small number of animals aimed at investigating the reproductive toxicity of hexavalent chromium, doses of 1.1 to 29.3 mg/kg-day of hexavalent chromium 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 limitation (short duration, small number of animals per dose and limitations in the toxicological evaluation), uncertainty factors appropriate to provide an adequate margin of safety for human exposure to hexavalent chromium 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.

$$\text{HPD} = 1.1 \text{ mg/kg-day} / 3,000 = 0.00037 \text{ mg/kg-day}$$

Mackenzie et al., 1958 - In a study focused on investigating tissue levels of chromium following oral exposure to hexavalent chromium, doses of 0.0045 to 2.5 mg/kg-day of hexavalent chromium 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 uptake

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 in a companion study of cadmium toxicity (that used the same protocol) failed to observed toxicity at doses levels of cadmium 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 limitation (small number of animals per dose and limitations in the toxicological evaluation, uncertainty factors appropriate to provide an adequate margin of safety for human exposure to hexavalent chromium 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 chromium VI in the water). Thus the aggregate uncertainty factor is 1,000.

$$\text{HPD} = 2.5 \text{ mg/kg-day} / 1,000 = 0.0025 \text{ mg/kg-day}$$

Chopra et al., 1996 - Only one dose level was used in this 22-week study of hexavalent chromium administered to female rats in their drinking water. The LOAEL for chromium 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 hexavalent chromium 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 chrome VI levels in the water were monitored). The aggregate factor is limited to 3,000.

$$\text{HPD} = 1.4 \text{ mg/kg-day} / 3,000 = 0.00046 \text{ mg/kg-day}$$

Acharya et al., 2001 – Only one dose level was used in this 22-week study of hexavalent chromium administered to male rats in their drinking water. The LOAEL for chromium 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 hexavalent chromium 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 chrome VI levels in the water were monitored). The aggregate factor is limited to 3,000.

$$\text{HPD} = 1.1 \text{ mg/kg-day} / 3,000 = 0.00037 \text{ mg/kg-day}$$

NTP 2007a - Doses of 1.6 to 21.4 mg/kg-day of chromium 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 hexavalent chromium 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.

$$\text{HPD} = 1.6 \text{ mg/kg-day} / 3,000 = 0.00053 \text{ mg/kg-day}$$

NTP 2007b - Female rats received 0.2, 0.9, 2.6 or 7.0 mg/kg-day of chromium 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, fatty changes). The uncertainty factors appropriate to provide an adequate margin of safety for human exposure to hexavalent chromium 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.

$$\text{HPD} = 0.2 \text{ mg/kg-day} / 1,000 = 0.0002 \text{ mg/kg-day}$$

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

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

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 taken from OEHHA (2000) for adult men, women, and children; dermal exposures are negligible, while inhalation of aerosols in showering is addressed separately (see below).

The maximum default relative source contribution of 0.8 is used in this case, based upon the assumption that the major source of hexavalent chromium is likely to be from drinking water. Little or no hexavalent chromium exposure is expected from air, food, and incidental 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 concentrations for hexavalent chromium based on non-cancer toxic endpoints (Table 17). In five of the studies, toxic effects were detected (NTP 1997a; NTP, 2007a, 2007b; Chopra *et al.*, 1966; Acharya *et al.*, 2001) although a NOAEL was not identified in four of these studies (NTP, 2007a, 2007b; Chopra *et al.*, 1966; Acharya *et al.*, 2001). 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 2007b NTP study is clearly the best of the available studies for deriving a health-protective concentration. Therefore it is proposed that the health-protective level for non-carcinogenic effects be based on the LOAEL from this study.

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

Study	HPD (mg/kg-day)	Health Protective Concentration (mg/L) ^a		
		Child, 1-10 yr	Male	Female
NTP, 2007a	0.00053	0.00483	0.00782	0.00797
NTP, 2007b	0.0002	0.0018	0.0030	0.0030
NTP, 1997a	0.00033	0.00301	0.00487	0.00496
Mackenzie <i>et al.</i> , 1958	0.0025	0.0228	0.0369	0.113
Chopra <i>et al.</i> , 1966	0.0005	0.00456	0.00738	0.00752
Acharya <i>et al.</i> , 2001	0.00033	0.00301	0.00487	0.00496

^aUpper 95 percentile water intakes for a child, adult woman, and adult male are 0.088, 0.054, and 0.053 L/kg-day, respectively.

