PUBLIC HEALTH GOALS FOR CHEMICALS IN DRINKING WATER

HEXAVALENT CHROMIUM (Cr VI)

July 2011

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

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PREFACE

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

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

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

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

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

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

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

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PUBLIC HEALTH GOAL FOR HEXAVALENT CHROMIUM IN DRINKING WATER

SUMMARY

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

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

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

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

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

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

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

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

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

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

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

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

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

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

INTRODUCTION

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

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

CHEMICAL PROFILE

Chemical Identity

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

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

Physical and Chemical Properties

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

Production and Uses

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

Sources

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

ENVIRONMENTAL OCCURRENCE AND HUMAN EXPOSURE

Air

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

Soil

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

Water

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

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

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

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

Food

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

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

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

Other Exposure Sources

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

EXPOSURE ASSESSMENT

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

Inhalation Route

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

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

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

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

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

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

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

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

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

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

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

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

$$Mrl = ML \times Vra$$

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

$$= 27 \text{ mg of water that is inhaled, or } 27\text{x}10^{-6} \text{ L}$$

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

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

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

The PHG for Cr VI will address both the inhalation and ingestion routes of exposure to water using the estimate of $3.86\text{x}10^{-7}$ L/kg-day exposure to inhaled water droplets in the shower (the reason for the preceding calculations) and an estimate of ingested tap water.

Dermal route

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

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

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

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

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

Comparison of the dermal to ingestion dose of Cr VI

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

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

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

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

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

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

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

METABOLISM AND PHARMACOKINETICS

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

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

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

Hexavalent Chromium Reduction by Saliva and Gastric Fluids

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

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

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

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

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

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

Absorption

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

<u>Urinary recovery</u>

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

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

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

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

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

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

<u>Blood/plasma and tissue levels of chromium</u>

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

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

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

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

Distribution

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Elimination

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

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

Pharmacokinetics of Trivalent versus Hexavalent Chromium

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

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

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

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

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

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

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

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

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

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

Figure 1. Toxicokinetic Model - Hexavalent Chromium

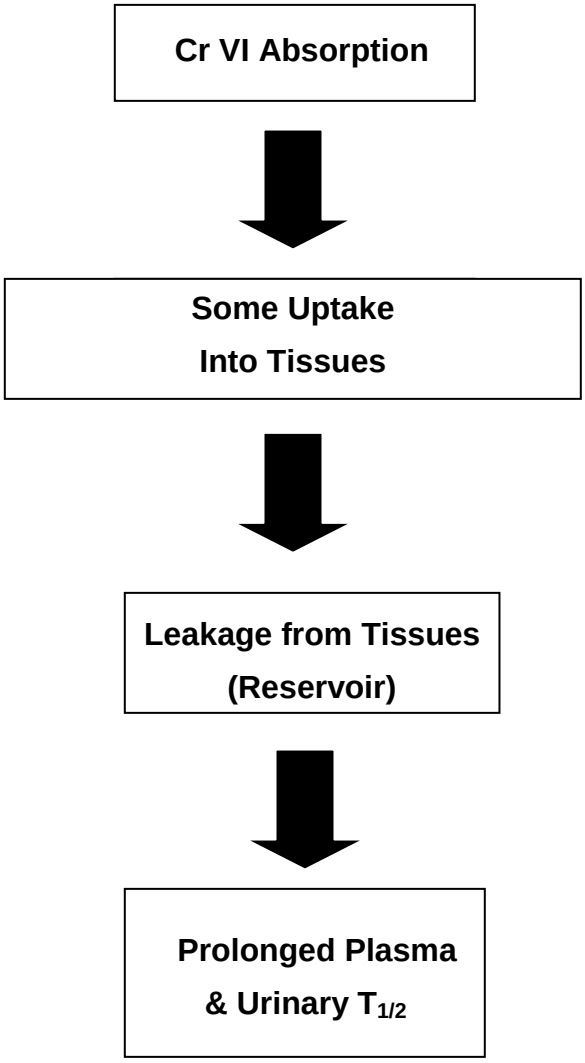
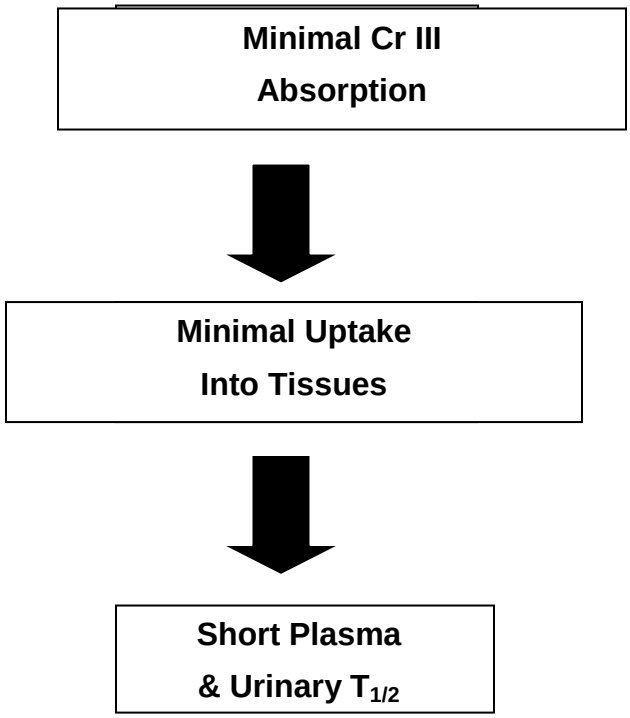


Figure 2. Toxicokinetic Model - Trivalent Chromium



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

Variability of the Human Toxicokinetics of Chromium

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

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

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

TOXICOLOGY

Toxicological Effects in Animals

Acute Toxicity

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

Developmental and Reproductive Toxicity

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Immunotoxicity

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

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

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

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

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

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

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

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

Subchronic Toxicity

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

NTP mouse and rat studies (1996 and 1997a)

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

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

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

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

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

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

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

Chopra et al., 1996

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

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

Acharya et al., 2001

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

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

NTP, 1997b

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

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

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

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

Vyskocil et al., 1993

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

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

NTP, 2007

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

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

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

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

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

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

Chronic Toxicity

MacKenzie et al., 1958

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

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

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

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

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

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

NTP, 2008

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

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

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

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

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

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

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

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

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

Strengths and weakness of subchronic and chronic animal studies

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

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

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

Table 1. Strengths and Weaknesses of Available Hexavalent Chromium Bioassays

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

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

Genetic Toxicity

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

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

Inhalation, intratracheal, intraperitoneal and intravenous exposures

Human

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

Animal

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

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

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

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

Oral exposures

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

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

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

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

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

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

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

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

Summary

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

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

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

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

Mechanism of Genotoxicity and Carcinogenicity

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

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

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

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

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

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

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

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

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

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

Carcinogenicity

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

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

Borneff *et al.*, 1968

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

NTP, 2008

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

Rat

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

Neoplasms

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

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

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

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

Figure 3. Survival curves for female rats

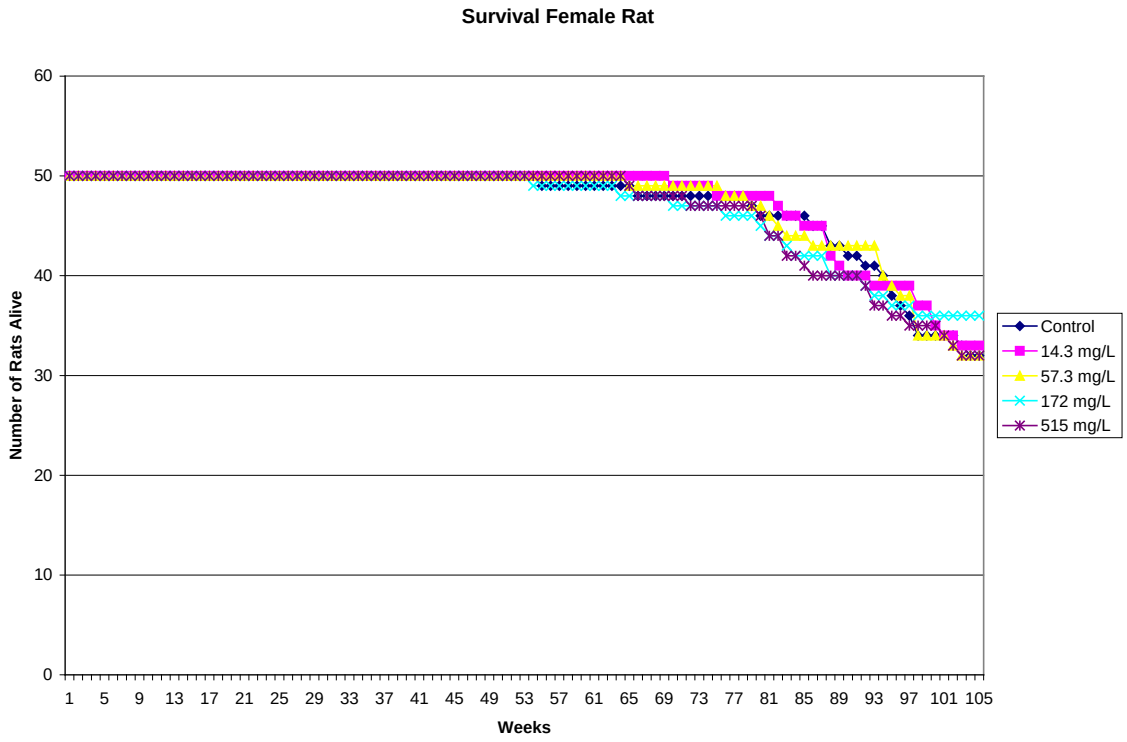


Figure 4. Survival curves for male rats

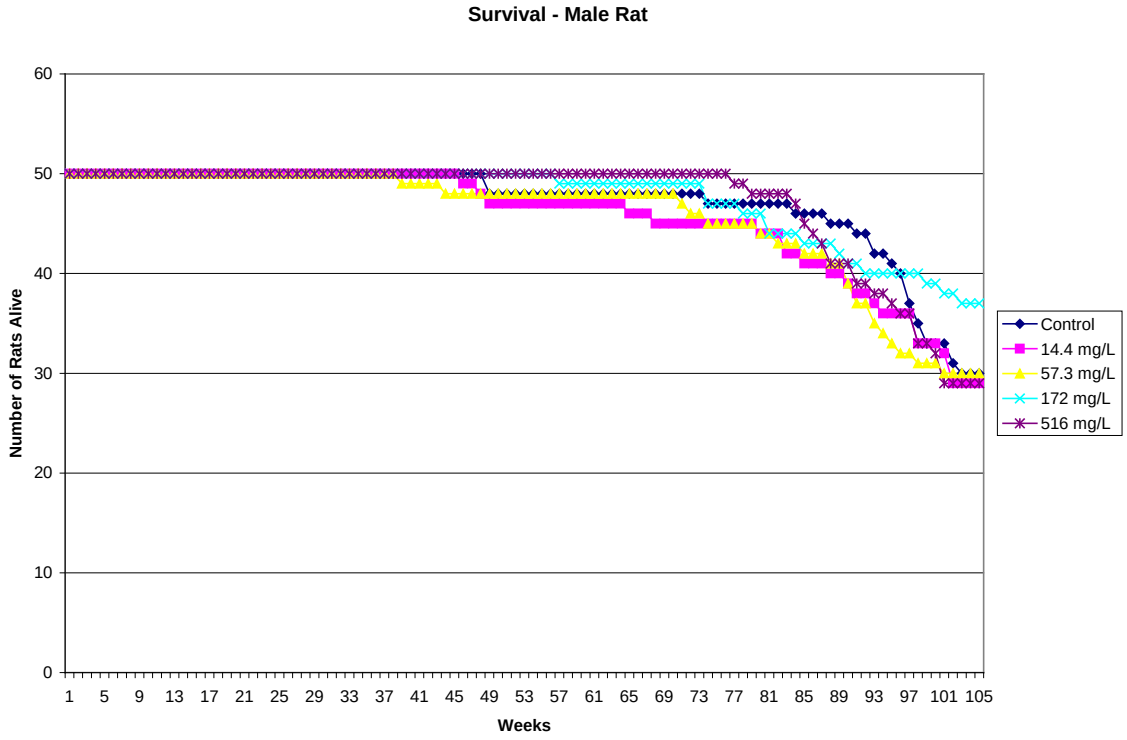


Figure 5. Female rats body weights, by week

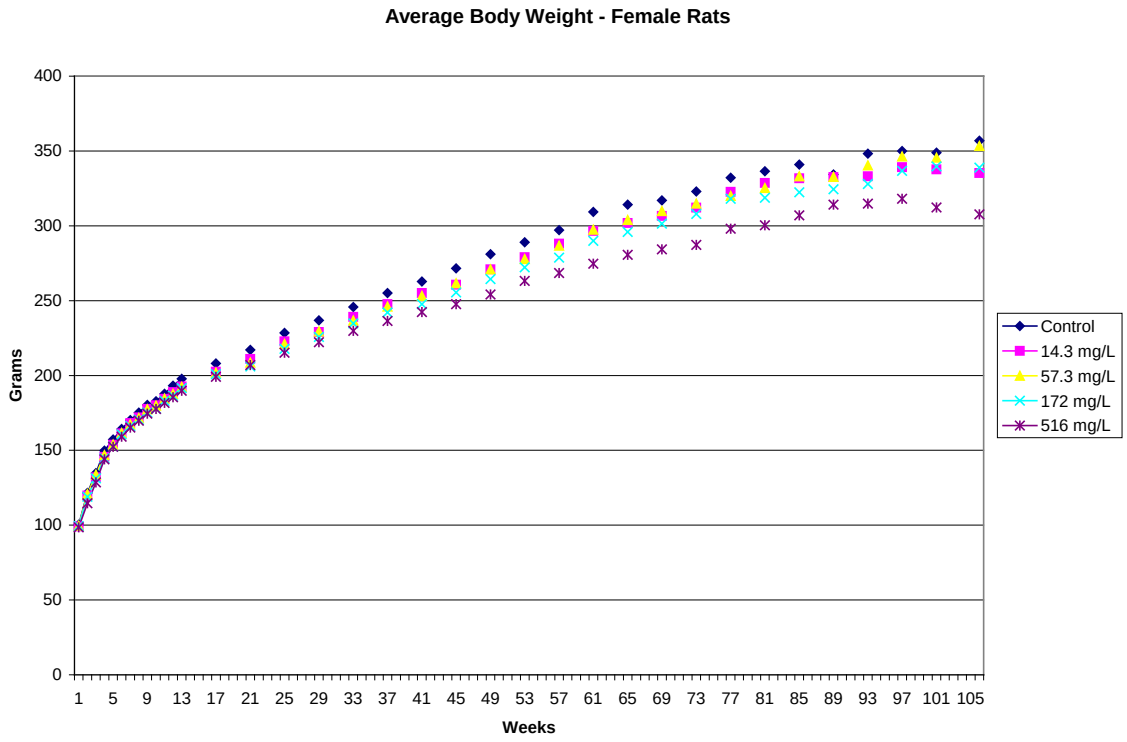
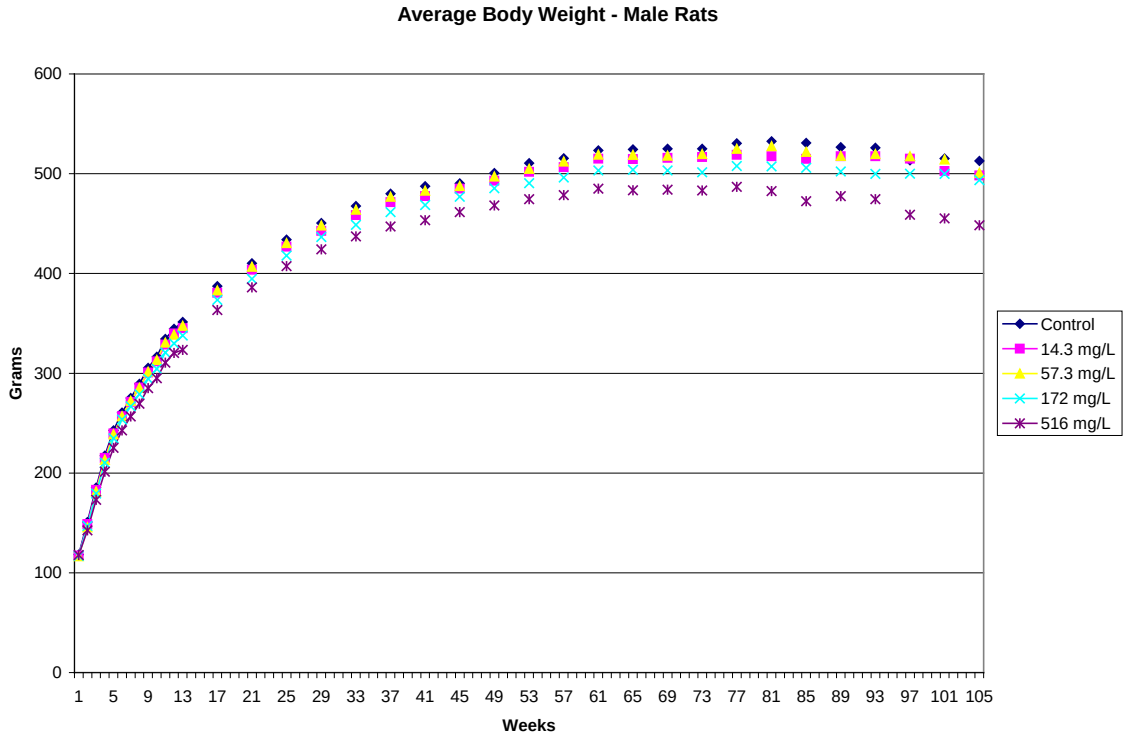


Figure 6. Male rat body weights, by week



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

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

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

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

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

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

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

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

Mouse

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

Neoplasms

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

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

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

Figure 7. Survival curves for female mice

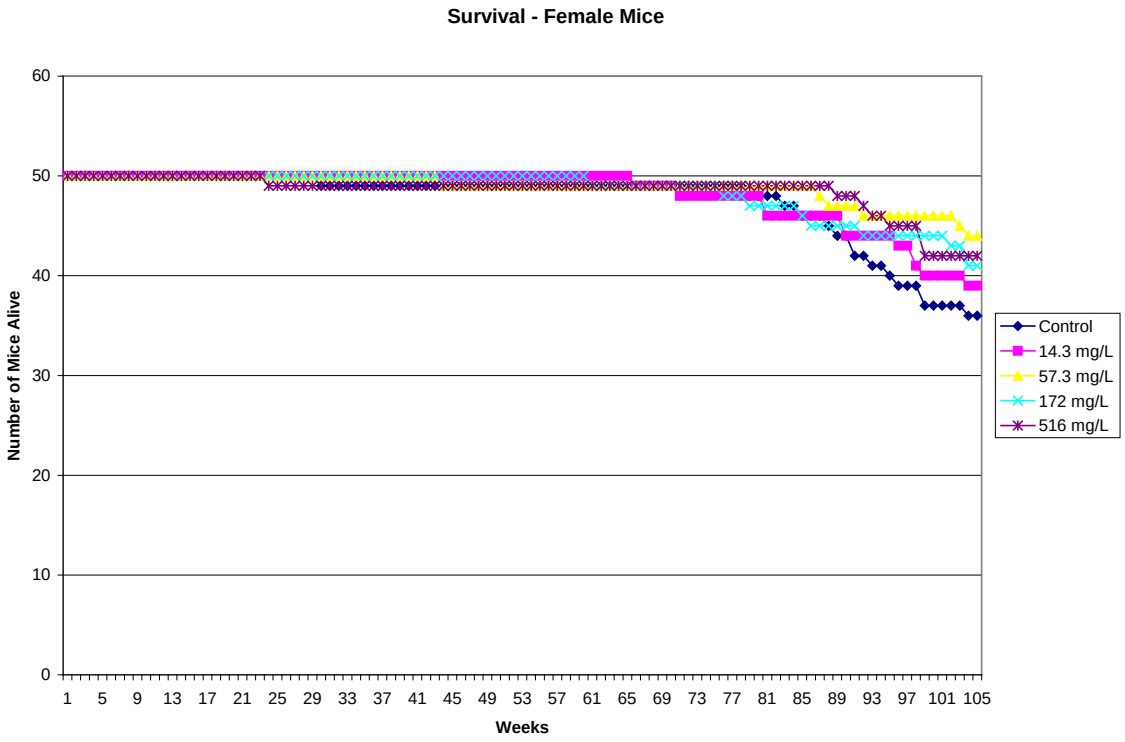


Figure 8. Survival curves for male mice

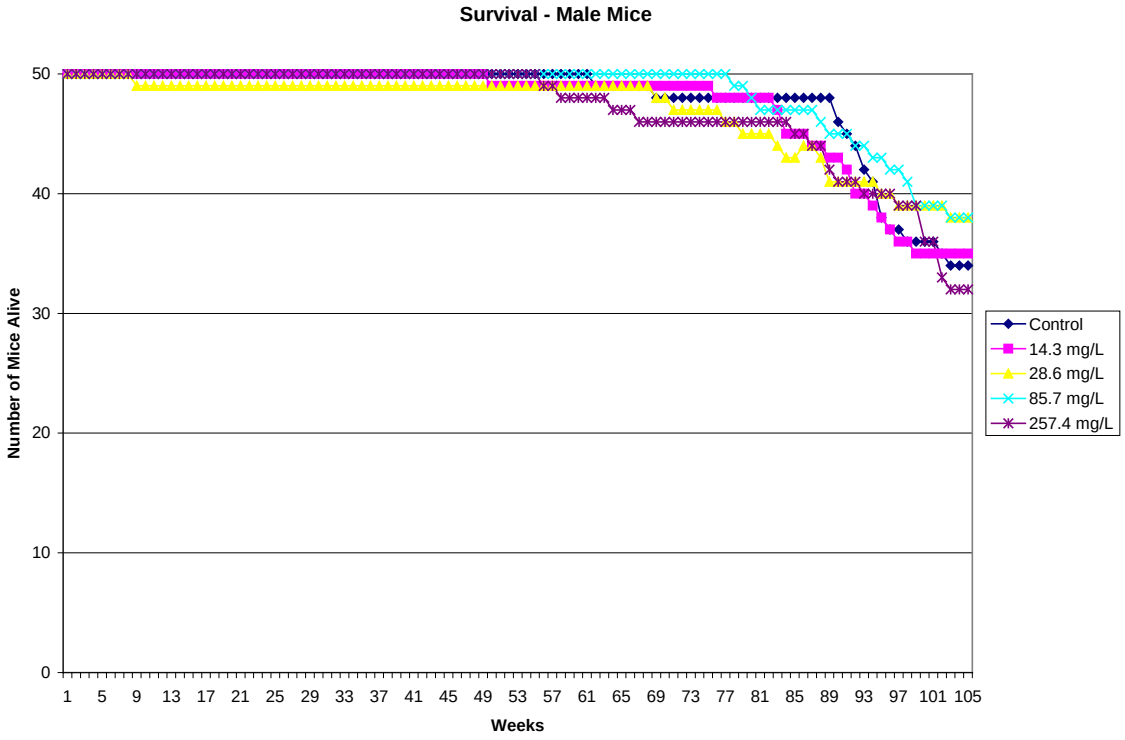


Figure 9. Male mouse body weights, by week

Figure 10. Female mouse body weights, by week

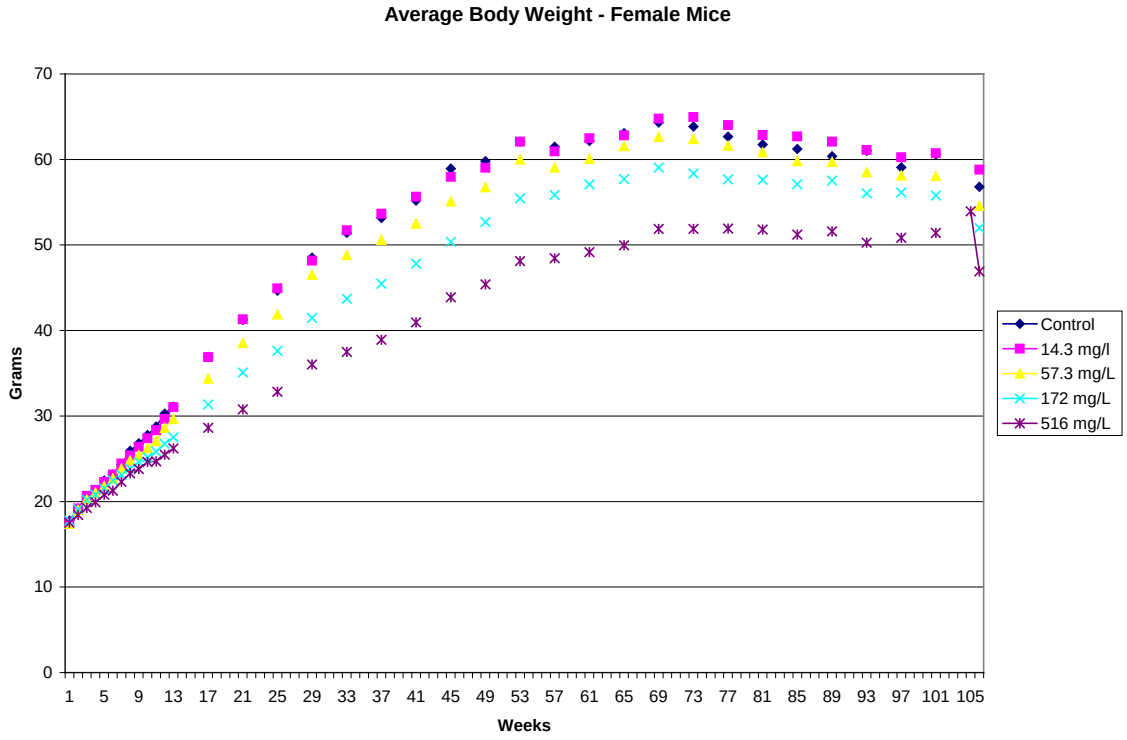


Figure 11. Body weights of male mice, compared to control

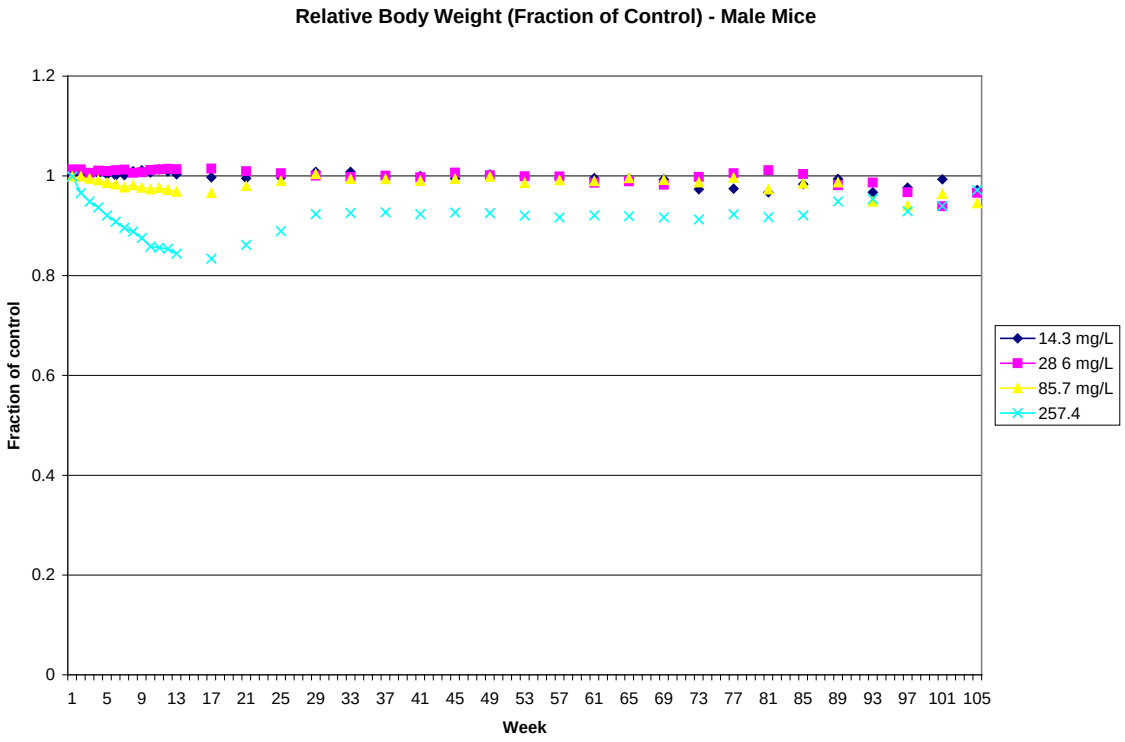


Figure 12. Body weights of female mice, compared to control

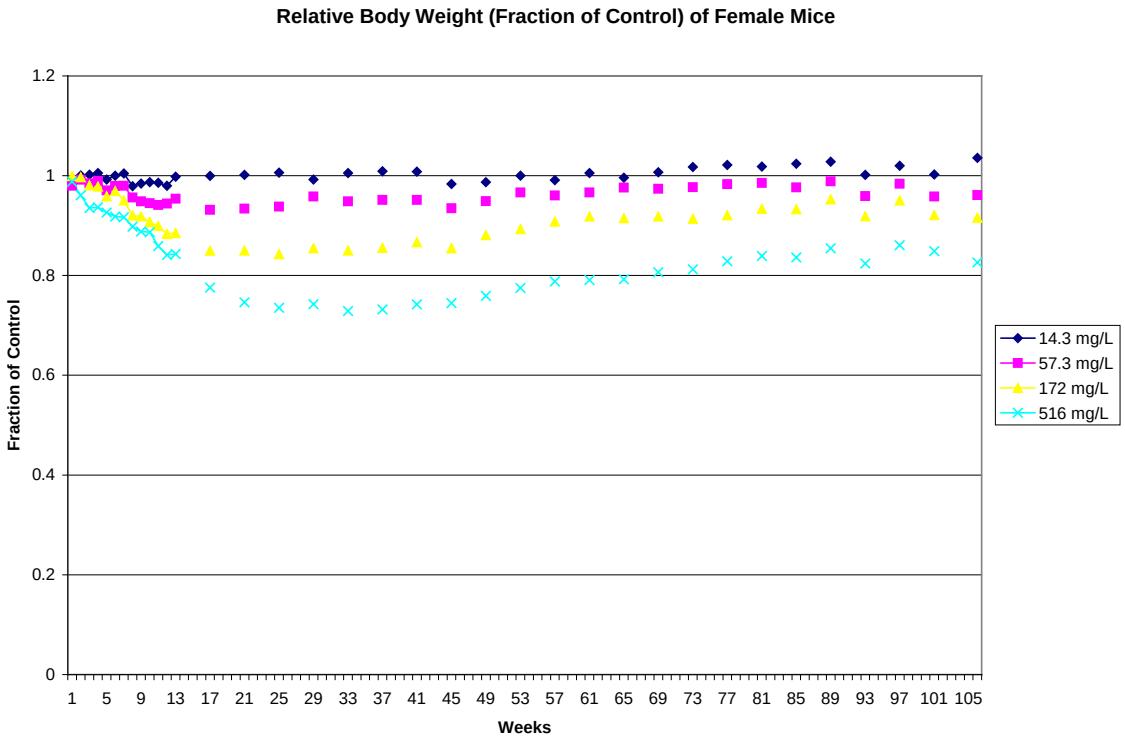


Table 5. Small Intestine Tumors in Male Mice Administered Hexavalent Chromium.

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

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

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

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

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

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

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

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

Maximum Tolerated Dose - Rats

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

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

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

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

Maximum Tolerated Dose - Mice

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

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

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

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

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

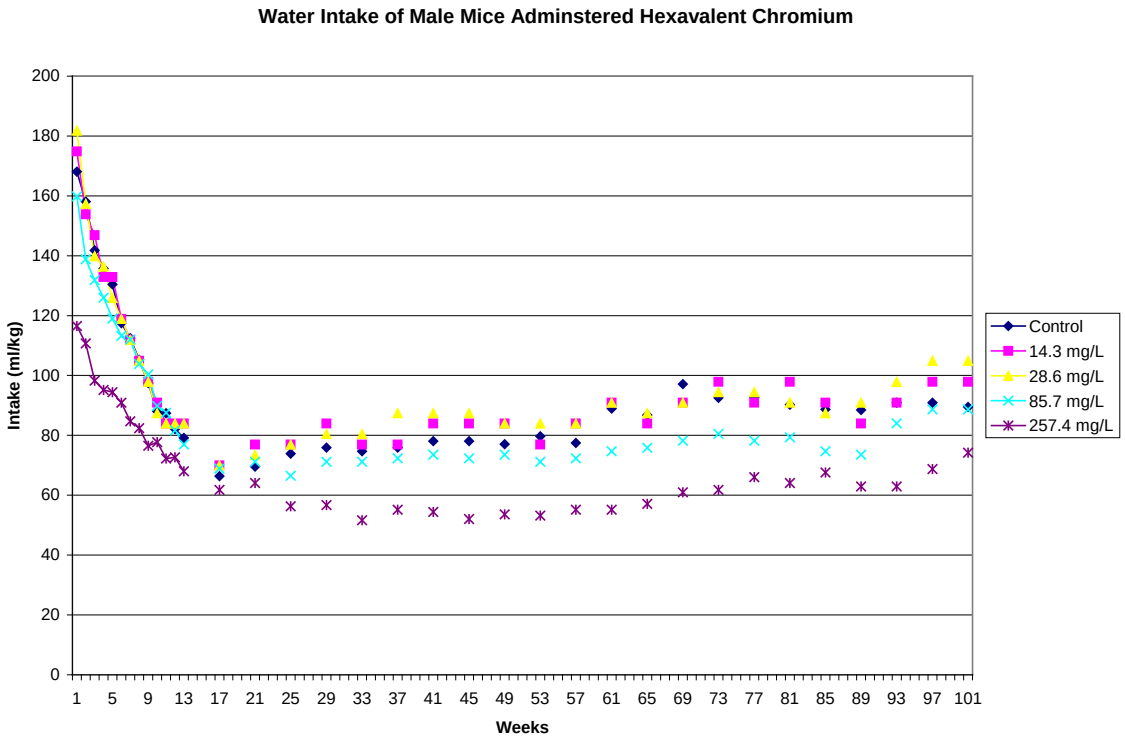
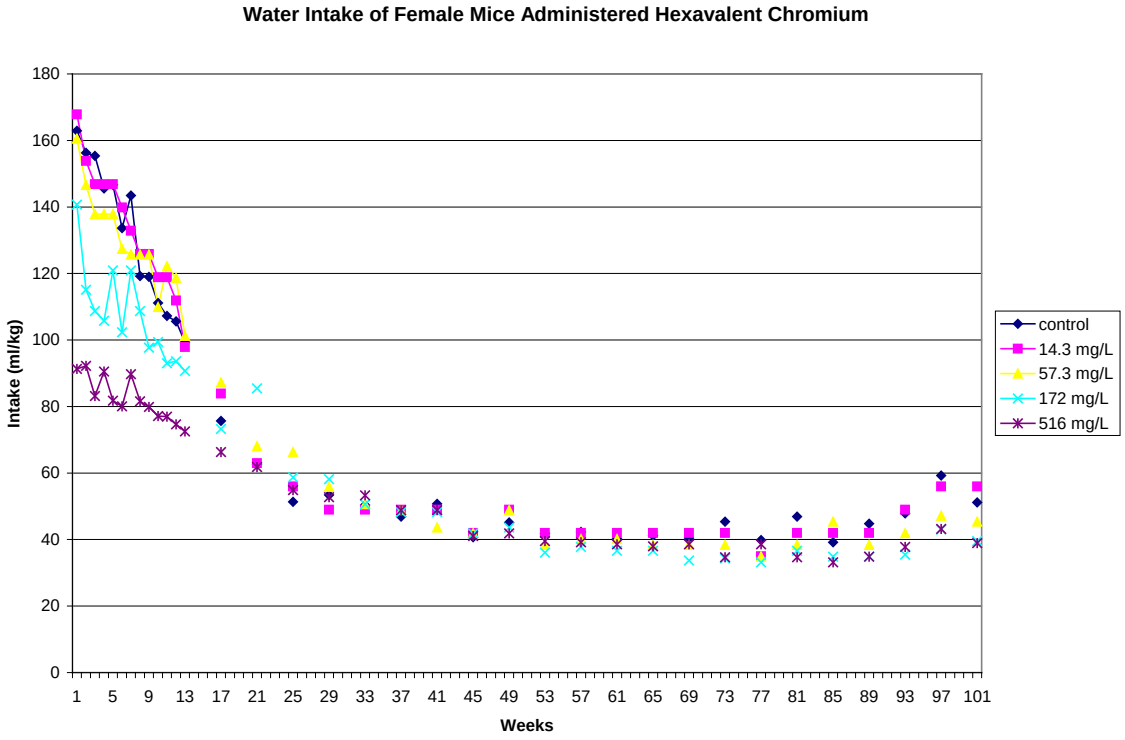


Figure 14. Effect of hexavalent chromium on water intake in female mice, by week.



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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Non-Oral routes/ Unorthodox Protocol

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

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

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

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

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

Toxicological Effects in Humans

Acute Toxicity

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

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

Developmental and Reproductive Toxicity

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

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

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

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

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

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

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

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

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

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

Immunotoxicity

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

Chronic Toxicity

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

Carcinogenicity

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

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

Inhalation studies

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

[*] Code ranges for the 9th ICD Revision are presented for illustration; the actual ICD revisions used in the studies ranged from 5th through 9th.
[@] Lung cancer is an indicator of Cr VI inhalation exposure and nonmalignant respiratory disease is an indicator of heavy cigarette smoking.
[1] SMR = standardized mortality ratio, SIR = standardized incidence ratio, PMR = proportionate mortality ratio, PCMR = proportionate cancer mortality ratio. "Control" is the comparison population.
[2] The liver cancer category in Satoh *et al.* 1981 was labeled "Liver" with no mention of gall bladder and an incompatible ICD code (157, which was pancreatic cancer in the 8th Rev. ICD used by the study).
[3] Liver cancer only (gallbladder excluded).
[4] Included cancer of the small intestine.
[5] Hayes *et al*. 1979 used a nonstandard ICD code grouping for all digestive cancer (ICD codes 140-154, instead of 150-159) which included buccal cavity and pharynx, and excluded biliary passages, liver, gall bladder, pancreas, and peritoneum.
[6] In Hayes *et al*., 1989 the nonmalignant respiratory diseases SMR was for all factory employees (not limited to workers with 1 month or more Cr+6 exposure).
[7] The Baltimore cohort studied by Hayes *et al.* (1979) was updated by Gibb *et al*. (2000), but only Hayes *et al*. reported findings for cancers of the buccal cavity, pharynx, or digestive system.
[9] Included laryngeal cancer.

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

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

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

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

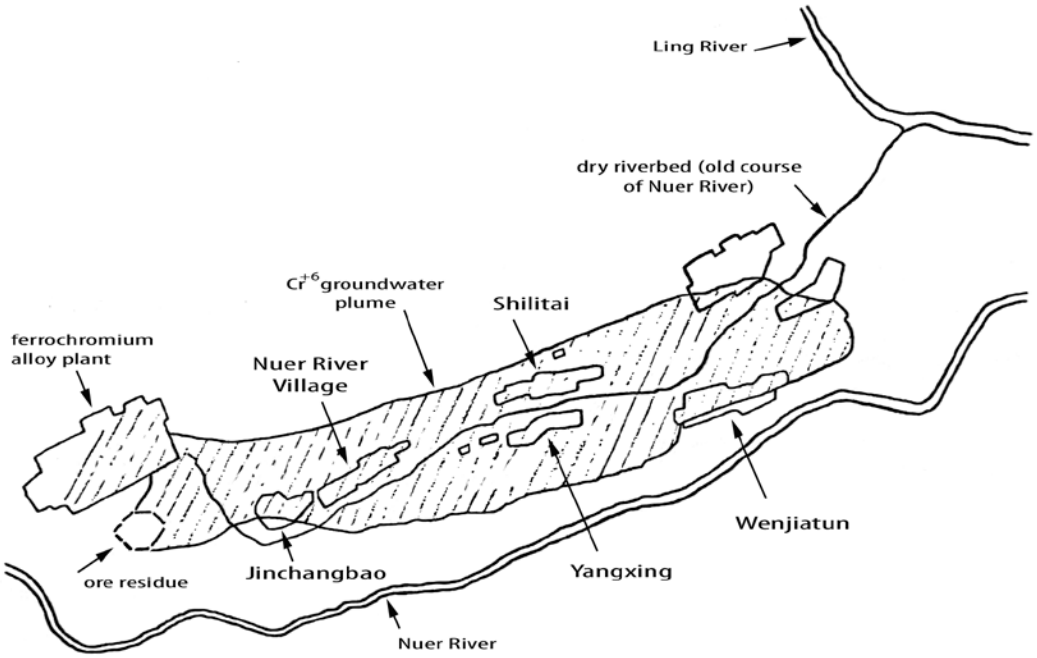
example, Rafnsson *et al.* (1986) reported Cr VI concentrations in the range of 5.8 – 9.4 mg/kg in cement used by masons in Iceland in 1983. A few studies have reported breathing-zone concentrations. Vestbo *et al.* (1991) in Denmark reported mean Cr VI concentrations (in dust by weight) of 9 mg/kg in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

Ingestion studies

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

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

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



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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Sensitive Subpopulations

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

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

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

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

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

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

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

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

Examination of Evidence for Chromium Carcinogenicity

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

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

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

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

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

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

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

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

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

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

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

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

DOSE-RESPONSE ASSESSMENT

Noncarcinogenic Effects

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

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

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

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

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

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

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

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

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

Carcinogenic Effects

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

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

Oral Potency Estimates Based on Animal Studies

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

Dose-Response Modeling

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

Male Mice

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

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

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

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

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

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

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

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

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

Female Mice

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

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

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

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

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

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

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

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

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

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

Cancer Potency for the Inhalation Route

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

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

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

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

Methods for Analysis I: Simple Dose-Response Analysis

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

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

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

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

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

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

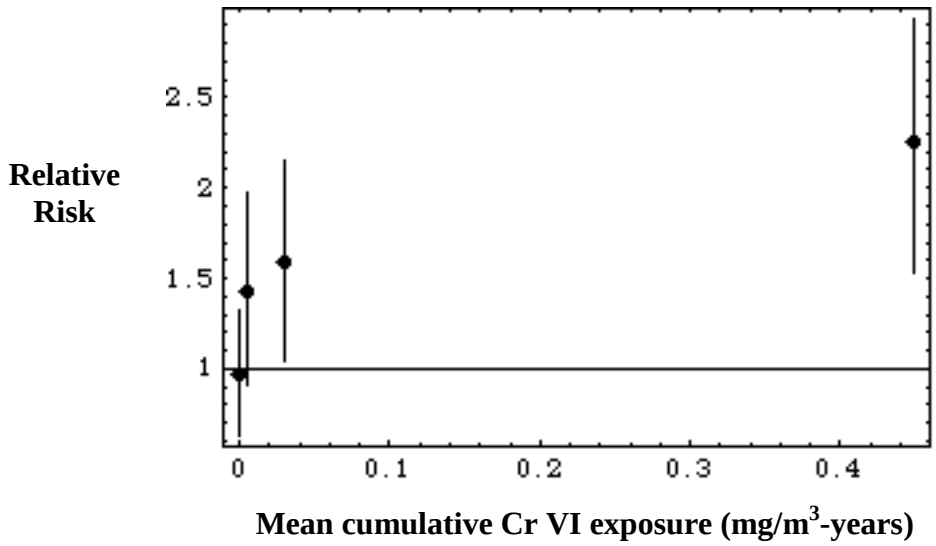
Table 12. Gibb *et al*. (2000) Lung Cancer Occupational Cohort Data; Quartiles of Exposure Without Categories for Age

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

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

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

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



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

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

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

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

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

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

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

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

Conversion Factors

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

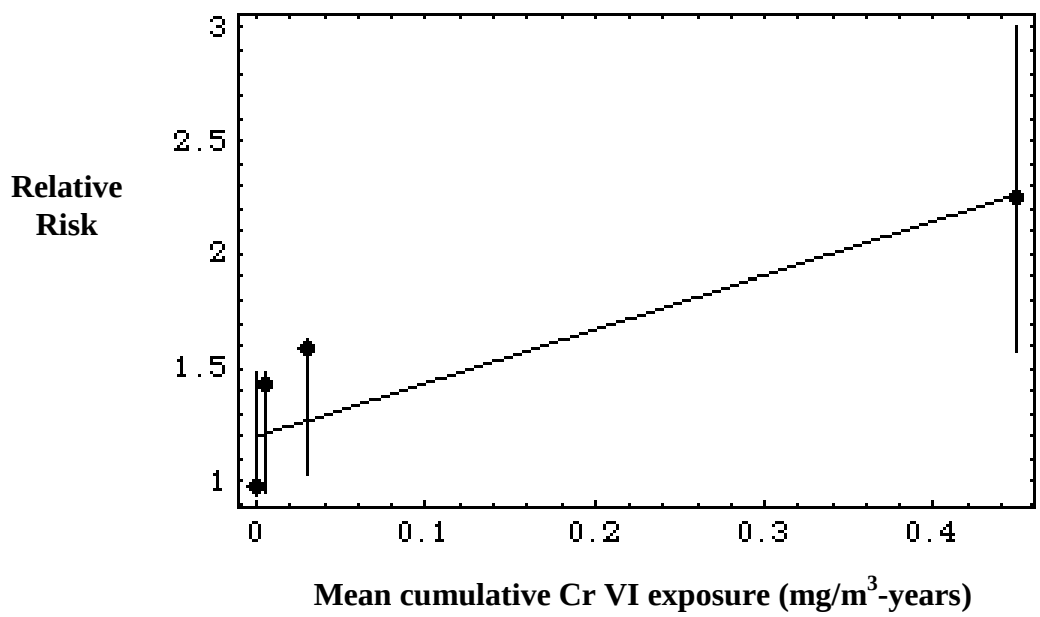
was then applied, described by the equation (10 m^3 / 20 m^3) * (240 day / 360 day) = 0.33, which was then divided into the aforementioned product.