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 men, women and children 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 0.005 mg/L with a median value of 0.004 mg/L (NTP, 1997a; NTP, 2007a,b; Chopra *et al.*, 1966; Acharya *et al.*, 2001). The health

protective concentration of 0.002 mg/L based on the 2007b 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 hexavalent chromium addresses three routes of exposure: water ingestion, 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 hexavalent chromium 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:

$$C = \frac{R \times BW \text{ (kg)}}{(P_{\text{oral}} \text{ (mg/kg-day)}^{-1} \times L_{\text{ingest}}/\text{day}) + (P_{\text{inhal}} \text{ (mg/kg-day)}^{-1} \times L_{\text{inhal}}/\text{day})}$$

where:

- R = a default risk level of one in one million, or 10^{-6} ;
- BW = body weight (a default of 70 kg);
- P_{oral} = oral cancer potency;
- P_{inhal} = inhalation cancer potency;
- $L_{\text{ingest}}/\text{day}$ = daily amount of water ingested (2 L/day);
- $L_{\text{inhal}}/\text{day}$ = daily amount of water droplets inhaled (27×10^{-6} L/day) (Keating and McKone, 1993).

Estimates of the oral potency of hexavalent chromium were obtained from the results of an animal study (NTP, 2007b) because a study of human exposure to hexavalent chromium in drinking water (Zhang and Li, 1987), and our analysis of stomach tumors associated with occupational exposure to hexavalent chromium (see earlier section) were judged to be unsuitable for deriving a dose-response relationship for hexavalent chromium. 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 hexavalent chromium 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. 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 B6C3F₁ mice following hexavalent chromium administration in drinking water (NTP 2007b). The findings in male mice were judged to yield the best dose-response relationship for oral exposure to hexavalent chromium and therefore are the basis of the oral slope factor. For the oral and inhalation

route, the risk can be calculated as follows, using the human cancer potency value of 0.6 (mg/kg-day)⁻¹ for the oral route and 510 (mg/kg-day)⁻¹ for inhalation as derived above in the dose response assessment section:

$$C = \frac{10^{-6} \times 70 \text{ kg}}{(0.6 \text{ (mg/kg-day)}^{-1} \times 2 \text{ L/day} + (510 \text{ (mg/kg-day)}^{-1} \times 27 \times 10^{-6} \text{ L/day})}$$

$$= 0.00006 \text{ mg/L or } 0.06 \text{ } \mu\text{g/L or ppb}$$

The proposed PHG for chromium VI+ is therefore set at 6 x 10⁻⁵ mg/L, or 0.06 ppb, representing a lifetime cancer risk of 1 in 1 million. Other toxic effects associated with chromium VI were observed at higher exposure levels. The PHG for carcinogenic effects is protective against these other toxic effects.

RISK CHARACTERIZATION

The proposed PHG for hexavalent chromium of 0.06 μg/L is based on risk associated with the ingestion of drinking water and 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 to hexavalent chromium have resulted in increased incidences of lung cancer, studies needed to characterize toxic effects associated with the oral exposure route are limited. Only one epidemiological study was identified that specifically addressed human exposure to hexavalent chromium in drinking water (Zhang and Li, 1987). Only two carcinogen bioassays were identified where animals were chronically exposed to hexavalent chromium in drinking water (Borneff *et al.*, 1968; NTP, 2007b). OEHHA's analysis of finding 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 bioassay (NTP, 2007b) revealed statistically significant dose related increase 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, hexavalent chromium has been shown to damage DNA. The finding of genotoxicity in the liver following oral administration of hexavalent chromium 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 reason for concern regarding the carcinogenic potential of this toxicant in humans.

The NTP studies in which hexavalent chromium was administered to rodents in the feed suggest that liver and blood-forming tissues may also be affected by hexavalent chromium (NTP, 1996, 1997a,b, 2007a). Three studies in male and female rats given hexavalent chromium orally for 22 weeks or two years suggest that the liver is a target organ (Acharya *et al.*, 2001; Chopra *et al.*, 1996; NTP 2007b). These studies appear to indicate that hexavalent chromium 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 hexavalent chromium. However, in one early study, no toxicity was reported in rats administered hexavalent chromium 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 hexavalent chromium 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 hexavalent chromium. The findings of the NTP 2007b study provided sufficient information for developing a dose-response relationship for hexavalent chromium. Statistically significant increases in tumors were observed in the small intestine of male and female mice. Acceptable fits to models in the BMDS were obtained using all dose groups in the male but not female mouse. Thus the findings in male mice were judged to be the most suitable for developing a dose-response relationship for hexavalent chromium.

Inhalation exposure - A dose-response relationship was derived from an occupational exposure to hexavalent chromium, 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 2007b 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 – An upper-bound estimate of drinking water consumption for children was employed to develop the health-based criterion for oral exposure to hexavalent chromium in drinking water. The proposed 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. This route of exposure contributed very little to the total exposure to hexavalent chromium in drinking water.

Risk Characterization - The many sources of uncertainty are reflected by the large combined uncertainty factor used in the calculation of the proposed PHG. When and if better studies of hexavalent chromium toxicity, dose-response, and exposure become available, the uncertainties associated with the risk assessment can be reduced. With the presently available information, the risk associated with exposure to hexavalent chromium may have been under- or overestimated. Protection of public health requires that health-based criteria be developed in a manner to ensure that risk is not markedly underestimated. OEHHA requests comment on the extent to which the proposed PHG level of 0.06 ppb provides adequate health protection to meet the goal of protecting California consumers of drinking water, including potential sensitive subpopulations, against the potential adverse effects of hexavalent chromium.

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 3×10^{-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 chromium III and chromium 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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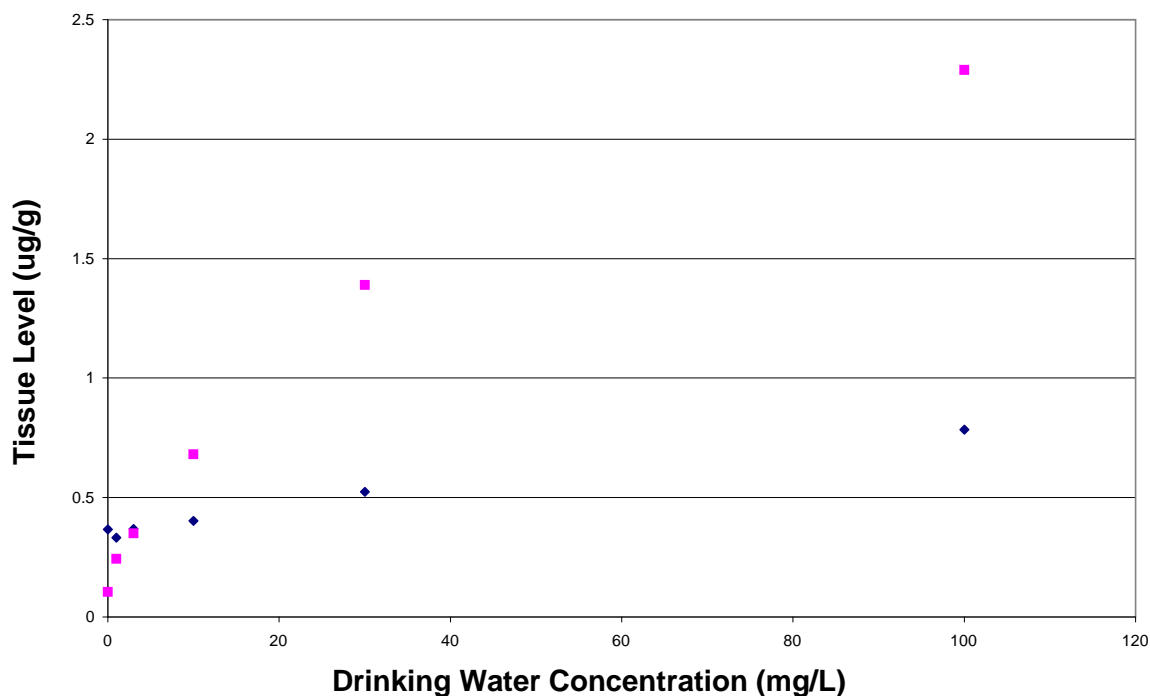
APPENDIX A

Carcinogenic Threshold?