Results

Analysis I: Simple Dose-Response Analysis

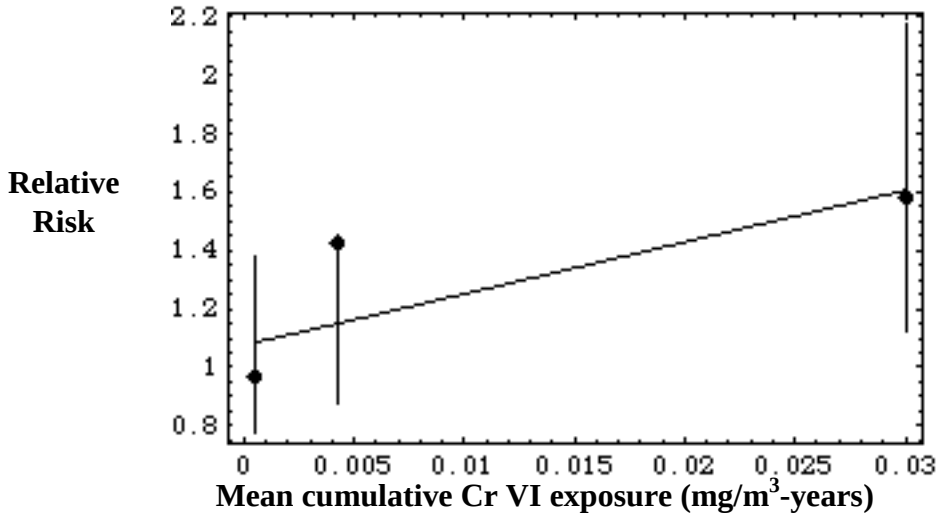
The dose-response data presented in Table 12 were fit to the model represented by Equation (2). Figures 17, 18, and 19 display the fits of the model to the observed data. Table 14 presents model parameter estimates (in terms of cumulative exposure), the 90 percent profile likelihood-based confidence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

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



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

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



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

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

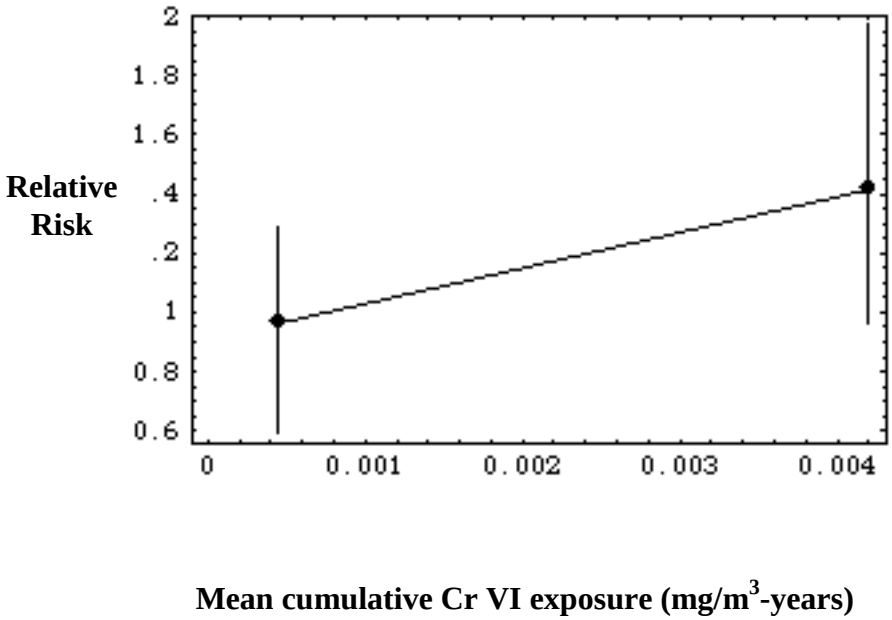


Table 14. Estimated Model Parameters for Gibb *et al*. (2000) Four Points, Analysis I

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

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

Analysis II: Stratified Person-Years Dose-Response Analysis

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

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

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

Discussion

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

Comparison with previous results

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

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

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

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

Usefulness of the new data

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

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

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

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

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

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

Correction for Early-in-Life Exposures

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

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

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

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

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

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

$cons^o{}_1$	=	Oral route, infancy;
$cons^o{}_2$	=	Oral route, childhood;
$cons^o{}_3$	=	Oral route, adult;

$cons^i_2$ = Inhalation route, childhood;

$cons^i_3$ = Inhalation route, adult.

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

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

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

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

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

Rearranging Equation 2:

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

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

CALCULATION OF THE PHG

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

Noncarcinogenic Effects

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

Choosing Appropriate Uncertainty Factors

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

where,

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

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

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

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

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

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

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

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

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

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

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

Carcinogenic Effects

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

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

where:

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

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

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

Drinking water exposure is estimated for this calculation for the age ranges used above in the Dose Response section. The drinking water consumption values utilized are upper 95th percentile values calculated by OEHHA of 0.125, 0.047, and 0.039 L/kg-day for infancy, childhood, and adult life stages using the "Consumers only" values from U.S. EPA (2008) and Kahn and Stralka (2009). The infant value is the time weighted average of the values for 0<2 months, 1<3 months, 3<6 months, 6<12 months, and 1<2 years. The childhood value is the time weighted average of the values for 2<3, 3<6, 6<11 and 11<16 years. The adult value is a time weighted average of the values for 16<18, 18<21 and >21 years. The adult (maternal) value was used for the 3rd trimester calculation. The value for exposure to water droplets in showering is 3.86×10^{-7} L/kg-day (Keating and McKone, 1993), which is applied to children and adults only, since infants are presumed not to take showers.

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

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

Life Stages	**ASF_j x d_j x $cons_j$**	
	Oral	**Inhalation**
3rd Trimester	0.0014	0.138×10^{-7}
Infant (0-2)	0.0357	--
Child (2-16)	0.0282	2.316×10^{-7}
Adult (16-70)	0.0300	2.978×10^{-7}
Exposure Totals (L/kg-day)	0.0953	5.432×10^{-7}

Inserting the exposure values in the equation above,

$$C = \frac{R}{0.5\ (mg/kg\text{-}day)^{-1} \times (0.0953)(L/kg\text{-}day) + 510\ (mg/kg\text{-}day)^{-1} \times (5.432 \times 10^{-7})\ (L/kg\text{-}day)}$$

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

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

RISK CHARACTERIZATION

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

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

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

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

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

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

Dose Response – cancer endpoint

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

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

Dose response – non-cancer endpoint

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

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

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

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

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

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

OTHER REGULATORY STANDARDS

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

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

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APPENDIX A

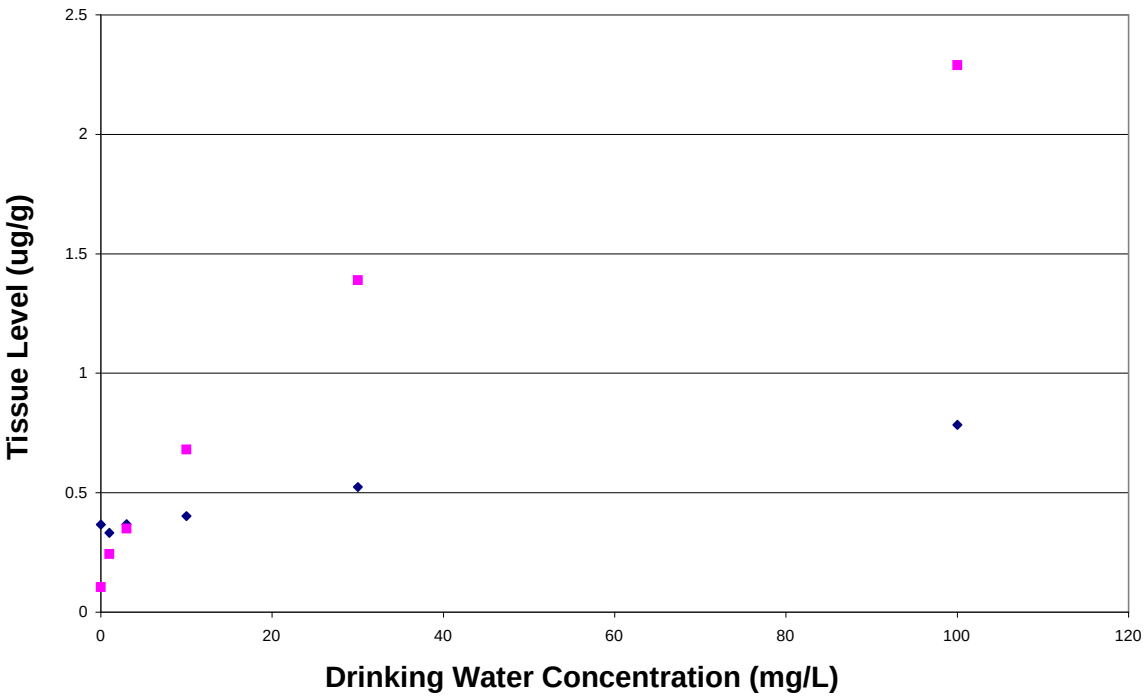
Carcinogenic Threshold: Was the reductive capacity of the rodent GI tract exceeded in the NTP (2008) two-year bioassay?

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

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

Studies in animals also do not indicate that the absorption of Cr VI was a consequence of the exhaustion of the capacity of the GI tract to reduce Cr VI to Cr III. Chromium blood and kidney levels were determined in male $B6C3F_1$ mice administered Cr VI in drinking water at 1 to 300 mg/L for 21 days (NTP, 2007). Blood and kidney chromium levels increased with the concentration of chromium in water with no threshold evident (Figures A1 and A2). Figure A2 is the same data as Figure A1 with the addition of the highest dose group.

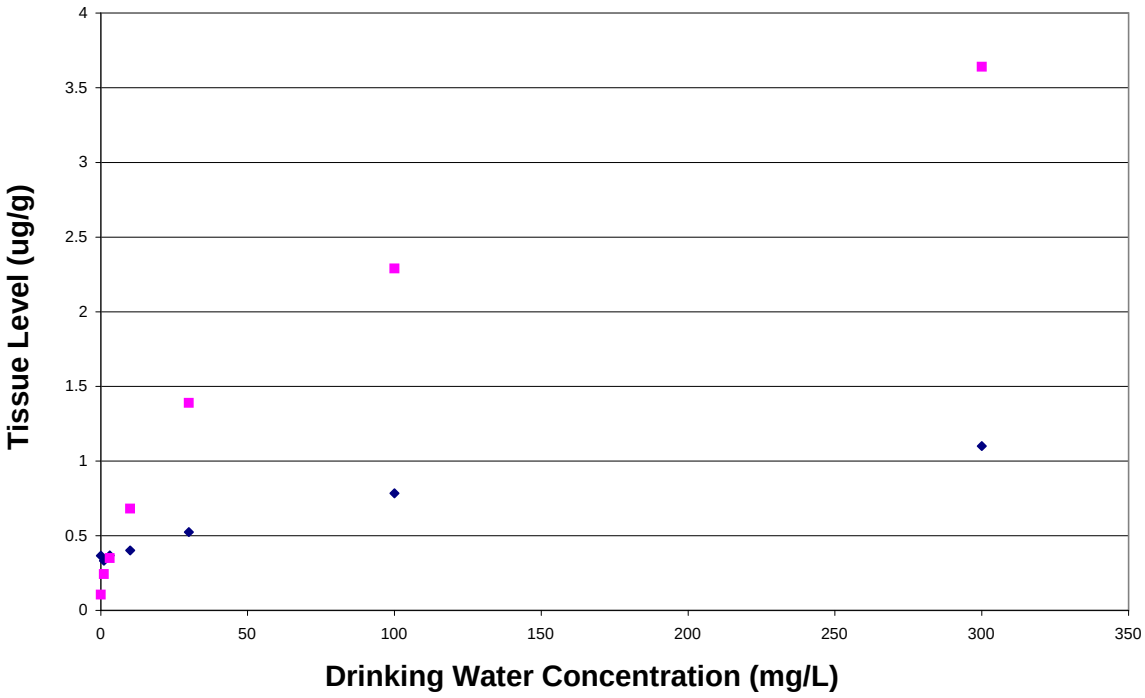
Figure A1. Blood and Kidney Chromium Levels in Male Mice



Square: Kidney chromium levels Diamond: Blood chromium levels

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

Figure A2. Blood and Kidney Chromium Levels in Male Mice



Square: Kidney chromium levels. Diamond: Blood chromium levels

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

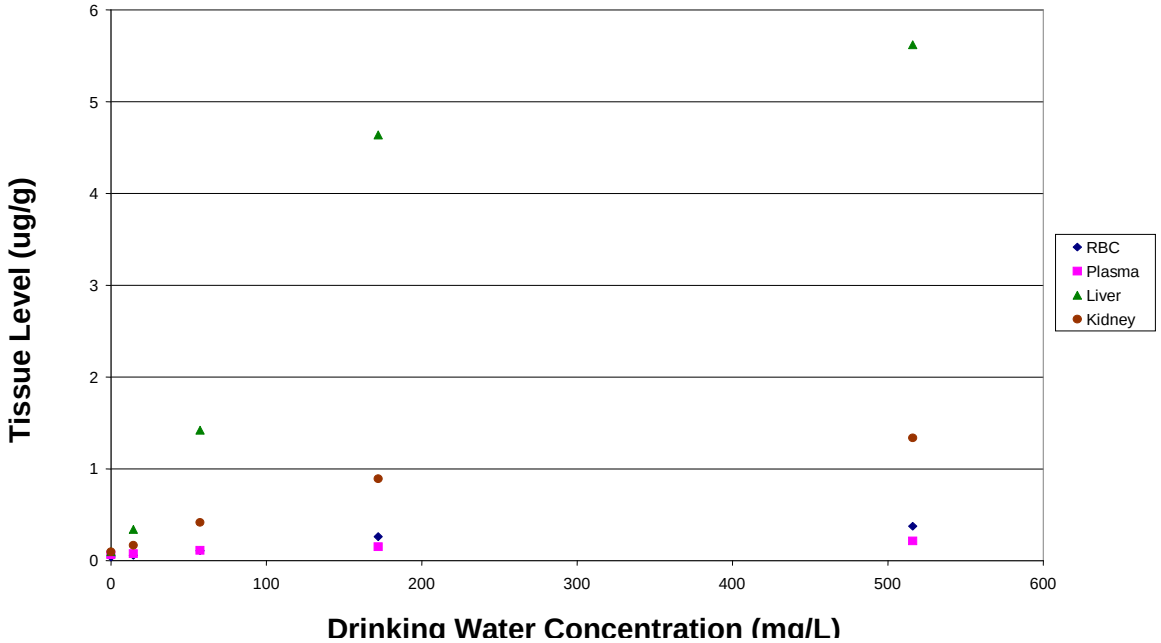


Figure A4. Chromium Tissue Levels on Day 13 in Female Mouse

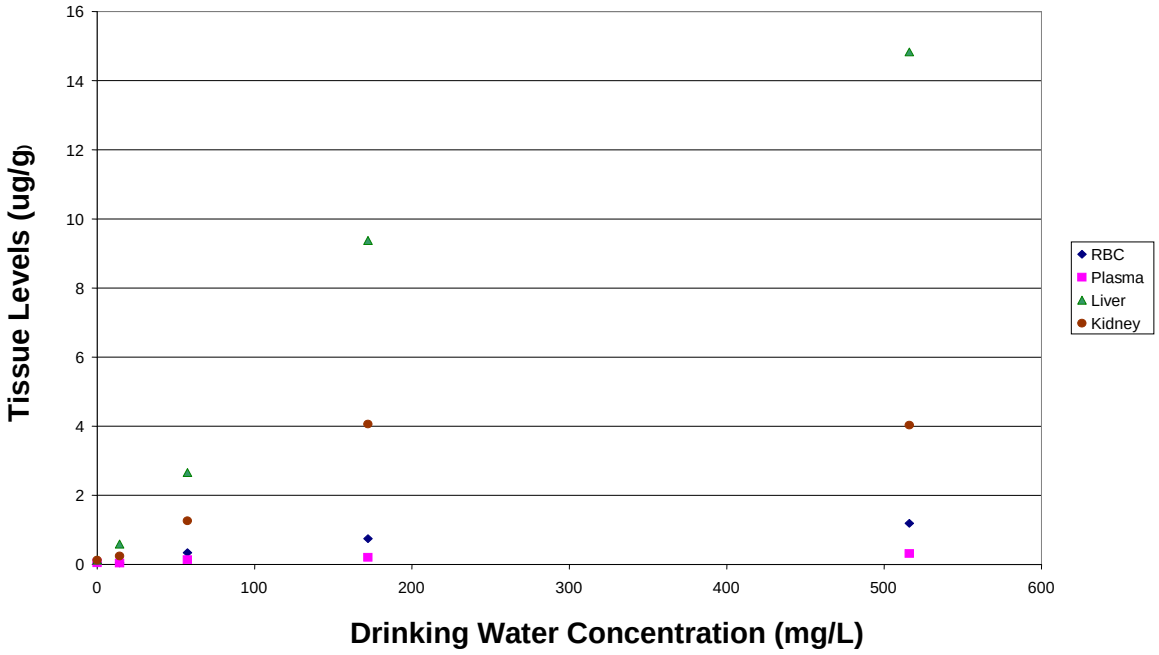


Figure A5. Chromium Tissue Levels on Day 18

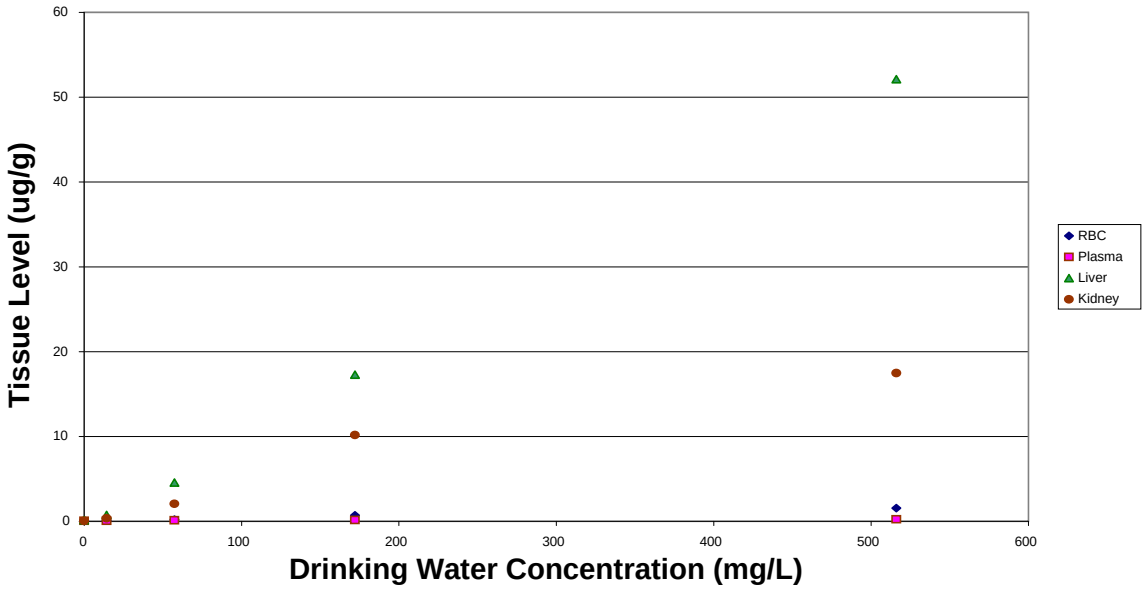
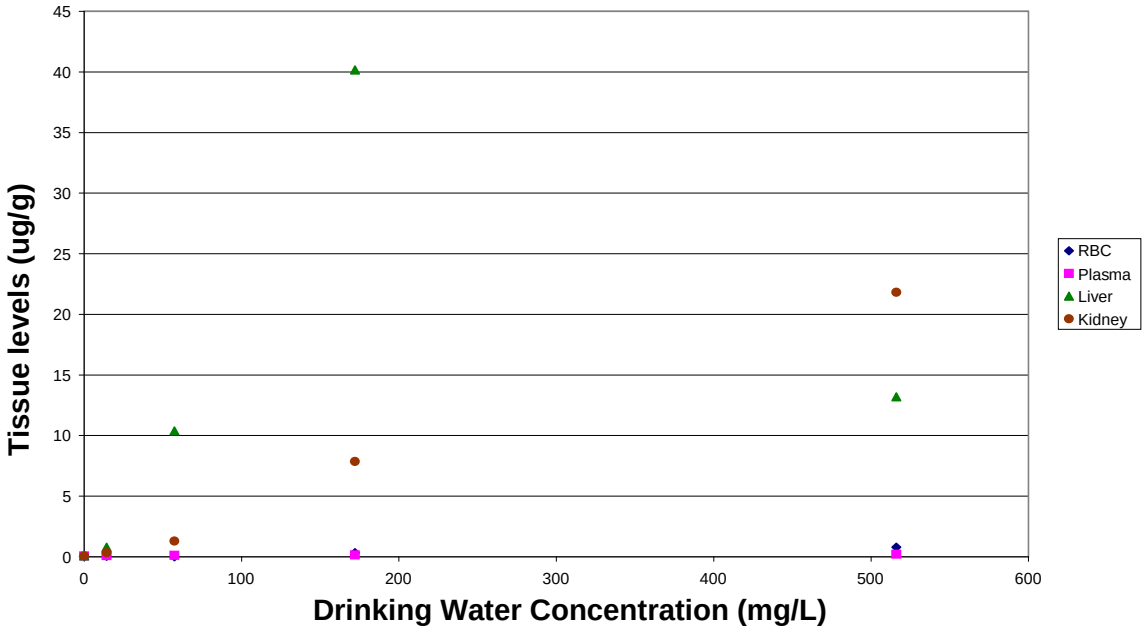


Figure A6. Chromium Tissue Levels on Day 371



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APPENDIX B

Mouse Cancer Study of Borneff et al. (1968)

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

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

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

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

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

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

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

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

Issues related to experimental design and adequacy of the animal model

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Potential Influence of Helicobacter Infections on Stomach Tumors

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

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

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

The Helicobacter Hypothesis

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

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

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

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

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

Helicobacter Pylori

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

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

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

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

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

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

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

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

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

Colonization and Transmission

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

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

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

Gastritis and Ulcers

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

Cancer

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

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

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

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

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

Animal Models.

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

The Forestomach

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

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

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

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

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

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

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

Chemical Carcinogens

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

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

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

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

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

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

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

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

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

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

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

Vagotomy

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

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

Stomach Irritation and Cancer

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

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

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

Hexavalent Chromium - Toxicity Studies

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

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

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

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

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

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

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

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

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

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

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

Toxicity Mechanisms

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

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

Future Studies

Stomach Cancer

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

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

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

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

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

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

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

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

Specific Areas of Investigation

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

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

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

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

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

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Responses to Major Comments on Technical Support Document

Public Health Goal
For
Hexavalent Chromium (Cr VI)
In Drinking Water

Prepared by

Pesticide and Environmental Toxicology Branch
Office of Environmental Health Hazard Assessment
California Environmental Protection Agency

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INTRODUCTION

The following are the combined responses to major comments received by the Office of Environmental Health Hazard Assessment (OEHHA) on a 2008 pre-release draft of the proposed public health goal (PHG) technical support document for hexavalent chromium and on two later public review drafts of that document. The pre-release draft was reviewed by three University of California peer reviewers. Their comments and OEHHA's responses were posted on the OEHHA Web site on September 10, 2009. They are included here for completeness. The first public review draft PHG document was released for public comment on August 20, 2009. A public workshop on the first PHG draft technical support document for hexavalent chromium was held on October 19, 2009. The public comment period on that draft document closed on November 2, 2009. The Association of California Water Agencies and Honeywell International, Inc. subsequently requested an external scientific peer review pursuant to Health and Safety Code section 116365(c(3)(D). Public comments on that draft document, including comments from five additional peer reviewers, are included here along with OEHHA's responses. A revised PHG draft document was released for public review on December 31, 2010. The public comment period on the second PHG draft technical support document for hexavalent chromium closed on February 12, 2011. Public comments received in response to that second draft document are also included here along with OEHHA's responses. Changes have already been made in response to these comments, and have been incorporated into the final PHG document posted on the OEHHA website. For the sake of brevity, we have selected the more important or representative comments for responses. Comments appear in quotation marks where they are directly quoted from the submission; paraphrased comments are in italics.

These comments and responses are provided in the spirit of the open dialogue among scientists that is part of the process under Health and Safety Code Section 57003. For further information about the PHG process or to obtain copies of PHG documents, visit the OEHHA Web site at www.oehha.ca.gov. OEHHA may also be contacted at:

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RESPONSES TO MAJOR COMMENTS ON 2008 PRE-RELEASE DRAFT RECEIVED FROM UNIVERSITY OF CALIFORNIA PEER REVIEWERS (POSTED ON OEHHA WEB SITE SEPTEMBER 2009)

Comments from Roberto Gwiazda, University of California, Santa Cruz

Comment 1: *Sensitive population issues.*

From page 1. "However, the weakest aspect of the estimate of the human protective level is the very crude approach followed to calculate it. The slope factor calculated via a linear extrapolation to zero of the lower boundary level of the ED_{10} ignores two issues that are not incorporated under this approach but that may yield a different protective level (lower or higher) if included: namely, the existence of sensitive populations and the extent to which the reducing capacity of the gastrointestinal tract may have different efficiencies in the conversion of CrVI to CrIII depending on the amount of CrVI in the stomach. Because of these unknowns it is uncertain whether the PHG provides adequate public health protection."

And from page 3 "There are two sensitive populations that are not included in the estimate of the one in a million lifetime cancer risk: carriers of Helicobacter pylori and people with anomalous stomach pH regulation. It is noted that animals in the NTP 2007 study were free of H. Pylori. As noted at the end of the document, a more realistic scenario, at least to evaluate the oral carcinogenicity of CrVI in carriers of H. pylori would utilize infected animals. This study would most likely yield a lower point of departure for linear extrapolation to zero and result in a lower PHG estimate.

The document recognizes the existence of other groups of sensitive individuals: those with a variety of conditions that result in reduced gastric capacity production. The equation of page 97 does not consider these sensitive subpopulations either. At this point there is no sufficient information to quantify the higher risks that these populations may be exposed to due to CrVI in drinking water. The only certainty is that their inclusion in the cancer risk estimate would yield a lower protective level of CrVI in drinking water than the current one that does not incorporate them specifically."

Response 1. OEHHA is mandated by statute to protect sensitive populations. The PHG identifies two sensitive populations; 1) individuals with high stomach pH, which may result in less reduction of Cr VI to Cr III in the stomach and therefore a likely increase in the amount of Cr VI in absorption in the intestine, and 2) individuals infected by Helicobacter pylori.

While OEHHA is mandated by statute to protect sensitive population, there are no studies found that specifically evaluate these identified sensitive populations, and therefore no data that could be used to develop a dose-response relationship in these populations. The results of the NTP animal bioassay (NTP, 2007) did not yield findings that are informative regarding a dose-response relationship in the sensitive populations. An adjustment to the potency estimate based on differences in absorption of chromium VI in sensitive humans and rodents is problematic given it is unclear how much

hexavalent chromium was absorbed in the mouse relative to how much would be absorbed in individuals with high stomach pH.

However, the methods employed to develop a slope factor, using the most sensitive tumor site, sex and species, and using the lower bound estimate of the dose associated with a 10 percent incidence of tumors (and not the mean), are aimed at protecting sensitive populations.

From U.S. EPA (2005) guidance:

> "Slope factors generally represent an upper bound on the average risk in a population or the risk for a randomly selected individual but not the risk for a highly susceptible individual or group. Some individuals face a higher risk and some face a lower risk. The use of upper bounds generally is considered to be a health-protective approach for covering the risk to susceptible individuals, although the calculation of upper bounds is not based on susceptibility data."

Comment 2: *Reduction capacity of saliva and gastric fluids.*

From page 1: "and the extent to which the reducing capacity of the gastrointestinal tract may have different efficiencies in the conversion of CrVI to CrIII depending on the amount of CrVI in the stomach."

From Page 2: "It was my opinion that in the process of calculating the oral cancer slope factor by extrapolating to zero a CrVI dose that is associated with a certain incidence of cancer in an animal study, there is an unwarranted assumption that the efficiency of saliva and gastric fluids to reduce CrVI to CrIII is the same in the presence of nanogram amounts of CrVI in the human stomach resulting from exposure to drinking water as it is in the presence of milligram amounts of CrVI in the rodent stomach resulting from high CrVI doses in the rodent studies. There is no information to support this assumption of linearity. … It is assumed with the approach followed in 2005 and here in this PHG estimate that the fraction of CrVI that is reduced to CrIII is the same at high exposures, at the point of departure, at lower exposures and at the protective level."

Response 2. The amount of reduction of Cr VI to Cr III in the stomach is a very important issue. Some risk assessors have suggested or concluded that the reducing capacity of stomach fluids is so vast that all Cr VI would be immediately reduced and therefore there is no cancer risk associated with oral exposure to hexavalent chromium. This opinion is not supported by the findings of pharmacokinetic studies in animals and humans (reviewed in the PHG document) and studies that have observed significant increases in tumors in animals and humans exposed to Cr VI (NTP, 2007; Borneff *et al*., 1968; Beaumont *et al*., 2007; Zhang and Li, 1987).

The rate of chromium reduction could be a function of concentration in the GI tract, but the reduction does not appear to be an enzymatic process and therefore not limited by the amount of an enzyme in the stomach. The reducing equivalents appear to be from dietary protein (and not the acid) in the stomach and in sufficient quantities that are not rate limiting. Thus mechanisms that would limit the rate of Cr VI reduction in the stomach (saturation of available enzymes or limited availability of reducing equivalents)

do not appear evident in the stomach. Studies by Donaldson and Barreras (1996), Kerger *et al*. (1996), Finley *et al*. (1996, 1997) do not indicate that the amount of absorption increases with increasing doses of hexavalent chromium in humans. A new paragraph in the absorption section of the PHG now discusses this issue.

This comment raises a concern that is similar to other concerns related to interpreting the results of animal cancer bioassays. Because of statistical considerations (the ability to detect tumors), high doses of agents are routinely tested in animal cancer bioassays. High doses may alter the rates of absorption, metabolism (activation and detoxification), and elimination as well as differences in ability to prevent or repair DNA damage, all of which could influence the occurrence of tumors. The use of high doses in bioassays and the consequences of using high doses have been discussed elsewhere (U.S. EPA, 2004); use of high doses is generally thought to help offset the statistical limitations of the relatively small animal study used to estimate human risk for the entire California population.

Comment 3, page 4: "The document extensively discusses the unknowns involved in many of the parameters that are to be considered and included in the PHG estimate. However, this discussion does not translate into a quantifiable measure of uncertainty. In other words: what is the degree of confidence in the PHG value? Can OEHAA quantify the uncertainty and say "There is X probability that a value as low as this PHG would protect 1 in a million"?."

Response 3. While there are many sources of uncertainty, the ability to quantify various sources of uncertainty (e.g., the uncertainty associated with using the findings in animals to predict effects in humans, extrapolating risk associated with high doses to low doses, etc.) is problematic given the lack of data. The PHG discusses uncertainty in the Risk Characterization portion of the document, but the PHG document does not attempt to quantify the uncertainty because there is no accepted method for carrying out such a calculation.

Comment 4, page 4: "The absorption section is muddled and could be improved. The paragraphs are not thematically separated nor are the arguments built consistently on the basis of the previous paragraphs. These could be rewritten by leading each paragraph with the main point that is being made and each conclusion built on the foundation set by the previous paragraph."

Response 4. This section of the PHG document has been rewritten to address the issue.

Comment 5, page 5: "The observation that there is absorption of CrVI when administered in the 6+ species is supported by a different tissue distribution and urinary half-lives after CrVI and CrIII administration. However, there is an apparent inconsistency in the fact that the half life of Cr in RBC's after intraperitoneal or intravenous CrVI dosing does not match the half life of Cr in RBC's after oral CrVI

administration. It is argued that blood carries Cr immediately from the point of oral absorption to the liver preventing a blood buildup of CrVI. Critics would argue that the Cr RBC time profile is not consistent with CrVI in blood and the increase in liver CrVI is in fact evidence for absorption of complexes of CrIII-organic ligands."

Response 5. The difference in the tissue distribution and half-life of Cr following oral vs intraperitoneal administration is not unexpected. Given that oral absorption is a slower process, most of the orally absorbed chromium VI is probably rapidly reduced to Cr III in the plasma before it can get into cells. Being relatively insoluble, Cr III associates with proteins in the plasma and proteins on the outside of the RBC. Thus immediately following oral administration, a larger fraction of Cr in the blood is Cr III, which does not move into cells (RBCs) and is rapidly eliminated by the kidney. Intraperitoneal injection delivers Cr VI much more rapidly and at higher concentrations so immediately after an ip injection, more Cr VI would be expected to have the opportunity to move into RBCs before it is reduced to Cr III in the plasma.

Neither of these observations provides any evidence that orally administered Cr VI is absorbed because it is converted in the stomach to a CrIII-organic ligand complex nor has such a ligand been identified or isolated. The revised absorption section in the PHG document highlights two studies where oral absorption of inorganic trivalent chromium and various organic complexes of trivalent chromium was about the same. If oral absorption occurred via such a ligand complex, then the amount of oral absorption of Cr III and Cr VI should be about the same given most Cr VI is reduced to Cr III in the stomach.

Comment 6, page 5: "The case is made that despite the fact that the reducing capacity of the stomach should completely reduce the dose a human receive from drinking California waters, genotoxic effects were observed in distant tissues in rodents chronically administered by gavage doses…not likely to overwhelm the reductive capacity of the stomach, intestines, and blood, … such as 1 mg/kg-d or 2.5 mg/kg-d. Further, at the end of the page this information is quoted again indicating that in these oral studies CrVI was not fully reduced, and DNA damage was observed. First, it is not known what the reducing capacity of the rodent stomach is. Second, this argument fails to account for the peculiarities of a gavage study."

Response 6. The findings of this study indicate that at the doses given, Cr VI administration resulted in a genotoxic effect. Given that Cr III is not associated with genotoxicity, this finding indicates that not all of the administered Cr VI was reduced or converted in the stomach to Cr III. Otherwise, no genotoxicity would have been observed.

Comment 7, page 6: "The document discusses extensively the Borneff et al., 1968, study. The amount of space devoted to this study is not justified and it appears that this extensive presentation and discussion are a leftover from previous PHG's documents were Borneff et al. 1968, was the only animal study that could be used to demonstrate that oral CrVI is carcinogenic and to calculate an oral cancer slope factor. This is not

the case anymore and it is puzzling that given the amount of uncertainty surrounding the results of this study so much space and speculation is devoted to it, in contrast to the study of Beaumont et al 2008, which is the only human study that shows a relationship between CrVI environmental exposure and oral cancer, but receives a mere two paragraphs of attention."

Response 7. Point taken. The extensive discussion of the Borneff *et al*. (1968) study has been removed from the body of the PHG document and placed in an Appendix. While there are more recent studies available, conducted with more current study guidelines, a weight of the evidence approach for evaluating the carcinogenicity of Cr VI necessitated considering the findings of Borneff *et al.* (1968). Understanding/explaining the findings of Borneff *et al.* (1968) can help us better understand why Cr VI is an oral carcinogen. The discussion of the CrVI exposure in China which is the subject of Beaumont *et al.* (2008) has been expanded.

Comment 8, page 6: "The analysis of the occupational studies is fairly inconclusive and at most suggestive of a link between CrVI exposure and stomach cancer. Given the very little weight that this analysis carries OEHHA should consider not including this analysis in the PHG document…"

Response 8. The text in the PHG was revised to indicate that evaluation was undertaken "to determine if there may be a link between occupational exposure to hexavalent chromium and cancers of the digestive organs." The results section of the analysis was changed to indicate that the rate ratio for stomach tumors exceeded 1 in a majority of studies (18/25) but was below 1 in some studies (7/25). Rate ratios for other sites in the digestive system are now included. The interpretation of the findings of this study was modified as suggested in the Examination of Evidence for Chromium Carcinogenicity section of the PHG document.

Comment 9, page 7: "The Beaumont et al. 2008 study deserves much more attention than two paragraphs and meaningless map!."

Response 9. The discussion of Beaumont *et al.* (2008) in the PHG and the underlying data has now been expanded in the PHG document.

Comment 10, page 7: "The modeling of the female data of the NTP 2007 study is not used for the calculation of cancer potency because "the male data used in the modeling was more robust". OEHAA should reconsider this. Examination of the cancer incidence response with dose from the NTP study suggests a different response according to gender, with males appearing to have a more linear response through the dose range and with female data showing an apparent higher sensitivity at lower doses and saturation in cancer incidence at a lower dose than the males. Does this indicate a gender specific difference in the response shape and sensitivity? Female data should be considered, the LED_{10}'s are lower than those derived from the male data, and the most conservative approach would suggest taking that data into account."

Response 10. The NTP bioassay consisted of three dose groups of male and female mice plus a control group. Statistically significant increases in tumors were observed in the two highest dose groups. Given the limited number of data points for each sex (only two points were significantly different than control), any comparison of the shape of the dose–response relationship in males and females is problematic, particularly in the low dose region where the incidence of tumors was no different than background.

None of the models yielded acceptable fits in female mice when all of the doses were used. After dropping the high dose, all of the models yielded acceptable fits with a LED_{10} similar to that obtained in male mice (which was based all dose groups). Given that in both sexes only the two high dose groups yielded statistically significant increases in tumors, a dose-response relationship based on both high dose groups (male mice) appeared to be preferable to a dose response relationship where one of the high dose groups had to be censored to obtain an acceptable fit (female mice). Thus the proposed PHG was based on the findings in male mice.

Saturation of the response is not evident in males or females, as at most 50 percent of the animals exhibited tumors in the highest dose groups.

Comment 11: "Page 60: 'The reduced water consumption appears to be consistent with the reduced weight gain in these animals…" This is not the case. Female mice drank as much as controls from week 15 and never gained enough weight. Male mice drank less than controls from week 15 but gained as much weight."

Response 11. The paragraph was rewritten.

Comments from Leonard Bjeldanes, University of California, Berkeley

Comment 1, page 2: "A further cautionary note in the interpretation of the human cancer data apparently comes from a study in 453 communities in Nebraska (Bednar CM and Kies C, J Am Water Resour Assoc. 1991;27:631-635). No association was found in this study between low levels of Cr(VI) in drinking water (up to 10 ppb) with total cancer mortality. This study, to which this reviewer does not have ready access, seems to be highly relevant for the development of safe standards for Cr(VI) in water with relatively low contamination levels, and without obvious exacerbating factors, but was not discussed in the current PHG proposal. Indeed, this latter study apparently can provide dose-response data that could test the validity of the various extrapolation methods used in the PHG proposal to project low dose effects in humans based on high dose exposures in rodents."

Response 1. The Nebraska study evaluated a number of inorganics including chromium. While the precise analytical methods used in this study are unclear, it is likely that the analysis (conducted by the Nebraska Public Health Department and not the authors) in 1986 and 1987 used standard U.S. EPA analytical methods of the time and therefore measured total chromium and not hexavalent chromium. Low levels of

chromium were detected in the municipal supplies (average level of 0.002 mg/L or twice the detection limit), 80 percent of which came from groundwater (authors). The Nebraska study did not find a relationship between chromium in drinking water and cancer. These data could be examined regarding statistical power and ability to detect an effect at the reported chromium levels, but lack of identification of the chromium species present makes it difficult to compare the findings to those of Beaumont *et al*. (2008) of a relationship between hexavalent chromium in water and increased risk of stomach cancer.

Comment 2, page 2: "The effort to develop a safe dose standard for Cr(VI) in drinking water, however, is complicated by the fact that the human and rodent cancer studies that were considered in the proposal involved only very high doses of Cr(VI). These high exposures are likely to overwhelm the strong reductive capacity of saliva and gastric juices that have been well documented (c.f. De Flora S, Carcinogenesis 2000;21; 533-541). Published work also suggests that rodents may be more sensitive to oral Cr(VI) toxicity that humans. Thus, published pharmacokinetic studies have reported a several fold greater level of gastric absorption of Cr(VI) in rodents compared to humans, possibly due to the higher pH of rodent gastric juice."

Response 2. The absorption portion of the pharmacokinetic section of the PHG was rewritten and Appendix A was added to the document to address this important issue. The available evidence does not support the notion that hexavalent chromium only is absorbed when GI reduction capacity is exhausted. No marked increase in oral absorption of hexavalent was observed with dose, which would be expected if the reducing capacity of the GI tract had been overwhelmed.

The oral absorption of hexavalent chromium appears to be quite similar in rodents and humans. From page 10 of the PHG document: "The amount of hexavalent chromium recovered in urine was below ten percent of the administered dose of hexavalent chromium 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)."

The pHs of the rodent and human stomach fluids are quite acidic and it is unclear if small differences in acidity would cause a difference in absorption given that the reducing equivalent appears to come from protein and not directly from the acid. Infusion of hexavalent chromium directly into the human jejunum (bypassing the stomach) resulted in considerable absorption of hexavalent chromium (roughly 30 percent). Preincubation of hexavalent chromium with HCl alone (which was then neutralized) did not prevent the absorption in the jejunum but preincubation with acidic stomach contents (and then neutralization) prior to infusion into the jejunum largely prevented the absorption (Donaldson and Barreras, 1966).