Because hexavalent chromium is rapidly converted to the trivalent form in the GI tract, several investigators have asserted that negligible amounts of hexavalent chromium are orally absorbed (because it would all be rapidly and completely reduced to trivalent chromium (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 hexavalent chromium to trivalent chromium at levels that ranged from 3 to 10 mg/L (Proctor *et al.*, 2002a).

In studies in humans where the oral administration of hexavalent chromium 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 trivalent chromium, were also observed in a study where hexavalent chromium was administered in an acidic vehicle (orange juice) (Kerger, 1996). Other studies by Kerger and associates indicated a rapid and essentially complete reduction of hexavalent chromium to trivalent chromium (*in vitro*) when added to orange juice (Kerger, 1996). Thus while considerable amounts of chromium are reduced to trivalent chromium 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.

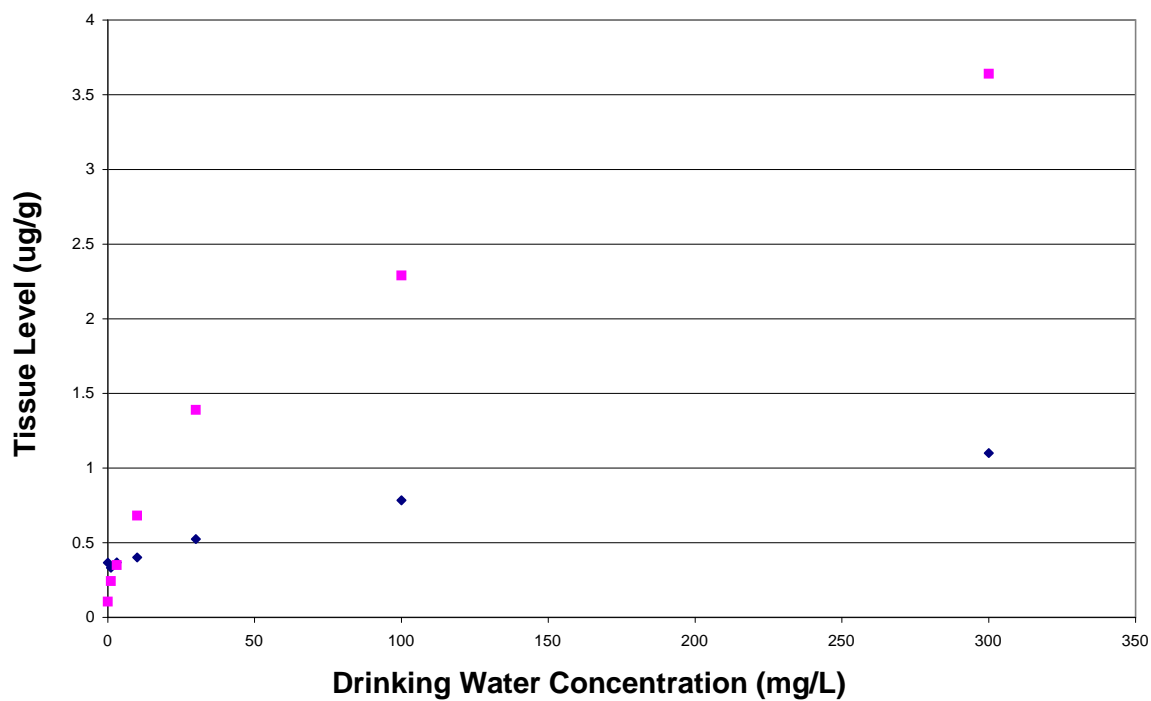
Studies in animals also do not indicate that the absorption of hexavalent chromium was a consequence of the exhaustion of the capacity of the GI tract to reduce Cr VI to Cr III. Chromium blood and kidney levels were determined in male B6C3F₁ mice administered hexavalent chromium in drinking water for 21 days (NTP 2007a). 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, hexavalent chromium was administered in drinking water to female B6C3F₁ mice for 6 to 371 days (NTP 2007b). 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 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 hexavalent chromium 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

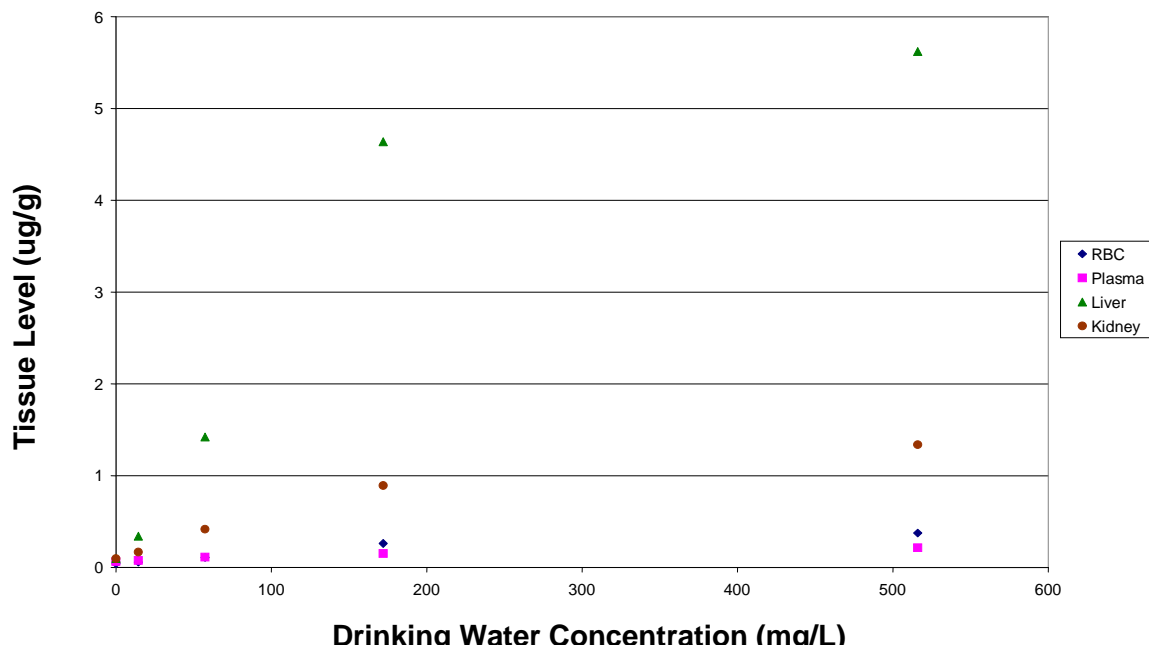


Figure A4. Chromium Tissue Levels on Day 13 in Female Mouse

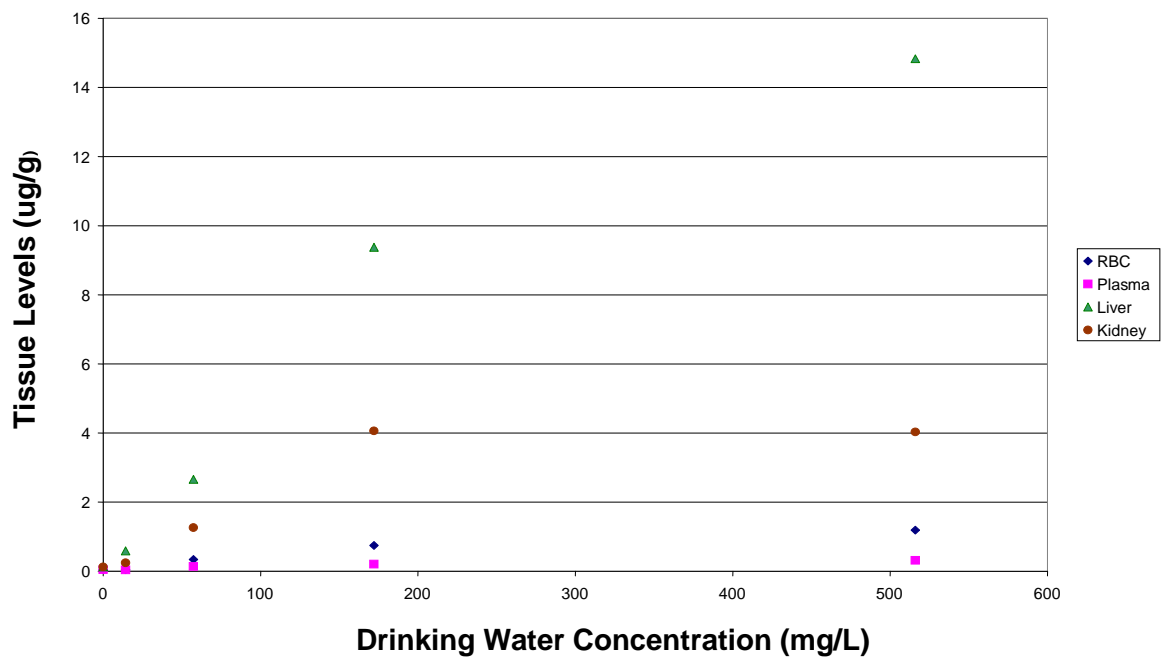