Comment 3, page 3: "[T]he proposed PHG for Cr(VI), which is fully six orders of magnitude lower than the active concentrations in mice, is well below current safety standards, appears to be lower than levels in uncontaminated waters, is near the limits

of detection with currently available analytical methods, and apparently does not consider the likelihood of a threshold for Cr(VI) biological activity, requires further justification."

Response 3. Carcinogens are routinely tested in rodent bioassays at high doses, orders of magnitude above levels where exposures typically occur. The need to use high doses in rodent bioassays, discussed elsewhere (Safe Drinking Water Committee, 1977; Committee on Risk Assessment Methodology, 1993), is due to the lack of sensitivity of these tests and mandates to protect public health from low levels of cancer risk (e.g., 10^{-6} risk).

PHGs, by statute, only consider health impacts. Development of the Maximum Contaminant Limit for Cr VI by the California Department of Public Health will address other issues such as background levels, detection limits and cost and feasibility. The possibility of a threshold for carcinogenic effects of Cr VI is an important consideration. For this risk assessment, OEHHA has followed the most recent carcinogen guidelines of the U.S. EPA (2005) and OEHHA's own principles (OEHHA, 2005). Basically, if there is evidence that an agent acts through a genotoxic mechanism (as there is for Cr VI), no threshold for effect is assumed.

Also, because Cr VI is reduced to Cr III in the GI tract, it has sometimes been asserted that no portion of a dose is absorbed in the Cr VI form. An inability to absorb Cr VI could be considered a pharmacokinetic threshold (independent of genotoxicity considerations). However, all the available pharmacokinetic studies indicate that a portion of the Cr VI <u>is</u> orally absorbed, at the doses studied, with results far too variable to indicate or estimate a threshold. Thus, while we acknowledge the possibility of a dispositional threshold, we have no quantitative basis for the extrapolation, and have felt constrained to utilize the standard cancer risk assessment methodology in this case.

Comments from Michael Kelner, University of California, San Diego

Comment 1, page 1: "The first [salient point] is that only selected data from the NTP studies is used (reference 2007b) to derive the target value. By selected data, I mean only one subset of data from a single study out of the entire NTP database is deemed relevant. This is the one study describing the combined incidence of adenomas and carcinomas in male B6C3F1 mice. The data from all other rodent studies involving chromium-6 ingestion is not utilized."

Response 1. Most cancer potency estimates that utilize animal data are derived based on the most sensitive species and strain. This is a health-protective assumption, intended to ensure that the cancer risk in humans is not underestimated. The most recent U.S. EPA guidelines (2005) acknowledge a variety of choices for selection of data for the potency calculation, including adding up tumors at various sites, combining data from different datasets (in various ways), presenting the potency as a range, choosing a single dataset "if it can be justified as most representative of the overall

response in humans," or a combination of these options (U.S. EPA, 2005, section 3.3.5).

OEHHA evaluated the cancer incidence in rats and mice from the NTP (2007) study and concluded that the rat data were inferior for dose-response modeling (poor fits with the common models). We calculated the cancer potency for male and female mice combined intestinal tumors using several different models, finding reasonably good fits and estimated cancer potencies within the same range for both data sets with the various models. The most common model, the linear multistage, gave LED_{10} values within the range of the other model outputs for both male and female mice, although the highest dose was eliminated from the model for female mice, to achieve best fit. These linear multistage estimates were selected as representative values; the slope factors calculated from them were nearly the same for males and females. Because the male mice data were statistically more robust (no discarded data points), we selected the cancer slope factor for males for calculation of the proposed PHG. The value derived from the female mice data would have been slightly smaller (0.04 versus 0.06), but in a statistical sense should not be thought of as any better or more accurate than the chosen approach. An average of the two values could also have been chosen for the proposed PHG, which would have been within the spirit of the U.S. EPA guidelines, but this seemed to us to add complexity with no added value. Thus, we believe that all the available data from the best studies were considered, and the most appropriate data set was chosen for calculation, with a result that is consistent with the intent of the U.S. EPA guidelines as discussed above.

Comment 2, page 1: "The second [point] is the equation on page 97. This is where the 0.06 ppb threshold is derived, from oral intake and 'shower inhalation.' … Contribution from 'shower inhalation' is negligible in comparison to oral (drinking intake), so one needs to focus primarily on the oral intake value and its derivation."

Response 2. OEHHA typically considers three possible pathways of exposure when developing a PHG: ingestion and dermal contact with water and inhalation in the shower. Because hexavalent chromium is carcinogenic by the inhalation pathway with a very high potency, inhalation exposure in the shower was a possible concern. Therefore, this pathway was addressed and the results showed that the inhalation exposure's contribution to the overall cancer risk was negligible.

Comment 3, page 2: "The third [point] is the oral intake value for the LED10 on page 80 of 1.1 mg/kg-day(mouse). It is this value that drives the 0.06 ppb limit. … Is it reasonable to use rodent data versus human? … The answer to the … question appears to be yes, based on the paucity and poor quality of human data."

Response 3. We agree. The only available human study with demonstrable exposure to hexavalent chromium is Zhang and Li (1987). The exposure was not adequately characterized for a dose-response determination.

Comment 4, page 2: "Should an LED10 be used (versus an ED10)? If so, is the LED10 derived appropriately? The answer to [these questions] appears to be "no" as their use and derivation appear to conflict directly with guidelines in the EPA publication 630/P-03/001B, Guidelines for Carcinogen Risk Assessment (March 2005)."

Response 4. The U.S. EPA (2005) guidelines extensively discuss use of various endpoints within the observable range, such as LED10, and we believe that the calculations in the PHG document are well within the scope of recommended options. The specific discussion in the U.S. EPA document uses LED01 for the example of extrapolation from an appropriate point of departure (POD), but this is clearly only an example:

> "The POD for extrapolating the relationship to environmental exposure levels of interest, when the latter are outside the range of observed data, is generally the lower 95% confidence limit on the lowest dose level that can be supported for modeling by the data. (Section 1.3.4, p. 1-14)"
>
> "The slope of this line, known as the *slope factor*, is an upper-bound estimate of risk per increment of dose that can be used to estimate risk probabilities for different exposure levels. The slope factor is equal to $0.01/LED_{01}$ if the LED_{01} is used as the POD." (Section 3.3.3, p. 3-23)"

Comment 5, page 2: *The approach appears to overestimate risk because:*

"#1) The mouse is a susceptible strain (vs even another rodent strain such as a rat that was concurrently tested by the NTP). Why was the data for the rat excluded? Furthermore, the results from this one single mouse experiment, used to derive all factors in the text, appears to be have a higher tumor incidence rate than even other mouse studies performed by the NTP. In essence, the data used represents the most sensitive gender of the most sensitive study of the most sensitive strain, and all other NTP results are discarded."

"#2) Linear extrapolation was used to derive an LED10 at 95% confidence interval (not an ED10)."

"#3) The largest of several slope factors was chosen as the sole parameter to derive the slope (rather than the mean of all experiments)."

"The latter two are critical as #2 vastly overestimates true risk even for the model used. Regarding #3, not only was the largest slope factor [chosen], but this factor is vastly higher than other slope factors for other rodent studies done by the NTP (perhaps by over a magnitude)."

Response 5. The methods used in a cancer dose-response assessment are intended to be health-protective, but whether the methods result in an underestimate or overestimate of "actual" risk is usually unknown. For example, it is not known whether the most sensitive strains of rats and mice have been chosen for the carcinogenicity study, since only one strain of each species was studied. All the applicable data were considered, as discussed above. The linear extrapolation method for calculating cancer potency is the method of choice when the mode of action is unknown (U.S. EPA, 2005),

and the 95th percentile lower confidence limit on the benchmark dose for a 10% tumor response (i.e., LED_{10}) is the most common benchmark for extrapolation.

OEHHA did not choose the largest available slope factor from the models evaluated, nor calculate the proposed PHG based on the most sensitive sex, as described in the response to comment 1 above. It is unclear whether the commenter may have been alluding to the NTP studies on chromium picolinate as other data available. OEHHA did not consider these data relevant because this compound is an organic complex of Cr III.

OEHHA sought examples to determine how the U.S. EPA is using the 2005 guidance (or an earlier draft version of this guidance) in conducting cancer risk assessment. Only one example was identified for an analogous situation (vinyl chloride, where tumors occurred in males and females of two species; U.S. EPA, 2000). The U.S. EPA developed four slope factors based on the results in male and female rats and mice. The most conservative estimate was recommended, with this statement:

> "The oral slope factor and inhalation unit risk calculated for VC are presented in Table 9 (LMS model) and Table 10 (95% lower bound on the ED10). The values calculated using these two methods were very similar. The oral slope factor using the LMS model was determined to be 7.2×10^{-1} per (mg/kg)/day. Inhalation unit risk estimates of 2.6, 2.1, 1.0, and 4.4×10^{-6} per g/m^3 for male mice, female mice, male rats, and female rats, respectively were derived. The more conservative estimate of 4.4×10^{-6} per·g/m^3 is recommended."

When developing health-based criteria, OEHHA routinely selects the data set from the most sensitive species and sex if multiple data sets (of sufficient quality) are available. In addition, when tumors are observed in more than one site, the site with the highest incidence of tumors or which yields the highest cancer potency is routinely selected. This approach is taken because the actual carcinogenic potency in humans is unknown, because of the variability of effects in humans, and because of the mandates to protect sensitive human populations.

Recommendations and guidelines supporting this approach include:

- "Since humans vary widely in sensitivity and some individuals are likely to be as sensitive as the most sensitive animal species, a common procedure is to use the most sensitive system as the basis for extrapolation. This procedure was explicitly recommended by the U.S. Inter-Agency Regulatory Liaison Group (IRLG) which stated, 'the use of data from less sensitive species is justifiable only if there are strong reasons to believe that the most sensitive animal model is completely irrelevant to a segment of the exposed human population.' OSHA justified the same procedure on grounds of prudence: It is prudent for public health reasons to use the data for the most sensitive system as the basis for extrapolation." From California's Guideline for Chemical Carcinogen Risk Assessments and Their Scientific Rationale (CDHS, 1985).
- "For a given chemical, the model was fit to a number of data sets. As discussed in the section above, the default was to select the data for the most sensitive target organ in the most sensitive species and sex, unless data indicated that this

was inappropriate." From OEHHA Air Toxics Hot Spots Program Risk Assessment Guidelines (OEHHA, 2005).

- "(3) Risk analysis shall be based on the most sensitive study deemed to be of sufficient quality. (4) The results obtained for the most sensitive study deemed to be of sufficient quality shall be applicable to all routes of exposure for which the results are relevant. (5) The absence of a carcinogenic threshold dose shall be assumed and no-threshold models shall be utilized. A linearized multistage model for extrapolation from high to low doses, with the upper 95 percent confidence limit of the linear term expressing the upper bound of potency shall be utilized. Time-to-tumor models may be appropriate where data are available on the time of appearance of individual tumors, and particularly when survival is poor due to competing toxicity." From California Code of Regulations, Title 27, Chapter 3. Safe Drinking Water and Toxic Enforcement Act of 1986, Article 7. No Significant Risk Levels, §25703. Quantitative Risk Assessment.

Comment 6, page 3: "However, all the NTP2007 studies need to be analyzed and slope factors derived for each study by an accepted methodology. Then the mean median (preferably) slope factor is to be utilized for subsequent calculations. NOT the 95% confidence interval."

"Note that the use of a mean or median ED10 (not a 95% confidence interval) is also described in the EPA document."

<u>"Furthermore, the average slope factor (not the upper and lower limits) is to be used to generate the slope factors. Thus, risk assessors should calculate, to the extent practicable, and present the central estimate and the corresponding upper and lower statistical bounds (such as confidence limits) to inform decision makers."</u>

"The ED10 used to generate a human equivalent dose) should be calculated by using all available rodent data considered reliable (e.g. all data in NTP2007B report). Do not restrict the data to one gender from one experiment from one species that is highly susceptible compared to other rodent species (or even other strains of the species)."

"Then the mean value for all studies determined and this value is used to derive the human equivalent dose, which is then used to generate the desired standard."

Response 6. As described earlier, the U.S. EPA (2005) guidance recommends that the LED_{10} value be employed to derive the slope factor. OEHHA presents the ED_{10} values (the "central estimate" referred to above) as well as the LED_{10} values in Tables 10 and 11, but in accordance with the U.S. EPA guidance, the LED_{10} value is employed as the point of departure (POD) to generate the slope factor. Given OEHHA's statutory mandate to be health protective and to protect sensitive populations, the LED_{10} is the appropriate value to use as the basis of the POD.

The discussion in U.S. EPA (2005) of central estimates is in the context of a formal uncertainty analysis, as follows:

> "For example, it may be appropriate to emphasize the central estimate in activities that involve formal uncertainty analysis that are required by OMB

Circular A-4 (OMB, 2003) as well as ranking agents as to their carcinogenic hazard. Thus, risk assessors should calculate, to the extent practicable, and present the central estimate and the corresponding upper and lower statistical bounds (such as confidence limits) to inform decisionmakers."

Guidelines for conducting such an uncertainty analysis for cancer risk extrapolation from animal data have never been provided, and no cancer risk assessment meeting the OMB criterion has yet been produced by U.S. EPA. However, OEHHA does acknowledge in the Risk Characterization section of the PHG document the various uncertainties inherent in cancer risk assessment.

Combining or pooling the results of individual studies can be appropriate under various conditions, especially when the endpoint appears to be a measure of the same effect in independent experiments. Combining or pooling data on different effects (different tumor sites in different species, for example) is very problematic. One could envision combining or pooling data from sites and studies where no significant increase in tumors was observed with sites where there were tumors. This approach would be subject to manipulation, as the dose-response relationship (and therefore the cancer potency) would be a function of the sites and experiments that were selected to be pooled.

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AUGUST 2009 PUBLIC-REVIEW DRAFT

Comments from Sharada Balakrishnan, Practical Innovators Inc.

General Comment and Response

Comment: "Overall, this draft report is a well documented compilation of information on the metabolism and toxicity of Cr(VI) and on the evidence of the carcinogenic potential of Cr(VI) via the oral route."

Response. Comment acknowledged. No response needed.

Specific Comments and Responses

Comment I-1: "Although the Felter and Dourson study did show no responses are likely to be detected below 4 ppm of Cr(VI), there are several factors that affect the interpretation of the results – individual susceptibility, different compounds used in the testing and the fact that levels required to elicit a reaction in previously sensitized persons will be quite variable."

Response I-1. The Immunotoxicity section has been revised as suggested.

Comment I-2: "The average value of Cr(VI) in the urine of the 4 volunteers is listed. What is the standard deviation of this value?"

Response I-2. The standard deviation (7.7 µg) has been added.

Comment I-3: In the Summary section, page 2, last paragraph, it is mentioned that "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." As detailed in the Results section (page 61) of Cancers of ingestion- and digestion-related organs reported in occupational studies, this data is indicative but not all-conclusive. Therefore, the summary statement must be revised to reflect that."

Response I-3. The Summary has been revised accordingly.

Comment I-4: "As presented in Appendix II, it is believed that infection with Helicobacter pylori is likely to increase susceptibility to the occurrence of stomach cancers, this hypothesis should not be relegated to the Appendix section or to a small comment in the Sensitive subpopulations section but at least a summary-synopsis should be discussed within Sensitive subpopulations."

Response I-4. OEHHA has received a number of different opinions concerning whether the discussion of Helicobacter and Cr VI is too speculative to include in the main body of the PHG. The current consensus is to keep it in the Appendix.

Comment I-5: "Comments by reviewer Dr. Robert Gwiazda, (Detailed review, point A) has raised an important point 'It is assumed that the fraction of Cr(VI) that is reduced to Cr(III) is the same at high exposures, at the point of departure, at lower exposures and at the protective level.' What is the authors' view on this? Currently it is not clear if low doses of Cr(VI) will also evade reduction and/or cause DNA damage in the oral cavity or GI tract."

Response I-5. It is true that the dose-response curve in mice for intestinal tumors versus ingested dose of Cr VI is assumed to be linear from the point of departure down to zero dose. It is also true that such an extrapolation assumes that the same fraction of ingested Cr VI dose is available (i.e., not reduced to Cr III) to induce tumors as the dose is decreased. Unfortunately, we do not have data in the mouse to know exactly what fraction of ingested Cr VI is reduced to Cr III at low dose levels. Therefore, in accordance with standard risk assessment practice for a genotoxic carcinogen (U.S. EPA, 2005; OEHHA, 2009), we assume a linear relationship between ingested dose and tumor incidence. The PHG document discusses a number of studies in which

doses of Cr VI below the point of departure (LED_{10}) resulted in toxicity and/or measurable tissue uptake of Cr VI, indicating that some fractions of administered Cr VI evaded reduction.

Comment I-6: "I think it is not correct to argue that the absorption [of Cr VI] is similar. Looking at the data, one can argue that there is high variability in humans and this is likely in rats as well. There is not enough evidence to assume that the absorption is the same."

Response I-6. We agree. The PHG document presents the limited data but makes no judgment as to whether absorption is similar or different between the two species.

Comment III-1: "Evidence in literature suggests increased susceptibility to cancer from early-life exposure, particularly for chemicals acting through a mutagenic mode of action (Barton et al, 2005, USEPA Supplemental Guidance 2005 and OEHHA 2009)…The PHG derivation should take into account an age dependent adjustment factor (ADAF) or age sensitivity factor (as also recommended by OEHHA's own guidelines). The PHG needs to be recalculated accordingly."

Response III-1. We agree. The PHG was revised to address early in life exposures to carcinogens. The mutagenic mode of action described by McCarroll et al. (2010) has been cited as well.

Comment III-2: "There is no explanation in the draft document on why the multistage model and corresponding LED 10 value was picked for derivation of the cancer slope factor when there are other LED 10 values with higher potency (such as the Quantal linear model in male and female mice) that could have been more conservative and health protective."

Response III-2. The document was revised to only present the multistage model results. As now stated in the document, this is the model preferred by OEHHA (2009) and U.S. EPA (2010) for conducting cancer dose-response assessments (U.S. EPA, 2005; OEHHA, 2009). This is primarily due to the multistage model's generally good fit of the data in the relatively high dose range used in rodent bioassays (Armitage and Doll, 1961).

Comment III-3: "On page 43 under the NTP 2007b study, the body weight gains of the rat are discussed but there is no mention about the water consumption. Please add a few sentences to explain the water consumption, which presumably is just like the mice data which was reduced in the highest dose groups (this is mentioned in the Mice section, Page 47)."

Response III-3. Water consumption was also reduced in the rat. This information has been added to the text.

Comment III-4: "As detailed on Page 46 under the section Neoplasms, it is indicated that in the NTP 2007b study there were other tumors in male rats (benign pheochromocytomas) and female rats (adenomas in the clitoral gland). The authors have not indicated how many animals were affected and what is the historical rate of such tumors in male and female rats? Has NTP addressed these tumors? Is there an

explanation on their occurrence and their significance? Can they be dismissed even though they were statistically significant?."

Response III-4. For both of these tumor types there were more tumors at lower Cr VI concentrations than at the higher concentrations. This precluded their use in dose-response assessment, as discussed in the PHG document. NTP stated "the relationship of these changes to exposure is uncertain." The historical occurrence of pheochromocytomas in male rats is 12 percent and adenomas of the clitoral gland in female rats is 4 percent.

Comment IV. None of these comments required a response.

Comment V-1: "The subheading Physiologic and Nutritional role (page 21) under the section on Metabolism and Pharmacokinetics seems unnecessary. The last sentence (about dietary intake of chromium) of this point on nutritional role has been discussed under Food (page 6). I think that there is no specific need to mention it again here. The first two sentences of this paragraph can also be mentioned under Food and this heading eliminated from this section."

Response V-1. The PHG document has been revised accordingly.

Comment V-2: "The heading on Page 22 is Toxicological effect in Animals and Plants but there are no effects in plants discussed anywhere in the document. The index should also be changed accordingly."

Response V-2. The PHG document has been revised accordingly.

Comment V-3: "On page 56, the last sentence in the first paragraph under the heading Non-oral routes reads "Although the data are rather sparse, it appears that rodents are relatively insensitive to hexavalent chromium when it is administered by inhalation." Did the authors mean to write trivalent chromium since it is apparent that hexavalent chromium causes toxicity via inhalation in rodents?."

Response V-3. Our reference to Cr VI is correct. Cr VI is clearly carcinogenic in humans via inhalation. Few studies have been performed with rodents exposed via inhalation. Those that have been performed suggest that it is not a potent carcinogen in rodents, but more data are required.

Comment V-4: "…for completeness, a small paragraph of Discussion/Conclusion/Summary should be included at the end of this section that indicates the conclusion of the authors based on the results i.e the presence of a "suggestive link between inhalation exposure in epidemiological studies and ingestion related cancer."

Response V-4. The PHG document has been revised as suggested.

Comment V-5: It appears as if undue time/space is devoted to the Helicobacter hypothesis and Borneff study…this can be represented in a short summary in the main document itself."

Response V-5. Discussion of Borneff et al. (1968) was moved to the Appendix per the recommendations of peer reviewers. We find it appropriate as it was not used directly for the calculation of the PHG, but its inclusion serves as a scientific resource and as a

record of the issues that have been addressed in the research for and preparation of this PHG document.

Comment V-6: "Under the section Vagotomy (page 133), it would be clearer to add 1-2 sentences to describe what it is and why it is used."

Response V-6. The PHG document has been revised as suggested.

Comments from Mitchell D. Cohen, New York University School of Medicine

General Comment and Response

No general comment was made.

Specific Comments and Responses

Comment 1: "In response to the 2008 Reviewer's comments, the OEHHA modified the PHG to contain a new section (c.f. Appendix A) dealing with the issue of Cr6+ absorption and its relation to any potential 'carcinogenic threshold', and to revise the 'Metabolism and Pharmacokinetics' portion of the document to better address this issue. OEHHA also indicated in these responses that the reducing equivalents that may be key to the reduction of Cr6+ to Cr3+ appear to come from dietary proteins rather than from gastric acid. This is an important point for deriving the PHG in light of the increased consideration of sensitive populations (i.e., those with anomalous pH regulation due to disease or medications). However, there is no mention of this potential alternative pathway for reduction of ingested Cr6+ in this version of the Draft."

Response 1. It is not an alternate pathway. The acidic environment appears to act like a catalyst for the reduction of Cr VI to Cr III. We used the word "appears" in our response to the 2008 Reviewer's comments because it is not certain what factor or factors limit Cr VI reduction in the human stomach. Therefore, we prefer not to speculate on this subject in the PHG document.

Comment 2: "This revised portion of the Draft, in citing the Finley et al. (1997) study showing that administration of Cr6+ (over a range of 0.1 – 10 mg Cr6+/d, for 4 d) did not cause dose-related changes in the percentage of Cr6+ in the urine of human subjects, concludes that "results of these studies do not indicate that oral absorption of administered Cr6+ begins to occur when the reducing capacity of the stomach is exhausted." This Reviewer questions if insertion of the term "only" before "begins" would be more in keeping with the intention of the OEHHA. As it currently reads, this statement could be interpreted to suggest that there is *always* some Cr6+ that will pass into the GI tract intact rather than only occurring if/when the local ability to reduce ingested Cr6+ is overwhelmed, an outcome with its own toxicologic ramifications (Editorial note: the Finley results are in 'total Cr present in urine', not Cr6+ as could be inferred from the corresponding sentence on Page 12)."

Response 2. The PHG document has been revised by inserting the word "only" and indicating that total chromium and not hexavalent chromium was measured in the urine.

Comment 3: "There clearly are more studies on the immunotoxicologic impact of Cr6+ exposure than are provided here – some of these unexplored studies have dealt with effects upon host resistance, changes in functionality of macrophages, etc. Many of the

Cr-induced alterations induced in phagocytes have the potential to also impact on host resistance against tumor cells. Thus, an expansion of the Immunotoxicity section of the document would have greatly strengthened the overall accuracy and completeness of the Draft."

Response 3. The Immunotoxicity section of the PHG document has been expanded. How effects on immunologic parameters such as host resistance and macrophage functions could affect tumor occurrence resulting from oral Cr VI exposure is not known.

Comment 4: "Nevertheless, as noted in some of the comments from the Public sector, the 'lack' of negative results could be disconcerting…However, it is also essential that non-toxic outcomes be reported to provide the proper context and completeness necessary for valid conclusions to be made about the overall toxicity of any given agent, including Cr6+."

Response 4. The Genotoxicity section of the PHG document has been rewritten. It now includes the in vivo studies that yielded negative results, as suggested in some of the Public Comments.

Comment 5: "The sentiment that only a full explanation of all MOA for Cr6+ should be presented before any PHG can be derived and accepted is illogical. This would be akin to stating that any government-based warnings about smoking and cancer should not be offered even at this point in time since the precise MOA are still evolving [after >50 years]."

Response 5. OEHHA agrees. It should be pointed out that the Cr VI cancer mechanism is discussed in detail in the document, in particular where the weight of the evidence carcinogenicity determination is made (see "Examination of Evidence for Chromium Carcinogenicity" section of the PHG).

Comment 6: "Another study discussed by the 2008 Reviewers for possible inclusion in the Draft was that of Bednar and Kies (1991) which found no relationship between exposure to "Cr6+ in drinking water" and total cancer mortality…This Reviewer believes that inclusion of this study (as an example of a 'non-outcome'-type study that the 2008 Reviewers felt necessary to include to provide scientific balance to all the other studies indicating Cr6+-induced effects and thereby mitigate any perceived "selective bias") would have greatly strengthened the overall accuracy and completeness of this Draft."

Response 6. Discussion of Bednar and Kies (1991) and Fryzek et al. (2001) has been added to the document.

Comment 7: "Optimally, the OEHHA should have presented calculated PHG values based upon both the ED_{10} and the LED_{10} (thereby giving rise to a PHG range of 0.06 - 0.11 ppb). However, as noted above, a most conservative approach should be used when dealing with a risk for the potential for causing cancer in exposed populations. Thus, **the determination has been made that use of the LED_{10} was appropriate for generating the PHG for human exposures to Cr^{6+} in drinking water."**

Response 7. Revised tables in the PHG document contain both the LED_{10} and ED_{10} values used for the cancer potency estimates in male (Table 10) and female (Table 11)

mice. Only the LED_{10} was used to develop the PHG. Developing multiple final PHG values was judged not useful.

Comment 8: "It is clear that the data presented in the Draft document (c.f. Figure 13; Editorial note: abscissa needs the addition of units as the values shown do not correspond to any of the reported doses in Table 5 and 6) shows that tumor formation in the mice as a function of Cr^{6+} level in drinking water is not linear."

Response 8. In the 2008 NTP study statistically significant increases in tumors of the small intestine were observed for both male and female mice at the two highest drinking water concentrations. Exact trend tests were positive for both sexes. The absence of statistically significant increases in tumors at the two lowest drinking water concentrations may be due to the small number of animals tested. The use of high doses in cancer bioassays is generally thought to offset the statistical limitations of using small numbers of animals (50 /sex/dose level) to measure a relatively rare event (tumors). Figure 13 from the draft PHG document referred to here has been removed.

Comment 9: "Reviewer comment: The information provided in the OEHHA response to the Reviewer noted here [2008 Peer Reviewer Michael Kelner] should also be placed in the Draft document to provide critical clarity for readers and others who will rely upon the Draft for making important decisions regarding Public Safety/Health matter."

Response 9. This information has been added to the "Dose-Response Assessment" section of the PHG document.

Comment 10: "The second issue is about the use of the 2 L/day value for modifying the 0.6 $(mg/kg\text{-}day)^{-1}$ slope factor value (originally derived on Page 78) in the final PHG estimate. This conflicts with the fact that in the Table 17 data displaying the HPD values for non-cancer endpoints, an adult consumption of water is presumed to be ~ 3.7 L/day (to yield 0.053 L/day value noted in the footnote)."

Response 10. The water consumption values associated with Table 17 (non-cancer endpoints) and Table 18 (cancer endpoint) are now correct.

Comments from Toby Rossman, New York University

General Comment and Response

Comment: "Attached is my review of the chromium document. I have some serious reservations about the mechanistic aspects, as you will see."

Response. See below for OEHHA's responses to each specific comment made by this reviewer.

Specific Comments and Responses

Comment 1-1: "DNA damage *per* se does not inform us about eventual heritable change, which is the true issue. Assays that do not depend on the survival of genetically-altered offspring (i.e. chromosome aberrations, SCE, micronuclei) are only suggestive."

Response 1-1. The *in vivo* genotoxicity studies reported in Table 2 of the PHG document are short-term assays that do not depend on the long-term survival of the

genetically-altered cells. For mutation induction by Cr VI, which generally does depend on long-term survival, see the first paragraph of the "Genetic Toxicity" section of the PHG document for references to recent reviews that discuss experiments with cultured mammalian cells and bacteria. Nonetheless, evidence of chromosome breakage implied by the appearance of chromosome aberrations, micronuclei or SCE (Table 2 of the PHG document) has traditionally been considered an important part of the discussion concerning mechanism of action.

Comment 1-2: "The description of an agent as a "genotoxic carcinogen" is out of date. What we really need to know is whether an agent has a mutagenic mode of action (MOA)."

Response 1-2. Genotoxicity continues to be an important consideration in discussions of MOA. The in vivo evidence for genotoxicity is presented in Table 2 of the PHG document. The evidence for mutation induction by Cr VI is presented in some recent review articles cited in the first paragraph of the "Genetic Toxicity" section of the PHG. We now note in the document that a mutagenic mode of action has been fully described and justified by McCarroll et al. (2010). Further, the described mode of action is consistent with extrapolation procedures used in the risk assessment.

Comment 1-3: "Cr(VI) is only weakly mutagenic in mammalian cells, rarely giving more than a 3-fold increase in mutant fraction over background levels (in endogenous genes), and in a very narrow (and toxic) dose-range with a strong threshold (reviewed in Nickens et al., in press)…Mutations can result from DNA damage, but can also result from loss of mismatch repair and other types of genomic instability, and in some cases "mutations" are actually epimutations resulting from altered DNA methylation…Salnikow and Zhitkovich also discuss the lack of p53 mutations in Cr-induced lung tumors (which usually have p53 mutations when associated with other agents such as tobacco smoke), and the fact that the few mutations found do not correspond to the types of mutations caused by Cr in *in vitro* systems…Thus, the actual increase in mutant fraction reached only about 1.5-fold over background."

Response 1-3. There are a number of published studies which report robust (in excess of 3-fold increases) mutagenic responses of cultured mammalian cells to Cr VI (see Paschin et al. (1983) Mut Res103(3-6):345-347; Mitchell et al. (1988) Environ Mol Mutagen 12(Suppl 13):37-101; Myhr and Caspary (1988) Environ Mol Mutagen 13(12):103-194; McGregor et al. (1987) Environ Mutagen 9(2):143-160; Oberly et al (1982) J Toxicol Environ Health 9(3):367-376). Reviews discussing these studies are cited in the first paragraph of the "Genetic Toxicity" section of the PHG document. Cr VI also caused mutations in bacteria, yeast, D. melanogaster and mice (U.S. EPA, 2010). Loss of mismatch repair could hardly be responsible for the findings reported in Table 2 of the PHG document. Lastly, we would not rule out the plausibility that Cr VI causes tumors by a mutagenic MOA as described by McCarroll et al. (2010).

Comment 1-4: "It is of interest that most of the *in vivo* drinking water experiment described in the document gave negative results for genotoxicity, except for the high dose of 100-200 ppm (Coogan et al., 1991)."

Response 1-4. Table 2 in the PHG document updated with the results of NTP (2007) now cites nine drinking water studies of which three are positive for genotoxicity, and 21

total studies (drinking water or gavage) of which 10 are positive for genotoxicity. OEHHA believes these data demonstrate that Cr VI is genotoxic by the oral route.

Comment 1-5: "In the case of Cr(VI), most of the micronuclei are kinetecore-positive, meaning that they arise from malsegregation (Seoane and Delout, Mutat. Res. 490:99-106, 2001; Figgitt et al., Mutat. Res. 688:53-61, 2010)."

Response 1-5. Ten *in vivo* studies were positive for genotoxicity (Table 2 of the PHG document). Of these, one was a micronucleus study (NTP, 2007). Micronuclei were not assayed for the presence of kinetochores in this study. In addition, both studies cited in this comment also reported increases in cells with chromosome fragments relative to controls, demonstrating genotoxicity.

Comment 1-6: "There is evidence that food contains Cr(VI) as well as Cr(III). In fact, all parts of grain contain Cr(VI) and 10% of the Cr in bread is Cr(VI) (Mishra et al., Food Chem. Toxicol. 33:393-397, 1995; Soares et al., J. Agric. Food Chem. 58:1366-1370). It is possible that dietary Cr(VI) is significant and should be evaluated."

Response 1-6. Mishira and associates studied the uptake of Cr VI into the corn plant but did not speciate the form of Cr in the plant. Soares and coworkers did speciate between total chromium and Cr VI in finished bread and this paper has been added to the "Food" section of the PHG document. For non-carcinogens, a relative source contribution (RSC) factor is used to derive the PHG. The choice of the RSC is based on how much exposure comes from sources other than drinking water. A public health-protective concentration for Cr VI in drinking water based on noncarcinogenic effects is based on 20 percent of the total daily exposure to Cr VI coming from other (non-water) sources. The health protective dose (HPD, referred to as acceptable daily dose or ADD in the final PHG document) of 0.0002 mg/kg-day or 14 µg/day was for an adult male. Twenty percent of the ADD is 2.8 µg/day which is considerably higher than the level of 0.6 µg/day due to intake from bread reported by Soares and coworkers (now discussed in the "Food" section of the PHG document).

Comment 1-7: "It is incorrect to say the relative contribution of the various species to DNA damage is unknown when the most recent reference given is 2000…The intracellular reduction of Cr(VI) is non-enzymatic. Reductants are ascorbate (major), GSH, other thiols, maybe NADH…The second paragraph on p. 42 should be deleted and replaced with up-to-date material."

Response 1-7. The section entitled "Mechanism of Genotoxicity and Carcinogenicity" has been revised and updated.

Comment 1-8: "The experiment by Davidson et al. is not a non-oral route, it is a cocarcinogenesis experiment with solar UV and Cr(VI) in drinking water."

Response 1-8. We reported this study together with non-oral studies because its protocol is not typical of oral studies (highlighting this fact). It is a co-carcinogen type study, many of which were done by skin painting of two carcinogens together.

Comment 2-1: "The data set is probably the best available. However, the calculations are confusing...Concerning the dose/response relationship in Fig. 13: What are the units on the axes? Where are the error bars or 95% C.I.?."

Response 2-1. Figure 13 from this draft has been dropped from the PHG document. The mouse tumor data are now presented in Tables 5 and 6. The discussion has been revised.

Comment 2-2: "The paragraph on the historic rate of small intestine tumors is confusing…In any case, the final sentence in the first paragraph is nonsense. Statistical analysis decides."

Response 2-2. This paragraph has been revised. A sentence has been added to clarify that tumors are for 1) duodenum, or, 2) for the entire small intestine. While statistical analysis is important, increases in rare tumors are often a concern even when the increase is not statistically significant. In this case the increase was for a rare tumor that also happened to be statistically significant.

Comment 3-1: "The assumption is that Cr(VI) in drinking water has a mutagenic MOA with no threshold."

Response 3-1. OEHHA does not know the mechanism by which Cr VI causes cancer in humans or animals. The review of possible mechanisms includes a number of genotoxicity studies that are consistent with a non-threshold mechanism. As the reviewer indicates, there are studies that suggest other mechanisms. The document now cites the mutagenic mode of action described by McCarroll et al. (2010).

Comment 3-2: "A "genotoxic" agent does not necessarily cause tumors by a mutagenic MOA. Cr(VI) is only weakly mutagenic in animal cells (it is more mutagenic to bacteria). Furthermore, the mutagenicity occurs only at toxic doses in a narrow range (i.e. it has a threshold)."

Response 3-2. It is not unusual for mutagens to also cause some cell killing in the dose range where mutations are induced. See Response 1-3 for a discussion of the magnitude of the mutagenic response of cultured mammalian cells to Cr VI. With regards to experimental thresholds, this is to be expected. As the dose is lowered, at some point the experimental system will not be sufficiently sensitive to measure the change.

Comment 3-3: "Other MOA's have not been considered. These include, for example, selection for Cr-resistance (involving epigenetic changes) and aneuploidy. These events generally show thresholds."

Response 3-3. Various effects of Cr VI on DNA are discussed in the PHG document. However, given the evidence of reactions between Cr VI and DNA that could result in a non-threshold dose response relationship, the dose response relationship for calculation of the PHG is based on a non-threshold mechanism.

Comment 3-4: "In the NTP study, there is no statistically significant increase in tumors below 85.7 mg/L. Is this taken into account in deriving the slope? What would happen if a threshold were included?."

Response 3-4. In many NTP studies, statistically significant increases in tumors are not detected in the lowest dose group. This may be due to the inability to detect a low number of tumors at the low dose levels (too few animals), not a mechanistic threshold. The multistage model used to derive the dose response relationship in male mice used

all dose groups. If a different model were used that gave a threshold at the lower dose levels, the PHG would be different.

Comment 3-5: "Please consider the recent meta-analysis of cancers of the G.I. tract among those occupationally exposed to Cr(VI), which concludes that these workers are not at greater risk than the general population (Gatto et al., Cancer Epidemiol. 34:388-399, 2010). Inhalation exposure usually also leads to G.I. exposure, so this also suggests a possible threshold if the ingested dose can be estimated."

Response 3-5. The study by Gatto et al. (2010) is discussed in the "Toxicological Effects in Humans" section of the PHG document. Its conclusions as quoted above may or may not be evidence of a threshold. The inability to detect significant increases in GI tract tumors may be due to the small population studied or the low doses of Cr VI swallowed. GI tract tumors may have been detected if the population comprising the study had been larger.

Comment 3-6: "Using the LED_{10} is overly conservative."

Response 3-6. Use of the LED_{10} is recommended for cancer risk assessment (U.S. EPA, 2005; OEHHA, 2009).

Comment 4: "I would just add that Cr(VI) in food may be more significant than assumed. The fact that Cr is essential also implies that oral Cr(VI) could supply the necessary Cr(III), again implying a threshold."

Response 4. The study by Soares et al. (2010) on the Cr VI content of bread has been added to the "Food" section of the PHG document. The knowledge on the essentiality of chromium in the mammalian diet is still being developed and chromium essentiality has recently been questioned by Di Bona et al. (2010, J Biol Inorg Chem 16(3):381-90).

Comments from William Shotyk, University of Heidelberg

General Comment and Response

Comment: "In general, the PHG report is excellent and I have no significant criticisms. However, I have some minor, general comments which are made below, and a few specific remarks about the PHG of 0.06 parts per billion of Cr in drinking water; these comments are based on my experience measuring Cr in natural freshwaters, including ground waters and surface waters."

Response. Specific responses to each comment are provided below.

Specific Comments and Responses

Comment 1: "The units employed for concentration are inconsistent, sometimes on the same page, including mg/L, mg/kg, and ppm (parts per million); this probably reflects the concentration units employed in the original publications and is a general problem in reviewing scientific literature, not something unique to this report."

Response 1. The reviewer is correct. The PHG document utilizes the units provided by the study's authors so there is no confusion as to which dose is discussed by OEHHA when describing the study. So when NTP presents tumors at various dose levels (in mg/L) the PHG document uses the same units to discuss the study. When OEHHA

then develops a dose response relationship from the study, the units are often transformed. For the NTP study, the dose in mg/kg-day was derived using water consumption rates and body weights provided by NTP.

Comment 2: "Also, given the number of abbreviations used throughout the report, a Table summarizing and defining these would also be helpful."

Response 2. Each abbreviation is spelled out the first time it is used in the document.

Comment 3: "On p.21 it is indicated that "trivalent chromium is an essential mineral", but "element" would be more appropriate than "mineral"…The occurrences of CrO_3 (there are at least two) should be replaced by Cr_2O_3."

Response 3. The PHG document has been revised accordingly.

Comment 4: "Again, I have no question about how the authors of this report arrived at the PHG value of 0.06 parts per billion hexavalent chromium. I simply wish to indicate that this concentration may be low, relative to the abundance of Cr in natural freshwaters, even when the natural waters are tested using "clean lab" methods."

Response 4. OEHHA will forward these data to the Department of Public Health so they have these data when they develop the MCL for hexavalent chromium.

Comment 5: "One final, personal remark about Cr and contact dermatitis…I have no data about Cr release rates from these materials, only these observations, but it is difficult to imagine parts per million levels of Cr being released from a stainless steel watch bracelet coated with either Au/Rh or Ti."

Response 5. In addition to chromium other metals such as Ni are components of stainless steel. Nickel is also associated with skin sensitization. Thus, it is difficult to know what is precipitating skin sensitivity. Also, it is not clear how much of the Cr in stainless steel is in the form of Cr VI.

Comments from Elizabeth Snow, University of Tasmania

General Comment and Response

Comment: "Having carefully read and evaluated the above mentioned document (PHG for Cr6) it is my considered opinion that the document is based on the best available scientific knowledge and that the conclusions reached are to the best of my knowledge and understanding both accurate and complete."

Response. Comment noted.

Specific Comments and Responses

Comment 1: "Based on these data it is clear that environmental exposure to Cr6 in drinking water can pose a potential risk for human carcinogenesis."

Response 1. OEHHA agrees.

Comment 2: "A cancer potency estimate of 0.6 $(mg/kg\text{-}day)^{-1}$ derived following standard guidance from the U.S. EPA and OEHHA (U.S. EPA 2005, OEHHA 2009), resulted in an extrapolated 1 in 10^6 lifetime cancer risk level for Cr6 in tap water of 0.06 ppb."

Response 2. The PHG document now has a slightly lower cancer potency estimate (calculated from the LED_{10}) of 0.5 $(mg/kg\text{-}day)^{-1}$ compared to 0.6 in the previous draft. This is due to rounding and use of the NTP (2008) study values for ingested dose of Cr VI in the December 2010 draft and final PHG documents rather than ingested dose as calculated by OEHHA in the August 2009 draft. The PHG value in the current document is 0.02 ppb due to correction for early-in-life exposures to carcinogens (as described in OEHHA, 2009).

Comment 3: "Based on this study, along with very limited evidence of tumor response at lower levels of Cr6, there is very limited evidence for a linear dose response."

Response 3. This is the case with most carcinogens. Dose-response data are not available in the low dose region where human exposures are expected.

Comment 4: "It is more likely, due to the high probability of extracellular conversion of the Cr6 to the much less toxic Cr3, that uptake and bioavailability of the Cr6, in itself, will exhibit a non-linear (threshold) dose response."

Response 4. This may or may not be true. The PHG document contains examples of Cr VI absorption at dose levels that are far below the calculated capacity of the GI tract of humans and rodents to reduce all ingested Cr VI to Cr III. The PHG document also discusses examples where Cr VI absorption was not concentration dependent.

Comment 5: "A low dose, linear response also assumes a lack of DNA repair and other protective mechanisms with an expected maximum protective effect at low dose."

Response 5. A linear cancer response at low dose levels is consistent with DNA repair. Consider radiation induced carcinogenesis, the best data set we have covering cancer induction by low dose levels of any genotoxic carcinogen (radiation-induced cancer in human A-bomb survivors). The cancer incidence responds linearly at low doses of radiation despite the well-characterized ability of mammalian cells to repair potentially lethal DNA damage (PLD repair). The linear dose response is also recommended by U.S. EPA Cancer Guidelines (2005) based on the described mutagenic mode of action (McCarroll et al., 2010).

Comment 6: "The essential nature of Cr3 as a required trace element should also be considered as oral Cr6 is expected to be reduced to Cr3 for which there must be some sort of uptake mechanism in order to supply this nutrient to the body."

Response 6. The presumption is that the chromium needed by the body is absorbed at a slow rate as Cr III and not as Cr VI. The understanding of the essentiality of Cr is still developing and this essentiality in the mammalian diet has recently been questioned by Di Bona et al. (2010, J Biol Inorg Chem 16(3):381-90).

RESPONSES TO MAJOR COMMENTS RECEIVED ON AUGUST 2009 DRAFT, FIRST COMMENT PERIOD (2009)

Comments from Silvio De Flora, University of Genoa

Comment 1: "Although it is evident that Cr(VI) detoxification mechanisms represent formidable barriers against Cr(VI) toxicity, genotoxicity, and carcinogenicity, I do not pretend that they are infinite and cannot be saturated. Under certain conditions, especially in animal models, they may be overwhelmed as a function of the dose and of the administration route (see Comment #9). Therefore, the statement, reported on page 17 of the OEHHA Document, that according to my studies the Cr(VI) detoxifying mechanisms in the organism are "essentially inexhaustive" does neither reflect my opinion nor what is written in my papers."

Response 1. The sentence was revised and quotes from the investigators are now included in the text.

Comment 2: "On page 37 is stated that no study to date has looked for DNA damage in the oral cavity or gastrointestinal tract following oral administration of Cr(VI). It is also stated that these studies are needed. The authors of the document overlooked our *ad hoc* study (S. De Flora *et al.*, *Mutat. Res.* 659, 60-69, 2008), in which we demonstrated that the daily administration of sodium dichromate to SKH-1 mice, at the doses of 5 or 20 mg/L for 9 consecutive months, failed to enhance the frequency of DNA-protein crosslinks and did not cause oxidative DNA damage, measured in terms of 8-oxo-dGuo, in mouse forestomach, glandular stomach, and duodenum."

Response 2. The Genotoxicity section of the document has been revised and now discusses De Flora et al. (2008). However, judging from the responses of the positive controls in De Flora et al. (2008), it is likely that their methodology lacked the sensitivity to measure DNA damage at the dose levels tested. In addition, McCarroll *et al*. (2010) noted that the levels tested in this study may in part explain the negative results because they are below the exposure levels used in the NTP 2-year drinking water study.

Comment 3: "Table 2 and pages 37-41 of the OEHHA Document summarize studies on the genotoxicity of Cr(VI) administered by the oral route. Again, relevant literature data were overlooked. In a study of mine (S. De Flora et al., Mutat. Res. 610, 38-47, 2006)…"

And "Surprisingly, unless I missed them somewhere else in the document, even the NTP studies evaluating the frequency of micronucleated erythrocytes in peripheral blood were not cited. … "

Response 3. The Genotoxicity section of the document has been revised and now includes these studies.

Comment 4: "As noted in the OEHHA Document, a statistically significant increase of oral cancers only occurred at the highest dose tested (516 mg/L sodium dichromate) in both male and female rats [NTP, 2008]. A statistically significant increase of small intestine tumors only occurred at the highest dose tested in male mice (257.4 mg/L) and at the two highest doses tested in female mice (172 and 516 mg/l) [NTP, 2008]. These are huge doses! One should go to the lab and see the color and appearance of water containing hundreds or even tens mg/L Cr(VI). Nobody would drink this water unless for suicidal purposes (which probably would be unsuccessful, see Comment #8). No effect was observed at the lowest doses tested in the NTP study, corresponding to 5-30 mg Cr(VI)/L water (which still are quite high doses), which is in agreement with the conclusions of our genotoxicity study (S. De Flora *et al*., *Mutat. Res.* 659, 60-67, 2008), ruling out that DNA damage may occur not only in the forestomach and glandular stomach but also in the duodenum of mice receiving sodium dichromate with the drinking water, at the doses of 5 and 20 mg Cr(VI)/L (see Comment #2)."

Response 4. In the 2008 NTP study statistically significant increases in tumors of the small intestine were observed for both male and female mice at the two highest drinking water concentrations. Exact trend tests were positive for both sexes. The absence of statistically significant increases in tumors at the two lowest drinking water concentrations should not be interpreted as a threshold for tumorigenicity, since the number of animals may have been too low to detect tumors at the two lowest drinking water concentrations. The use of high doses in cancer bioassays is generally thought to offset the statistical limitations of using small numbers of animals (50 /sex/dose level) to measure a relatively rare event (tumors). In addition, the study by De Flora *et al.* (2008) assayed only a narrow subset of the different types of DNA damage Cr IV can cause (discussed in the PHG). Also, judging from the responses of their positive controls, it is likely that their methodology lacked the sensitivity to measure DNA damage at the dose levels tested. Lastly, McCarroll *et al*. (2010) noted that the levels tested in this study may in part explain the negative results because they are below the exposure levels used in the NTP 2-year drinking water study.

Comment 5: "It should be noted that in the NTP study there were significant decreases of certain tumors in Cr(VI)-treated rodents, such as a decrease of total benign tumors in both rats (females only) and mice (males only), which by the way was the only concomitant change in the two rodent species, a decrease of pituitary gland tumors in both male and female mice, and a decrease of liver adenomas in both male and female mice, which was the only effect observed at 2 or 3 Cr(VI) concentrations. Clearly, although these decreases are statistically significant, they do not mean that Cr(VI) is protective but highlight the fact that, likewise, significant increases at high doses are not biologically significant and do not bear relevance to the human situation."

Response 5. First, OEHHA agrees that a decrease in tumor incidence does not mean that Cr VI protects against cancer. Second, biological significance does not follow from occasional increases or decreases in tumors. Rather, OEHHA looks for a dose-responsive change exhibiting statistical significance. In mice such a pattern was observed for intestinal tumors. Decreased tumor incidences at higher dose levels have been observed for a number of chemicals tested at the NTP (Haseman and Johnson, 1996; Haseman et al., 1997). In many cases the decreases in tumors were significantly correlated with decreased bodyweights, also commonly observed at higher dose levels. This may well be the reason for the decreases in some tumors observed in the two-year Cr VI bioassay.

Comment 6: "On page 58, last paragraph, it is stated that IARC (1990) concluded that Cr(VI) is a 'strong' carcinogen for the respiratory system. This statement is not correct. As quoted on page 42 of the OEHHA Document, the IARC concluded that 'there is sufficient evidence in humans for the carcinogenicity of Cr(VI) compounds as encountered in the chromate production, chromate pigment industry and chromium plating industries.'"

Response 6. This reference to IARC (1990) has been deleted. IARC conclusions regarding carcinogenicity are reported in the "Toxicological Effects in Animals, Carcinogenicity" section of the PHG document.

Comment 7: "The need for high Cr(VI) doses to induce lung cancer is confirmed by more recent study, such as the Gibb *et al.* (2000) study, which is extensively reported and discussed in the OEHHA Document."

Response 7. Exposures are often higher in occupational studies than what occur in the ambient environment. The ability to detect effects such as cancer in such a small population typically the subject of occupational studies is likely related to the high levels of exposure. This does not suggest that cancer would not occur at lower levels of exposure, but rather one may not be able to detect an increased incidence of cancer in a small population. This is similar to the problem of conducting bioassays in small populations of animals and is addressed by using high doses in these bioassays.

Comment 8: "As everybody knows, the Mancuso's data, that U.S. EPA used for the potency estimate, are highly biased.

Response 8. The strengths and weakness of both the Mancuso (1997) study and the Gibb et al. (2000) study are discussed in cancer potency for the inhalation route section of the PHG document. Our analysis indicates that the two studies are consistent.

Comment 9: "In the last paragraph of page 72, the OEHHA Document concludes that "a summary of the findings of multiple studies where workers were exposed to Cr(VI) by the inhalation route (conducted by OEHHA) was suggestive of a link between inhalation exposure to Cr(VI) and cancer of the digestive organs". This conclusion is surprising and

contrasts with the actual results of the OEHHA study, which are reported in Tables 7 and 8 on pages 62-69. In fact, taking into account statistically significant variations, the analysis of 30 studies led to the following results for cancers of the digestive system ..."

Response 9. A new Table 8 shows cancers of a variety of organs as well as nonmalignant respiratory diseases, all in persons occupationally exposed to Cr VI via inhalation. As indicated in the PHG document, for several studies the rate ratios for stomach cancer exceeded one, and in three the associations were statistically significant.

Comment 10: "In addition to the considerations on the carcinogenic potency (see Comment #5) and on the link between inhalation exposure to Cr(VI) and cancer of digestive organs (see Comment #7), the OEHHA Document relies on the Chinese study, whose limitations are extensively discussed on pages 69-71. Note that this controversial study was further examined in a recent article (B.D. Kerger *et al.*, *J. Toxicol. Environ. Hlth*, 72, 329-44, 2009), which is not quoted in the Document."

Response 10. Discussion of the Kerger et al. (2009) study has been added to the PHG document. It is not clear what is meant by "the OEHHA document relies on the Chinese study." Cancer potency was calculated from the rodent data in NTP (2008). Zhang and Li (1987) was one of two studies identified in which humans were exposed to Cr VI via their drinking water and in which organ-site-specific results were available. The exposed population exhibited a statistically significant increase in stomach cancer mortality. These findings suggest that Cr VI is carcinogenic in humans via the oral route.

Comment 11: "As to the Borneff *et al.* (1968) study, which is extensively reported and discussed both in the text and in Appendix D of the OEHHA Document, this study was so obsolete, inadequate and full of problems that the IARC Working Group (including myself and other 20 scientists) decided not even to cite it in the 1990 Monograph. Incidentally, it is noteworthy that the Borneff *et al.*, study suggested an increase of forestomach tumors in mice (that even the author interpreted with a great caution) while the NTP study suggested an increase of small intestine tumors in mice. Who is right?."

Response 11. It is not uncommon that different studies detect tumors at difference sites, particularly if different genders and strains were employed.

Comment 12: "Genotoxicity. As previously discussed (Comments #2 and #3), the data reported in the OEHHA Document are largely incomplete."

Response 12. This section of the PHG document has been updated to include discussions of the studies mentioned in Comments #2 and #3 above.

Comment 13: "Toxicokinetics. As previously discussed (Comment #1), I do not pretend that detoxification mechanisms are infinite. In any case, they are formidable barriers that imprint a threshold character to Cr(VI) carcinogenesis (see Comment #9)."

Response 13. OEHHA agrees that the ability to reduce CrVI to Cr III is not infinite. However, there appears to be sufficient reducing capacity (84 mg/day according to the estimates of DeFlora and coworkers) to adequately reduce the amount of chromium VI that was administered to humans in several pharmacokinetic studies. Therefore, the observed absorption of hexavalent chromium in these studies did not occur because the reducing capacity of the GI tract was exhausted. In a recent study of rats and mice exposed to Cr VI via their drinking water, there was no threshold for its accumulation in a variety of tissue (Collins *et al*., Tox Sci 118: 368-379, 2010). Rather, its accumulation was either linearly related to its concentration in the drinking water over the entire concentration range tested, or linearly related at low concentrations with indications of a plateau at higher concentrations. These data are discussed in Appendix A of the PHG.

Comment 14: "This section of the Document summarizes some mechanisms of Cr(VI). Regarding the meaning of the intracellular Cr(VI) reduction, when in 1989 I prepared a review (cited in the Document) together with the late Karen Wetterhahn, the best researcher on Cr(VI) biochemical toxicology ever, we agreed on the interpretation that when Cr(VI) reduction occurs close to DNA target molecules, it is an activation mechanism (uptake-activation theory). However, when Cr(VI) reduction occurs in the cell cytoplasm or in any case far away from DNA, it is a detoxification (uptake-detoxification theory), due to the myriad of intracellular ligands that block Cr(VI) or its derivatives before reacting with DNA. Here is a further mechanism responsible for the occurrence of thresholds in Cr(VI) toxicology."

Response 14. OEHHA has considered your work in the PHG document and finds it informative. While considerable mechanistic research has yielded several plausible mechanisms by which Cr VI may be causing tumors (reviewed in the PHG document), the exact mechanism remains unclear.

Comment 15: "It is surprising that this chapter reaches the conclusion that 'the findings of available human, animal, genotoxic, and toxicokinetics studies all indicate that Cr(VI) is a possible human carcinogen by the oral route.' It is intriguing that all data that were evaluated to be either incomplete or heavily criticized in the document itself now become the starting point to reach the above conclusion and to develop a proposal of PHG."

Response 15. The document does discuss the weaknesses of key studies such as the Zhang and Li (1987) study in China. However, the evidence that Cr VI is carcinogenic by the oral route is compelling. The evidence from various types of studies (toxicokinetic, genotoxic, mechanistic, animal bioassays and epidemiology) is internally consistent, and points to carcinogenesis. Toxicokinetic studies indicate absorption and cellular uptake of Cr VI, the genotoxic and mechanistic studies provide a plausible

mechanism for carcinogenesis and both animal and humans studies reveal evidence of an increased incidence of tumors. In particular, four recent animal bioassays meeting quality standards were positive for tumors in two rodent species, in both males and females, at two sites (NTP, 2008).

Comment 16: "The lack of thresholds, as claimed in APPENDIX A of the OEHHA Document, would imply that even a single Cr(VI) molecule, introduced in the organism, would be able to reach the DNA of target cells, which is unbelievable. It should be added that threshold mechanisms occur not only at toxicokinetic and metabolic levels but also after DNA damage, e.g., due to DNA repair and apoptosis. My lab investigated these processes by analyzing *in vivo* both transcriptome (A. Izzotti *et al.*, *Mol. Carcinogenesis*, 35, 75-84, 2002) and proteome (A. Izzotti *et al.*, *Int. J. Oncol.*, 24, 1513-22, 2004)."

Response 16. The issue of thresholds in carcinogenesis has been discussed in detail (U.S. EPA, 2005; OEHHA, 2009). It may be difficult to envision a single molecule of Cr VI in a liter of drinking water causing significant damage to a human. However, at the PHG for Cr VI of 0.02 micrograms per liter, there would be 2.3×10^{14} molecules of Cr VI per liter of drinking water, any one of which has the potential to damage DNA. While detoxification and DNA repair can reduce the potency of genotoxic carcinogens, it is not evident that these mechanisms result in a threshold for Cr VI or other carcinogens.

Comment 17: "However, starting from inconsistent epidemiological and experimental data and denying the occurrence of threshold mechanisms in Cr(VI) toxicity and carcinogenicity lead to unrealistic figures."

And,

"The results of the NTP carcinogenicity study in mice and rats that, as noted in Comment #7, were not consistent with the results of epidemiological studies, were used as a major conceptual base for claiming that Cr(VI) is carcinogenic also by the oral route and for calculating the proposed PHG."

Response 17. As discussed above, both the findings of animal studies and the results of epidemiological studies were consistent with a statistically significant increase in tumors associated with exposure to Cr VI. This suggests that Cr VI is carcinogenic by the oral route.

Comment 18: "The concentrations of Cr(VI) in water that produced significant variations of tumor incidence in the NTP study were in the range of hundreds mg/L, i.e., millions of times higher than the proposed 0.06 µg/L PHG."

Response 18. The use of high doses in cancer bioassays is traditionally used in toxicological testing to offset the statistical limitations of using small numbers of animals (50 /sex/dose level) to measure a relatively rare event (e.g., tumor occurrence).

OEHHA employed standard procedure to estimate the dose associated with 10^{-6} risk which was employed to derive the proposed PHG (U.S. EPA, 2005; OEHHA, 2009).

Comments from Andrew DeGraca, San Francisco Public Utilities Commission

Comment 1: Our principal concern is that OEHHA has relied on the results of National Toxicology Program's 2007 study to derive the PHG for carcinogenic effects. Although this study found that chromium VI in drinking water was carcinogenic in mice and rats, there are unresolved questions about the applicability of these results to humans. It is well accepted that chromium VI is reduced to non-toxic chromium III in the human stomach, a transformation that does not occur in rodents."

Response 1. Cr VI is also reduced to Cr III in the rodent stomach. This is discussed in detail in the PHG document in the sections "Hexavalent Chromium Reduction by Saliva and Gastric Fluids", "Absorption" and "Pharmacokinetics of Trivalent versus Hexavalent Chromium." See also Appendix A. While Cr VI reduction in the GI tract of rodents compared to humans has not been fully described, the U.S. EPA (2010), the New Jersey Department of Environmental Protection (NJDEP, 2009) and OEHHA (this PHG document) have all found that they are similar enough to allow calculation of a human cancer slope factor for Cr VI based on the NTP two-year bioassay.

Comments from Michael Rogge, California Manufacturers and Technology Assc.

General Comment 1: "Update the literature review, addressing the many errors and omissions identified herein and by others during public and peer review, and also correct the scientific deficiencies and substantially revise the current draft."

General Response 1. The literature considered in the PHG document has been updated and expanded by the addition of relevant journal articles that we could identify and retrieve in the update, and were either missing from the August 2009 draft or were published after that draft was posted. All recognized errors have been corrected and omissions rectified where warranted.

General Comment 2: "Revise the PHG document to address the spirit and specific content of the UC peer reviewers and comments of DTSC."

General Response 2. OEHHA has carefully reviewed the peer reviewer comments and the DTSC memorandum. Responses to the UC peer reviewers are included in this document. Responses to specific CMTA comments regarding the UC peer reviewers and the DTSC memorandum are included below in OEHHA's responses to those comments.

General Comment 3: "Include a MOA/HRF evaluation for cancers of the mouse small intestine. There are recognized uncertainties in the currently available MOA data, so we recommend that OEHHA utilize the research that is currently under development at The Hamner Institute for Health Sciences to fill data gaps in the MOA and provide additional information to quantify the differences in reducing capacity across doses and species. With these data, it is possible to develop a PHG that utilizes the best available science and is protective of public health for Californians exposed to low levels of Cr(VI)

in drinking water; however, without the MOA/HRF evaluation, the analysis is fatally flawed."

General Response 3. The mechanism of action (MOA) of Cr VI is discussed in the following sections of the PHG document: "Pharmacokinetics of Trivalent versus Hexavalent Chromium," "Genetic Toxicity," "Mechanism of Genotoxicity and Carcinogenicity," and "Examination of Evidence for Chromium Carcinogenicity." The section entitled "Mechanism of Genotoxicity and Carcinogenicity" concludes:

> "OEHHA could not discern a consistent pattern of histiocytic infiltration, inflammation, hyperplasia and the occurrence of tumors in the mouse or rat duodenum, oral cavity or liver in the NTP (2008) study. Therefore, an MOA other than that of genotoxicity or mutagenicity is not supported by these findings. The standard approach for carcinogens operating via a genotoxic or mutagenic MOA is to apply a linearized multistage model to calculate the cancer potency (U.S. EPA, 2005; OEHHA, 2009)."

The human relevance of the mouse tumor data are discussed in the section entitled "Examination of Evidence for Chromium Carcinogenicity." The section concludes:

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

Note that OEHHA employed the same allometric scaling methodology as NTP (Stout et al., 2009) and U.S. EPA (2010) to extrapolate from mice to humans.

OEHHA acknowledges that new research is on-going and looks forward to the new data when available for consideration. The Safe Drinking Water Act of 1996, amended 1999 (Health and Safety Code [H&SC], Section 116365) contains an important provision that addresses new scientific research when it becomes available: "(e) (1) Public health goals established by the office shall be reviewed at least once every five years and revised, pursuant to the provisions of subdivision (c), as necessary based upon the availability of new scientific data". When new research data are published in a finalized, peer-reviewed format, OEHHA will consider them in the development of a revised PHG for hexavalent chromium. OEHHA acknowledges that new studies may alter a PHG. From the risk characterization section of the 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."

OEHHA will review papers and materials relating to the Hamner Institutes study when they are published. If the study produces compelling information that should be reflected in the PHG document, OEHHA will take appropriate action.

General Comment 4: "Focus the literature review and analysis in all sections on data that are most relevant to current human exposures to Cr(VI) in California drinking water. Specifically, concentrate on data collected using *ad libitum* drinking-water administration

and at concentrations that most closely represent reasonable drinking-water exposures in California."

General Response 4. The NTP (2008) study, an *ad libitum* drinking water study, was used to calculate the cancer slope factor for Cr VI. It was performed at Cr VI concentrations that are generally much higher than those to which Californians are exposed in their drinking water. The use of high concentrations in cancer bioassays is designed to offset the statistical limitations of using small numbers of animals

(50 /sex/dose level) to measure a relatively rare event (tumors) (U.S. EPA, 2005; OEHHA, 2009).

General Comment 5: "Provide a balanced review of the epidemiologic literature, using accepted methods, specifically addressing the human relevance of cancers consistent with the findings of the NTP study by focusing on oral cavity and small-intestine cancers, and evaluating epidemiologic findings for exposures to Cr(VI) at levels that are relevant for the California drinking-water supply."

General Response 5. Two epidemiologic studies were located which measured organ-specific mortality from cancer in humans exposed to Cr VI in their drinking water. The study by Zhang and Li (1987) was already in the August 2009 draft document, while a recent study by Linos et al. (2011) was added. Both studies are evaluated in detail in the PHG document according to standard epidemiologic methods. The older study was judged to have accurately identified the exposed population, but the magnitude of the exposure was considered unclear. The more recent study provided exposure concentrations that varied widely, with the five highest concentrations being 44, 48, 51, 53, 54, and 156 µg/l. Comparing these values to those measured in California drinking water and presented in the "Environmental Occurrence and Human Exposure" section of the PHG document demonstrates that this study's results are relevant to persons in California.

General Comment 6: "Use benchmark dose (BMD) modeling for the non-cancer PHG, and a weight-of-evidence analysis to determine appropriate uncertainty factors, which is consistent with OEHHA guidance."

General Response 6. Only a single PHG is developed for each chemical that is evaluated. For Cr VI in drinking water one PHG was developed in this document: that of 0.02 µg/L based on tumor data. A health protective concentration of 2.0 µg/L was calculated based on non-cancer effects. However, this latter value was not adopted as the PHG because the value protective of both cancer and non-cancer effects is 100-fold lower (i.e., 0.02 µg/L).

OEHHA will be applying the BMD approach in future analyses of the non-cancer data. Our preliminary analysis applying the BMD approach to the non-cancer data followed by an uncertainty factor of 100 yielded a final value that was more than 100-fold higher than the current PHG (0.02 µg/L) based on cancer effects. Thus, the current PHG is more protective and would not change.

General Comment 7: "Use PBPK modeling tools under development at The Hamner Institutes or U.S. EPA to evaluate tissue dose, extrapolate between species, and evaluate sensitive subgroups."

General Response 7. OEHHA acknowledges that new research is on-going and looks forward to the new data when available for consideration. The Safe Drinking Water Act of 1996, amended 1999 (Health and Safety Code [H&SC], Section 116365) contains an important provision that addresses new scientific research when it becomes available: "(e) (1) Public health goals established by the office shall be reviewed at least once every five years and revised, pursuant to the provisions of subdivision (c), as necessary based upon the availability of new scientific data". When new research data are published in a finalized, peer-reviewed format, OEHHA will consider them in the development of a revised PHG for hexavalent chromium. OEHHA acknowledges that new studies may alter a PHG. From the risk characterization section of the 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."

General Comment 8: "Include a quantitative and expanded qualitative uncertainty analysis. This should include an evaluation of the uncertainties in each significant science policy choice that is made in the derivation of the PHG and the quantitative impact of science policy choices and the scientific support for alternatives."

General Response 8. The uncertainty associated with the development of the PHG for Cr VI in drinking water is discussed in the "Risk Characterization" section of the document. OEHHA is not aware of an established methodology for quantifying the uncertainty associated with cancer risk extrapolation.

Specific Comment 1: "The PHG document does not adequately address the comments of the UC peer reviewers [of the 2008 pre-release draft]."

Specific Response 1. The PHG document has been revised as appropriate in accordance with each substantive comment from each UC peer reviewer (see below).

Specific Comment 1-1: "The peer reviewers [of the 2008 pre-release draft] repeatedly emphasized that the approach used to extrapolate from high-dose animal data to set a PHG was crude and overestimated risk in the low dose range. We strongly agree with this general observation, and with the specific points identified in the peer reviews with regard to this issue."

Specific Response 1-1. The approach taken in the PHG document to calculate a cancer slope factor using high dose rodent tumor data is the up-to-date approach used by U.S. EPA (2005; Davis et al., 2010) and OEHHA (2009). One University of California peer reviewer (Dr. Gwiazda) suggested that this approach may have ***underestimated*** the cancer potency of Cr VI, not overestimated it.

Specific Comment 1-2: "Dr. Gwiazda, of UC, pointed out that all the studies presented in the documents that were specifically cited to support the PHG, administered Cr(VI) at doses that are several orders of magnitude higher than drinking-water exposures in California and the proposed PHG. OEHHA's response and arguments provided in the PHG document do not respond to Dr. Gwiazda's comments, because: 1) systemic absorption is not necessary for tumors of the GI tract to occur, because these tumors resulted from direct contact of high concentrations of Cr(VI) in lumen with epithelial tissues of the small intestine, and 2) OEHHA relied on studies of chromium

administered at >5 mg Cr(VI)/L, but Sutherland et al. (2000) found no increase in chromium tissue levels following drinking-water exposures of 0.5 mg Cr(VI)L for 44 weeks in rats."

Specific Response 1-2. 1) Systemic absorption of Cr VI may or may not be required for tumor induction, as it simply is not known at present and 2) Part of OEHHA's analysis of the relationship between GI tract reduction capacity and carcinogenicity is presented in Appendix A. Data from NTP (2008) in Figures A.3-A.6 show increased Cr accumulation in a variety of mouse tissue at drinking water concentrations ranging from 5 to 180 mg/L of Cr VI. The dose response for Cr accumulation was generally linear over the dose range tested, indicating that Cr VI reduction did not saturate. Collins et al. (2010) reached the identical conclusion. Importantly, this range of Cr VI concentrations includes the drinking water concentrations causing increased mouse intestinal tumors in the two-year bioassay (30 to 180 mg/L). Sutherland et al. (2000) measured significant increases in tissue Cr of rats relative to controls at drinking water concentrations of 3 and 10 mg/l Cr VI. No increases were observed at the lowest concentration tested:

0.5 mg/L of Cr VI. Some have interpreted this as an indication that at low Cr VI concentrations in drinking water, reduction of ingested Cr VI to Cr III is sufficient to prevent significant tissue accumulation of Cr VI or toxicity (Proctor et al., 2011; Thompson et al., 2011). However, since the amount of tissue Cr in control animals was near or below the detection limit in the study by Sutherland et al. (2000), an increase in tissue Cr at this low drinking water concentration (0.5 mg/L of Cr VI) may not have been measurable. The currently available data do not permit evaluation of whether reduction of Cr VI in the GI tract is sufficient to prevent significant entry of Cr VI into tissue at drinking water concentrations below approximately 1 mg/L. As discussed in the "Metabolism and Pharmacokinetics" section of the PHG document, there are a number of studies, some performed with radioactively labeled chromium compounds administered at dose levels below 1 mg/L, in which oral administration of Cr VI or Cr III (to rodents or humans) resulted in different patterns of absorption, distribution and excretion, indicating that not all Cr VI was reduced to Cr III following ingestion.

Specific Comment 1-4: "Further, in response to Dr. Bjeldanes, OEHHA states (Response 2), 'No marked increase in oral absorption of hexavalent Cr was observed with dose, which would be expected if the reducing capacity of the GI tract had been overwhelmed.' However, as noted above, OEHHA overlooked the findings of Sutherland et al. (2000)-another paper cited in the PHG but not carefully considered-of no chromium absorption at exposures of 0.5 mg/L, administered *ad libitum*, which is still far higher than exposures to Cr(VI) in drinking water in California and the proposed PHG. At higher exposures of >3 mg/L, Sutherland et al. (2000) observed chromium accumulation in tissues consistent with the observations of other researchers for high-dose exposures. OEHHA used only high-dose exposure data to justify its position of linear extrapolation from high to low doses, which is a critical flaw in the evaluation"

Specific Response 1-4. See Response 1-2. More discussion of the study by Sutherland et al. (2000) has been added to the PHG document.

Specific Comment 2: "The PHG document does not adequately address the comments offered by the Department of Toxic Substances Control (DTSC)."

Specific Response 2. See below for responses to specific issues raised by DTSC and cited by this commenter.

Specific Comment 2-1: "PBPK modeling should be used to quantify the effective dose at the target organ. Use of a PBPK model refines both the cancer and the non-cancer risk assessments, because it can address the interspecies scaling uncertainty factor. Simple allometric scaling is inadequate for a site-of-contact-based carcinogenic MOA, because target tissue dose cannot be quantified adequately for the purpose of scaling to humans."

Specific Response 2-1. We agree that development of the PHG would be enhanced if more information were available on the target tissue dose of Cr VI in mice and humans. Absent such data, we have performed allometric scaling according to standard risk assessment practice to extrapolate between species. A similar approach was taken by the New Jersey Department of Environmental Protection (2009; see PHG document) and the U.S. EPA (2010).

Specific Comment 2-2: "Risk assessment should be based on an MOA evaluation that considers the key events necessary for carcinogenicity and whether those key events, such as inflammation and hyperplasia, can occur at environmentally relevant exposures to Cr(VI) in California's drinking water."

Specific Response 2-2. No inflammation was reported in the key tumor bioassay in mice. Hyperplasia is consistent with the appearance of tumors and is considered a precursor to tumor formation. These issues are discussed in the text describing Table 7 of the PHG document.

Specific Comment 2-3: "OEHHA's highly conservative approaches substantially overestimate the carcinogenic potency of ingested Cr(VI)."

Specific Response 2-3. OEHHA used standard procedures to derive potency (U.S. EPA, 2005; OEHHA 2009; Davis et al., 2010).

Specific Comment 2-4: "Historical occupational exposure to Cr(VI) resulted in exposure of oral cavity tissues to Cr(VI), yet no study has reported a significant excess of oral-cavity tumors among workers, an observation consistent with OEHHA's review of the epidemiology literature presented in the PHG document. This lack of concordance in tumor sites between humans and rats should be discussed. The oral-cavity tumors in rats, which did not occur in mice, appear to be a species-specific observation."

Specific Response 2-4. The discussion of Table 8 now includes a statement that cancer of the oral cavity and pharynx was not significantly elevated in exposed workers.

Specific Comment 2-5: "The MOA for small-intestine tumors in mice has not been adequately addressed. The NTP data suggest that an MOA associated with chronic inflammation, induced by chronic tissue damage, resulting from direct contact with high-dose Cr(VI), is a promotional mechanism that is likely not relevant at environmental exposure levels."

Specific Response 2-5. The PHG document contains a detailed discussion of the relationship between tissue damage, inflammation, hyperplasia and tumors (see Table 7

and its accompanying discussion). It is noted that tissue damage was not observed in the 2-year bioassay in either the mouse or the rat. Also, chronic inflammation was not observed in tissues where tumors increased. Lastly, the hyperplasia that was observed in the mouse duodenum was not regenerative in nature. The NTP scientists who performed the 2-year bioassay published a report in which they stated, "We observed no increase in non-neoplastic histopathology lesions in either species suggestive of overt tissue damage due to the oxidant properties of Cr (VI)" (Stout et al., 2009).

Specific Comment 2-6: "Tumors in the small intestine appear to be related to direct contact of the small-intestine epithelial tissues with high doses of Cr(VI); therefore, species-specific variability of GI anatomy and physiology are critical to understanding the relationship between observations in mice and relevance to low-concentration exposure in humans."

Specific Response 2-6. We agree that the risk assessment of Cr VI via the oral route would benefit from more information on its pharmacokinetics in humans and mice. Absent these data, OEHHA has utilized allometric scaling to extrapolate dose from mice to humans. A similar approach has been taken by the New Jersey Department of Environmental Protection (NJDEP, 2009) and the U.S. EPA (2010).

Specific Comment 2-7: "The inhalation cancer slope factor is based on dated information and an inadequate review of the published literature. Published risk estimates, developed from the original data sets, are available for estimating the lung cancer risk associated with inhalation exposure, and these estimates should be used in developing the PHG."

Specific Response 2-7. See the "Carcinogenic Effects" section of the PHG document located within the "Calculation of the PHG" section for the relative contributions of the inhalation and ingestion risks to the total risk posed by Cr VI in drinking water. Inhalation contributes less than 1 percent of the total risk. Therefore, refinements of the inhalation potency of Cr VI, as suggested in this comment, will not significantly affect the final PHG value.

Specific Comment 2-8: "OEHHA's analysis of the mouse stomach's reductive capacity and tissue accumulation (Appendix A) does not provide a clear scientific basis to discount a threshold-based dose-response. The NTP studies clearly demonstrate that over-burdening the GI tract's ability to reduce Cr(VI) to Cr(III) results in overt tissue damage at the site of contact, as well as chronic inflammation and regenerative hyperplasia."

Specific Response 2-8. The title of Appendix A has been revised to indicate that the discussion pertains to whether thresholds were observed over the dose range used in the NTP (2008) bioassay. The data in NTP (2008) suggest that the GI tract's ability to reduce Cr VI to Cr III was not exceeded over the dose range tested. As discussed in the PHG document (see discussion of Table 7) no overt tissue damage was observed in this study, and inflammation was not observed at the sites where tumors arose. These findings also indicate that the observed hyperplasia was not regenerative.

Specific Comment 2-9: "The analysis of the Borneff et al. (1968) study and the Helicobacter hypothesis is highly speculative, lacks relevance, and should be deleted."

Specific Response 2-9. The PHG does not rely on the Helicobacter hypothesis or Borneff et al. (1968) in the development of the PHG. The hypothesis is located in Appendix B and clearly indicated as such. The Helicobacter hypothesis was formulated by OEHHA as part of an effort to obtain a better understanding of the findings of diverse studies such as the occurrence of tumors in the first generation of the Borneff et al. (1968) study and stomach tumors following a relatively short term exposure to Cr VI in rural China. These discussions serve as a scientific resource and are attached in the Appendix as records of the issues that have been addressed in the research for and preparation of this PHG document.

Specific Comment 2-10: "The Hamner Institute's ongoing studies, which have progressed significantly since the DTSC's comments of last year, are definitely 'prerequisites' to any revisions to the OEHHA PHG for Cr(VI)."

Specific Response 2-10. OEHHA acknowledges that new research is on-going and looks forward to the new data when available for consideration. The Safe Drinking Water Act of 1996, amended 1999 (Health and Safety Code [H&SC], Section 116365) contains an important provision that addresses new scientific research when it becomes available: "(e) (1) Public health goals established by the office shall be reviewed at least once every five years and revised, pursuant to the provisions of subdivision (c), as necessary based upon the availability of new scientific data". When new research data are published in a finalized, peer-reviewed format, OEHHA will consider them in the development of a revised PHG for hexavalent chromium. OEHHA acknowledges that new studies may alter a PHG. From the risk characterization section of the 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."

Summary and Introduction

Specific Comment 3: "Page 2 of August 2009 draft PHG document, '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).' This statement, and several others like it in the text of the PHG document, misrepresent the papers cited, and importantly, do not discriminate between observations at very high doses and at lower doses. Neither Proctor et al. or De Flora et al. indicate that all Cr(VI) is completely reduced at any dose. OEHHA has misunderstood and misrepresented this research."

Specific Response 3. Quotes from papers published by these investigators have been added to the text of the PHG document.

Environmental Occurrence and Human Exposure

Specific Comment 4: "This information (water monitoring data in California) is out of date. CDPH data for Cr(VI) monitoring is current through February of 2009 and is available on the CDPH website at…"

And,

”Finally, and most importantly, Cr(VI) in California drinking water occurs widely in the low parts per billion range. OEHHA has relied on studies of animals and humans exposed in the high part per million (ppm) range to develop a PHG that is in the part per trillion range (60 ppt).”

Specific Response 4. The data covering Cr VI concentrations in California drinking water have been updated.

The use of high doses in cancer bioassays is traditionally used to offset the statistical limitations of using small numbers of animals (50 /sex/dose level) to measure a relatively rare event (tumors). OEHHA employed standard procedure to estimate the dose associated with 10^{-6} risk which was employed to derive the proposed PHG (U.S. EPA, 2005; OEHHA, 2009). As required by law, PHGs are based on scientific assessments of health risks posed by drinking water contaminants, and not the actual contaminant levels measured in drinking water.

Metabolism and Pharmacokinetics

Specific Comment 5-1: “(Page 11of August 2009 draft PHG document) ‘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.’ First, it is not reasonable to assume that the maximum plausible level of Cr(VI) that would be ingested by humans is 5 mg/L. This level is 100 times higher than the current MCL in California and far higher than the levels of Cr(VI) measured in drinking water, as discussed in the PHG document and shown by the most current monitoring data.”

Specific Response 5-1. The reference Kerger et al. (1996a) has been added to the text. Although OEHHA used a level a little lower than Kerger and coworkers (1996a), it is consistent with their statement, "The findings of this study may have important mechanistic implications applying to detoxification of Cr(VI) at plausible concentrations in drinking water (i.e., <10 mg/liter)."

Specific Comment 5-2: “OEHHA needs to consider the half-life of Cr(VI) reduction in these biological media in order to understand the tissue dose of Cr(VI) at the target tissue. These kinetic processes can be quantified only by using a PBPK model.”

Specific Response 5-2. The concentrations of Cr VI at the target tissues are not known. In the absence of such data, OEHHA has followed the standard procedure of calculating cancer potency using the drinking water concentrations of Cr VI (U.S. EPA, 2005; OEHHA, 2009).

Specific Comment 5-3: “Finley et al. (1997) found no dose-related increases in plasma and red-blood-cell chromium at ingested concentrations of 0.1 mg/L, and Sutherland et al. (2000) found no increase in chromium concentrations of any tissue in rats exposed to 0.5 mg/L for 44 weeks.”

Specific Response 5-3. In the study by Finley et al. (1997), five subjects received a dose of 100 ug/day of Cr VI for 3 days. Red blood cell (RBC) Cr levels increased in 4 out of the 5 subjects while the plasma Cr level increased only slightly in only 2 individuals. As for dose-response relationships, the changes were too variable even within the same individual, so not much can be concluded regarding the effect of dose. But increases in RBC Cr levels were observed following the administration of as little as 100 ug VI for 3 days. Sutherland et al. (2000) measured significant increases in tissue Cr of rats relative to controls at drinking water concentrations of 3 and 10 mg/l Cr VI. No increases were observed at the lowest concentration tested: 0.5 mg/L of Cr VI. Some have interpreted this as an indication that at low Cr VI concentrations in drinking water, reduction of ingested Cr VI to Cr III is sufficient to prevent significant tissue accumulation of Cr VI or toxicity (Proctor et al., 2011; Thompson et al., 2011). However, since the amount of tissue Cr in control animals was near or even below the detection limit in the study by Sutherland et al. (2000), an increase in tissue Cr at this low drinking water concentration (0.5 mg/L of Cr VI) may not have been measurable.

Specific Comment 6: "Page 12 of August 2009 draft PHG document), '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." This statement is incorrect. Finley et al. (1997) reported average absorption of 1.7% at 0.1 mg/L and 3.5% at 10 mg/L, which is an increase, and they suggest increased absorption with increased exposure."

And,

"Also, it is clear that one of the three participants absorbed considerably more when given chromium at 10 mg/L, with 8% absorption. These data demonstrate variability in absorption and are not useful for evaluating reduction capacity."

And,

"Further, Kerger et al. (1996) found temporarily increased levels of chromium in RBCs and plasma following Cr(III) administration; thus, it is questionable whether these data can be used, as OEHHA has done, to surmise whether Cr(VI) or Cr(III) is being absorbed in these studies. Absorption and reduction are competing kinetic processes that, for the purposes of risk assessment, can best be addressed using a PBPK model."

Specific Response 6. From Finley et al. (1997): urinary Cr recovery (means) of 1.7 % at 100; 1.2 % at 500; 1.4 % at 1000; 1.7 % at 5000 and 3.5 % at 10,000 ug/day. To test if there is a dose related increase of urinary excretion, a comparison using only the high dose and low dose is inappropriate because it ignores all the intermediate doses. Comparisons should use all of the dose levels. No increase in absorption was evident (in all the intermediate doses) with the possible exception of the high dose, and absorption at the high dose still remained quite low. The text has been revised to include urinary recoveries at the various dose levels.

As indicated in the PHG document, there is considerable variability between individuals, which makes modeling these limited data using PBPK particularly problematic.

As noted in the PHG document both Cr VI and Cr III are absorbed. Kerger et al. (1996) demonstrated that a lot more Cr VI is absorbed, demonstrating that Cr VI is not completely converted to Cr III in the stomach. Otherwise the amount of absorption should have been the same.

Specific Comment 7: "The Kerger et al. (1996a) study demonstrates different pharmacokinetic patterns with different forms of ingested chromium, which finding is consistent with other research related to chromium-containing vitamins. Further, all exposures in the Kerger et al. study were to 10 mg Cr/L, which is far more than would be expected in California drinking water, and as such, the findings are of questionable relevance to drinking-water exposures."

Specific Response 7. The different forms being referred to are Cr VI versus Cr III. OEHHA agrees that there is a difference in pharmacokinetic patterns which indicates that Cr VI is being absorbed as Cr VI and is not all reduced to Cr III in the stomach prior to its absorption. Otherwise, the pharmacokinetic patterns would be the same.

Specific Comment 8: "(Page 12 of the August 2009 draft document) '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).' OEHHA is not correctly citing this study."

Specific Response 8. The text of the PHG document has been revised to read, "Finley and associates observed marked increases in RBC chromium levels in some individuals (but not in others) that ingested three daily doses of Cr VI, at total doses as low as 0.1 mg/day, while plasma chromium levels were less affected (Finley et al., 1997)."

Specific Comment 9-1: "First, the kinetics of Cr(VI) following inhalation, intratracheal instillation, subcutaneous injection, and intraperitoneal injection is substantially different from that associated with exposure to Cr(VI) in drinking water at environmentally relevant concentrations, and therefore, is of no relevance to the development of a PHG for Cr(VI) in drinking water. It is not appropriate to summarize these findings as if there is no difference."

Specific Response 9-1. There is no statement in the PHG document that there is no difference in the kinetics associated with differing routes of exposure. For most xenobiotics, the route of administration will influence the pharmacokinetics.

Specific Comment 9-2: "The important study of Sutherland et al. (2000) found no increase in chromium in tissues following drinking-water exposures of 0.5 mg/L of Cr(VI) for 44 weeks in rats…The entire discussion of toxicokinetics should be refocused and rewritten."

Specific Response 9-2. Sutherland et al. (2000) measured significant increases in tissue Cr of rats relative to controls at drinking water concentrations of 3 and 10 mg/L

CrVI. No increases were observed at the lowest concentration tested: 0.5 mg/L of Cr VI. Some have interpreted this as an indication that at low Cr VI concentrations in drinking water, reduction of ingested Cr VI to Cr III is sufficient to prevent significant tissue accumulation of Cr VI or toxicity (Proctor et al., 2011; Thompson et al., 2011). However, since the amount of tissue Cr in control animals was near or below the detection limit in the study by Sutherland et al. (2000), an increase in tissue Cr at this low drinking water concentration (0.5 mg/L of Cr VI) may not have been measurable.

Specific Comment 9-3: "Second, the discussion of Cr(III) binding on page 14…indicates that there is considerable uncertainty in concluding whether Cr(VI) or Cr(III) is systematically absorbed…In light of this discussion, OEHHA should reconsider whether the profile of total chromium in RBCs and plasma can be used as a measure of systematic absorption of Cr(VI)."