Figure A5. Chromium Tissue Levels on Day 18

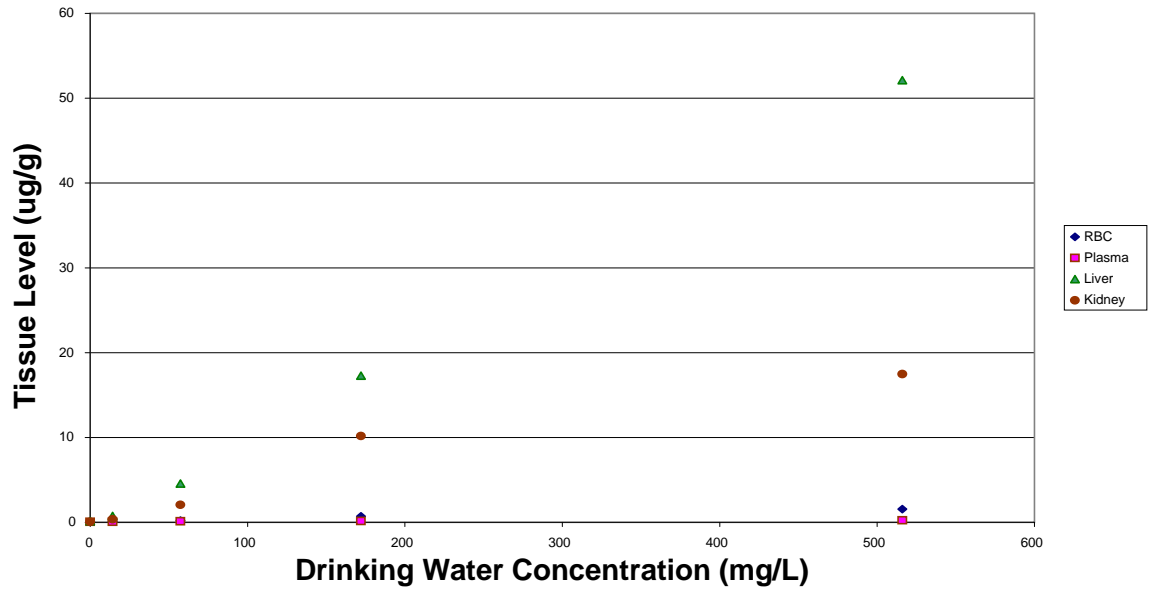
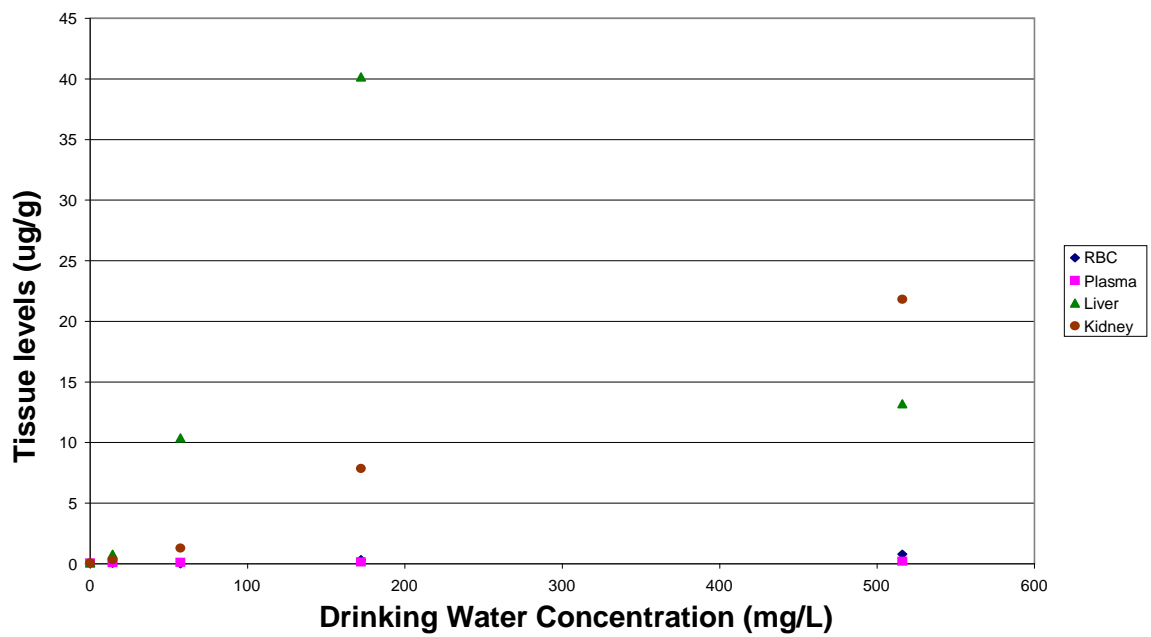