Specific Response 9-3. The levels of Cr excreted in the urine and its urinary half life are the key data that demonstrate that Cr VI is absorbed as Cr VI. As indicated in the PHG document ("Metabolism and Pharmacokinetics" section), little increase in RBC Cr levels occur when Cr VI is administered by the oral route in humans or animals. It appears that non-RBC sites are the depots for absorbed Cr VI.

Specific Comment 10-1: "First, if OEHHA carefully examines the tissue accumulation data of the NTP study, they would recognize that there are notable differences between rodent species, the basis for which has not been explained, nor has its relevance to humans been described. The data suggest that far more Cr(VI) was absorbed in the mouse than the rat, and that the rat had increased capacity to reduce Cr(VI) to Cr(III) in the stomach."

Specific Response 10-1. It is not unusual to observe differences in absorption between different species. With regard to differences in reducing capacity, NTP addressed this issue and indicated no data are available but calculations based on allometric considerations indicate no marked differences.

Specific Comment 10-2: "Humans, with greater gastric acid production capacity than a rat, and a greater volume of gastric acid in the stomach, are expected to be able to reduce more Cr(VI) to Cr(III) in the stomach, and thus to have increased ability to detoxify Cr(VI)."

Specific Response 10-2. We were unable to identify documentation in support of the commenter's statement that humans will have an increased ability to reduce ingested

Cr VI relative to the rat. However, the ability to reduce Cr VI to Cr III does not appear to be strictly a function of pH, as the reducing equivalents come from small molecules such as ascorbate, GSH and proteins. Reduction appears to be facilitated by low pH.

Specific Comment 10-3: "Second, in this paragraph, OEHHA sites Sutherland et al. (2000) but ignores the findings at the lowest dose by the relevant route of exposure."

Specific Response 10-3 Sutherland et al. (2000) measured significant increases in tissue Cr of rats relative to controls at drinking water concentrations of 3 and 10 mg/L

Cr VI. No increases were observed at the lowest concentration tested: 0.5 mg/L of Cr VI. Some have interpreted this as an indication that at low Cr VI concentrations in

drinking water, reduction of ingested Cr VI to Cr III is sufficient to prevent significant tissue accumulation of Cr VI or toxicity (Proctor et al., 2011; Thompson et al., 2011). However, since the amount of tissue Cr in control animals was near or below the detection limit in the study by Sutherland et al. (2000), an increase in tissue Cr at this low drinking water concentration (0.5 mg/L of Cr VI) may not have been measurable.

Specific Comment 11: "The profile of chromium in the blood and urine in the Kerger et al. (1996) study is, as the author notes, more consistent with absorption of Cr(III) than Cr(VI); however, we recognize that this conclusion includes uncertainty."

Specific Response 11. OEHHA disagrees. The prolonged urinary half-life following

Cr VI administration compared to following Cr III administration is consistent with the absorption of Cr VI, not Cr III.

Specific Comment 12: "Consistent with the authors' report, the observation of a prolonged half-life is also evidence that there is a difference in the toxicokinetics of Cr(III) bound to an organic matrix."

Specific Response 12. We know of no evidence that "Cr III bound to an organic matrix" exists. However, the occurrence of Cr VI absorption does explain a prolonged half-life for chromium. See the discussion of the work of O'Flaherty et al. (2001) in the "Metabolism and Pharmacokinetics" section of the PHG document.

Specific Comment 13: "(Page 17 of the August 2009 draft PHG document) '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…' This paragraph mischaracterizes this research. It has been well recognized for decades that Cr(VI) is reduced to Cr(III) in the stomach and other tissues, which reduces the toxicity of Cr(VI). To our knowledge, no researcher has claimed that the capacity of these tissues to reduce Cr(VI) to Cr(III) is 'inexhaustible' at any dose."

Specific Response 13. The phrase "essentially inexhaustible" has been removed.

Specific Comment 14: "(Page 17 of the August 2009 draft document) 'Proctor and coworkers investigated the reducing capacity of stomach secretions using human gastric fluid and a simulated stomach fluid (Proctor et al., 2002a). The findings of these investigators appear to be consistent with estimates of De Flora and others that gastric fluids are capable of rapidly reducing large quantities of hexavalent chromium. Both human stomach fluid and simulated stomach fluid reduced from 300 to 1000 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.'

This statement misquotes the paper cited. The abstract actually states that real human gastric fluid reduced 0.3 to 1 mg Cr(VI) per liter of gastric fluid within 2 minutes. Increasing the pH from 1.5 to 4.5 reduced both the rate and capacity of Cr(VI) reduction

by approximately one-third. However, further increasing the pH to 8.2 by adding Rolaids did not affect the reduction rate or capacity, as compared to that at a pH of 4.5. Thus, between a pH of 4.5 and 8.2 there was no affect of pH on reduction rate or capacity, but between a pH of 1.5 and 4.5, there clearly was.

In the presence of food within simulated gastric fluid, 10 mg of Cr(VI) per liter of simulated stomach fluid is reduced in 4 minutes, and Proctor et al. concludes that, under fasting conditions, Cr(VI) at 1 ppb would exist in the stomach for less than 1 minute before being reduced to Cr(VI). This study found that dilution does make a significant difference in reduction capacity. The mass reduced is proportional to the level of dilution. The study reported half-lives of 0.7 to 10 minutes. Dilution did have a significant impact on reduction capacity, as did pH. OEHHA should cite the study correctly, and advance its discussions of kinetics to focus on rates of reduction and rates of absorption, rather than speculation regarding absolute quantities."

Specific Response 14. The discussion of this paper has been expanded. However, the original discussion in the PHG document quoted above was accurate. Antacid had little effect on the reducing properties of the simulated stomach fluid. Regarding dilution, the authors of Proctor et al. (2002a) stated, "Thus, diluted stomach fluid reduces approximately the same amount of Cr (VI) as full strength stomach fluid when put in terms of actual gastric fluid/enzymes."

Specific Comment 15: "Although OEHHA speculates at length about the findings of the Kerger et al. paper – most of which are contrary to the author's conclusions – this dialog is entirely unnecessary, because the observations of cancer in the NTP study, and other studies that OEHHA deems of value (Borneff et al. 1968; Zhang and Li 1987), occur only at the site of exposure in the GI tract, not in distant tissues. Systemic absorption is not necessary for these tumors to occur."

Specific Response 15. The tumor studies cited above may well be site of contact tumors. Ingested Cr VI also causes non-carcinogenic toxicity following absorption and systemic distribution. The PHG document discusses many of these types of toxicity in the "Toxicological Effects in Animals" section. The work of Kerger and colleagues is useful in understanding the pharmacokinetics of Cr VI and these types of systemic toxicity.

Specific Comment 17: "The most meaningful data by Sutherland et al., which finds no increase in chromium in any tissues following prolonged exposure to Cr(VI) at 0.5 mg/L, are very important for understanding the kinetics of Cr(VI) from lower-level Cr(VI) exposure and should be highlighted in the OEHHA document."

Specific Response 17. Sutherland et al. (2000) measured significant increases in tissue Cr of rats relative to controls at drinking water concentrations of 3 and 10 mg/L Cr VI. No increases were observed at the lowest concentration tested: 0.5 mg/L of Cr VI. Some have interpreted this as an indication that at low Cr VI concentrations in drinking water, reduction of ingested Cr VI to Cr III is sufficient to prevent significant tissue accumulation of Cr VI or toxicity (Proctor et al., 2011; Thompson et al., 2011). However, since the amount of tissue Cr in control animals was near or below the detection limit in the study by Sutherland et al. (2000), an increase in tissue Cr at this low drinking water concentration (0.5 mg/L of Cr VI) may not have been measurable.

Specific Comment 19: "OEHHA provides a reasonable summary of the results for these two studies (NTP 1996, 1997a) that are intended to evaluate reproductive effects of oral Cr(VI) exposure. However, the apparent differences in interspecies sensitivity to liver effects from these exposures should be discussed. Although mice seemed to be sensitive to cytoplasmic vacuolization in hepatocytes at doses as low as 50 ppm in diet, this effect was not seen in rats under the same conditions at doses as high as 400 ppm in diet. Further, these effects were not observed in mice in the 2008 NTP study. These findings are not reproducible and, as such, should not be used for risk assessment."

Specific Response 19. The PHG document now mentions the commenter's point that cytoplasmic vacuolization in hepatocytes in NTP (1997a) was not observed in the chronic mouse study (NTP, 2008). Note that the Health Protective Concentration for non-carcinogenic effects calculated in Table 17 of the PHG document is based on inflammation and fatty changes to the livers of females rats treated for two years with drinking water containing Cr VI, not on the effects discussed in this specific comment.

Specific Comment 20: "Note that the Chopra et al. (1996) and Acharya et al. (2001) studies were conducted by the same laboratory using nearly identical study protocol, with the exception that Chopra et al. (1996) evaluated female Wistar rats while Acharya et al. (2001) evaluated male Wistar rats, and the same weaknesses that are apparent in the Chopra et al. study (e.g., lack of detail about study-group size, lack of detail about histopathological findings) are apparent in the Acharya et al. (2001) study.

Specific Response 20. The strengths and weaknesses of both studies are discussed in Table 1.

Specific Comment 22: "The use of doses in excess of the MTD in toxicity studies is undesirable for a variety of reasons, including lack of relevance to expected environmental exposure levels. Notably, chronic inflammation of the liver was reported in female rats in this study in only the highest (1000 mg/L) dose group, and in none of the male rat dose groups, and fatty liver was not reported for any dose group."

And,

"These data suggest that at least the four highest dose groups (125, 250, 500, and 1000 mg/L) likely exceeded the MTD…As discussed above, interpretation of study results associated with doses above the MTD and reduced water consumption is problematic."

And,

"It is also important to recognize that health-effect findings above a dose level at which water consumption is decreased may or may not be due to the test substance (Campbell et al. 2009). In the sodium dichromate studies, high concentrations in water are presumably unpalatable to rodents, such that comparisons to control animals may be confounded because controls consume standard volumes of drinking water. This issue can complicate interpretation of study results."

Specific Response 22. The MTD is discussed in detail in the "Carcinogenicity" section of the document in the discussion of the NTP (2008) study. This study (NTP, 2008), and not the subchronic study described in Specific Comment 22, was used to develop the health protective concentrations of hexavalent chromium for cancer and non-cancer

effects. Considering water intake (Figure 13), body weights (Figure 11) and various other indicators of animal hydration, the dose levels of Cr VI administered to male mice appear not to have exceeded the MTD. A similar conclusion was reached by NJDEP (2009) and U.S. EPA (2010).

Chronic Toxicity

Specific Comment 24: "(Page 32 of the August 2009 draft PHG document) 'Groups of 50 male and female rats…and 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)…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).'

As evidenced by the water consumption and body weight data from this and the 3-month study (NTP 2007a), the highest dose administered to rats and mice likely exceeded the MTD. As discussed above, interpretation of study results associated with doses above the MTD and reduced water consumption is problematic, and may not reflect toxic effects of the chemical agent itself. If a BMD model is used with these study results to calculate a non-cancer PHG in the future, the uncertainty regarding the effects seen at the higher dose levels would need to be considered."

Specific Response 24. A detailed discussion of the two-year bioassay in rodents (NTP, 2008) is presented in the "Carcinogenicity" section of the draft PHG. The specific issue of whether the MTD was exceeded in either rats or mice is included. OEHHA determined that the MTD was not exceeded in male mice. This is important because male mouse tumor incidence was used to calculate the cancer potency of ingested Cr VI in the PHG document. NJDEP (2009) and U.S. EPA (2010) also concluded that the MTD was not exceeded in the male mice in NTP (2008).

Specific Comment 25: "(Page 33 of the August 2009 draft PHG document) 'The animals appeared to recover from the anemia by 12 months.' This statement is not supported by the data in the study and should be revised."

Specific Response 25. We have changed "appeared to recover" to "appeared to be recovering."

Specific Comment 26-1: "First, given the relationship between the reducing capacity of the GI tract and Cr(VI) toxicity, the high dose levels used in the NTP study are of questionable relevance to much lower environmental exposure levels. The fact that responses may be qualitatively, as well as quantitatively, different at high vs. low dose levels is well recognized in toxicology. This issue was discussed in regard to interpretation of the carcinogenicity data, as well as by the UC peer reviewers and the DTSC. Specifically, the animal studies used doses that overwhelmed the test animals' capacity to reduce Cr(VI) to Cr(III) in the gastrointestinal tract, resulting in tissue damage in the small intestine (including chronic inflammation)."

Specific Response 26-1. See Appendix A of the PHG document for a discussion of the data indicating that the capacity of the rodent GI tract to reduce Cr VI to Cr III was not exceeded in the NTP (2008) two-year bioassay.

Specific Comment 26-2: "For example, Finley et al. (1997) found no dose-related increases in plasma and RBC chromium at ingested concentrations of 0.1 mg/L, and Sutherland et al. (2000) found no increase in chromium concentrations of any tissue in rats exposed to 0.5 mg/L for 44 weeks."

Specific Response 26-2. With regard to Finley et al. (1997), humans exposed to drinking water concentrations of Cr VI as low as 0.1 mg/L exhibited increased urinary chromium compared to controls, indicating that Cr VI had been absorbed. The authors suggested that this was due to absorption of Cr III. These data are discussed in the "Absorption" subsection of the "Metabolism and Pharmacokinetics" section of the PHG document. In rats, Sutherland et al. (2000) found no increased tissue chromium after giving rats drinking water containing 0.5 mg/L Cr VI. The PHG document discusses the likelihood that the rat study's detection limit was too high to detect increased tissue chromium at 0.5 mg/L (see Specific Response 1-2).

Specific Comment 26-3: "Second, the NTP (2007b) expressed clear reservations concerning the biological significance of the chronic liver inflammation observed in the Cr(VI) study animals…NTP's statements about the significance of these findings raise questions about the suitability of these data for use as the point of departure in derivation of the PHG. NTP's statements are supported by examination of historical control data from other NTP studies, which show that liver inflammation and fatty changes are common in these species of rat and mouse."

Specific Response 26-3. We did not find any statements in the NTP study that expressed reservations concerning its findings of an increased incidence of minimal to mild inflammation in the livers of female rats. It is true that similar changes were observed in the livers of aged, control animals. However, when a concurrent control population is available, as was the case here, OEHHA would not discount an effect that was significant relative to the concurrent control, even if the values fell within the historical control range.

Specific Comment 26-4: "These points all lead to significant uncertainty in OEHHA's non-cancer PHG calculation. The administration of high doses that overwhelm the reductive capacity of the GI tract in animal studies likely overestimates risks at environmental exposure levels. The use of a different point of departure due to uncertainty regarding the validity of the liver effects would result in a corresponding increase in the PHG. Finally, the use of a different uncertainty factor (less than 10) to account for the apparent greater sensitivity of rats to oral Cr(VI) exposure would also result in an increase in the non-cancer PHG."

Specific Response 26-4. OEHHA acknowledges that there is uncertainty associated with the calculation of the health-protective concentration of Cr VI for non-cancer effects. This is discussed in the "Risk Characterization" section of the PHG document. Chronic liver inflammation in female rats, measured in the two-year bioassay (NTP, 2008), was the most sensitive non-cancer endpoint. As discussed in Collins et al. (2010) and in Appendix A of the PHG document, the dose responses for chromium

accumulation in tissue (NTP, 2008) indicate that the reductive capacity of the rat GI tract did not become saturated over the concentration range used in the study (5 to 180 mg/L of Cr VI). In addition, we know of no data indicating that the liver effects observed in rats in a number of Cr VI drinking water studies (discussed in the PHG document) are not valid. Lastly, we know of no data demonstrating that rats are either more or less sensitive than humans to the non-cancer, chronic effects of Cr VI in drinking water.

Specific Comment 27: "The majority of studies cited by OEHHA for the noncancer assessment do not meet this criterion. A NOAEL was reported for only two of the noncancer studies (NTP 1997a and Mackenzie et al., 1958), highlighting the fact that most of the studies examined excessively high doses that overwhelmed the test animals' capacity to reduce Cr(VI) to Cr(III) in the gastrointestinal tract, allowing mechanisms of toxicity to emerge than are not relevant at lower, environmentally relevant doses."

Specific Response 27. Part of OEHHA's analysis of the relationship between GI tract reduction capacity and carcinogenicity is presented in Appendix A. Data from NTP (2008) in Figures A.3-A.6 show increased Cr accumulation in a variety of mouse tissue at drinking water concentrations ranging from 5 to 180 mg/L of Cr VI. The dose response for Cr accumulation was generally linear over the dose range tested, indicating that Cr VI reduction did not saturate. Collins et al. (2010) reached the identical conclusion. Importantly, this range of Cr VI concentrations includes the drinking water concentrations causing increased mouse intestinal tumors in the two-year bioassay (30 to 180 mg/L). Sutherland et al. (2000) measured significant increases in tissue Cr of rats relative to controls at drinking water concentrations of 3 and 10 mg/l Cr VI. No increases were observed at the lowest concentration tested: 0.5 mg/L of Cr VI. Some have interpreted this as an indication that at low Cr VI concentrations in drinking water, reduction of ingested Cr VI to Cr III is sufficient to prevent significant tissue accumulation of Cr VI or toxicity (Proctor et al., 2011; Thompson et al., 2011). However, since the amount of tissue Cr in control animals was near the detection limit in the study by Sutherland et al. (2000), an increase in tissue Cr at this low drinking water concentration (0.5 mg/L of Cr VI) may not have been measurable. Due to the absence of data, OEHHA is currently unable to evaluate whether reduction of Cr VI in the GI tract is sufficient to prevent significant accumulation of Cr in tissue at drinking water concentrations below approximately 1 mg/L. As discussed in the "Metabolism and Pharmacokinetics" section of the PHG document, there are a number of studies in which oral administration of dose levels below 1 mg/L of Cr VI or Cr III (to rodents or humans) resulted in different patterns of absorption, distribution and excretion, indicating that not all Cr VI was reduced to Cr III following ingestion.

Specific Comment 30: "The "weaknesses" for NTP (2007a), the subchronic study, should note that the higher doses administered to rats (at least the 1000-mg/L dose group) and mice (at least the 125-, 250-, 500-, and 1000-mg/L dose groups) likely exceed the MTD."

Specific Response 30. The subchronic rodent study (NTP, 2007) was partly performed as a range-finding study prior to the two-year bioassay. Good range-finding studies should exceed the MTD. This is not a weakness of the study.

Genetic Toxicity

Specific Comment 32: "The weight of evidence strongly supports that Cr(VI) is not genotoxic from drinking water exposures. OEHHA should revise this section to reflect this highly relevant observation.

Specific Response 32. The genotoxicity data presented by NTP (2007) have been added to Table 2. Table 2 now cites nine drinking water studies of which three are positive for genotoxicity, and 21 total studies (drinking water or gavage) of which 10 are positive for genotoxicity. See the "Genotoxicity" section of the PHG document for a discussion of these studies and presentation of OEHHA's reasons for concluding that Cr VI is genotoxic via the oral route.

Specific Comment 33: "De Flora et al. (2008) evaluated DNA damage in the stomach, forestomach, and small intestine of mice exposed to Cr(VI) at 5 and 20 mg/L administered in drinking water for 9 months. As noted in the general comments, there was no evidence of DNA oxidative damage or DNA cross-linkage, two key indicators indicating that genotoxicity in mice, in the target tissues of the small intestines or other portions of the GI tract evaluated, is not part of the mode of action."

Specific Response 33. The results of De Flora (2008) have been added to the "Genotoxicity" section. However, judging from the responses of their positive controls, it is likely that their methodology lacked the sensitivity to measure DNA damage at the dose levels tested. In addition, McCarroll *et al*. (2010) noted that the levels tested in this study may in part explain the negative results because they are below the exposure levels used in the NTP 2-year drinking water study.

Specific Comment 34: "OEHHA provides no basis for concluding that genotoxic effects occur at doses that do not overwhelm the reductive capacity of the stomach."

And,

"In addition, the Bagchi et al. findings of genotoxicity in the brain are either limited to extreme high-dose exposures of the Bagchi et al. study or are of questionable reliability, because Sutherland et al. (2000) did not observe increased levels of chromium in brain tissue of rats exposed to Cr(VI) at 10 ppm for 44 weeks."

Specific Response 34. The basis for the statement that genotoxicity has been observed at drinking water concentrations not likely to overwhelm the reductive capacities of the stomach, intestines and blood is discussed in De Flora (2000), as cited in the PHG document in the "Genetic Toxicity" section.

Sutherland et al. (2000) were not able to detect chromium in the brain tissue of any of their rats, including controls. Clearly, the amount of chromium in brain tissue was below the detection limit of their methodology, and their failure to measure increases in the chromium levels of brain tissue from dosed animals is uninformative.

Carcinogenicity

Specific Comment 35-1: "First, it is important to emphasize that most of the tumors did occur in the duodenum, the portion of the small intestine in closest proximity to the stomach, and with greater distance from the point where the stomach empties into the small intestine, fewer tumors were observed."

Specific Response 35-1. This information has been added to the text where Tables 5 and 6 are discussed.

Specific Comment 35-2: "Second, the denominator data in Tables 5 and 6 are not consistent with those presented by NTP for the 28.6-mg/L and 257.4-mg/L dose groups of the male mice, and for all dose groups of the female mice. OEHHA should provide a more detailed description as to why the numbers are inconsistent, or use the results presented by NTP if the result is "essentially the same…We recommend that OEHHA follow the direction of NTP and use the number of animals in each dose group as the denominator for calculation of the oral cancer slope factor, or at least subtract only the number of animals that died within the first year of the study."

Specific Response 35-2. Calculation of the denominators of the tumor incidence data now conforms to standard procedure (U.S. EPA, 2005; OEHHA, 2009). This is discussed in the text in the "Carcinogenicity" section, subheadings "NTP, 2008; Mouse; Neoplasms." The intestinal tumor data were normalized to the number of mice alive at the time of occurrence of the first tumors in the small intestine: day 451 for males and day 625 for females.

Specific Comment 37: "(Page 53 of the August 2009 draft PHG document) '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.'

This statement is incorrect. The LOAEL for the female rat for chronic liver inflammation was identified as 14.3 mg/L…Rates of chronic inflammation of the liver were also high in the control groups of females and males (24% and 28% respectively)."

Specific Response 37. The discussion of chronic inflammation observed in rat liver has been revised. Reference to liver inflammation in control animals has been added to this discussion.

Specific Comment 38: "Regenerative hyperplasia, secondary to previous epithelial cell injury, is consistent with a non-mutagenic mode of action, especially when combined with the findings of no genotoxicity in these tissues (De Flora et al., 2008). For a non-mutagenic mode of action, a non-linear dose response in the low dose range is consistent with EPA guidance (U.S. EPA 2005). If the MOA is as NTP indicates, the resulting PHG would be at least 200-fold higher than that calculated with the assumption of a linear dose-response. Further, the observations of the NTP study are consistent with a direct-irritation effect of the small-intestinal epithelium, which occurred with the greatest severity where the stomach empties, indicating that, at the doses administered in the NTP study, Cr(VI) was not reduced in the stomach but passed the duodenum and was reduced as it passed through the intestines of the animals."

Specific Response 38. No epithelial cell injury or irritation of the small-intestinal epithelium was observed in the two-year bioassay conducted by NTP. These observations, and their implications for an MOA, are discussed in the "Carcinogenicity"

section of the draft PHG (see Table 7). In addition, judging from the responses of their positive controls, it is likely that the methodology used by De Flora et al. (2008) lacked the sensitivity to measure DNA damage at the dose levels tested. Also, McCarroll *et al*. (2010) noted that the levels tested in this study may in part explain the negative results because they are below the exposure levels used in the NTP 2-year drinking water study.

Specific Comment 39: "The Davidson et al. (2004) study has several methodological flaws that render it inapplicable to human exposures. First, the UV radiation to which the mice were subjected was not consistent with natural sunlight and included UV-C radiation, which is a highly potent carcinogen. The authors never measured dose, but only reported the drinking-water concentration administered, not how much water the animals consumed. They reported the total number of tumors in each dose group and did not report the number of tumor-bearing mice nor the numbers of tumors per animal, both of which are the appropriate parameters for reporting results."

And,

"Hence, the conclusion that a higher fraction of tumors were malignant among Cr(VI)-dosed animals is not supported by the data."

And,

"Finally, studies of Cr(VI)-exposed humans, including studies of Cr(VI)-exposed workers who presumably have also been exposed to sunlight, have never reported a statistically significant increase in skin cancer; thus, the relevance to humans is highly questionable. OEHHA should provide an analysis of the Davidson et al. paper to put the findings into the context of the significant limitations of the study."

Specific Response 39. With regards to the study limitations cited in Specific Comment 39: 1) The paper states that the UV radiation contained less than one percent in the UVC range, 2) The discussion of the study in the PHG document states that the observed dose-response was for chromate concentration, not chromate dose. To make this clearer the phrase "concentration in the drinking water" has been added to the document, 3) The PHG states that the observed dose-response was for skin tumor formation. To make this clearer the phrase "increased numbers of skin tumors" has been added, 4) The PHG document does not discuss the study's findings regarding what fractions of tumors were malignant, 5) With regards to skin effects in humans, note the "Toxicological Effects in Humans", subheading "Carcinogenicity" section of the PHG document, which discusses dermatitis in Portland cement workers due to exposure to Cr VI in the cement. We located very few epidemiological data to either support or refute a link between exposure to Cr VI and increased risk of skin cancer. Thus, we would not discount the observations of Davidson et al. (1994) in mice and their potential implications for human exposures to Cr VI.

Toxicological Effects in Humans

Specific Comment 40: "The discussion of allergic contact dermatitis is dated and incomplete."

Specific Comment 41: "Many pertinent papers are missing from this review. It is not clear how OEHHA selected only three papers for discussion of carcinogenicity by inhalation. Other studies that should be included are Boice et al. (1999), Birk et al. (2006), and Luippold et al. (2005). For a more complete discussion of carcinogenicity from inhalation, the OSHA hexavalent chromium rule (2006) provides a highly detailed review of the literature."

Specific Responses 40 and 41. PHG documents are not intended to be comprehensive reviews of the literature and in the case of Cr VI focuses on the oral pathway. The inhalation studies discussed were judged to be the best available at the time of the calculation. To clarify that the review is not comprehensive, OEHHA has added the phrase "selected studies."

Specific Comment 42: "Gibb and colleagues studied cancer mortality at all sites, but reported only that for lung, prostate, and all cancer. Observed and expected cancers for all sites are available from the original authors. The SMR for stomach cancer in this cohort is 0.48 (CI: 0.13, 1-24), demonstrating that even among this highly exposed cohort of chromate production workers, stomach cancer rates were not elevated. This observation should be included in OEHHA's review of GI-tract cancers among occupationally exposed populations."

Specific Response 42. The commenter makes a valid scientific suggestion that the unpublished results for stomach cancer from the Gibb et al. (2000) study be included in the OEHHA review. At this time, however, the review contains only published results. If unpublished results were to be included, an effort would have to be made to contact the investigators of all published studies and ask them if they can provide unpublished results. The scope of that effort is beyond the resources of OEHHA.

Specific Comment 43-1: "Although there are limitations to the Cole and Radu (2005) study, those limitations identified by OEHHA are not meaningful."

Specific Response 43-1. OEHHA believes that the limitations are meaningful because, as discussed in the PHG document, the analysis included studies in which there was no exposure to Cr VI, did not include studies in which there was Cr VI exposure, and included a study that was retracted by the journal that published it.

Specific Comment 43-2: "OEHHA should include a review of SES and stomach cancer, or cite the findings of Cole and Radu (2005)".

Specific Response 43-2. The following sentence was added to the PHG document: "A common limitation of the studies was lack of data on socioeconomic status, which may be associated with stomach cancer as noted by Cole and Radu (2005)."

Specific Comment 44-1: "OEHHA's review of occupational epidemiology data is riddled with errors and does not reflect a thorough review."

Specific Response 44-1. OEHHA acknowledges that it was not able to identify with 100 percent certainty all occupational studies with relevant exposure and excluded all studies with little exposure. A sentence about this has been added to the PHG document.

Specific Comment 44-2: "Virtually all of the papers identified in this section are not included in the reference section."

Specific Response 44-2. OEHHA has updated the PHG document. All cited occupational studies are now listed in the reference section.

Specific Comment 44-3: "OEHHA states…there is a 'suggestive link between inhalation exposure to hexavalent chromium and cancer of the digestive organs; however, such an evaluation is premature at best.'"

Specific Response 44-3. To lessen the implication of causality, the PHG document now says "consistent with an association between" instead of "suggestive of a link between."

Specific Comment 45-1: "OEHHA missed many papers…These include {studies of} tannery workers."

Specific Response 45-1. Since approximately World War II tanneries have switched to a single bath process that does not involve exposure to Cr+6. OEHHA has examined the tannery papers mentioned by the commenter and has concluded that Cr+6 exposure was unlikely for the workers studied in those papers.

Specific Comment 45-2: "OEHHA…missed the Guberan (1989) study of painters."

Specific Response 45-2. OEHHA carefully examined the Guberan (1989) study of painters in the general population of Geneva, Switzerland. The painters were identified during a national census. Based on information provided in the article, OEHHA's best professional estimate by two staff members with degrees in industrial hygiene is that less than 50% of the painters in Geneva were significantly exposed to Cr+6.

Specific Comment 45-3: "Also not included were a study of mild-steel and stainless-steel welders in France (Moulin et al 1993).

Specific Response 45-3. The Moulin 1993 article did not include results for digestive system cancers for stainless-steel welders, and mild-steel welders are not of interest to the PHG document because mild steel contains very little chromium compared to stainless steel.

Specific Comment 45-4: "{Also not included was} a study of deaths among die-casting and electroplating workers in the U.S. (Silverstein et al 1981)."

Specific Response 45-4. The Silverstein (1981) population included chrome platers, but results were not presented for chrome platers and they were probably a small portion of the entire population. OEHHA judged the study to be not useful.

Specific Comment 45-5: "{Also not included was} a study of stainless-steel, mild-steel, and shipyard welders in nine European countries (Simonato et al. 1991)."

Specific Response 45-5. The Simonato (1991) article did not include results for digestive system cancers for stainless-steel welders, and mild-steel welders and shipyard welders are not of interest to the PHG document because mild steel contains very little chromium compared to stainless steel.

Specific Comment 45-6: "{Also not included was} a study of chrome platers in Japan (Takahashi et al. 1990)."

Specific Response 45-6. The Takahashi (1990) cohort was updated by Itoh et al. (1996) and those results are already included in the PHG document.

Specific Comment 46: "How did OEHHA determine that at least half of the population likely has been exposed to Cr+6? What papers/findings were included or excluded on this basis?"

Specific Response 46. OEHHA made its best professional estimate as to which papers/findings should be included or excluded based on industrial hygiene-related information provided in the articles.

Specific Comment 47: "{The category} 'all digestive system cancers'…should be removed from the summary…This represents a broad category… OEHHA's review should consider only individual cancers, not the digestive system as a whole."

Specific Response 47. OEHHA agrees that the broad category of digestive system cancers is much less useful than organ-specific results. While less useful, OEHHA believes that the results for all digestive system cancers combined should be included in the PHG document because Cr VI could cause cancers in multiple digestive organs.

Specific Comment 48: "The assumption that underlies rate standardization methods is often questioned, because age, race, and gender distributions of occupational cohorts are unlikely to parallel those of the standard population."

Specific Response 48. OEHHA does not understand this concern about standardization, because the whole point of standardization is to control for differences in age, race, and gender distributions. Rate standardization methods are not "often questioned" in the opinion of OEHHA.

Specific Comment 49-1: "OEHHA twice included the same occupational cohort in the German chromate industry – Korallus et al. (1993) and Birk et al. (2006)… Birk et al. represents the most recent follow-up."

Specific Response 49-1. OEHHA agrees that there was overlap of populations between the two studies and has added a footnote to the entries for these studies in the PHG document.

Specific Comment 49-2: "OEHHA reported data from an unknown paper (Raffnsson 1984") concerning concrete mixers in Iceland…There is a more recent publication to Raffnsson et al. (1997) that could be the same cohort… Data from the more recent study should be included."

Specific Response 49-2. The commenter is correct that Raffnsson et al. (1997) is a more recent update and OEHHA has updated Table 8 to reflect the newer data.

Specific Comment 50: "OEHHA appears to have extracted relative risk estimates for more highly exposed subcohorts within the individual studies, but the approach taken appears random."

Specific Response 50. OEHHA has attempted to be consistent in judging the relative exposure levels of subcohorts within studies. With regard to the Axelsson *et al.*(1980) paper, for clarification OEHHA has added the following text to the PHG document: " In the Axelsson *et al.*(1980) study of ferrochromium manufacturing, arc furnace workers

were exposed to higher levels than other workers (0.25 mg/m^3 Cr VI versus a maximum of 0.05 mg/m^3 in other subcohorts), thus the results for the arc furnace workers were abstracted." With regard to Horiguchi et al. (1990) chrome platers, OEHHA confirmed that it abstracted the results for all chrome platers. With regard to Sorahan et al., (1987), OEHHA added text as follows: "In the Sorahan *et al.* (1987) study of metal platers, chrome bath workers were said to be 'more heavily exposed,' thus the results for the subcohort of workers whose first employment was 'chrome bath' were abstracted."

Specific Comment 51-1: "It should be recognized that OEHHA's literature review is incomplete."

Specific Response 51-1. OEHHA has attempted to perform a complete literature review, but acknowledges possible limitations in any literature searches. Thus OEHHA has added the following statement to the PHG document: "OEHHA cannot say with 100% certainty that all occupational studies with relevant exposures were included and that no studies with little exposure were included".

Specific Comment 51-2: "A conclusion regarding the number of studies with risk ratios less than or greater than one is not reliable."

Specific Response 51-2. OEHHA agrees and has removed all text and a table in which risk ratios were compared to 1.00.

Specific Comment 51-3: "Exposures to Cr+6 are low compared to other industries…Mixtures…added to cement…are sometimes carcinogenic—for example asbestos."

Specific Response 51-3. While exposures to Cr+6 may have been relatively low, OEHHA believes that the exposures were significant enough to warrant inclusion of cement industries. With regard to asbestos, the PHG documents states that the review excluded studies with workers exposed to asbestos-containing cement.

Specific Comment 51-4: "Rosenman and Stanbury (1996)…is a proportionate mortality ratio (PMR) study. OEHHA reported the PMR for stomach cancer. However, the PCMR (proportionate cancer mortality ratio) from the same study was not significantly increased for stomach cancer."

Specific Response 51-4. The commenter raises a valid scientific issue about whether the PMR or PCMR in a study is preferable. In this study, the overall risk of cancer was substantially elevated (~40%), primarily due to large excesses of lung cancer. OEHHA concluded that comparing proportions within all cancers (the PCMR method) would cause the ratio for stomach cancer to be biased downward because of the overall excess of cancer. Comparison to all other deaths (the PMR method) was judged by OEHHA to be the preferred measure of association in this study.

Specific Comment 52: "Evaluating the risk by counting the number of studies with risk ratios greater or less than one is not a valid scientific method."

Specific Response 52. OEHHA agrees and has removed text and a table in which the numbers of rate ratios above and below 1.00 were compared.

Specific Comment 53-1: "OEHHA has ignored..{studies of} environmental exposure to Cr+6 via ingestion, {including} Armienta-Hernandez and Rodriguez-Castillo (1995), Fryzek *et al.* (2001), and Bednar and Kies 1991)."

Specific Response 53-1. All three of these studies are now discussed in the PHG document.

Specific Comment 53-2: "The recent paper by Kerger *et al.* (2009) should be added to the discussion."

Specific Response 53-2. Kerger *et al.* (2009) has been added to the discussion.

Specific Comment 54-1: "Beaumont et al (2008) argues that Cr+6 exposure hastened the mortality of villagers with cancers initiated by other causes, which is a possibility given the timeline, but it is not reasonable to assume the Cr+6 exposure caused the cancers reported."

Specific Response 54-1. OEHHA agrees that Cr+6 might have promoted rather than initiated cancer. The point of Beaumont's argument was that it may not be reasonable to assume that Cr+6 exposure initiated the cancers. This agrees with the commenter's view.

Specific Comment 54-2: "An ecological measurement of exposure … was used to assign a level of Cr+6 exposure to the individuals included in the study."

Specific Response 54-2: OEHHA did not assign levels of Cr+6 exposure; rather, it only classified geographic regions as to yes or no with regard to contaminated water having been present.

Specific Comment 54-3: "There is reason to question the assignment of exposure status because of discoloration and poor taste. It is questionable whether residents continued to consume the affected water."

Specific Response 54-3. OEHHA agrees that the exposure may have been self limiting because of color and taste, and has added a paragraph to the PHG document regarding data on the color of the water in the Armienta-Hernandez and Rodriguez-Castillo (1995) study.

Specific Comment 54-4: "OEHHA should…focus on those studies most representative of California drinking water exposures (Fryzek et al.2001; Bednar and Kies 1991)."

Specific Response 54-4. Those studies did not provide useful results as explained in new text in the PHG document.

Specific Comment 55: "OEHHA is attributing these effects {oral ulcers, diarrhea, and abdominal pains in the villagers} to Cr+6 exposure. It can be surmised that the exposures of the villagers are not representative of Cr+6 exposures in California."

Specific Response 55. OEHHA has removed the paragraph about the acute symptoms reported by the Chinese investigators because the methods used for the symptom study are not clear. OEHHA agrees that the concentrations of Cr+6 in groundwater encountered by the villagers were not representative of typical exposures in California.

Specific Comment 56: "What evidence exists that Cr+6 in water causes an increase in lung cancer {among the Chinese villagers}?"

Specific Response 56. The PHG document has never claimed that Cr+6 in water causes lung cancer. OEHHA has added to the PHG text that cigarette smoking is an uncontrolled risk factor in the Chinese study.

Specific Comment 57: "We concur that more information is clearly needed to provide an adequate exposure assessment for Cr+6 {exposure in drinking water in the Chinese study}, and without such the study is of questionable reliability… Is OEHHA going to conduct more research to better assess exposure?"

Specific Response 57. OEHHA has no plans to further investigate exposure to the Chinese villagers. OEHHA did not use data from the Chinese study to calculate the PHG.

Specific Comment 58: "OEHHA failed to cite the study that shows human variability of Cr(VI) absorption due to differences in stomach pH; we are not aware of any such data…Hence, this statement appears to be speculative and should be supported or struck."

Specific Response 58. See Donaldson and Barreras (1966) for data showing increased Cr VI absorption in humans with pernicious anemia and achlorhydria. This citation has been inserted in the PHG document after the statement referred to in Specific Comment 58.

Examination of Evidence for Chromium Carcinogenicity

Specific Comment 59: "Neither the rats nor mice of the NTP study developed forestomach tumors, but the epithelial tissue of the forestomach was exposed to Cr(VI) under conditions of naturally higher pH. Hence, OEHHA's hypothesis that higher stomach pH is associated with an increased risk is not supported by the findings of the NTP study. Further, the Borneff et al. study does not provide adequate evidence that tumors of the forestomach occurred due to higher pH, because two of the three generations in the Borneff et al. study did not have an increased risk of forestomach tumors, despite the naturally high pH of the forestomach."

Specific Response 59. It is true that the mice in the NTP study did not develop tumors of the forestomach. However, different strains of mice were used in the NTP and Borneff et al. (1968) study. Mouse strain differences in tumor induction have been reported for other chemicals. As discussed in Appendix B of the PHG document, Borneff et al. (1968) suggested that tumor growth may have been inhibited in the F_1 and F_2 generations due to exposure to mousepox vaccine.

Specific Comment 60: "For accuracy and clarity, OEHHA should insert the word "lung" before "cancer" in this statement."

Specific Response 60. This change has been made.

Specific Comment 61: "As described above in detail, this conclusion is based on a flawed analysis and is not correct."

Specific Response 61. To lessen the implication of causality, the PHG document now says “consistent with an association between” instead of “suggestive of a link between.”

Specific Comment 62: “OEHHA ignored the more relevant studies by Fryzek et al. (2001) and Bednar and Kies (1991). These studies are more relevant, because they evaluated populations and exposures more consistent with Cr(VI) exposures of Californians. The OEHHA analysis of Zhang and Li (1987) is very uncertain and limited and should be considered, as Beaumont et al. (2008) described, as the basis for ‘hypothesis-generating,’ because it does not provide strong evidence that Cr(VI) exposures in drinking water are associated with cancer, and although it may be true for that population of rural Chinese villagers, it is not relevant for assessing the risk of cancer due to Cr(VI) in California drinking water.”

Specific Response 62. The studies cited in this comment (Fryzek et al, 2001; Bednar and Kies, 1991) are both discussed in the PHG document in the sub-section “Carcinogenicity” located in the “Toxicological Effects in Humans” section. Also discussed is a new epidemiology study of a geographic population exposed to Cr VI in drinking water in Greece. The limitations of these studies, discussed in the PHG document, did not allow conclusions to be drawn concerning organ-specific cancers.

Specific Comment 63: “OEHHA should heed the advice of the expert peer reviewers of this draft and the previous draft, and discontinue its relentless and unjustified position that the Borneff et al. study constitutes evidence that Cr(VI) causes forestomach (not stomach) tumors.”

Specific Response 63. The discussion of Borneff et al. (1968) was moved to the Appendix on the advice of some reviewers. OEHHA provided extensive analysis of its findings in its endeavor to consider all available scientific data when evaluating chemicals and developing PHGs aimed at protecting public health. The weight of evidence approach taken by OEHHA necessitated a discussion of Borneff et al. (1968).

Specific Comment 64-1: “OEHHA should focus its conclusions regarding genotoxicity on findings from drinking-water exposures that are more representative of environmental exposures. The findings of genotoxicity in these studies do not correspond to tumors in these tissues and are of questionable relevance for understanding the MOA. It is critical that OEHHA include the negative genotoxicity data of De Flora et al. (2008), because this data set is specific to the target tissue (small intestine) where tumors were observed in the NTP mice, which is the basis of the cancer PHG.”

Specific Response 64-1. When making MOA determinations, OEHHA, U.S. EPA and others commonly consider the results of genotoxicity tests in tissue not yielding tumors (OEHHA, 2009; U.S. EPA, 2005). The mutagenic MOA described by McCarroll et al. (2010) has also been added to the document. The data of De Flora et al. (2008) have been added to the PHG document. It is now noted in the PHG document that De Flora et al. (2008) are the only investigators known by OEHHA to have looked for genotoxicity in the GI tract of rodents exposed to Cr VI in drinking water. However, judging from the responses of their positive controls, it is likely that their methodology lacked the sensitivity to measure DNA damage at the dose levels tested. In addition, McCarroll *et al*. (2010) noted that the levels tested in this study may in part explain the negative

results because they are below the exposure levels used in the NTP 2-year drinking water study.

Specific Comment 64-2: "Further, OEHHA cites the genotoxicity study of Bigaliev et al. (1977), conducted by gavage dosing, seven times in the PHG document, and not in a manner questioning the findings. Yet Footnote 2 of Table 2 (page 39) indicates that OEHHA does not understand what the authors were originally reporting in the study, presumably in part because the paper is in Russian. Yet a far superior study, Mirsalis et al. (1996), of mice and rats exposed by drinking water, and conducted using well-recognized and accepted scientific methods, is cited only three times. Another example of this biased reporting of the literature is the genotoxicity study of Kuykendall et al. (1996), which is a study in humans exposed by drinking water is also cited only three times in the PHG document. Clearly, the data produced by Kuykendall et al. and Mirsalis et al. are far more meaningful for understanding risk assessment than the data on which OEHHA relies. OEHHA must revise the analysis to remove bias and provide a balanced review of the literature, focusing on those studies that provide the most meaningful information for Californians exposed to Cr(VI) in drinking water."

Specific Response 64-2. The ambiguity in Bigaliev et al. (1977) related to the part of the study that was conducted for one year. The uncertainty regarding exactly how the dose was applied is described in the footnote to Table 2. The part of that study in which the animals were administered a single dose by gavage was clearly understandable from the translation. Table 2 presents all the genotoxicity studies considered in the PHG document. Some of these were positive for genotoxicity and some were negative. The PHG document summarizes these results as follows:

> Fifteen primary studies of the potential genotoxic effects following ingestion of Cr VI by humans or other mammalian species were located. A summary of these studies is provided in Table 2. Nine of the fifteen studies reported positive genotoxicity findings in various tissues.

We believe this is an even-handed evaluation of the findings of the available studies. The studies listed in Table 2 were judged to have been of sufficient quality to be included in the weight-of-evidence consideration of whether Cr VI is genotoxic. It is not clear why the studies by Mirsalis et al. (1996) and Kuykendall et al. (1996) are considered "far more meaningful" than the other studies cited in Table 2.

Specific Comment 65: "(Page 73 of the August 2009 draft PHG document) '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'."

As noted above, the NTP study authors (Stout et al. 2009) recognized that the administered dose in the NTP study exceeded the reductive capacity of the stomach. They stated, "Under the conditions of this study, at least a portion of the administered Cr(VI) was not reduced in the stomach." OEHHA should accept the NTP authors' conclusions, rather than repeatedly asserting the opposite based on an obviously flawed attempt to calculate reductive capacity in a mouse based on a crude scaling of human data."

Specific Response 65. A recent publication by the NTP authors (Collins et al. 2010) is discussed in Appendix A of the PHG document. Figures A3 to A6 show the accumulation of chromium in different tissues of female mice administered drinking water containing Cr VI ranging from 5 to 180 mg/L. Chromium accumulation was either linearly related to the concentration of Cr VI in the drinking water over the entire concentration range tested, or linearly related at low concentrations with indications of a plateau at higher concentrations. These data suggest that the reductive capacity of the GI tract was not exceeded in the two-year bioassay (NTP, 2008). The conclusion that the reductive capacity was not exceeded is compatible with the statement of Stout et al. (2009) that a portion of Cr VI escaped reduction since, as recognized earlier in these comments from the CMTA, Cr VI reduction and absorption are considered to be competing processes.

Mechanism of Genotoxicity and Carcinogenicity

Specific Comment 66-1: "As noted by DTSC, the NTP study provides ample evidence for inflammation, chronic tissue damage, and regenerative hyperplasia as key promotional events that are necessary for tumor development. Further, De Flora et al. (2008) examined the potential for genotoxicity in the mouse small intestine (the target tissue) and found that at drinking-water exposures of 5 and 20 mg/L, oxidative DNA damage and DNA-protein crosslinks did not occur. While target tissue data in the small intestine at the doses that caused tumors in the NTP study do not exist, the only available target tissue genotoxicity data demonstrate that, at exposures far higher than current drinking-water exposures in California, a mutagenic MOA is not operative because necessary key events were not observed. Consistent with the current state of the science, these and other questions in the mechanism of carcinogenicity should be addressed with an MOA/HRF analysis."

Specific Response 66-1. See the discussion of NTP (2008) and Table 7 in the PHG document. No inflammation or chronic tissue damage (including intestinal epithelial cell injury) was observed in the tumor-bearing tissue in either mice or rats. Hyperplasia was observed in mouse intestine but there were no indications that it was regenerative. With regard to De Flora et al. (2008), the DNA-protein crosslinks and 8-oxo-dG adducts they assayed (see Table 2 of the PHG document) comprise only a small subset of the types of DNA damage caused by Cr VI. In addition, judging from the responses of their positive controls, it is likely that their methodology lacked the sensitivity to measure DNA damage at the dose levels tested. Lastly, McCarroll *et al*. (2010) noted that the levels tested in this study may in part explain the negative results because they are below the exposure levels used in the NTP 2-year drinking water study.

Specific Comment 66-2: "OEHHA cites no data that would indicate that Cr(VI) is genotoxic in target tissues, but rather, relies on genotoxicity data developed from animals exposed to extremely high concentrations of Cr(VI) and doses delivered by non-drinking water routes. The tissues evaluated in these studies included the liver and circulating blood lymphocytes, but evidence of tumors or the potential for a carcinogenic response in these tissues were not seen in the NTP study. Thus, these observations are of questionable usefulness, because they are not "anchored" in observations of tumors in these tissues. Further, studies that had negative findings for genotoxicity in humans following drinking-water exposures at 10 mg/L (Kuykendall et al. 1996), and in

mice and rats at drinking-water exposures of 1, 5, and 10 mg/L (Mirsalis et al. 1996) were not given adequate consideration."

Specific Response 66-2. The short-term tests presented in Table 2 of the PHG document were designed to determine whether ingested Cr VI is genotoxic in a variety of different tissues. Such genotoxicity data stand on their own, irrespective of whether tumors were detected in the same tissues in a two-year bioassay. We believe that the positive and negative studies comprising Table 2 are presented in an evenhanded manner.

Specific Comment 67: "The response of mice is likely due to non-genotoxic processes related to regenerative hyperplasia, which is secondary to epithelial injury and not operative at low doses."

Specific Response 67. See the discussion of NTP (2008) and Table 7 in the PHG document. No inflammation or chronic tissue damage (including intestinal epithelial cell injury) was observed in the tumor-bearing tissue in either mice or rats. Hyperplasia was observed in mouse intestine but there were no indications that it was regenerative.

Specific Comment 68: "The current method for evaluating whether the collection of available data is relevant for humans is by using the MOA/HRF. This must be included in the PHG document."

Specific Response 68. The mechanism of action of Cr VI and its relevance to humans is discussed at length in the PHG document, especially in the following sections: "Mechanism of Genotoxicity and Carcinogenicity," "Carcinogencity (Animals), Non-neoplastic findings – Possible relationships between tissue damage, inflammation, hyperplasia and tumors in rats and mice," and "Examination of Evidence for Chromium Carcinogenicity." The document now cites the mutagenic mode of action described by McCarroll et al. (2010).

Dose-Response Assessment

Specific Comment 69: "The descriptions for these studies [Chopra et al., 1996 and Acharya et al., 2001] should indicate that the reported LOAEL was the only dose tested. Further, as discussed above (Comment 20), histological findings in the liver and kidney are not quantified or otherwise clearly reported."

Specific Response 69. These study shortcomings are listed in Table 1 of the PHG document entitled "Strengths and Weaknesses of Available Hexavalent Chromium Bioassays."

Specific Comments 71 and 72: "In the two-year NTP study of sodium dichromate, the only nonneoplastic lesion observed in the liver at the lowest dose was mild chronic inflammation in female rats."

Specific Responses 71 and 72. The discussion of the NTP (2008) study in the "Chronic Toxicity" section of the document has been revised. It now states that female rats exhibited another change in addition to inflammation at the lowest dose level; that of increased fatty changes to the liver. This discussion also notes that while fatty changes were increased at all dose levels, the increase was not statistically significant compared to the control at the lowest dose level.

Specific Comment 73: "OEHHA should use the current version of the BMD model for their PHG slope factor derivation."

Specific Response 73. BMDS versions 1.4.1 and 2.1 gave identical risk estimates.

Specific Comment 74-1: "Comparisons of the results from Table 11 to those generated by the New Jersey Department of Environmental Protection, for the same data set, yields a different LED_{10} and p-value for most models (see Table 4a of NJDEP 2009). OEHHA should check the calculations and results to ensure their correctness, and should provide the detailed output information from the BMD modeling work…Also, it is unnecessarily confusing to present the results for just the duodenum in Table 9, when the results for total cancers of the small intestine are the basis for the PHG."

Specific Response 74-1. The cancer slope factors for male and female mice, as shown in Tables 10 and 11, are now the same as those calculated by both Stern (2010) and U.S. EPA (2010). The results for the duodenum only were dropped from both tables. The BMD modeling work consisted only of the results for the multistage model. The Chi-square statistics, P-values, ED_{10} values and LED_{10} values for that model are presented in Tables 10 and 11. Only the multistage model was used to model the tumor incidence data because this is the model preferred by OEHHA (2009) and U.S. EPA (2010) for conducting cancer dose-response assessment. This is primarily due to the multistage model's generally good fit of the data in the relatively high dose range used in rodent bioassays (Armitage and Doll, 1961).

Specific Comment 74-2: "It is important to recognize that the uncertainty in the assumption of a linear dose-response is readily quantified here. The LED_{10} can be used with a 30-fold uncertainty factor (3-fold factor for toxicdynamics and 10-fold for intraspecies variability) to derive a HPD that is 200-times higher than the value developed from the linear dose-response."

Specific Response 74-2. OEHHA does not consider this a quantification of the uncertainty associated with its method for calculating the cancer risk. Rather, this is a choice to use a different model based on a different mechanism of action.

Specific Comment 75-1: "It is interesting to note that the findings of the NTP study for the mouse were not consistent with those for the rat, yet these two species have very similar GI anatomy and physiology, both of which are substantially different from that in humans. Hence, it is not reasonable to assume that findings in one rodent species (tumors in the small intestine) could be extrapolated to humans, when the same outcome did not occur, at the same and higher dose, in another rodent species (rat)."

Specific Response 75-1. Standard cancer risk assessment practice is to assume that humans are at least as sensitive as the most sensitive species tested (U.S. EPA, 2005; OEHHA, 2009). It is not uncommon for a chemical to cause tumors in one rodent species and not in a second species. In the case of Cr VI, both rodent species tested yielded tumors.

Specific Comment 75-2: "The PHG uses a customary approach to scale between species, but this approach does not adequately account for differences in Cr(VI) reduction in the human GI as compared to rodents."

Specific Response 75-2. OEHHA employed the same interspecies scaling methodology used by NTP (Stout et al., 2009) and U.S. EPA (2010) for the mouse tumor data.

Specific Comment 76: "New Jersey DEP (2009) used yet a different approach and applied the time-weighted average body weight at zero dose, which coincidently equals 0.05 kg. OEHHA should provide justification for the approach used to scale to humans."

Specific Response 76. The PHG document has been revised and now uses the time-weighted average bodyweight of the control male or female mice in the NTP (2008) study to scale from mice to humans (OEHHA, 2009). This is shown in the "Dose-Response Modeling" section of the document, where Tables 10 and 11 are discussed.

Specific Comment 77: "If there is no evidence of saturation, OEHHA provides no basis for excluding the high dose group. More information regarding this analysis is needed. What is the basis for dropping the high-dose group? We could not reproduce the stated results for female mice without dropping the highest dose group. OEHHA should provide greater detail on how the BMD model parameters were set, so that the modeling analysis can be reproduced."

Specific Response 77. As shown in Table 11 of the PHG document, the small intestine tumor data for female mice were modeled after excluding the high dose value. That the high dose tumor incidence data from female mice were dropped prior to BMD modeling is stated in the text and in the title of Table 10. As stated in the discussion accompanying Table 10, the high dose value was excluded because this yielded an acceptable fit of the model (according to Chi-square statistic and P value) to the data in the low dose region of the dose response curve. The low dose region is the region of importance for modeling the cancer risk, since the point of departure is in this region. There are many possible reasons for the bending of a dose response curve in the high dose region including pharmacokinetic, toxicodynamic and others. Dose response modeling, including dropping a high dose data point, is commonly performed when the biological basis for the bending over of the dose response curve is not known.

Specific Comment 79: "Hence, the upper confidence interval on the more refined dose-response assessment by Crump et al. (2003) is more than an order of magnitude lower than the value used by OEHHA of 0.15 $(\mu g/m^3)^{-1}$…OEHHA should delete its evaluation of an inhalation cancer slope factor using the Gibb et al. (2000) published data, and use the published risk assessments of Crump et al. (2003) and Park et al. (2004), because these risk assessments used far superior data sets, as compared to that used by CalDHS in 1985; they were not forced to rely on only the published findings in the mortality studies, but had access to all the original data from which to do their assessment."

And,

"The most robust modeling analyses rely on larger numbers of data points. Park et al. modeled five, and Crump et al. modeled seven. OEHHA should use the published risk assessments, because they are far superior to the analysis presented."

And,

"We urge OEHHA to review the OSHA 2006 rule and utilize the published risk assessments as the basis for a new Cr(VI) inhalation cancer risk assessment in this document."

Specific Response 79. As shown in Table 18 of the PHG document, the proportion of the total cancer risk contributed by inhalation is very small: less than ~0.6%. Using a decreased estimate of the inhalation potency of Cr VI, as suggested in the comment, will have no significant effect on the final PHG value.

Calculation of the PHG

Specific Comment 82: "For consistency with OEHHA's most current guidelines for development of chronic toxicity criteria for noncancer effects, *Technical Support Document for the Derivation of Noncancer Reference Exposure Levels* (June 2008), OEHHA should use BMD modeling, rather than LOAELs and NOAELs, as the basis for determining an HPD…Use of this approach, as in ATSDR (2000), would reduce uncertainty and likely increase the non-cancer PHG; e.g., the ATSDR Health Protective Dose (HPD)-equivalent is seven times higher than the current PHG."

Specific Response 82. At present the Cr VI PHG based on cancer effects is 100-fold lower than if it were based on non-cancer effects (see "Calculation Of The PHG" section of the document). OEHHA will be applying the BMD approach in future analyses of the non-cancer data. Our preliminary analysis applying the BMD approach to the non-cancer data followed by an uncertainty factor of 100 yields a final value that is more than 100-fold ***higher*** than the proposed PHG based on cancer effects. Thus, the proposed PHG (0.02 ppb) for protecting against both cancer and non-cancer effects would not change.

Specific Comment 84: "For example, OEHHA (2008) recommends a UF of 6 to extrapolate from a LOAEL for a mild effect to a NOAEL, and an uncertainty factor of 1 if the study duration is greater than 12% of lifetime, or $\sqrt{10}$ if the study is 8%-12% of estimated lifetime."

Specific Response 84. The noncancer liver effects in female rats at the LOAEL were chronic inflammation and fatty changes (NTP, 2008). The incidence of animals with fatty changes was increased relative to controls at the lowest dose level, but was statistically significant only at the three higher dose levels (discussed in the PHG document). Since the health consequences of these effects are not known, OEHHA hesitates to classify them as "mild." Even were they to be classified as "mild," OEHHA would still use an uncertainty factor of 10 to extrapolate from a LOAEL to a NOAEL ***for a chronic study*** (see Table 4.4.1 in OEHHA, 2008). Since the study lasted two years, an uncertainty factor for less-than-lifetime duration is not needed.

Specific Comment 85: "As discussed previously, the inclusion of this study in the dose-response assessment or calculation of a PHG is problematic due to conflicting information from at least two other studies. The liver effects seen in this mouse study at relatively low concentrations (50 ppm in diet) were not seen in another mouse study or another rat study at doses up to 400 ppm in diet, and were not seen in the 2007b NTP study."

Specific Response 85. The "problematic" study referred to in Specific Comment 85 is the subchronic study by NTP (1997a). We have expanded the discussion of NTP (1997a) and have now pointed out that similar liver effects were not observed in NTP (1997b), a subchronic study using the same strain of mouse and similar levels of Cr VI in the feed, or in the two year drinking water study (NTP, 2008).

Specific Comment 88-1: "For clarity, the only effect seen at the 0.2-mg/kg-day dose was mild chronic inflammation of the liver. Fatty changes were seen in the female rat liver at the next-higher dose (0.9 mg/kg-day)."

Specific Response 88-1. Fatty changes to the liver were also increased relative to controls at the lowest dose level of 0.2 mg/kg-day; however, the fatty changes at the lowest dose level were not statistically significant relative to controls. This is now stated in the PHG document.

Specific Comment 88-2: "OEHHA should use the benchmark dose modeling approach for consistency with its own guidance and that of ATSDR."

Specific Response 88-2. At present the Cr VI PHG based on cancer effects is 100-fold lower than if it were based on non-cancer effects (see "Calculation Of The PHG" section of the document). OEHHA will be applying the BMD approach in future analyses of the non-cancer data. Our preliminary analysis applying the BMD approach to the non-cancer data followed by an uncertainty factor of 100 yields a final value that is more than 100-fold ***higher*** than the proposed PHG based on cancer effects. Thus, the proposed PHG (0.02 ppb) for protecting against both cancer and non-cancer effects would not change.

Specific Comment 88-3: "The endpoint of liver inflammation may not be biologically relevant to humans, because the NTP study rats had high rates of liver inflammation among control animals (24% of control female rats and 38% of male controls had liver inflammation), suggesting that these rodents are more prone to liver inflammation than humans."

Specific Response 88-3. We have not been able to locate data on the incidence of chronic liver inflammation in otherwise healthy humans. Without such data it is not possible to conclude that, "the endpoint of liver inflammation may not be biologically relevant to humans."

Specific Comment 88-4: "NTP (2008) described the rat liver histology data as providing "an indication of a chronic inflammatory process of minimal severity in the liver." Thus, inflammation would likely be considered a "mild" effect (as identified in OEHHA 2008), and a UF of 6, as opposed to 10, is appropriate."

Specific Response 88-4. As shown in Table 4.4.1 of OEHHA (2009), for extrapolating from a LOAEL to a NOAEL in a chronic study OEHHA recommends use of an uncertainty factor of 10.

Specific Comment 90-1: "The inhalation cancer risk assessment section does not conclude that OEHHA is using its inhalation slope factor from 1985 for this PHG until it is presented here. We note our earlier comments that this is not the most scientifically advanced position, nor is it based on the best scientific data."

Specific Response 90-1. As shown in Table 18 of the PHG document, the proportion of the total cancer risk contributed by inhalation is very small: less than ~0.6%. Using a decreased estimate of the inhalation potency of Cr VI, as suggested in the comment, will have no significant effect on the final PHG value.

Specific Comment 90-2: "The PHG document would benefit considerably from inclusion of a quantitative, and expanded qualitative, uncertainty analysis. This was also specifically requested by Dr. Roberto Gwiazda in his peer-review comments, but OEHHA responded, 'While there are many sources of uncertainty, the ability to quantify various sources of uncertainty (e.g., the uncertainty associated with using the findings in animals to predict effects in humans, extrapolating risk associated with high doses to low doses, etc.) is problematic given the lack of data. The PHG discusses uncertainty in the Risk Characterization portion of the document, but the PHG document does not attempt to quantify the uncertainty because there is no accepted method for carrying out such a calculation.' This response is not accurate, and a quantitative assessment is feasible."

"Numerous methods are available for quantitatively assessing uncertainty in risk assessments. Further, uncertainty regarding extrapolating from animals to humans and high to low doses in the CR(VI) risk assessment can be addressed using data that are generated by PBPK modeling. Although we strongly recommend that OEHHA complete the PHG document using the refined PBPK models currently under development at The Hamner Institutes, the currently available models developed by O'Flaherty could be used for a quantitative evaluation of toxicokinetics between species."

Specific Response 90-2. PBPK modeling based on the limited data we currently have would add more uncertainty to the derivation of the PHG.

Specific Comment 90-3: "Other assumptions, such as linear extrapolation from cancer risk at high doses to that at low doses, can be quantified. Using OEHHA's current analysis, it is possible to quantify the PHG using standard U.S. EPA (2005) methods, with the assumption of a threshold dose-response, and the resulting cancer PHG is at least 200 times higher. Also, a non-cancer PHG using BMD modeling similar to that conducted by ATSDR results in a cancer PHG that is seven times higher than the current value."

Specific Response 90-3. The issue of whether or not to use linear extrapolation to estimate the cancer risk is not an issue in uncertainty quantification. It is not clear what is meant by, "it is possible to quantify the PHG." Also, it is not clear how a "non-cancer PHG" can "result in a cancer PHG."

Specific Comment 90-4: "Further, OEHHA guidance indicates that a qualitative discussion of the sources and potential impact of uncertainty is important to include in risk assessments (OEHHA 2003). While the draft document includes a brief discussion of uncertainty in the development of the PHG (p. 98), the document does not discuss the impact of OEHHA's compounded conservative assumptions on the resulting calculated PHG. Guidance on how to perform and interpret uncertainty analyses for risk assessment is found in numerous publications and regulatory documents (Hammonds et al. 1994; U.S. EPA 1997). OEHHA should perform a more extensive qualitative and quantitative uncertainty analyses and include these in the next draft document."

Specific Response 90-4. The results of this risk assessment are more certain than most PHGs. Cr VI is a known human carcinogen. A state-of-the-art chronic bioassay demonstrated carcinogenicity in two animal species in the relevant route of exposure, drinking water. There are extensive studies demonstrating the genotoxicity of Cr VI in vitro and in vivo. The description of uncertainty in the document is sufficient for the purpose of developing this PHG.

Appendix A – Carcinogenic Threshold?

Specific Comment 92-1: "Comment 92 OEHHA's analysis of the lack of a carcinogenic threshold is flawed and should be removed."

Specific Response 92-1. The use of the term "carcinogenic threshold" in the title of Appendix A has created confusion. It was meant to apply only to the results of the NTP (2008) bioassay and the dose range tested in that study. Over that dose range, the rodent GI tract's ability to reduce Cr VI to Cr III was not exceeded. Therefore, the title has been modified to "Carcinogenic Threshold: Was the reductive capacity of the rodent GI tract exceeded in the NTP (2008) bioassay?"

Specific Comment 92-2: "Because the doses that resulted in cancer in the NTP study were below OEHHA's estimated reduction capacity threshold, OEHHA incorrectly concluded that there is no threshold for carcinogenicity."

Specific Response 92-2. OEHHA did not draw such a conclusion. However, the NTP (2008) study results do show that the apparent threshold for increased tumor incidence in that study was not due to exceeding the reductive capacity of the rodent GI tract over the dose range tested. This has been added to the discussion in Appendix A. Whether the rate of reduction of Cr VI to Cr III changes markedly over a much lower dose range is not known (discussed in the "Metabolism and Pharmacokinetics" section of the PHG document).

Specific Comment 92-3: "Only through the use of a PBPK model could one quantify a threshold dose, because it is necessary to consider the rate of reduction and the rate of absorption, not absolute quantities, and quantify the differences in rates and volumes between species. The O'Flaherty et al. (2001) PBPK model makes this point quite clearly. However, the published O'Flaherty et al. (2001) model is not sufficiently sophisticated to accommodate the requirements of the current risk assessment, because it does not have a small-intestine compartment and is limited to humans and rats. The Hamner Institutes is currently expanding and refining the PBPK model for humans and rats and developing a model for mice using the NTP data and the preliminary results of ongoing studies. This work will allow for interspecies extrapolations and evaluations of tissue dose in the low dose range, which is relevant to environmental exposures."

Specific Response 92-3 (repeat of General Response 4). Health and Safety Code Section 116365.5 required the department (now the Department of Public Health) to adopt a Cr VI Maximum Contaminant Level (MCL) by January 1, 2004. Section 116365 also mandates the development of PHGs as part of the process of adopting drinking water standards. Given the mandate to adopt a standard for hexavalent chromium by

January 2004 it is very difficult to justify additional delays in the development of a PHG for hexavalent chromium.

It is also difficult to predict the impact of future research on the development of PHGs. OEHHA does not know when the results of future research will become available, nor can OEHHA predict the outcome of future research, nor does OEHHA know how future findings could impact the development of PHGs in the future.

Section 116365 contains an important provision that addresses new scientific research when it becomes available: "(e) (1) Public health goals established by the office shall be reviewed at least once every five years and revised, pursuant to the provisions of subdivision (c), as necessary based upon the availability of new scientific data". When new research is completed and published in a peer-reviewed format, OEHHA will consider it in the development of a revised PHG for hexavalent chromium. OEHHA acknowledges that new studies may alter a revised PHG. From the risk characterization section of the 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."

OEHHA will review the Hamner Institutes PBPK model upon its completion. If the model produces compelling information that should be reflected in the PHG document, OEHHA will take appropriate action.

Specific Comment 93: "Further, OEHHA must consider that, because tumors occurred only in the alimentary tract of the rodents, whether the observed tumors occurred at exposures that exceeded the reductive capacity of the rodent GI is actually not relevant (i.e., systemic absorption was not necessary for the observed effects to occur)."

Specific Response 93. If the reductive capacity is exceeded one would expect both more Cr VI to be absorbed into the systemic circulation and more to be transported directly from the intestinal contents into the epithelial cells that line the small intestines.

Specific Comment 94: "Proctor et al. (2002a) measured the rate of reduction of Cr(VI) in gastric acid; hence, the mg/L is of stomach acid, not mg/L of water. Further, the study tested Cr(VI) concentrations primarily in the ppb range. It is not clear where the 3 to 10 mg/L comes from. However, OEHHA uses this inaccurate statement to assume that the 10 mg/L of Cr(VI) administered in the Kerger et al. (1996) study is below a reductive threshold of the stomach. That clearly misrepresents the work quoted."

Specific Response 94. The text has been revised accordingly.

Specific Comment 95: "(From page 115 of the August 2009 draft PHG document) '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.' OEHHA should recognize that this argument holds only at the exposure levels tested (>5 mg/L) and in the species tested (rodents). There is no basis to assume that the reductive rate and capacity of humans are the same as rodents, and further, the exposure levels tested clearly do exceed the capacity of the rodent stomach to reduce Cr(VI) to Cr(III). If OEHHA actually believes that looking at tissue accumulation will identify a threshold, it should further consider the findings of

Sutherland et al. (2000), wherein chromium accumulated in tissues among rats exposed at 3 mg/L and 10 mg/L but not at 0.5 mg/L."

Specific Response 95. The results of Sutherland et al. (2000) have been cited by some as a demonstration of a threshold for Cr VI absorption of between 0.5 and 3 mg/L. For reasons discussed in the PHG document, including methodological limitations and results from other studies performed with radioactive Cr VI, the Sutherland et al. (2000) data should not be used as a basis for concluding that essentially all Cr VI is reduced to Cr III at drinking water concentrations of 0.5 mg/L and below.

Appendix B

Specific Comment 96-1: "It is not necessary to provide the detailed justification for considering the Borneff et al. study results, because the NTP study is used for risk assessment in the PHG document…In reality, what OEHHA has provided is a series of guesses to support their previous work. The study does NOT contribute to our understanding of why Cr(VI) is an oral carcinogen."

Specific Response 96-1. The discussion of the Borneff et al. (1968) study was moved to the Appendix on the advice of some reviewers. OEHHA provided extensive analysis of its findings in its endeavor to consider all available scientific data when evaluating chemicals and developing PHGs aimed at protecting public health. The weight of evidence approach taken by OEHHA necessitated a discussion of Borneff et al. (1968).

Specific Comment 96-2: "OEHHA has not considered in this entire dialog that Borneff et al. (1968) tested Cr(VI) at a dose more than two times higher than any of the NTP doses, so it is entirely possible that the effect, if it is real (which we don't think it is) is due to the higher dose."

Specific Response 96-2. We have added this information to the discussion of Borneff et al. (1968) in Appendix B.

Comments from Mark Johnson, Coachella Valley Water District (CVWD)

Comment 1: "CVWD understands other studies exist and are referenced in the document providing evidence that complete reduction may not always occur, but believes the administered doses in the NTP study are so large they easily overwhelmed the reductive capacity of both the oral cavity and the stomach in the rodents."

Response 1. See Appendix A in the PHG document for a discussion of the data showing that the Cr VI reducing capacity of the rodent GI tract was not saturated over the dose range tested in the NTP (2008) two-year study.

Comment 2: "This is especially significant as the NTP study did not find excess cancers at the lowered doses in both rats and mice."

Response 2. The absence of excess tumors at the lower dose levels may have been due to the use of too few animals to detect a relatively rare event (tumor formation).

Comment 3: "Equally as important, the stomach composition of humans and rodents is very different, with humans having a much more sophisticated and higher level of gastric juices than rodents."

Response 3. See the "Metabolism and Pharmacokinetics" section and Appendix A of the PHG document for discussions of human data indicating that Cr VI escapes reduction and is absorbed into the circulation at drinking water concentrations below those used in the NTP (2008) two-year bioassay in rodents.

Comment 4: "After extensive review, an expert panel report concluded this study [Borneff et al., 1968] was seriously flawed due primarily to poor hygiene, which killed most of the parent and first generation mice and could have been the cause of the specific adverse effects that the authors attributed to hexavalent chromium. This study has no merit and should not be used to support the subject PHG."

Response 4. The Borneff et al. (1968) study is not a key study in the derivation of the PHG for Chromium VI and was moved to the Appendix for that reason. OEHHA provided extensive analysis of its findings in its endeavor to consider all available scientific data when evaluating chemicals and developing PHGs aimed at protecting public health. The weight of evidence approach taken by OEHHA necessitated a discussion of Borneff et al. (1968).

Comment 5: "In the work completed in 1987 and 1997 by Zhang and Li, the data shows a negative dose-response between chromate exposure in drinking water and cancer rates found in about 10,000 villagers exposed to groundwater contaminated with hexavalent chromium levels as high as 2,600 ppb. The authors concluded there was no association between chromate exposure and any form of cancer in this population. Using a selective re-analysis of this study, the PHG document concludes a statistically significant increase in stomach cancer occurred based on unsupported assumptions about water consumption practices, plume migration and population distributions."

Response 5. The commenter is in error in saying that the 1987 Zhang and Li paper concluded there was no association; only the 1997 paper, which has since been withdrawn by the journal that published it, concluded that there was no association. Although the names of the second authors of the two papers appeared to be the same, they are different individuals. The findings of Beaumont et al. (2008) replicated the findings of the original study: a statistically significant increase in stomach cancers. This is discussed in detail in the "Toxicological Effects in Humans, Carcinogenicity" section of the PHG document. See that discussion as well as the original publication by Beaumont et al. (2008) for a description of the assumptions and limitations of the OEHHA analysis. Another recent reevaluation of the Zhang and Li (1987) study by Kerger et al. (2009) has been added to the PHG document.

Comments from C. L. Stathos, Department of Defense

Cover Letter Comment 1: "To ensure complete transparency and improve understanding of the science underlying the proposed PHG, we urge the California Office of Environmental Health Hazard Assessment (OEHHA) to publish an analysis of the available weight-of-evidence for (a) the determination that Cr6+ is genotoxic and (b) epidemiological evidence of gastrointestinal cancer causation."

Cover Letter Response 1. The issue of genetic toxicity is discussed at length in the PHG document in the "Genetic Toxicity" section. This discussion focuses on studies

performed *in vivo*, some of which were negative and some positive for genotoxicity. The PHG document also provides citations for a number of review articles which discuss Cr VI-induced genotoxicity in bacteria and cultured mammalian cells. Epidemiological studies of GI tract cancers in humans are discussed for exposure by inhalation (Table 8) in the "Cancers of ingestion- and digestion-related organs reported in occupational studies" section of the PHG, and for exposure by ingestion in the "Ingestion studies" section (Figure 15).

Cover Letter Comment 2: "We also recommend the publication of statistical analyses of the correlations between the State's Cr6+ drinking water data and incidence of gastrointestinal cancers. We further recommend that OEHHA make available a comparison of these data to the cancer incidences predicted by the risk assessment on which the draft PHG is based. This will provide needed perspective on the proposed PHG for the public."

Cover Letter Response 2. OEHHA is not aware of any published study or report comparing statewide Cr VI ingestion rates and incidences of GI tract cancers other than the very limited study of twelve families living in Hinkley, California. That study (DHHS, 2000) is discussed in the PHG document in the "Ingestion studies" section. We are unsure that a study such as that proposed in Cover Letter Comment 2 is feasible.

General Comment 1: "These data also strongly suggest that Cr+6 is a site-of-contact carcinogen. The data do not suggest that Cr+6 is a systemic carcinogen because…"

General Response 1. Given the types of tumors observed in the NTP (2008) bioassay (oral cavity in rats and small intestine in mice), Cr VI may be a site-of-contact carcinogen. However, Cr VI also caused systemic toxicity (see "Chronic Toxicity" section of the PHG) including genotoxicity in the liver (Table 2), indicating it was absorbed and bioavailable to distant tissues. Thus, we would not rule out the potential of Cr VI to cause cancer at sites distant from the GI tract.

General Comment 2: "Nevertheless, the data provide information that is useful and provide a biologically plausible alternative to the standard, default analysis that assumes systemic carcinogenicity. This alternative analysis could be part of the risk characterization that is presented to the decision-maker."

General Response 2. Given the available data OEHHA is not able to distinguish between a point of contact or a systemic mechanism of carcinogenesis by Cr VI. We have added this information to the "Examination of Evidence for Chromium Carcinogenicity" section of the PHG document.

General Comment 3: "The dose-response curves for both cancer and mutagenicity are highly nonlinear, with statistically significant increases observed at only the highest doses, i.e., not at the lower doses. These would support a nonlinear extrapolation from the point of departure."

General Response 3. The absence of statistically significant increases in tumors at the two lowest drinking water concentrations in NTP (2008) should not be interpreted as a threshold for tumorigenicity, since the number of animals may have been too low to detect tumors at the two lowest drinking water concentrations. The use of high doses in

cancer bioassays is generally thought to offset the statistical limitations of using small numbers of animals (50 /sex/dose level) to measure a relatively rare event (tumors).

General Comment 4: "Point-of-contact carcinogens are usually caused by triggering events that only occur at high doses, e.g., irritation or cellular toxicity, rather than low-dose mutagenicity that is the historical basis for the linear extrapolation as a default for carcinogenesis."

General Response 4. See discussion of Table 7 in the "Non-neoplastic findings – Possible relationships between tissue damage, inflammation, hyperplasia and tumors in rats and mice" section of the PHG document. Neither tissue damage nor inflammation was observed in the oral cavity of the rat or small intestine of the mouse, both sites where tumors were observed.

General Comment 5: "We should inquire as to whether OEHHA is considering Cr+6 to be acting by a mutagenic mode of action for carcinogenesis, as this would have additional implications for its risk assessment."

General Response 5. As discussed in various parts of the PHG document ("Examination of Evidence for Chromium Carcinogenicity", "Risk Characterization" sections), OEHHA finds that Cr VI is probably inducing tumors via a genotoxic/DNA-damaging mechanism of action that may or may not include mutations. The mutagenic mode of action described by McCarroll et al. (2010) has been added to the document.

General Comment 6: "Thus, if even 1% of the dietary chromium is Cr+6, our typical diet would expose a person to almost 10 times the proposed PHG. Information on the percentage of chromium in the diet that is Cr+6 should be obtained so that the previous estimate can be made. In particular, if typical, dietary exposure to Cr+6 greatly exceeds the draft PHG, one would expect higher GI tract tumors in the general population. This is a good and relatively easy method for determining how much the risk estimate (based on significant, but limited data) may overestimate the actual risk."

General Response 6. As discussed in the PHG document in the "Food" section, the measurements of Cr in food rarely provide information on speciation. Were these data available, it would indeed be useful to use them to test an association between Cr VI intake and cancer.

General Comment 7: "The conversion performed by OEHHA from exposure to dose is only referenced as "OEHHA calculations." If this was performed by a standard OEHHA procedure, that method should be publicly available and the reference provided. If it was specific to this study, it should be provided, perhaps as an appendix."

General Response 7. The revised PHG document now uses the original daily Cr VI intake values provided by NTP (2008). That citation is now provided where the intake values are quoted.

General Comment 8: "Most of the human, non-lung cancers that were reported in the tables in the draft PHG document have a lower confidence limit of <1, indicating an absence of statistical significance. Four did not. Two of those involved cement or concrete workers that would have exposures to other potential carcinogens. The remaining two involved production of chromium materials. Nine other studies of similar

worker populations were negative. The weight of the evidence for carcinogenicity from epidemiological studies, therefore, is less than definitive."

General Response 8. OEHHA agrees. The discussion of the worker studies in Table 8 has been revised to read, "These results are consistent with an association between occupational exposure to Cr VI (via inhalation) and stomach cancer."

General Comment 9: "In all studies presented throughout the document, either human or animal studies, the doses of administered chromate are orders of magnitude higher than the doses that would be taken if drinking water were to meet the PHG guidelines."

General Response 9. The use of high doses in cancer bioassays is generally thought to offset the statistical limitations of using small numbers of animals (50 /sex/dose level) to measure a relatively rare event (tumors).

Specific Comment 1: "Therefore, it appears plausible that these NTP findings may support a nongenotoxic mode of action for initiation of the small intestine tumors observed in mice, as hyperplasia is usually associated with chronic tissue irritation in the "continuum-of-change"…Therefore, we believe that despite the data presented that Cr+6 can have "systemic" genotoxic effects distant from the site of carcinogenicity, the data presented in the draft document is not convincing that it operates via a mutagenic MOA for carcinogenesis during exposure to low environmental concentrations in drinking water."

Specific Response 1. OEHHA found no evidence that the tumors observed in the two-year bioassay (NTP, 2008) were associated with epithelial cell damage or chronic inflammation (see discussion of Table 7 in the draft PHG). Also, it is not uncommon for carcinogens to stimulate cellular proliferation in the absence of cell killing. With regards to genotoxicity, ingested doses of Cr VI similar to those used by NTP (2008) caused a variety of genotoxic damage in rats and mice (Table 2). Thus, a genotoxic MOA is the only mode of action that is consistent with the available data.

Specific Comment 2: "As there is significant scientific concern associated with the results of the Borneff et al., 1968 animal study, it is not clear why this particular study is singled out and cited in the Summary and expanded upon at length in Appendix B; and why the draft PHG document does not elaborate on the weaknesses identified…Available studies, such as 2007 and the previous NTP rodent studies, and human population studies of drinking water ingestion reporting negative findings of increased population carcinogenicity (for example, June 2009 Texas Department of State Health Services, Evaluation of Chromium in Private Wells in Midland County Texas, ATSDR Letter Health Consultation and others), and on mode of action for digestive tract carcinogenicity, genotoxicity and mutagenicity, etc. should have been considered."

Specific Response 2. The weaknesses of Borneff et al. (1968) are discussed in detail in Appendix B of the PHG document. The well contamination in Midland County Texas was discovered in 2009. The Letter Health Consultation states, "The site investigation and discovery has just started in the area. The source of contamination is not known, and the groundwater contamination has not been delineated or fully characterized at this time. Additionally, well water is being further assessed to determine if chromium is

the only contaminant of concern." The letter goes on to cite the Texas Cancer Registry. For the period of 1997 to 2006, there were no excess cancers reported for the zip code of interest in Midland County. Since the concentrations of Cr VI were poorly characterized at the time this report was made, and the cancer monitoring period preceded the time-frame of contamination monitoring, OEHHA did not include this report in the PHG document.

Specific Comment 3-1: "Comparing the data derived from the 2007 NTP drinking water ingestion studies, and the potential exposures to Cr+6 from maintenance operations, such as welding stainless steel, with ingesting low levels of Cr+6 in drinking water, it appears that humans may be much less susceptible than other animals to Cr+6-induced gastrointestinal (GI) tract cancers, since the adenomas or carcinomas of the duodenum, jejunum, or ileum are only reported in mice exposed to about 6 orders of magnitude higher active concentrations of Cr+6 and rats stomach tumors at even higher administered doses then that for mice (NTP, 2007)."

Specific Response 3-1. The use of high doses in cancer bioassays is designed to offset the statistical limitations of using small numbers of animals (50 /sex/dose level) to measure a relatively rare event (tumors) (U.S. EPA, 2005).

Specific Comment 3-2: "As stated in the 'Comments Regarding NTP Technical Report on the Toxicology and Carcinogenesis Studies of Sodium Dichromate Dihydrate for May 16-17, 2007 Peer Review,' 'Many differences exist in the physiology and anatomy of the rat and mouse gastrointestinal tracts, with even greater differences in humans. One such difference of particular importance is basal rate of gastric acid secretion, which is approximately 1,200 times greater in the rat compared to the mouse (Friis-Hansen et al. 1998; Runfola et al. 2003; Wang et al. 2003). The human basal gastric acid secretion rate is approximately 8-times higher than that of the rat (Friis-Hansen et al. 1998).' Thus, a more in-depth discussion of potential interspecies variability, as seen in the NTP 2007 rodent studies, is important to increase understanding of potential implications for human increased potential for carcinogenicity…Peer reviewers' comments have further suggested that interspecies variability may be due to differences in the pH of human salivary glands (6.5-7.5) compared to the mouse (9.0-10.0). Other interspecies differences such as (a) acid secretion rate differences in humans 8,000-20,000 (µEq/4h) compared to 1-168 (µEq/4h) in the mouse; (b) stomach bacteria and protozoan species indigenous in the mouse and rat; and (c) a much larger stomach fraction of GI tract compartments compared to rodents, may result in greater conversion of Cr+6 to Cr+3 than in the human stomach at low environmental concentrations."

Specific Response 3-2. Calculation of the human oral cancer slope factor in the PHG document was performed with tumor data collected from the mouse. Due to inadequate toxicokinetic and toxicodynamic data, it was only possible to adjust the mouse dose to a human equivalent dose with default methodology; i.e., scaling by bodyweight$^{3/4}$ (U.S. EPA, 2005; OEHHA, 2009; Stern, 2010; U.S. EPA, 2010). The interspecies differences between rodents and humans in reduction, absorption and distribution of Cr VI are discussed in the "Metabolism and Pharmacokinetics" section of the PHG document. More discussion of interspecies differences has been added to the "Calculation Of The PHG," subheading "Choosing Appropriate Uncertainty Factors" section and the "Risk Characterization" section of the document.

Specific Comment 3-3: "Regarding absorption, the 2008 ATSDR Draft *Toxicological Profiles for Chromium* states that less than 10% of Cr+6 ingested is absorbed from the stomach; the majority of ingested Cr+6 is absorbed from the stomach as Cr+3 via reduction by the acidic juices; and 0.5-2% of Cr+3 ingested is absorbed from the gastrointestinal tract."

Specific Response 3-3. The PHG document contains extensive discussion of Cr VI and Cr III absorption and reduction.

Specific Comment 4: "Figure 12 indicates that, at the highest dose level, female mice had a body weight approximately 20% less than controls. This suggests that the maximum tolerated dose (MTD) was exceeded. Even if this decrease in body weight was due to a lower consumption of water, a decrease in body weight of > 10% for any reason is generally considered sufficient to raise concerns about the toxicity observed in those animals."

Specific Response 4. The possibility that the MTD was exceeded in the high dose female mice is now discussed in the "Carcinogenicity," subheading "Neoplasms" section of the draft PHG.

Specific Comment 5: "Therefore, we recommend that the document more clearly states that a causal link between exposure to Cr+6 in drinking water and tumors of the digestive tract has not been confirmed based on the data derived from human studies; and that the human data are considered "suggestive" of such a link, but not compelling."

Specific Response 5. The final PHG document states, "In the only two studies of human exposure to Cr VI in drinking water that specifically measured organ-specific cancer, statistically significant increases in stomach cancer mortality (Zhang and Li, 1987; statistical analysis conducted by OEHHA) and primary liver cancer mortality (Linos *et al*., 2011) were detected in the exposed population."

Specific Comment 6: "It appears that CA OEHHA simply counted the numbers of human studies with relative risk ratios less than or greater than one, without giving any consideration to the range of the confidence intervals for each study. Generally, epidemiological studies with a lower confidence limit that includes "1" are not considered to be statistically significant. Thus, we recommend that CA OEHHA consider a more rigorous statistical approach to better understand the strength of these studies. The need for a statistical approach was also a recommendation made by one of the three university external peer reviewers on the 2008 PHG Draft, Dr. R. Gwiazda, Environmental Toxicology, University of California, Santa Cruz."

Specific Response 6. We have added more discussion of Table 8 that includes the suggestions of Dr. Gwiazada to: 1) not compare the rate ratios to 1.00, and 2) conclude that the results are consistent with an association between occupational exposure to Cr VI (via inhalation) and stomach cancer.