Figure A6. Chromium Tissue Levels on Day 371



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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 K₂CrO₄ 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 K₂CrO₄ in drinking water were included in the study. Animals were mated six weeks after the start of treatment. Two mice from each litter were selected as the first generation (F₁) 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 K₂CrO₄, or benzo[a]pyrene or benzo[a]pyrene + 500 ppm K₂CrO₄] in their drinking water as did the parent (F₀) 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 (F₁) mice were mated after the mousepox epidemic had ended. The numbers of offspring from the mating of F₁ mice were much less than after the breeding of the F₀ animals. The F₂ generation mice received the same food and concentration of test substance [0 or 500 ppm K₂CrO₄, or benzo[a]pyrene or benzo[a]pyrene + 500 ppm K₂CrO₄] in their drinking water as did the F₀ and F₁ generations. The F₂ 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, F₂ 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 stomach were observed in female mice exposed to K₂CrO₄. No malignant stomach tumors were found in control mice. Nine benign stomach tumors were observed in female mice exposed to K₂CrO₄. 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 F₀ generation, 1 tumor in the F₁ generation and 2 tumors in the F₂ 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 stomach tumors (11/66) in K₂CrO₄-exposed-female mice (all three generations combined) was significantly different than the combined incidence of tumors in control female mice (2/79) [Fishers Exact test, p<0.05, (OEHHA analysis)]. Analysis of tumor incidence by generation finds that in F₀ animals, 22 percent of K₂CrO₄-exposed mice had stomach

tumors compared to 3.6 percent of controls. In the F₁ and F₂ animals, tumor incidence was similar to controls.

Borneff and coworkers suggest that the mousepox epidemic may have delayed tumor growth in the F₁ 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 F₁ generation, tumor growth had already begun in F₀ mice at the time that the mousepox epidemic occurred (experimental month eight). The F₂ generation was not exposed to mousepox virus; however, they were vaccinated and this could have affected tumor development

Borneff and coworkers calculated that the K₂CrO₄-exposed mice who developed stomach 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 F₂ 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 were 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 hexavalent chromium in drinking water for their lifetime and the drinking water solution containing K₂CrO₄ 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 stomach tumors that occurred in female animals administered hexavalent chromium compared to the vehicle/negative control group would appear to be due to the administration of hexavalent chromium. 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 F₀ generation. (3) Only one dose level of hexavalent chromium 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 F₀ 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 F₁ generation resulting from the mousepox epidemic and the shorter lifespans of the F₁ and F₂ 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 hexavalent chromium in the study. Only one dose level of hexavalent chromium 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 K₂CrO₄ treatment.

The level of hexavalent chromium 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 states 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) states that the high dose is one that produces some toxicity during the course of the study. Regarding lower doses IARC (1980) states: “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 states that “an adequate high dose would generally be one that produces some toxic effects without unduly affecting mortality from effects other than cancer or producing significant adverse effects on the nutrition and health of the test animals.” It further states 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 hexavalent chromium appears to be reduced to trivalent chromium 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 (Browning *et al.*, 1983; Browning *et al.*, 1984; Kunstyr *et al.*, 1976; Ward *et al.*, 1986). Browning *et al.* (1984) reported that forestomach pH in male rats was 4.3 ± 0.1 except in starving animals where it was much more acidic ($\text{pH } 2.3 \pm 0.5$). Similarly 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.