Specific Comment 7-1: "The Beaumont et al 2008 study of the same Chinese villagers reported a statistically significant relationship between Cr+6 environmental exposure and oral cancer in 5 villages in China with high concentrations of Cr+6 in well water…The PHG Draft has also reported that this population was found to have been infected with *Helicobacter pylori* bacteria, which is much more prevalent in developing

countries and may be associated with the increased risk of stomach cancer in the entire province (even in regions without Cr+6 contaminated drinking water."

Specific Response 7-1. *Helicobacter pylori* was not measured in the Zhang and Li (1987) study. Also, the study reported stomach cancer mortality, not oral cancer.

Specific Comment 7-2: "The draft PHG document does not emphasize in the main portion of the text the fact that well documented dietary and other environmental and genetic factors have been shown to lead to stomach cancer itself, in the absence of Cr+6 in drinking water…It would be beneficial to provide additional pertinent information concerning the prevalence of gastrointestinal cancer in developing countries versus the U.S…We also recommend including additional information on other potential environmental confounders that also may be associated with stomach cancer in humans in addition to those already discussed, such as ingestion of asbestos particulates in drinking water, etc., and should be discussed in greater detail, to help account for other potential confounders in future research designs and study evaluations."

Specific Response 7-2. These suggestions for an expanded general discussion of human stomach cancer go beyond the scope of the PHG document.

Editorial Comment 1: "We believe it would increase clarity if the text were changed to indicate whether the chromium analysis in blood and plasma was speciated to differentiate between hexavalent chromium (Cr+6), trivalent chromium (Cr+3), or total chromium following administration of Cr+6.

Editorial Response 1. We have revised the "Summary" section of the PHG document accordingly.

Editorial Comment 2: "The reference JHAS (1979) is mentioned as one of the papers with findings as the basis for OEHHA's re-evaluation of PHG but it is not discussed in the non-carcinogenic Effects Section under Choosing Appropriate Uncertainty Factors. It should be added to this section, added to the reference list, and the acronym should be defined."

Editorial Response 2. The acronym has been added to the citation in the "References" section of the PHG document.

Editorial Comment 3: "Figure 13 has no units on the x-axis…Thus, it must be something like "tumor-bearing animals" but the actual title and how the data were calculated should be transparent."

Editorial Response 3. The mouse tumor data are now presented in Tables 5 and 6 of the PHG document.

Comments from Robert Hollander, The Western Coalition of Arid States

Comment 1: "WESTCAS understands other studies exist and are referenced in the document providing evidence that complete reduction may not always occur, but believes the administered doses in the NTP study are so large they easily overwhelmed the reductive capacity of both the oral cavity and the stomach in the rodents. This is

especially significant as the NTP study did not find excess cancers at the lowered studied doses in both rats and mice."

Response 1. See Appendix A in the PHG document for a discussion of the data showing that the Cr VI reducing capacity of the rodent GI tract was not saturated over the dose range tested in the NTP (2008) two-year study. The absence of excess tumors at the lower dose levels may have been due to the use of a small number of animals to detect a relatively rare event (tumor formation).

Comment 2: "Equally as important, the stomach composition of humans and rodents is very different, with humans having a much more sophisticated and higher level of gastric juices than rodents."

Response 2. Cr VI is reduced to Cr III in both the rodent and human stomach. This is discussed in detail in the PHG document in the sections "Hexavalent Chromium Reduction by Saliva and Gastric Fluids", "Absorption" and "Pharmacokinetics of Trivalent versus Hexavalent Chromium." See also Appendix A. While Cr VI reduction in the GI tract of rodents compared to humans has not been fully described, the U.S. EPA (2010), the New Jersey Department of Environmental Protection (NJDEP, 2009) and OEHHA (this PHG document) have all found that they are similar enough to allow calculation of a human cancer slope factor for Cr VI based on the NTP two-year bioassay.

Comment 3: "The first of these studies was completed in 1968 by Borneff et al…This study has no merit and should not be used to support the subject PHG."

Response 3. The Borneff et al. (1968) study is not a key study in the derivation of the PHG for Chromium VI and was moved to the Appendix for that reason. OEHHA provided extensive analysis of its findings in its endeavor to consider all available scientific data when evaluating chemicals and developing PHGs aimed at protecting public health. The weight of evidence approach taken by OEHHA necessitated a discussion of Borneff et al. (1968).

Comment 4: "In the work completed in 1987 and 1997 by Zhang and Li, the data shows a negative dose-response between chromate exposure in drinking water and cancer rates found in about 10,000 villagers exposed to groundwater contaminated with hexavalent chromium levels as high as 2,600 ppb. The authors concluded there was no association between chromate exposure and any form of cancer in this population. Using a selective re-analysis of this study, the PHG document concludes a statistically significant increase in stomach cancer occurred based on unsupported assumptions about water consumption practices, plume migration and population distributions."

Response 4. The commenter is in error in saying that the 1987 Zhang and Li paper concluded there was no association; only the 1997 paper, which has since been withdrawn by the journal that published it, concluded that there was no association. Although the names of the second author of the two studies appeared the same, they are actually different individuals. The findings of Beaumont et al. (2008) published in 2008 replicated the findings of the original study (Zhang and Li, 1987): a statistically significant increase in stomach cancers. This is discussed in detail in the "Toxicological Effects in Humans, Carcinogenicity" section of the PHG document. See that discussion

as well as the original publication by Beaumont et al. (2008) for a description of the assumptions and limitations of the OEHHA analysis. Another recent reevaluation of the Zhang and Li (1987) study by Kerger et al. (2009) has been added to the PHG document.

Comments from Andria Ventura, Clean Water Action California

Comment 1: "If anything, OEHHA's analysis is not adequately conservative in that it actually does not go far enough in considering the impacts on specific vulnerable populations. Their studies do not reflect the department's guidelines on accounting for early-life susceptibility to carcinogens, putting pregnant women, their fetuses, and young children at greater risk."

Response 1. The PHG document has been revised to account for increased early-life susceptibility to carcinogens. See "Correction for Early-in-Life Exposures" section of the document.

Comment 2: "Furthermore, we would suggest greater consideration of the large portion of the population whose ability to transform hexavalent chromium into less toxic trivalent chromium may be impaired. One only has to review the wide range of over the counter medications to address common gastrointestinal problems that can impact millions of people's ability to convert hexavalent chromium to understand the potential threat to the population at large."

Response 2. These potentially sensitive subpopulations are discussed in the "Sensitive Subpopulations" section of the PHG document. For calculation of the acceptable daily dose (ADD) for noncarcinogenic effects ("Calculation OF The PHG, Noncarcinogenic Effects" section of the PHG), an uncertainty factor of 10 was judged sufficient for protecting potentially sensitive human subpopulations, such as antacid users. Methodology does not currently exist for incorporating such an uncertainty factor into the calculation of the ADD for carcinogenic effects.

Comments from Kristy L Morrison, American Chemistry Council

Comment 1: "In July 2009, Toxicology Excellence for Risk Assessment (TERA) convened a Science Advisory Board (SAB) to provide guidance on research to investigate the potential mode(s) of action (MOA) of hexavalent chromium based on the US EPA Guidelines for Carcinogen Risk Assessment (2005). Based on TERA's scientific recommendations, The Hamner Institute for Health Sciences was commissioned to conduct research on five key areas integral to assessing the MOA(s) for chromium…"

And "We urge OEHHA to await additional research findings anticipated in 2010 before finalizing the draft PHG."

And "In a memo dated October 23, 2008, from Dr. David Berry, Senior Toxicologist with the Human and Ecological Risk Division of the Department of Toxic Substances Control, to Dr. Jeff Wong, Chief Scientist of the Department of Toxic Substances Control, the Hamner research program was recognized as critical in addressing the mode of action of chromium and the studies should be 'prerequisites to any revisions to

the OEHHA public health goal for Cr^{+6}.' In issuing the draft PHG prematurely; however, OEHHA has failed to consider the anticipated mode of action research (*See* Appendix A). We agree with DTSC comments regarding how important it is to use current scientific principles and recent advances such as incorporating mode of action are preferable to using outdated default assumptions."

Response 1. Neither the August 2009 draft document nor the final PHG document is premature. Health and Safety Code Section 116365.5 required the Department of Public Health to develop a primary drinking water standard for Cr VI by January 1, 2004. Health and Safety Code Section 116365 requires the development of a PHG by OEHHA before the department can adopt a primary drinking water standard. In light of this statutory mandate, it would be very difficult for OEHHA to justify further delay to finalizing the PHG in order to incorporate any appropriate findings from the Hamner Institutes research program.

Health and Safety Code Section 116365 contains an important provision that addresses new scientific research when it becomes available: "(e) (1) Public health goals established by the office shall be reviewed at least once every five years and revised, pursuant to the provisions of subdivision (c), as necessary based upon the availability of new scientific data". OEHHA acknowledges that new studies may alter a revised PHG. From the risk characterization section of the 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."

OEHHA will review papers and materials relating to the Hamner Institutes study when they are published. If the study produces compelling information that should be reflected in the PHG document, OEHHA will take appropriate action.

Comments from Renee Sharp, Rebecca Sutton and Gina Solomon, Environmental Working Group

Comment 1: "<u>OEHHA's proposed PHG should be revised to more adequately protect sensitive populations (emphasis in original)</u>...Conversion of hexavalent to trivalent chromium can be impaired in individuals with low-acid stomachs, a condition brought about by several widely used medications, like antacids and proton pump inhibitors, which treat disorders including gastroesophageal reflux disease, peptic ulcer disease, and chronic gastritis. Other health conditions that can result in reduced stomach acid production include pernicious anemia, pancreatic tumors, infection with *Helicobacter pylori*, mucolipidosis type IV, and some autoimmune diseases."

Response 1. These potentially sensitive subpopulations are discussed in the "Sensitive Subpopulations" section of the PHG document. For calculation of the acceptable daily dose (ADD) for noncarcinogenic effects ("Calculation OF The PHG, Noncarcinogenic Effects" section of the PHG), an uncertainty factor of 10 was judged sufficient for protecting potentially sensitive human subpopulations, such as antacid users.

Comment 2: “One of the peer reviewers (Dr. R. Gwiazda) aptly noted how OEHHA had overlooked some of these concerns in its proposed PHG in the following statement: “There are two sensitive populations that are not included in the estimate of the one in a million lifetime cancer risk: carriers of Helicobacter pylori and people with anomalous stomach pH regulation” (Gwiazda 2008).”

Response 2. Methodology does not currently exist for incorporating the potentially heightened sensitivity of these subpopulations into the calculation of the acceptable daily dose (ADD) for carcinogenic effects.

Comment 3: “From the excerpts above, it is clear that OEHHA should revise its proposed hexavalent chromium PHG to reflect the agency’s own recently published guidelines to take into account the special concerns about early-life susceptibility to carcinogens.”

Response 3. The PHG document has been revised to account for the heightened sensitivity of infants and children to carcinogens as described in the OEHHA (2009) guidelines.

Comments from Christy Marani, Central Water District

Comment 1: “According to the Association of California Water Agencies (ACWA), the administered doses in the NTP study are so large that they easily overwhelmed the reductive capacity of both the oral cavity and the stomach in the rodents. This is especially significant as the NTP study did not find excess cancers at lower doses in both rats and mice.”

Response 1. See Appendix A in the PHG document for a discussion of the data showing that the Cr VI reducing capacity of the rodent GI tract was not saturated over the dose range tested in the NTP (2008) two-year study. The absence of excess tumors at the lower dose levels may have been due to the use of too few animals to detect a relatively rare event (tumor formation).

Comment 2: “Equally important, the stomach composition of humans and rodents is very different, with humans having a much more sophisticated digestive process.”

Response 2. See the “Metabolism and Pharmacokinetics” section and Appendix A of the PHG document for discussions of human data indicating that Cr VI escapes reduction and is absorbed into the circulation at drinking water concentrations below those used in the NTP (2008) two-year bioassay in rodents.

Comment 3: “Although we recognize all the efforts at research made to date, the NTP study and other referenced studies do not address, for example, the effects of prescription medications and over-the-counter antacids on gastric juices.”

Response 3. These issues are discussed in the PHG document in the section “Toxicological Effects in Humans,” subheading “Sensitive Subpopulations.”

Comments from Gary Buchanan, New Jersey Department of Environmental Protection

Comment 1: "We agree with your conclusion (echoing the NTP conclusion) that decreased water consumption was a contributing factor to decreased body weight compared to controls in the high-dose male and female mice. Additional information supplied to us by NTP provides evidence that for the high dose female mice there was also a systemic component to decreased body weight."

Response 1. We have added text to the discussion of Figures 9-14 suggesting that the approximate 20 percent decrement in female mouse bodyweight in high dose animals may indicate that the MTD was exceeded in that dose group (NJDEP, 2009).

Comment 2: "In addition, since the issue of possible dehydration and the possibility of its contribution to the neoplasia was raised in the initial peer review of the NTP study, the NJDEP document addresses this question."

Response 2. We have added text to the discussion of NTP (2008) stating that there were no indications that the rats or mice became dehydrated during the study.

Comment 3: "There are some small and essentially non-significant differences between the values you identified for the denominator of the incidence ratio and those identified in the NJDEP analysis."

Response 3. The denominators in the tumor incidence values shown in Tables 5 and 6 of the PHG document now correspond to the animals alive at the time of the first occurrence of tumor (day 451 for male mice and day 625 for female mice; OEHHA, 2009).

Comment 4: "You may want to include the observations in our discussion that support self-restriction of water intake in high dose males, but not high dose females, and the related conclusion that the significant decrease in body weight in the high-dose females was a systemic effect indicating a possible exceedance of the MTD rather than a result of palatability issues."

Response 4. We have added text to the "Maximum Tolerated Dose – Mice" section of the PHG document citing the NJDEP (2009) suggestion that the MTD may have been exceeded in the high dose females.

Comments from Thomas LaHue, Soquel Creek Water District

Comment 1: "We are concerned that the human carcinogenicity of low levels of chromium 6 in drinking water has not yet been clearly established with a significant body of solid scientific evidence."

Response 1. In the absence of conclusive data in humans, carcinogenicity in rodents is sufficient justification for developing a human protective dose (U.S. EPA, 2005; OEHHA, 2009).

Comments from Anthony Zampiello, Raymond Basin Management Board

Comment 1: "The California Health and Safety Code specifically requires that OEHHA employ the most current practices and methods used by health science experts when proposing a new PHG, Cal H&SC Sec.l16365(c)(I). OEHHA did not comply with its own and EPA's procedures for calculating the PHG. As pointed out by the Department of Toxic Substances Control (DTSC) in an October 23, 2008 memo on the PHG, the method employed by OEHHA to calculate the PHG ignored recent advances in assessing carcinogenesis. EPA guidance specifically requires alternate means of assessing the results of cancer bioassays where appropriate scientific data is available. In contrast, OEHHA ignored all other options for calculation of cancer potency and simply adopted the EPA's default "linear extrapolation" procedure for this PHG. In fact, the DTSC and scientific peer reviewers from the University of California suggested that an analysis of alternative approaches should have been included in the draft PHG documents."

Response 1: OEHHA employed currently accepted procedures for calculating the PHG for Cr VI (U.S. EPA, 2005; OEHHA, 2009; Davis et al., 2010). Multiple means for calculating the cancer potency were considered including choice of animal species (rat versus mouse), sex, tumor site (oral cavity versus small intestine) and mathematical model (such as linear multistage, logistic, probit, Weibull). An alternative approach was also tested based on the most sensitive change detected in any drinking water study: that of mild chronic inflammation in the livers of female rats (NTP, 2008) (see "Calculation Of The PHG, Noncarcinogenic Effects" section). Other alternative approaches were suggested by the peer reviewers, some of which were discussed in OEHHA's Response to Major Comments on Technical Support Document (OEHHA, 2009b).

Comment 2: "However, the OEHHA has used exactly the kind of overly speculative theories that it was warned not to use by both the Risk Assessment Advisory Committee and the prior peer reviewers. DTSC has indicated this so-called Helicobacter Hypothesis is speculative, lacks relevance to developing the PHG and it should be eliminated from the document as it is speculation. However, OEHHA with absolutely no scientific basis, use this as the primary basis for linking tumor findings in animal studies to the possible occurrence of stomach cancer in humans ingesting chromium in water."

Response 2: The PHG for Cr VI in drinking water is based on tumors in rodents (NTP, 2008). The Helicobacter hypothesis plays no role in the derivation of the PHG. Note that the hypothesis is not discussed in the technical document but only in the Appendix. The hypothesis was formulated by OEHHA in order to obtain a better understanding of the findings of diverse studies. Some unexplained findings such as the occurrence of tumors in the first generation of the Borneff et al. (1968) study could be explained by the presence of Helicobacter bacteria. Also the hypothesis may explain why stomach tumors occurred following a relatively short term exposure to Cr VI in rural China. It is a scientific resource to keep these discussions attached in the Appendix as records of the

issues that have been addressed in the research for and preparation of this PHG document.

Comment 3: “OEHHA only relied on studies that have been superseded by more recent findings. It also chose to reinterpret other studies that do not fit its own conclusions while also ignoring data that did not support its conclusion. For example, OEHHA's evaluation of the 1987 Zhang J and Li X assessment of chromium pollution of water supplies in China and in 1997, the lead co-author of the 1987 study expanded the assessment of the data found no statistically relevant link between stomach cancer in humans and consumption of water containing chromium 6. There are other examples where OEHHA similarly reevaluated published data and studies to support OEHHA's hypothesis which the 2008 Peer Reviewers noted as "overreaching" and DTSC's memo concluded inadequately addressed the with of (sic) evidence. This subjective process of picking and choosing data regardless if there is a scientific basis to obtain a predetermined answer should not be the process to develop the PHG giving the importance of this task.”

Response 3: The findings of Beaumont et al. (2008) published in 2008 (well after the original studies, Zhang and Li**,** 1987) replicated the findings of the original study and found a statistically significant increase in stomach cancers. The referenced 1997 study was withdrawn in 2006 by the publisher, The Journal of Occupational and Environmental Medicine. The second authors of the 1987 and 1997 studies were different individuals although they showed the same name. Another recent reevaluation of the Zhang and Li (1987) study by Kerger et al. (2009) has been added to the PHG document.

Comment 4: “In fact, DTSC recognized the importance of the Hamner Research program in addressing the "mode of action" (MOA) of chromium and said that the studies should be ‘prerequisites to any revisions to the OEHHA public health goal for chromium 6.’ By issuing a PHG without waiting for this information, OEHHA is not taking account of the most up-to-date science.”

Response 4: Health and Safety Code Section 116365.5 required the Department of Public Health to develop a primary drinking water standard for Cr VI by January 1, 2004. Health and Safety Code Section 116365 requires the development of a PHG by OEHHA before the department can adopt a primary drinking water standard. In light of this statutory mandate, it would be very difficult for OEHHA to justify further delay to finalizing the PHG in order to incorporate any appropriate findings from the Hamner Institutes research program.

Health and Safety Code Section 116365 contains an important provision that addresses new scientific research when it becomes available: “(e) (1) Public health goals established by the office shall be reviewed at least once every five years and revised, pursuant to the provisions of subdivision (c), as necessary based upon the availability of new scientific data”. OEHHA acknowledges that new studies may alter a revised PHG.

From the risk characterization section of the 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.”

OEHHA will review papers and materials relating to the Hamner Institutes study when they are published. If the study produces compelling information that should be reflected in the PHG document, OEHHA will take appropriate action.

Comments from J. Eric Tynan, Castroville Community Services District

Comment 1: “According to the Association of California Water Agencies (ACWA), the administered doses in the NTP study are so large that they easily overwhelmed the reductive capacity of both the oral cavity and the stomach in rodents. This is especially significant as the NTP study did not find excess cancers at the lowered studied doses in both rats and mice.”

Response 1. See Appendix A in the PHG document for a discussion of the data showing that the Cr VI reducing capacity of the rodent GI tract was not saturated over the dose range tested in the NTP (2008) two-year study. The absence of excess tumors at the lower dose levels may have been due to the use of too few animals to detect a relatively rare event (tumor formation).

Comment 2: “Equally as important, the stomach composition of humans and rodents is very different, with humans having a much more sophisticated and higher level of gastric juices than rodents.”

Response 2. See the “Metabolism and Pharmacokinetics” section and Appendix A of the PHG document for discussions of human data indicating that Cr VI escapes reduction and is absorbed into the circulation at drinking water concentrations below those used in the NTP (2008) two-year bioassay in rodents.

Comment 3: “It is our understanding that the Borneff et al study is seriously flawed and should not be considered in the development of the PHG. In the work completed by Zhang and Li, it is our understanding that not all factors were considered when the authors reached their conclusions, including the extremely high levels of hexavalent chromium and the presence of a particular bacterial infection potentially affecting the results.”

Response 3. The Borneff et al. (1968) study is not a key study in the derivation of the PHG for Chromium VI and was moved to the Appendix for that reason. OEHHA provided extensive analysis of its findings in its endeavor to consider all available scientific data when evaluating chemicals and developing PHGs aimed at protecting public health. The weight of evidence approach taken by OEHHA necessitated a discussion of Borneff et al. (1968).

The Zhang and Li (1987) study was thoroughly analyzed (see Beaumont et al., 2008). Although the magnitude of the exposure to Cr VI in drinking water is unclear, the population did appear to be exposed to high levels of chromium VI. A statistically

significant increase in stomach tumors was detected when compared to a nearby unexposed population and when compared to the province. The high levels of chromium VI in the wells is what triggered the study (lower levels probably would have gone undetected). Lower levels of chromium VI may still cause cancer but at a rate that would have been undetected in a population of several thousand. The possible infection of the study population by *Helicobacter pylori* is discussed in Appendix B of the PHG document.

Comments from David Koch, City of Watsonville

Comment 1: "The City understands other studies exist and are referenced in the document providing evidence that complete reduction may not always occur, but believes the administration doses in the NTP study are so large they easily overwhelmed the reductive capacity of both the oral cavity and the stomach in the rodents. This is especially significant as the NTP study did not find excess cancers at the lowered studied doses in both rats and mice."

Response 1. See Appendix A in the PHG document for a discussion of the data showing that the Cr VI reducing capacity of the rodent GI tract was not saturated over the dose range tested in the NTP (2008) two-year study. The absence of excess tumors at the lower dose levels may have been due to the use of a small number of animals to detect a relatively rare event (tumor formation).

Comment 2: "Equally as important, the stomach composition of humans and rodents is very different, with humans having a much more sophisticated and higher level of gastric juices than rodents."

Response 2. Cr VI is reduced to Cr III in both the rodent and human stomach. This is discussed in detail in the PHG document in the sections "Hexavalent Chromium Reduction by Saliva and Gastric Fluids", "Absorption" and "Pharmacokinetics of Trivalent versus Hexavalent Chromium." See also Appendix A. While Cr VI reduction in the GI tract of rodents compared to humans has not been fully described, the U.S. EPA (2010), the New Jersey Department of Environmental Protection (NJDEP, 2009) and OEHHA (this PHG) have all found that they are similar enough to allow calculation of a human cancer slope factor for Cr VI based on the NTP two-year bioassay.

Comment 3: "The Borneff et al. study is seriously flawed and should not be considered in the development of the PHG. In the work completed by Zhang and Li, not all factors were considered when the authors reached their conclusions including the extremely high levels of hexavalent chromium and the presence of a particular bacterial infection potentially affecting the results."

Response 3. The Borneff et al. (1968) study is not a key study in the derivation of the PHG for Chromium VI and was moved to the Appendix for that reason. OEHHA provided extensive analysis of its findings in its endeavor to consider all available scientific data when evaluating chemicals and developing PHGs aimed at protecting public health. The weight of evidence approach taken by OEHHA necessitated a discussion of Borneff et al. (1968).

The Zhang and Li (1987) study was thoroughly analyzed (and published, see Beaumont et al., 2008). Although the magnitude of the exposure to Cr VI in drinking water is unclear, the population did appear to be exposed to high levels of chromium VI. A statistically significant increase in stomach tumors was detected when compared to a nearby unexposed population. The high levels of chromium VI in the wells is what triggered the study (lower levels probably would have gone undetected). Lower levels of chromium VI may still cause cancer but at a rate that would have been undetected in a population of several thousand. There was no indication that the study population was infected by bacteria that influenced its sensitivity to Cr VI.

Comments from Danielle Blacet, Association of California Water Agencies

Comment 1: "The Office of Environmental Health Hazard Assessment's (OEHHA) draft PHG of 60 parts per trillion (ppt) was based largely on the findings of a recent National Toxicology Program (NTP) study that concluded there is sufficient data to classify hexavalent chromium as a carcinogen through the oral route of exposure. The researchers reached this conclusion through selected evidence that hexavalent chromium, when ingested in very high doses, causes cancer of the oral cavity and small intestine in rats and mice."

Response 1: OEHHA used a weight of the evidence approach, based on toxicokinetic studies, genotoxicity and mechanism studies and animal and human studies to evaluate the carcinogenic risk associated with exposure to hexavalent chromium in drinking water. The evidence was internally consistent and compelling that oral exposure to Cr VI results in cancer. The NTP study provided acceptable data for a quantitative cancer risk assessment.

Comment 2: "As indicated in the draft PHG document, several studies previously estimated that saliva and stomach fluids have the capacity to reduce hexavalent chromium to trivalent chromium in amounts much larger than the 'maximum plausible levels of hexavalent chromium in water that would likely be ingested by humans…' The document further asserts that '…exhaustion of the capacity of saliva and gastric fluids to reduce hexavalent chromium appears unlikely.' ACWA understands other studies exist and are referenced in the document providing evidence that complete reduction may not always occur, but believes the administered doses in the NTP study are so large they easily overwhelmed the reductive capacity of both the oral cavity and the stomach in the rodents. This is especially significant as the NTP study did not find excess cancers at the lowered studied doses in both rats and mice. Equally as important, the stomach composition of humans and rodents is very different, with humans having a much more sophisticated and higher level of gastric juices than rodents."

Response 2: All available evidence indicates that the stomach reduction capacity is not overwhelmed (see Appendix A of the PHG document). The lack of tumors at lower doses in the NTP bioassay is not surprising and is typical of many bioassays where tumors were not detected at lower doses. The absence of tumors at lower doses

indicates a good study design. The need for higher doses in carcinogenic bioassays is primarily to offset the statistical limitations of using small numbers of animals (50/sex/dose level) in experimental studies to measure a relatively rare event (e.g., tumor occurrence), and it has been discussed in detail (U.S. EPA, 2005; OEHHA, 2009).

Comment 3: "In addition, we have concerns with the interpretation and use of data from two key studies submitted as evidence that hexavalent chromium in drinking water is a human carcinogen. The Borneff *et al* study is seriously flawed due to the fact there was only a single-dose level examined and an ectromelia epidemic affected both control and treated groups with significant loss of mice. This study should not be considered in the development of the PHG. In the work completed by Zhang and Li, not all factors were considered when the authors reached their conclusions including the extremely high levels of hexavalent chromium."

Response 3: The Borneff et al. (1968) study is not a key study in the derivation of the PHG for Chromium VI and was moved to the Appendix for information purposes. OEHHA provided extensive analysis of its findings in its endeavor to consider all available scientific data when evaluating chemicals and developing PHGs aimed at protecting public health. The weight of evidence approach taken by OEHHA necessitated a discussion of Borneff et al. (1968).

The Zhang and Li (1987) study was thoroughly analyzed (see Beaumont et al., 2008) and was not used in the final derivation of the PHG. Although the magnitude of the exposure to Cr VI in drinking water is unclear, the population did appear to be exposed to high levels of chromium VI. A statistically significant increase in stomach tumors was detected when compared to a nearby unexposed population. The high levels of chromium VI in the wells is what triggered the study (lower levels probably would have gone undetected). Lower levels of chromium VI may still cause cancer but at a rate that would have been undetected in a population of several thousand.

Comment 4: "An internal Department of Toxic Substances Control (DTSC) memo recently obtained by our members titled 'Hexavalent Chromium Public Health Goal' also expressed some concerns with the conclusions reached in the OEHHA document. ACWA would like to know how those comments have been or will be taken into consideration by OEHHA staff prior to finalizing a draft PHG for hexavalent chromium."

Response 4: OEHHA does not include internal memoranda within the Agency in public comments. However, OEHHA did consider the information in the memorandum and has responded to all specific scientific issues relevant to the PHG document in the revised final document. The same scientific issues are also included in the discussions in this response document.

Comments from Ronald Gastelum, Southern Califonia Water Committee.

Cover letter

Comment 1: "California law requires the agency to prepare the risk assessment 'using the most current principles, practices, and methods used by public health professionals who are experienced practitioners in the fields of epidemiology, risk assessment, and toxicology.' Cal. Health & Safety Code § 116365(c)(1). Unfortunately, OEHHA has failed to do so in preparing this Draft PHG, as also recognized by the Department of Toxic Substances Control ("DTSC") in a memorandum dated October 23, 2008, analyzing the pre-release version of the Draft PHG. Furthermore, OEHHA has not rectified past mistakes made in previous draft PHGs for chromium, which were pointed out by past reviewers of these documents. For example, three reviewing bodies - the 1996 Risk Assessment Advisory Committee (made up of 34 nationally renowned scientists), the 2001 external peer review panel (comprising University of California experts), and the 2005 external scientific peer reviews (also consisting of University of California experts) were critical of previous draft PHGs because they contained hypotheses that were too speculative and did not constitute good science."

Response 1. OEHHA employed the most current, up-to-date procedures in developing the proposed PHG for Cr VI (U.S. EPA, 2005; OEHHA, 2009; Davis et al., 2010). With regard to rectifying past mistakes pointed out by reviewers of PHG documents for chromium, the 1996 Risk Assessment Advisory Committee (RAAC) did not review any PHGs because that program had not yet begun. However, the committee was supportive of OEHHA's risk assessment procedures in the report's Executive Summary, stating "Our general finding is that Cal/EPA's risk assessment products are of good quality, both from the perspective of scientific credibility and professional practice", "Overall, the best practices of Cal/EPA are equal to, if not better, than those of US EPA" and "Similar approaches are used by Cal/EPA and US EPA programs in evaluating the dose-response relationship of carcinogens and non-carcinogens" (RAAC, 1996). In 2001 the primary recommendation of the Chromate Toxicity Review Committee (CTRC, 2001) that a drinking water standard for Cr VI ***not*** be based on the study by Borneff et al. (1968) was followed by OEHHA. In 2008 UC reviewers Roberto Gwiazda, Leonard Bjeldanes and Michael Kelner provided comments on the draft PHG which included the recommendation to move discussion of Borneff et al. (1968) and the Helicobacter hypothesis to the Appendix. The draft PHG was revised accordingly.

Comment 2: "These mistakes pointed out by the external peer reviewers, including University of California bodies mentioned above, have been repeated in the current Draft PHG. Although its own procedures and California statutes require it to respond to the issues raised by external peer reviewers, OEHHA has not adequately done so in the Draft PHG. Importantly, by continuing to ignore EPA's 2005 Guidelines for Cancer Risk Assessment by failing to provide a range of risks associated with the PHG, OEHHA ignores the recommendation of University of California reviewer Dr. Michael Kelner that OEHHA comply with these guidelines by providing a range of risks rather than just one value for risk, which provides a sense of certainty that does not actually exist."

Response 2. In accordance with the U.S. EPA 2005 Guidelines for Cancer Risk Assessment and other U.S. EPA documents (cited below), the following are some reasons OEHHA developed a single PHG value for Cr VI in drinking water to be protective against cancer rather than a range of PHG values:

- University of California reviewer Dr. Michael Kelner suggested that different rodent data sets from the NTP (2008) study could be used to calculate a range of ED_{10} values. As discussed in the "Dose-Response Assessment" and "Calculation of the PHG" sections of the PHG document, the rat and mouse data sets for both sexes were considered for dose-response modeling. For reasons discussed in the PHG document and in our Response 1 to Dr. Kelner (in this document, see Table of Contents) the best data set was selected, that of intestinal tumors in the male mouse. This tumor data set was used to calculate a single cancer slope factor and a single PHG value for Cr VI in drinking water. Selection of the best data set for dose-response modeling was done in compliance with the U.S. EPA 2005 Guidelines for Cancer Risk Assessment.
- The phrase in this comment, "providing a range of risks" has been interpreted by some as meaning calculation of cancer risk based on linear extrapolation down to zero dose along with calculation of cancer risk based on a non-linear/threshold model (see DTSC memorandum in Comment 3 below). First, it should be emphasized than none of the three University of California peer reviewers recommended that OEHHA perform a non-linear extrapolation of the cancer risk from the point of departure down to zero dose. Second, the U.S. EPA 2005 Guidelines for Cancer Risk Assessment only recommend development of multiple risk models when data exist constituting "significant biological support" for such alternative models. As discussed in detail in the PHG document, there is an absence of "significant biological support" for threshold models of cancer induction by Cr VI. In contrast, evidence of genotoxicity and mutagenicity by Cr VI supports a linear extrapolation to estimate the cancer risk at low dose levels.
- The U.S. EPA draft Toxicological Review of Hexavalent Chromium (U.S. EPA, 2010) develops a single cancer risk value for ingested Cr VI by linear extrapolation. OEHHA used the identical methodology. The draft U.S. EPA document does not develop a range of cancer risk values based on alternative MOAs for Cr VI. We believe U.S. EPA drafted a Toxicological Review for Cr VI that is in compliance with its own 2005 Guidelines for Cancer Risk Assessment.
- The California Safe Drinking Water Act of 1996 (Health and Safety Code, Section 116365) requires OEHHA to develop a Public Health Goal (PHG) for environmental contaminants in drinking water, not a range of PHGs for each contaminant.

With regard to uncertainty, many issues of uncertainty are discussed throughout the PHG document as they arise. There is also a concentrated discussion of uncertainty in the "Rick Characterization" section. Similar to OEHHA, the U.S. EPA draft Toxicological Review of Hexavalent Chromium (U.S. EPA, 2010) discusses a number of issues of uncertainty associated with the cancer risk assessment. The draft document does not address uncertainty by developing a range of cancer risk values according to different models and MOAs.

Comment 3: “DTSC, in its memorandum, also remarks on OEHHA's non-compliance with EPA guidance in preparing the Draft PHG. EPA guidance requires that where appropriate scientific data is available, an agency use other methodologies to assess carcinogenesis. Both DTSC and University of California peer reviewers recommended that OEHHA include in the Draft PHG an analysis of alternative approaches to calculate cancer risk, as set forth in EPA guidance. OEHHA did not use any other methods to do so. OEHHA instead improperly used a default linear extrapolation procedure, in which the results of a study in which rodents are exposed to high doses are linearly extrapolated across five orders of magnitude of dose to estimate the risk to humans from much lower environmental exposures. Such a linear extrapolation method is extremely conservative, which leads to inappropriate overestimation of the cancer risk of ingested hexavalent chromium, as DTSC points out. OEHHA should have analyzed all available data to determine whether alternatives such as a non-linear analytical approach would have been appropriate.”

Response 3. OEHHA considered a number of varying parameters in estimating the cancer risk. Parameters included choice of data set (mouse versus rat, male versus female, tumor type, tumor site) and choice of mathematical model (multistage, logistic, probit, Weibull and others). Some but not all of these approaches are presented in tables and discussed in the “Dose-Response, Carcinogenic Effects” section of the PHG. Selection of the LED_{10} and extrapolation to 0 dose were performed according to published U.S. EPA and OEHHA methodology (U.S. EPA, 2005, 2010; OEHHA, 2009; Davis et al., 2010).

Comment 3 suggests that OEHHA’s use of linear extrapolation to estimate the cancer risk at low dose levels was contrary to the advice of the UC reviewers and the U.S. EPA 2005 Guidelines for Cancer Risk Assessment. This is incorrect. None of the three UC reviewers suggested that linear extrapolation from the point of departure down to zero dose was inappropriate, and that other models such as non-linear/threshold models should be included. Dr. Bjeldanes did ask for further justification for the use of a linear model, which has been added to the PHG document (see especially the discussion of Table 7 in the PHG document). The U.S. EPA 2005 Guidelines for Cancer Risk Assessment call for inclusion of alternate models when sufficient biological support exists. The PHG document contains ample discussion of why support is lacking (see especially discussion of Table 7) for other than a genotoxic MOA requiring linear extrapolation to zero dose. This comment is correct in citing the DTSC memorandum as recommending non-linear methods for analyzing the tumor data.

Comment 4: “OEHHA's error in defaulting to the linear extrapolation procedure instead of determining whether other alternatives could have been more appropriate is compounded by its failure to push back release of the Draft PHG until the release of currently ongoing studies that will provide additional information. For example, EPA is using its Integrated Risk Information System (‘IRIS’) program to evaluate human health risk from chromium on an expedited basis, and the Hamner Institute is evaluating a non-linear ‘mode of action’ (‘MOA’) approach for the same purposes. These studies, when

available, will provide additional scientific data to OEHHA to help it determine whether the best and most scientifically valid method to analyze risk from chromium is linear extrapolation, as OEHHA prematurely decided, or a non-linear MOA approach. DTSC appreciated the significance of the Hamner Institute's MOA studies, stating in its memorandum that they 'are prerequisites to any revisions to the OEHHA public health goal for [hexavalent chromium.' By refusing to wait for release of EPA's and the Hamner Institute's information, OEHHA further violates the California Health & Safety Code requirement to 'use the most current principles, practices, and methods' in its risk assessment. This information soon will be readily available, and the short time delay in obtaining it is well outweighed by its value. Furthermore, it is possible, if not probable, that OEHHA may have made decisions that could have led to calculation of a more reasonable standard than 60 parts per trillion, undetectable through standard commercial laboratory procedures, had it used the most current information. A more appropriate PHG that still protects public health is possible, as evidenced by the Agency for Toxic Substances and Disease Registry's recent calculation of a daily dose that is five hundred times the amount calculated in the Draft PHG."

Response 4. Neither the August 2009 draft document nor the final PHG document is premature. Health and Safety Code Section 116365.5 required the Department of Public Health to develop a primary drinking water standard for Cr VI by January 1, 2004. Health and Safety Code Section 116365 requires the development of a PHG by OEHHA before the department can adopt a primary drinking water standard. In light of this statutory mandate, it would be very difficult for OEHHA to justify further delay to finalizing the PHG in order to incorporate any appropriate findings from the Hamner Institutes research program.

Health and Safety Code Section 116365 contains an important provision that addresses new scientific research when it becomes available: "(e) (1) Public health goals established by the office shall be reviewed at least once every five years and revised, pursuant to the provisions of subdivision (c), as necessary based upon the availability of new scientific data". OEHHA acknowledges that new studies may alter a revised PHG. From the risk characterization section of the 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."

OEHHA will review papers and materials relating to the Hamner Institutes study when they are published. If the study produces compelling information that should be reflected in the PHG document, OEHHA will take appropriate action.

OEHHA's decision to perform linear extrapolation with the tumor incidence data was not a default decision. Rather, linear extrapolation was chosen as the model best supported by the extensive data base for Cr VI, including ample evidence of genotoxicity and mutagenicity.

Comment 5: "In addition to these flaws, OEHHA overlooks the advice of the Risk Assessment Advisory Committee and the two university of California external peer

review panels not to rely on hypotheses that are excessively speculative. One such hypothesis that completely lacks scientific basis regards speculation that the hypothesized presence of bacteria in the digestive tracts of some humans, but not others, may aggravate health effects from chromium. In its memorandum, DTSC stated that this bacterial infection hypothesis "is speculative, lacks relevance to developing the PHG and it should be eliminated from the document as it is speculation."

Response 5. The Helicobacter hypothesis was formulated by OEHHA in order to obtain a better understanding of the findings of diverse studies. It was not used as a basis for developing the PHG. Some unexplained findings such as the occurrence of tumors in the first generation of the Borneff et al. (1968) study could be explained by the presence of Helicobacter bacteria. Also the hypothesis may explain why stomach tumors may have occurred following a relatively short term exposure to Cr VI in rural China. It serves as a scientific resource to keep these discussions attached in the Appendix as records of the issues that have been addressed in the research for and preparation of this PHG document.

Comment 6: “Reliance on this speculative hypothesis causes OEHHA to make improper findings regarding ingestion-caused cancer in humans based on tumor findings in animal studies, as well as to avoid reconciliation of incongruent studies that do not support OEHHA's decision to default to the linear extrapolation method.”

Response 6. As stated above in Response 5, the PHG does not rely on the Helicobactor hypothesis in the development of the PHG. The hypothesis is located in Appendix B and clearly indicated as a hypothesis.

Comment 7: “OEHHA's evaluation of the value of the scientific data upon which it relies is faulty. Not only does OEHHA rely on scientific studies that have been superseded by more recent studies, it also reinterprets or ignores other analyses that do not support its own conclusions. As just one example, in the Draft PHG, OEHHA relies heavily upon the 1987 Zhang and Li analysis of the human health effects of chromium in water supplies in China. The lead co-author of this study further assessed the data in a 1997 study, finding no statistically relevant relationship between stomach cancer in humans and consumption of hexavalent chromium-containing water. In addition, in a peer-reviewed study recently published in 2009, Kerger et al, further evaluates the original 1987 data and failed to identify a dose-response relationship or even a rational pattern of association of cancer related mortality with exposure to chromium in the water. Despite this, OEHHA continues to reject this 1997 study, for which it was criticized by the University of California external peer review panel in 2005, and ignores the Kerger study, as neither support its conclusion. OEHHA continues to rely - inappropriately - on the 1987 study to support its position. Multiple other similar examples exist.”

Response 7. The findings of Beaumont et al. (2008) replicated the findings of the original study (Zhang and Li, 1987): a statistically significant increase in stomach cancers. The referenced 1997 study has been withdrawn by the publisher. Another

recent reevaluation of the Zhang and Li (1987) study by Kerger et al. (2009) has been added to the PHG document.

From the Attachment –I Executive summary section

Comment 8: "California Health and Safety Code Section 116365(c)(l) specifically requires that OEHHA employ the most current practices and methods used by health science experts when proposing a new PHG. In the past, OEHHA has been criticized for not using sound science in the development of PHGs. Three recent examples are the 1996 Risk Assessment Advisory Committee (comprised of 34 nationally known scientists), the 2001 Scientific Review and 2005 peer review provided by scientists at California universities on earlier draft chromium PHGs. Each of these bodies of scientists and their reviewers criticized OEHHA for using overly speculative hypotheses or for not using sound science as the basis for public health decisions..."

Response 8. OEHHA employed the most current, up-to-date procedures in developing the proposed PHG for Cr VI (U.S. EPA, 2005; OEHHA, 2009; Davis et al., 2010). With regard to rectifying past mistakes pointed out by reviewers of PHG documents for chromium, the 1996 Risk Assessment Advisory Committee (RAAC) did not review any PHGs because that program had not yet begun. However, the committee was supportive of OEHHA's risk assessment procedures in the report's Executive Summary, stating "Our general finding is that Cal/EPA's risk assessment products are of good quality, both from the perspective of scientific credibility and professional practice", "Overall, the best practices of Cal/EPA are equal to, if not better, than those of US EPA" and "Similar approaches are used by Cal/EPA and US EPA programs in evaluating the dose-response relationship of carcinogens and non-carcinogens" (RAAC, 1996). The objectivity of the "2001 Scientific Review" cited by the commenter came into question after two 2003 legislative hearings concerning allegations that some members of the committee had not properly disclosed their economic interests. Nevertheless, OEHHA carefully reviewed the committee's report (CTRC, 2001) and followed its primary recommendation that a drinking water standard for Cr VI ***not*** be based on the study by Borneff et al. (1968). In 2005 UC reviewers Roberto Guizda, Leonard Bjeldanes and Michael Kelner provided comments on the draft PHG which included the recommendation to move discussion of Borneff et al. (1968) and the Helicobacter hypothesis to the Appendix. The draft PHG was revised accordingly.