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, *Helicobacter pylori* infection) and due to medications (e.g., proton pump inhibitors, histamine receptor blockers). Infant’s stomachs are also neutral pH during the first days to weeks after birth. A more detailed discussion can be found in the sensitive population section of the PHG.

5) Lack of preneoplastic lesions. If oral exposure to hexavalent chromium 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 hexavalent chromium 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.”

For hexavalent chromium, perinatal exposure would not be expected to make much of a difference because of the reducing ability of the dam’s stomach, blood and the placenta. Little hexavalent chromium would be expected to get to the conceptus because of all the reduction in the intervening maternal organs. In addition, the pups were fed on dam’s milk and were not directly consuming drinking water, so the pups were not likely to be receiving much exposure to hexavalent chromium until weaned. Since the additional perinatal exposure period would not be expected to result in a significant increase in exposure of the offspring, a detectable increased incidence of stomach tumors is unlikely.

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 F₀ generation that survived the mousepox virus received a greater cumulative dose of hexavalent chromium because they lived the longest, which perhaps explains the occurrence of tumors primarily in the F₀ 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 F₀ generation, while no significant increases were observed in the F₁ and F₂ generations. Why the increase was only detected in the F₀ generation is unclear. OEHHA hypothesizes that this effect may have occurred because of the presence of *helicobacter* in the stomach of the F₀ 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 *helicobacter* 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 hexavalent chromium may have prevented the transmission of this agent to the F₁ and F₂ generations, thereby accounting for the lack of tumors in the F₁ and F₂ generation (the newborn stomach is characterized by lower acidity which may have substantially reduced the conversion of chromium VI to chromium 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 *Helicobacter 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 hexavalent chromium, 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 hexavalent chromium (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 administering 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 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. *Helicobacter pylori* 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 hexavalent chromium 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 hexavalent chromium 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 hexavalent chromium 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). A statistically significant increase in the incidence of tumors of the forestomach was observed in female mice exposed to 500 ppm of potassium dichromate in drinking water. The study employed a multigenerational design where the F₀ generation was exposed to hexavalent chromium in drinking water. The age of mice in the F₀ generation when the dosing commenced was not reported, but the average weight of mice was reported as 21.9 g when the dosing began. This indicates that the mice were around 4.5 weeks old (M & B Laboratory, 2001). The F₀ generation was bred six weeks after the start of the study and the F₁ generation was bred after approximately 11 months. Three weeks after birth, the offspring were separated (weaned) and were administered food and water containing hexavalent chromium.

The stomach tumors in this study were found almost exclusively in the F₀ generation. This generation was characterized by a slightly later onset of exposure, a slightly longer duration of exposure than the F₁ generation, and a significantly longer duration of exposure than the F₂ generation. While tumor incidence was markedly increased only in the F₀ generation, exposure duration was markedly shorter only in the F₂ generation and not in the F₁ generation. Thus, differences in the duration of exposure do not appear to explain why tumors occurred primarily in the F₀ generation.

We postulate that an earlier exposure of mice to hexavalent chromium in the F₁ and F₂ generations (which occurred following weaning) may have “prevented” tumors in these generations. This finding could have resulted from a combined exposure to hexavalent chromium and a *Helicobacter* infection, analogous to the studies in which MNNG or MNU was administered to Mongolian gerbils.

Mice in the F₀ generation infected with *Helicobacter* and exposed to hexavalent chromium developed stomach tumors. The lack of tumors in subsequent generations in the Borneff study may simply reflect the elimination of *Helicobacter* from the stomach at an early age by the high concentration of hexavalent chromium in their drinking water. Mutagenicity tests have revealed that hexavalent chromium 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 trivalent chromium in the stomach at the time of weaning in the Borneff *et al.* (1968) study may have been reduced. Higher hexavalent chromium levels in the stomach may have prevented colonization or eliminated *Helicobacter* from the stomachs of the mice in the F₁ and F₂ generations. The elimination of a *Helicobacter* infection from the stomach 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 F₀ generation may have been refractory to the bactericidal effects of hexavalent chromium 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 F₁ and F₂ 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 F₀ and F₁ 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 stomach tumors observed in the Borneff *et al.* (1968), and Zhang and Li (1987) studies 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 trivalent chromium 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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