Comment 9: "First, OEHHA did not comply with its own and the U.S. Environmental Protection Agency's (EPA) procedures for calculating the draft PHG. As pointed out by the Department of Toxic Substances Control (DTSC) in an October 23,2008 memorandum on the PHG (Berry, 2008) (the DTSC memorandum), the method employed by OEHHA to calculate the PHG ignored recent advances in assessing carcinogenesis. EPA guidance specifically requires alternate means of assessing the results of cancer bioassays where appropriate scientific data is available. In contrast, OEHHA ignored all other options for calculation of cancer potency and simply adopted the EPA's default "linear extrapolation" procedure for this draft PHG. This default

procedure linearly extrapolates the results of a high-dose exposure rodent study across five orders of magnitude of dose to estimate the human cancer risk from far lower environmental exposures. According to DTSC, the default methods employed by OEHHA are highly conservative and improperly overestimate the carcinogenic potency of ingested hexavalent chromium. If OEHHA followed the appropriate procedures, it would analyze all the available data to determine whether the weight of evidence favored alternative conclusions such as a nonlinear analytical approach. In fact, DTSC and scientific peer reviewers from the University of California (UC) suggested that an analysis of alternative approaches should have been included in the draft PHG documents. OEHHA improperly refused to do so.

Response 9. OEHHA complied with both U.S. EPA (2005) and OEHHA (2009) guidelines for cancer risk assessment in developing the PHG for Cr VI. Specifically:

- The most up-to-date methods were utilized in developing the PHG. This was substantiated when both the U.S. EPA (2010) and the New Jersey Department of Environmental Protection (NJDEP, 2009) chose the same methodology as OEHHA for calculating the cancer potency of Cr VI.
- The U.S. EPA 2005 Guidelines for Carcinogen Risk Assessment recommend including alternate methods of calculating the cancer risk when significant biological support exists for alternative MOAs. As discussed in the PHG document, this was not the case for Cr VI.
- OEHHA's decision to perform linear extrapolation with the tumor incidence data was not a default decision. Rather, linear extrapolation was chosen as the model best supported by the extensive data base for Cr VI, including ample evidence of genotoxicity and mutagenicity.
- The comment states that OEHHA did not consider all the available data in developing the PHG. This is incorrect. All available data of sufficiently quality were considered. The best data set (intestinal tumors in male mice) from the best long-term cancer study (NTP, 2008) was selected for calculation of the cancer potency. The identical data set was selected by U.S. EPA (2010) and the New Jersey Department of Environmental Protection (NJDEP, 2009).
- Only the DTSC memorandum recommended that OEHHA include a non-linear approach for analyzing the tumor data. None of the three UC reviewers recommended such an approach, although Dr. Bjeldanes asked for more justification for the linear approach. The U.S. EPA (2010) and the New Jersey Department of Environmental Protection (NJDEP, 2009) utilized the same linear approach as OEHHA. Neither group presented alternative, non-linear approaches.
- OEHHA also varied a number of parameters in estimating the cancer risk. Parameters included choice of data set (mouse versus rat, male versus female, tumor type, tumor site) and choice of mathematical model (such as linear multistage, logistic, probit, Weibull and others). Some but not all of these approaches are presented in tables and discussed in the "Dose-Response, Carcinogenic Effects" section of the PHG. Selection of the LED_{10} and extrapolation to 0 dose were performed according to published U.S. EPA and OEHHA methodology (U.S. EPA, 2005, 2010; OEHHA, 2009; Davis et al., 2010).

Comment 10: **"**Second, OEHHA did not adequately respond to several important issues raised by the University of California peer reviewers of the draft PHG. One key example regards the comments of Dr. Michael Kelner of UC San Diego's Medical Center. Dr. Kelner strongly recommended that "all the NTP National Toxicology Program] 2007 studies need to be analyzed and slope factors derived for each study by an accepted methodology. Then the mean median (preferably) slope factor is to be utilized for subsequent calculations. NOT the 95% confidence interval." Essentially, Dr. Kelner was urging OEHHA to follow EPA's 2005 Guidelines for Carcinogen Risk Assessment and to provide a range of risks to inform decision makers. By not doing so, OEHHA's work projects a false sense of certainty. The issues raised by the peer reviewers point to fundamental flaws in OEHHA's approach. OEHHA needs to address these important issues. In fact, it is required to so by its own procedures

Response 10. This comment does not interpret Dr. Kelner's peer-review comments correctly. Dr. Kelner suggested calculating different points of departure using a range of animal tumor data sets. He also suggested using a range of ED_{10} values rather than LED_{10} values followed by calculation of a mean ED_{10} value for use as a point of departure. He did ***not*** recommend that OEHHA "provide a range of risks to inform decision makers." OEHHA's responses to Dr. Kelners peer-review comments are provided in this document (see Table of Contents).

Comment 11: "Third, OEHHA has relied on exactly the kind of overly speculative theories that it was warned not to use by both the Risk Assessment Advisory Committee and the prior peer reviewers in the 2005 peer review. With absolutely no scientific basis, OEHHA speculates that adverse effects of chromium may be exacerbated by the hypothesized presence of bacteria in the digestive tracts of some human populations, but not others. DTSC has said this so-called Helicobacter Hypothesis "is speculative, lacks relevance to developing the PHG and it should be eliminated from the document as it is speculation." For OEHHA, this pure speculation is the primary basis for linking tumor findings in animal studies to the possible occurrence of stomach cancer in humans ingesting chromium in water. Further, OEHHA uses this speculation to avoid acknowledging the disparate results of the various studies that would otherwise call into question OEHHA's decision to default to a linear dose-response extrapolation. Without this guess work about bacteria, OEHHA would not have an adequate basis for choosing a 60 ppt PHG. Instead it would have come to the same conclusion as DTSC, i.e., "that ingested doses of Cr^{6+} at are insufficient to produce local irritation, tissue damage, inflammation and regenerative hyperplasia are also without additional carcinogenic risk."

Response 11. With regard to warnings about "overly speculative theories," the 1996 Risk Assessment Advisory Committee (RAAC) did not review any PHGs because that program had not yet begun. However, the committee was supportive of OEHHA's risk assessment procedures in the report's Executive Summary, stating "Our general finding

is that Cal/EPA's risk assessment products are of good quality, both from the perspective of scientific credibility and professional practice", "Overall, the best practices of Cal/EPA are equal to, if not better, than those of US EPA" and "Similar approaches are used by Cal/EPA and US EPA programs in evaluating the dose-response relationship of carcinogens and non-carcinogens" (RAAC, 1996).

In 2005 UC reviewers Roberto Gwiazda, Leonard Bjeldanes and Michael Kelner provided comments on the draft PHG which included the recommendation to move discussion of Borneff et al. (1968) and the Helicobacter hypothesis to the Appendix. The PHG document was revised accordingly.

The PHG does not rely on the Helicobactor hypothesis in the development of the PHG. The hypothesis is located in Appendix B and clearly labeled that it is a hypothesis. The Helicobacter hypothesis was formulated by OEHHA in order to obtain a better understanding of the findings of diverse studies. Some unexplained findings such as the occurrence of tumors in the first generation of the Borneff et al. (1968) study could be explained by the presence of Helicobacter bacteria. Also, the hypothesis may explain why stomach tumors may have occurred following a relatively short term exposure to Cr VI in rural China. We prefer to keep these discussions attached in the Appendix as records of the issues that have been addressed in the research for and preparation of this PHG document.

Comment 12: "Fourth, for this draft PHG, OEHHA erred in its scientific evaluation of the data in published studies in several ways. OEHHA relied on studies that have been superseded by more recent findings. It also chose to reinterpret other studies that do not fit its own conclusions. And OEHHA ignored data that did not support its conclusion. A good example of all three of these problems is OEHHA's evaluation of the 1987 Zhang and Li assessment of chromium pollution of water supplies in China. This was one of the major studies relied upon by OEHHA in developing the draft PHG. In 1997, the lead co-author of the 1987 study expanded the assessment of the data and found no statistically relevant link between stomach cancer in humans and consumption of water containing Cr(VI) (Zhang and Li, 1997). OEHHA did an internal reevaluation of the 1987 study data (which was not peer reviewed). The 2005 PHG scientific peer reviewers criticized OEHHA's rejection of the 1997 study, noting OEHHA's effort to explain the comparative decrease in cancers in areas in the closest proximity to the plant as 'the subject of speculation.' Since the re-analysis of the 1987 Zhang and Li study was a cornerstone of the OEHHA case for the carcinogenic activity of oral Cr(VI) in humans, their 'analysis too, must be subjected to full peer review by specialists in the field.' OEHHA subsequently published a peer-reviewed internal OEHHA reevaluation (Beaumont *et* al., 2008), and while this evaluation has been cited for its 'serious limitations in the data and the methods of analysis' (Smith, 2009), OEHHA cites its own study and continues to rely on the original 1987 brief report. A recent peer-reviewed and published study further evaluating the original 1987 data for the exposed villages and comparing the cancer rates to nearby areas with no Cr(VI) in groundwater did not find a dose-response relationship or a coherent pattern of association of lung-, stomach-, or all-cancer mortality with exposure to Cr(VI)-contaminated groundwater (Kerger *et*

al., 2009). Thus, OEHHA apparently disregards the more recent studies - both of which did not support OEHHA's hypothesis on an association of stomach cancer in humans drinking Cr(VI)-impacted water. There are other examples where OEHHA similarly reevaluated published data and studies to support OEHHA's hypothesis that the 2008 Peer Reviewers noted as 'overreaching' and that the DTSC memorandum concluded inadequately addressed the weight of evidence.

Response 12. The findings of Beaumont et al. (2008) published in 2008 (well after the original studies, Zhang and Li**,** 1987) replicated the findings of the original study and found a statistically significant increase in stomach cancers. The second authors of the 1987 and 1997 studies were different individuals although they shared the same surname. The referred to 1997 study was withdrawn in 2006 by the publisher, the Journal of Occupational and Environmental Medicine. Another recent reevaluation of the Zhang and Li (1987) study by Kerger et al. (2009) has been added to the PHG document.

Comment 13: "Fifth, OEHHA has ignored the fact that analyses and studies are underway that could call into question their adoption of the default linear extrapolation procedure. EPA is evaluating chromium risk on an expedited basis through its Integrated Risk Information System (IRIS) program. In addition, OEHHA is monitoring studies by the Hamner Institutes that will help determine whether the linear extrapolation method it chose or a more scientifically valid nonlinear "mode of action" (MOA) approach is the more appropriate risk analysis method for chromium. DTSC recognized the importance of the Hamner Institutes program in addressing the mode of action of chromium and said that the studies should be "prerequisites to any revisions to the OEHHA public health goal for Cr^{6+}. By issuing a draft PHG without waiting for this information, OEHHA is not taking account of the most up-to-date science."

Response 13. Health and Safety Code Section 116365.5 required the Department of Public Health to develop a primary drinking water standard for Cr VI by January 1, 2004. Health and Safety Code Section 116365 requires the development of a PHG by OEHHA before the department can adopt a primary drinking water standard. In light of this statutory mandate, it would be very difficult for OEHHA to justify further delay to finalizing the PHG in order to incorporate any appropriate findings from the Hamner Institutes research program.

Health and Safety Code Section 116365 contains an important provision that addresses new scientific research when it becomes available: "(e) (1) Public health goals established by the office shall be reviewed at least once every five years and revised, pursuant to the provisions of subdivision (c), as necessary based upon the availability of new scientific data". OEHHA acknowledges that new studies may alter a revised PHG. From the risk characterization section of the 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."

OEHHA will review papers and materials relating to the Hamner Institutes study when they are published. If the study produces compelling information that should be reflected in the PHG document, OEHHA will take appropriate action.

Comment 14: “OEHHA has drafted a PHG that is not scientifically reliable and therefore is not helpful to public health and water agencies trying to protect the public. These flaws are not without cost. As DTSC stated, "there are serious consequences associated with overly conservative analysis that fail to account for a carcinogenic MOA." As discussed by EPA's Dellarco and Baetcke (2005), application of an MOA framework to data generated from appropriate studies can also be very informative to risk assessors and policy makers. OEHHA's failure to use the latest risk assessment methods accepted by health science experts can dangerously skew future decisions regarding water supply, water quality treatment technology, and testing and monitoring methodology. By proposing a draft PHG that is so far below currently detectable levels, OEHHA has unnecessarily called into question the safety of California's water supply. Given the potentially enormous consequences to the State of California, it is essential that OEHHA be required to rigorously follow the most current procedures and apply the most up-to-date science before adopting a PHG for chromium. Accordingly, OEHHA should re-evaluate its draft PHG, consistent with its processes, "using the most current principles, practices, and methods used by public health professionals" and the absolute best science. Once that is done, a new and scientifically valid draft PHG should be reissued and peer reviewed.”

Response 14. OEHHA used the best and most up-to-date science and methodology available to calculate the cancer potency for ingested Cr VI. Thus, it is not surprising that the U.S. EPA (2010 draft document) and the New Jersey Department of Environmental Protection (Stern, 2010) recently developed cancer potencies for Cr VI by the oral route that are identical to that developed in the PHG document. A discussion of the mutagenic MOA proposed by McCarroll et al. (2010) has been added to the document. The proposed MOA supports our approach.

From the Attachment –II. Background

Comment 15: “The National Toxicology Program (NTP) conducted a carcinogenic and toxicological study of Cr(VI) in response to requests from members of the California Congressional delegation. California health and regulatory agencies also supported NTP conducting this study. California officials were concerned that they lacked information on the oral route of exposure for Cr(VI), as what information was available was insufficient to set a safe drinking water standard. The NTP study was aimed at determining carcinogenic impacts from high-dose chronic exposures to rats and mice. The NTP study, completed in July 2008, was not intended to, and did not, recommend a particular dose or regulatory exposure level. Going beyond risk assessment of oral chromium exposures to management of the risk of chromium in drinking water, OEHHA, in the draft PHG, applied certain key assumptions about dose-response relationships

and other factors and then extrapolated the NTP results to calculate a draft PHG of 60 ppt for Cr(VI) that would give a theoretical risk level of 1 x 1 0-6 (one in a million)."

Response 15. We agree that the NTP did not recommend a particular dose or regulatory exposure level. OEHHA employed the result of this state of the art cancer bioassay and standard procedure to derive a dose associated with 10^{-6} risk, a level of risk that is protective of public health. The use of high doses in cancer bioassay to estimate low levels of risk has been discussed above and in related documents (U.S. EPA, 2005; OEHHA, 2009).

Note that OEHHA was one of the entities that petitioned the NTP to perform these studies precisely to provide guidance in developing a PHG for Cr VI.

From the Attachment - III. OEHHA Did Not Apply State-of-the-Art Principles and Practices for Assessing Potential Carcinogenic Risk To Humans, Nor Did It Follow Current National and International Regulatory Program Guidelines.

Comment 16: "In summary, prolonged exposure to Cr(VI) above 14.3 mg/L sodium dichromate induces sustained cytotoxicity and cell proliferation that, as described by NTP, is regenerative hyperplasia secondary to epithelial injury. In the 2008 peer review comments of Dr. Bjeldanes, he noted his concerns about the high level of Cr(VI) in the drinking water in the NTP study and recognized that the lesions identified in the small intestine of the mouse are often considered to be pre-cancerous."

And,

"The genetic changes are postulated to be secondary to the cytotoxicity, metaplasia, and hyperplasia that are clearly induced by Cr(VI). Cr(VI) has been found to be genotoxic in some in *vitro* and ***in vivo*** test systems but was not acting as a direct mutagen. "

"This postulated MOA for Cr(VI) is mainly based on observations of consistent, nonlinear dose-response relationships for all three key events (sustained cell injury, cell proliferation, and tumors) and concordance of incidence of diffuse hyperplasia in other regions of the intestinal tract (NTP, 2007)".

Response 16. Neither sustained cytotoxicity nor inflammation was observed in the small intestine of mice during the two-year bioassay (NTP, 2008). Preceding the two-year study, a subchronic toxicity study in mice (NTP, 2007) reported similar findings; i.e., the absence of cytotoxicity and inflammation in the small intestine of mice. Therefore, this paradigm of cell injury leading to cell proliferation and tumorigenesis is not indicated by the findings of both NTP studies. These and related issues are discussed in detail in the section of the PHG document entitled "Non-neoplastic findings – Possible relationships between tissue damage, inflammation, hyperplasia and tumors in rats and mice," located in the "Carcinogenicity" section of the document.

Comment 17: “Oral exposure of animals to Cr(VI) but not Cr(III) results in irritation and histopathological changes to tissues including cell injury, death, and regeneration (NTP, 2007; NTP, 2008). Following three-month exposures to Cr(VI), dose-responses in duodenal histiocytic infiltration of the duodenum in rats and epithelial hyperplasia and histiocytic cellular infiltration of the duodenum in mice were observed (NTP, 2007). After two years of exposure, dose-responses in duodenal histiocytic infiltration in rats and duodenal epithelial hyperplasia and histiocytic cellular infiltration in mice were observed.”

Response 17. NTP did not report oral exposure to Cr VI resulted in irritation or histopatholical changes to tissue such as cell injury, death and regeneration. Histiocytic infiltration in the small intestine of rats and mice was reported and tumors (adenomas and carcinomas) were only detected in the mouse small intestine. From NTP (2008): “In the 3-month toxicity study (NTP, 2007a) and in the current 2-year studies of sodium dichromate dehydrate, histiocytic cellular infiltration was consistently observed in several tissues including the liver, duodenum and mesenteric and pancreatic lymph modes of rats and mice. The severities of these lesions were generally minimal to moderate and were characterized by the presence of individual small clusters, and sometimes syncytia of macrophages. The significance of these lesions is not known.”

Comment 18: “Appendix A - Carcinogenic Threshold? [note: there is no discussion of threshold in Appendix A. This is a discussion of the reducing capacity of the stomach documenting Cr(VI) absorption into the body. As questioned by the DTSC memorandum, it ‘is unclear how this discussion contributes to the understanding of a threshold-based dose-response relationship for ingested chromate.’ It is DTSC's position that ‘the most likely threshold effect is the ability of the hexavalent chromium to elicit dose-dependent overt tissue damage, chronic inflammation and local regenerative hyperplasia.’]”

Response 18. DeFlora proposed a threshold of carcinogenic effect based on the reducing capacity of the stomach. OEHHA agrees that the ability to reduce CrVI to Cr III is not infinite. However, there appears to be sufficient gastric reducing capacity (84 mg/day according to the estimates of DeFlora and coworkers) to adequately reduce the amount of chromium VI that was administered to humans in several pharmacokinetic studies. Therefore, absorption of hexavalent chromium in these studies was not due to the exceedance of the reducing capacity of the GI tract. In a recent study of rats and mice exposed to Cr VI via their drinking water, there was no threshold for its accumulation in a variety of tissue (Collins *et al*., Tox Sci 118: 368-379, 2010). Rather, its accumulation was either linearly related to its concentration in the drinking water over the entire concentration range tested, or linearly related at low concentrations with indications of a plateau at higher concentrations. These data are discussed in Appendix A of the PHG. As discussed in Response 16 above, intestinal tissue damage was not observed in the two-year bioassay in mice (NTP, 2008).

Comment 19: “Contrary to current cancer risk assessment guidance, no specific mode of action was identified or discussed to support the dose-response model used by OEHHA for the draft PHG. OEHHA states that 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’ (p. 97). Based on this, OEHHA assumed the default model to be a linear dose-response.

Response 19. It is correct that the mechanism of action of Cr VI in the etiology of cancer in the small intestine in mouse and the oral cavity in the rat is currently not known. However, when evaluating the evidence of carcinogenicity, hexavalent chromium displayed genotoxic activity in *in vitro* and *in vivo* bioassays. Mechanistic studies yielded evidence of the generation of reactive species that are associated with oxidative damage of DNA. Thus, the use of a linear dose response model was appropriate.

Comment 20: “It should be noted that OEHHA did not differentiate between genotoxicity and mutagenicity - which is a very important distinction. If OEHHA had utilized the concepts in EPA's Framework for Determining a Mutagenic Mode of Action for Carcinogenicity, it would have helped them determine whether or not the data support a finding of a mutagenic mode of action for carcinogenicity. The Framework also addresses the adverse endpoints of mutagenicity. OEHHA does not make one reference to Cr(V1)'s mutagenicity in the entire document - with the exception of Appendix A, where it was noted that 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, 1 987)."

Response 20. Mutagenicity is included under the section entitled “Genetic Toxicity.” Search of the PHG document indeed revealed that the term mutagenicity was not used until Appendix A. In the first paragraph of the “Genetic Toxicity” section it is noted that Cr VI induced gene mutations in multiple species. References to that work are provided. Discussion of mutagenicity has been added to other parts of the PHG document including where MOA is discussed. The mutagenic mode of action described by McCarroll et al. (2010) has been added to the document.

Comment 21: “While not discussed by OEHHA, the finding of cytotoxicity is also important when considering different modes of action that may be operating over different dose ranges, as stated in EPA's 2005 risk assessment guidelines and referenced in the draft PHG. Such cytotoxicity supports application of a nonlinear dose-response model per EPA's 2005 risk assessment guidelines. Specifically, the guidelines state that ‘depending on the strength of the suggestion of mutagenicity, the assessment may justify a conclusion that mutagenicity is not operative at low doses and focus on a nonlinear approach, or alternatively, the assessment may use both linear and nonlinear approaches.'"

Response 21. Given there is no report of cytotoxicity in the oral cavity of rats nor the intestine of mice in the NTP bioassay for Cr VI, it would be difficult to justify including cytotoxicity in a discussion of cancer mechanism.

Comment 22: *In vivo* genotoxicity studies indicate that there are exposures below which DNA damage would not be produced locally or systemically following ingestion of Cr(VI). Daily administration of Cr(VI) as chromate for up to 20 mg/L for nine months did not increase the frequency of DNA-protein crosslinks or produce oxidative DNA damage in mouse forestomach, glandular stomach, or duodenum (De Flora *et al.,* 2008). Micronucleus formation in bone marrow or peripheral blood in mice administered up to 500 mg/L (chromate) in drinking water for up to 210 days was not increased. No genotoxic effects in fetal liver or peripheral blood were observed in treated pregnant mice receiving up to 10 mg/L (chromate) in drinking water (De Flora et al., 2006). The results of incidences of four micronucleus tests conducted in the three strains of mice from 2007 NTP were predominately negative. In Study I (up to 1000 mg/L dichromate in drinking water for three months), no significant increases were seen in micronucleated normochromatic erythrocytes in peripheral blood samples from male or female B6C3F1 mice. In Study 2 (up to 250 mg/L chromate in drinking water for three months), a significant exposure concentration-related increase (P< .00 1) in micronucleated normochromatic erythrocytes was seen in am3-C57BLl6 male mice (transgenic for PhiX17am3). An equivocal increase in micronucleated erythrocytes was noted in male B6C3F1 based on a small increase in micronucleated normochromatic erythrocytes that did not reach statistical significance. No increase in micronucleated changes normochromatic erythrocytes was observed in male BALB/c mice. No significant effect of sodium dichromate dihydrate exposure on the percentage of and polychromatic erythrocytes was observed in any of the three micronucleus tests conducted in Study 2 (Bucher, 2007). None of this information is discussed in the draft PHG."

Response 22. The PHG "Genotoxicity" section has been updated.

Comment 23: "Clearly, OEHHA recognized the important role of irritation/inflammation, cytotoxicity, hyperplasia in tumor formation (pp. 42, 134), yet it failed to develop the logical and well established hypothesis for Cr(V1) mode of carcinogenic action, *ie.*, sustained cell injury, death, and repair (Figure 1). OEHHA mentioned the NTP's findings of a significant and dose-related increase in diffuse hyperplasia in mice duodenum. OEHHA cited the NTP's findings "that collectively, these lesions are considered consistent with regenerative hyperplasia secondary to previous epithelial cell injury."

Response 23. No irritation/inflammation or evidence of cytotoxicity was reported in the oral cavity of rats or intestine of mice in the 2-year NTP bioassay. NTP noted that the stomach irritation (not a tissue where an increase in tumors was observed) seen in the 3 month study was not evident in the 2 year bioassay (see discussion of NTP, 2008 in "Toxicological Effects in Animals, Carcinogenicity" section of the PHG document). Note

that the stomach irritation observed in the 3-month study occurred only in the high dose animals. This dose (1000 ppm sodium dichromate dihydrate) exceeded all the dose levels used in the 2 year bioassay to measure tumor induction.

Comment 24: "While not stated, if OEHHA's evaluation of the weight of evidence of 'all available data were insufficient to establish the mode of action' (EPA, 2005), then OEHHA should have presented alternative analyses. Specifically, OEHHA should have presented results based on both a linear and nonlinear approach as part of its risk characterization process. Such an analysis would help provide risk managers and decision-makers with a perspective on the uncertainty inherent in the numerical value of OEHHA's draft PHG. A calculation based solely on the linear dose-response model presents the draft PHG as if it were 'the number' that would be protective of human health. Specifically, OEHHA should have followed the 2005 EPA guidance, i.e., 'where alternative approaches with significant biological support are available for the same tumor response and no scientific consensus favors a single approach, an assessment may present results based on more than one approach.'"

Response 24. After reviewing all the data relating to Cr VI's mode of action, OEHHA made the determination that a linear extrapolation was the correct approach for predicting tumor response in the low dose region of the dose- response curve. The well-documented ability of Cr VI to cause genotoxicity (including mutation induction) in test animals, cultured mammalian cells, insects, yeast and bacteria was an important factor in that determination. Other groups including the New Jersey Department of Environmental Protection (Stern, 2010) and the U.S. EPA (2010) have come to similar conclusions, supporting a scientific consensus. Presumably, U.S. EPA followed their own guidelines for cancer risk assessment when they made the decision to base the cancer slope factor on a linear model and not on a non-linear/threshold model. Various sources of uncertainty associated with development of the PHG for Cr VI are discussed throughout the document, with a more concentrated discussion in the "Risk Characterization" section.

Comment 25: "Applying the benchmark dose (BMD) approach (a nonlinear dose extrapolation) to the NTP mouse duodenal hyperplasia data, as was done by ATSDR (2008) and as provided for in EPA's 2005 guidelines, results in BMDL10 values of 0.09 to 0.13 mg Cr VI/kg/day. Consistent with EPA practices, an uncertainty factor of 100-fold could be applied to account for extrapolation from animals to humans (10x) and for intra-human sensitivity (10X). The resulting reference dose would be approximately 0.001 mg/kg/day. The results of applying the linear and nonlinear dose-response models yield values with more than a 500-fold difference in daily doses, with proportional differences in the corresponding drinking water criteria, e.g., OEHHA's draft PHG of 60 ppt (using the linear model) and ATSDR's Minimum Risk Level of 35,000 ppt (using the BMD approach)."

Response 25. ATSDR's Minimum Risk Level for Cr VI referenced above was based on non-cancer effects, to be protective against such effects. It should not be compared to the value calculated in the PHG that is also protective against cancer (20 ppt in the current draft).

Comment 26: "OEHHA did not use the most current principles and practices in determining the non-cancer health-protective dose (HPD). Rather than using the benchmark dose/nonlinear approach on the mouse data for the noncancer risk assessment, OEHHA identified the lowest adverse effect level (LOAEL) from the rat study (female liver – mild chronic inflammation, fatty changes) and applied a 1,000-fold uncertainty factor that included 10x to account for the lack of a no observed adverse effect level (NOAEL). OEHHA guidance calls for the use of BMD over NOAEL/LOAEL. The DTSC memorandum on the draft PHG found that the NTP subchronic data incorrectly identified the NOAEL as an LOAEL, and DTSC criticized OEHHA for applying 1,000-fold uncertainty factors in developing the HPD."

Response 26. At present the Cr VI PHG based on cancer effects is 100-fold lower than if it were based on non-cancer effects (see "Calculation Of The PHG" section of the document). OEHHA will be applying the BMD approach in future analyses of the non-cancer data. Our preliminary analysis applying the BMD approach to the non-cancer data followed by an uncertainty factor of 100 yields a final value that is more than 100-fold ***higher*** than the proposed PHG based on cancer effects. Thus, the proposed PHG (0.02 ppb) for protecting against both cancer and non-cancer effects would not change.

Comment 27: "In particular, Dr. Kelner strongly recommended that 'all the NTP 2007 studies need to be analyzed and slope factors derived for each study by an accepted methodology. Then the mean median (preferably) slope factor is to be utilized for subsequent calculations. NOT the 95% confidence interval.' Dr. Kelner was urging OEHHA to use the mean or median ED10 as described in EPA's 2005 guidelines, e.g., 'risk assessors should calculate, to the extent practicable, and present the central estimate and the corresponding upper and lower statistical bounds (such as confidence limits) to inform decision makers.'"

Response 27. Current methodology followed by both the U.S. EPA (2005; Davis et al., 2010) and OEHHA (2009) is to use the LED_{10} to derive the cancer slope factor.

Comment 28: "OEHHA states that it acknowledges the 'various uncertainties inherent in cancer risk assessment' in the Risk Characterization section of the draft PHG document (pp. 96-98), but there is no quantitative assessment of uncertainty in the value of the draft PHG, nor is there any discussion or quantitative estimate of the large 'uncertainty factor' in the equation used to calculate the PHG (pp. 95-96)."

Response 28. OEHHA is not aware of an established methodology for quantifying the uncertainty of uncertainty factors or the uncertainty associated with cancer risk extrapolation.

Comment 29: "Contrary to the recommendations in this report and peer reviewers' comments on the 2005 draft PHG, OEHHA has developed a widely speculative hypotheses that inflammation caused by bacteria may be additive to, or synergistic with, adverse effects of hexavalent chromium-produced irritation on the stomach such that inflammation may "help push an individual along the path to stomach tumors" (OEHHA, Comments for the NTP Cr(VI) Public Meeting, July 24, 2002). Furthermore, OEHHA's approach is far from valid or conventional and dismisses valid scientific data (see Section IV). Although OEHHA's Bacterial Infection Hypothesis is overly speculative, it serves as the basis for developing the draft PHG for Cr(VI) in drinking water of 60 ppt.

Nevertheless, in the draft PHG documentation, OEHHA included the 2007 NTP study results and revived their Bacterial Infection Hypothesis to ultimately tie three Itey [sic] studies together to support OEHHA's preconception that Cr(VI) is carcinogenic to humans at environmentally relevant doses."

Response 29. The PHG does not rely on the Helicobactor hypothesis in the development of the PHG. The hypothesis is located in Appendix B and clearly labeled that it is a hypothesis. The Helicobacter hypothesis was formulated by OEHHA as part of an effort to obtain a better understanding of the findings of diverse studies, such as the occurrence of tumors in the first generation of the Borneff et al. (1968) study and stomach tumors following a relatively short term exposure to Cr VI in rural China. These discussions serve as a scientific resource and are attached in the Appendix as records of the issues that have been addressed in the research for and preparation of this PHG document.

Comment 30: "OEHHA's 2005 PHG peer reviewers discounted the use of the Borneff et al. (1968) study for cancer risk assessment purposes for multiple reasons, including the high mortality associated with the mouse pox outbreak in the study animals… However, OEHHA discounts the authors' explanation of the study findings and promotes their own speculative hypothesis that an unidentified bacterial infection in the parental generation (F_0) was in part responsible for stomach tumor formation. Stomach cancer was not produced in their first generation (F_1) because the bacterial infection was not passed to offspring due to Cr(VI) bactericide activity (p. 126)."

Response 30. The Borneff et al. (1968) study is not a key study in the derivation of the PHG for Chromium VI and is now placed in the Appendix for information purposes. OEHHA provided extensive analysis of its findings in its endeavor to consider all available scientific data when evaluating chemicals and developing PHGs aimed at protecting public health. The weight of evidence approach taken by OEHHA necessitated a discussion of Borneff et al. (1968).

Comment 31: "OEHHA relies on the original publication regarding the Chinese population associated with drinking water contaminated with Cr(VI) (Zhang and Li, 1987)… The overall findings in the studied population do not indicate a dose-response relationship or a coherent pattern of association of lung-, stomach-, or all-cancer mortality with exposure to Cr(VI)-contaminated groundwater (Kerger et al., 2009). Kerger et al. is not even referenced or discussed."

Response 31. Kerger et al. (2009) is now discussed in detail along with Beaumont et al. (2008) in the "Toxicological Effects in Humans, Carcinogenicity" section of the PHG.

Comment 32: "Furthermore, Dr. Bjeldanes submitted for OEHHA's consideration a study (Bednar and Kies, 1991) where 'no association was found between low levels of Cr(VI) in drinking water (up to 10 ppb) with total cancer mortality.' OEHHA dismissed this study and Dr. Bjeldane's criticism of OEHHA's selectivity stating 'the analysis was not specific to Cr(VI)' and that this makes it 'difficult to compare the findings to those of Beaumont et al. (2008) of a relationship between hexavalent chromium in water and increased risk of stomach cancer.'

Response 32. The study by Bednar and Kies (1991) is discussed in more detail in the final PHG document. An important limitation of this study was that sampling in the study occurred for only two years. Unlike the Zhang and Li (1987) study, no specific source of Cr VI exposure was identified. Also, while overall cancer mortality rates were evaluated, tumors at specific sites were not.

Comment 33: "OEHHA does recognize that the data underlying Zhang and Li (1987) have several important limitations that included lack of exposure data and a short observation time (14 years) after residents first noticed the yellow color of the water. However, OEHHA draws on the Bacterial Infection Hypothesis to overcome some of these limitations in order to continue relying upon the positive association of increased stomach and over all cancer rates with drinking Cr(VI)-tainted water."

Response 33. Zhang and Li (1987) is discussed in the "Toxicological Effects in Humans, Carcinogenicity" section of the PHG. This detailed analysis of the study, much of it taken from Beaumont et al. (2008), stands on its own, without any reference to the Helicobacter Hypothesis. That hypothesis, and its ***possible*** relation to the 14 year observation time cited above, is only discussed in Appendix B.

Comment 34: "When confronted with lack of concordance in tumor locations between NTP (2007), Borneff et al. (1968), and Zhang and Li (1987) (mouse intestine, mouse forestomach and human stomach, respectively), OEHHA evokes the Bacterial Infection Hypothesis… In addition to noting that the NTP mice that developed intestinal cancer were bacteria-free, OEHHA ties the intestinal tumor location to the Helicobacter infection since it is characterized by the occurrence of metaplasia in the stomach – 'a transformation of the stomach into a tissue that resembles intestine' (p. 136)."

Response 34. The PHG does not rely on the Helicobactor hypothesis in the development of the PHG. The hypothesis is located in Appendix B and clearly indicated as such. The Helicobacter hypothesis was formulated by OEHHA as part of an effort to obtain a better understanding of the findings of diverse studies such as the occurrence of tumors in the first generation of the Borneff et al. (1968) study and stomach tumors following a relatively short term exposure to Cr VI in rural China. These discussions serve as a scientific resource and are attached in the Appendix as records of the issues that have been addressed in the research for and preparation of this PHG document.

Comment 35: "It should be noted that all OEHHA's speculation on tumor formation is focused on the occurrence of tumors in mice and humans but does not mention or attempt to explain the tumors produced in the oral cavity of the rat in the NTP study."

Response 35. The rat tumor data from NTP (2008) are discussed in detail in the "Toxicological Effects in Animals, Carcinogenicity" section of the PHG.

Comment 36: "OEHHA states that the International Agency for Research on Cancer (IARC) (1990) concluded that CR(VI) is a 'strong' carcinogen for the respiratory tract, while the document concluded that 'there is sufficient evidence in humans for the carcinogenicity of Cr(VI) compounds as encountered in the chromate production, chromate pigment industry and chromium plating industries.'"

Response 36. The word "strong" has been deleted.

Comment 37: "Based on Dr. Gwiazda's 2008 peer review comments on the peer review version of the draft PHG document, OEHHA 'ignored' the confidence intervals of the epidemiological rate ratios in reaching its (OEHHA's) conclusion that most occupational studies showed an increase risk of stomach cancer. Dr. Gwiazda commented that if OEHHA chose to include the analysis, it should be "consistent and address the contradictory observation that on the basis of the rate ratios alone, e.g., 25% of the studies would support a protective role of Cr(VI) exposure against stomach cancer! But this logical conclusion was ignored."

Response 37. We have added more discussion of Table 8 that includes the suggestions of Dr. Gwiazda to: 1) not compare the rate ratios to 1.00, and 2) conclude that the results are consistent with an association between occupational exposure to Cr VI (via inhalation) and stomach cancer.

Comment 38: "As pointed out in the DTSC memorandum, analyses of these same data by Cole and Rodu (2005) indicated there were no significant increases in stomach or gastrointestinal tumors associated with Cr(VI) exposure."

Response 38. The study by Cole and Rodu (2005), along with some of its shortcomings, is discussed in the PHG document.

Comment 39: "As discussed above, OEHHA relies on the original publication on the Chinese population associated with drinking water contaminated with Cr(VI) (Zhang and Li, 1987) and OEAA's reassessment of the Chinese data (Beaumont et al., 2008) to draw the connection between exposure to Cr(VI) and stomach cancer in humans and various cancer sites in experimental laboratory animals. In doing so, OEHHA ignores other publications that do not support OEHHA's contention of the causal link between oral exposure to Cr(VI) and cancer in humans, including the recent publication on the study population by Kerger et al. (2009). Furthermore, in his 2008 peer review of the draft PHG, Dr. Bjeldanes brought the Bednar and Kies (1991) drinking water study to OEHHA's attention. In this study of 453 communities in Nebraska, no association was found between low levels of chromium in drinking water and total cancer mortality. OEHHA affirmed the finding and agreed that the data could be examined but cast doubts on the results. OEHHA believes the analytical method likely did not measure Cr(VI) but rather total chromium (Cr(VI) and Cr(III)). OEHHA ignores similar problems with the exposure assessment of the Chinese study population."

Response 39. Kerger et al. (2009) is now discussed in the PHG document. With regard to the study by Bednar and Kies (1991), a number of its limitations are now discussed in the PHG document including that sampling in the study occurred for only two years. Unlike the Zhang and Li (1987) study, no specific source of Cr VI exposure was identified. Also, while overall cancer mortality rates were evaluated, tumors at specific sites were not.

Comment 40: "Another example of OEHHA's selective interpretation of the literature can be found in its description of the role of Cr(VI) reduction to Cr(III) in the stomach and in cells – the subject of Appendix A, 'Carcinogenic Threshold?' to OEHHA's draft PHG document. Appendix A is intended to provide support of OEHHA's default to a linear extrapolation model because a fraction of ingested Cr(VI) is absorbed into the body – escaping the body's first line of defense, i.e., gastrointestinal reduction of Cr(VI)

to Cr(III). OEHHA points to the 2007 NTP study showing the dose-related systemic absorption of orally administered Cr(VI) in mice being inconsistent with the research of De Flora and others that OEHHA characterizes as the 'assertion that hexavalent chromium absorption occurs only when the reducing capacity of the GI tract is exhausted.' Contrary to OEHHA's interpretation of the literature, the studies published by researchers such as De Flora and others do not suggest that the detoxification pathways are 100% efficient or unsaturable. These researchers' contributions to the literature indicate that the reduction of Cr(VI) to Cr(III) in the gastrointestinal tract limits the bioavailability and attenuates the potential for adverse effects of Cr(VI) compounds *in vivo*. Apparently, OEHHA takes the position that since Cr(VI) can be absorbed into the body, inferring that there is no threshold for Cr(VI) carcinogenicity via ingestion." This is a critical OEHHA determination that ignores other mechanisms that attenuate the bioavailability and potential adverse effects of Cr(VI), including DNA damage (Sedman et al., 2006). The high rate of reduction of very low concentrations of Cr(VI) to Cr(III) effectively detoxifies Cr(VI) since Cr(III) is not readily taken up by cells, i.e., it is not bioavailable. This markedly changes the shape of the dose-response curve at low doses because the reduced Cr(VI) is no longer bioavailable."

Response 40. Appendix A addresses the Cr VI concentration range used in the NTP studies. Appendix A states, "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." This can be inferred by the absence of thresholds in Figures A1 through A6. Thus, the original title of Appendix A, "Carcinogenic Threshold?" This title was not meant to rule out the possibility that there is a threshold for carcinogenesis at much lower concentrations of Cr VI. We do not know the answer to this, since we do not have dose response data for either Cr VI absorption or tumorigenesis at low concentrations of Cr VI. We have modified the title of Appendix A to "Carcinogenic Threshold: Was the reductive capacity of the rodent GI tract exceeded in the NTP (2008) two-year bioassay?"

Comment 41: "OEHHA overlooked relevant in vivo genotoxicity data published in peer-reviewed journals during the development of the draft PHG… Curiously, the results of the four genotoxicity tests (micronucleus) conducted by NTP on three strains of mice receiving Cr(VI) in the drinking water at chromate concentrations up to 1,000 mg/L for three months were not considered by OEHHA (Bucher, 2007)."

Response 41. The results of both De Flora et al. (2006) and De Flora et al. (2008) are presented in Table 2. The mouse erythrocyte micronuclei data from NTP (2007) have been added to Table 2.

Comment 42: "VII. Rather than Applying 'Uncertainty Factors' Ranging Over Five Orders of Magnitude in Developing the Draft PHG, OEHHA Should Identify the Key Data Gaps and Acknowledge Ongoing Studies That Would Reduce the Uncertainty in OEHHA's Risk Assessment. VIII. OEHHA Should Take No Action to Finalize the PHG Until It Can Review the Results of the Ongoing Research, Including the Hamner Institute's Research Into the MOA."

Response 42. First, the PHG for Cr VI in drinking water is based on tumor induction. No uncertainty factors were used to develop this number (0.02 ppb).

Second, Health and Safety Code Section 116365.5 required the Department of Public Health to develop a primary drinking water standard for Cr VI by January 1, 2004. Health and Safety Code Section 116365 requires the development of a PHG by OEHHA before the department can adopt a primary drinking water standard. In light of this statutory mandate, it would be very difficult for OEHHA to justify further delay to finalizing the PHG in order to incorporate any appropriate findings from the Hamner Institutes research program.

Health and Safety Code Section 116365 contains an important provision that addresses new scientific research when it becomes available: "(e) (1) Public health goals established by the office shall be reviewed at least once every five years and revised, pursuant to the provisions of subdivision (c), as necessary based upon the availability of new scientific data". OEHHA acknowledges that new studies may alter a revised PHG. From the risk characterization section of the 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."

OEHHA will review papers and materials relating to the Hamner Institutes study when they are published. If the study produces compelling information that should be reflected in the PHG document, OEHHA will take appropriate action.

RESPONSES TO MAJOR COMMENTS RECEIVED ON DECEMBER 2010 DRAFT, SECOND COMMENT PERIOD (2011)

Comments from Ann Mason and Laura Brust, American Chemistry Council

Cover Letter

Comment 1: "The most important point in the ACC comments is that OEHHA improperly applies age sensitivity factors, without consideration of mode of action (MOA), to reduce the PHG from 0.06 ppb to 0.02 ppb."

Response 1. See "Correction for Early-in-Life Exposures" section of the PHG document. The Age Sensitivity Factor (ASF) for modifying cancer potency was used as described (OEHHA, 2009). This approach applies to all carcinogens, regardless of purported mechanism of action, unless chemical-specific data exist that could be used to make more specific adjustments to risk. Such chemical-specific data are not available for Cr VI. A mutagenic mode of action described by McCarroll et al. (2010) has been added to the document.

Comment 2: "OEHHA also fails to support significant changes from the 2009 draft PHG and provide the documentation and rationale for some of its calculations and assumptions used to support the revised draft PHG."

Response 2. The revised PHG document has provided additional support for calculations and assumptions used.

Comment 3: “Moreover, in many cases, OEHHA does not adequately consider the comments of peer reviewers in the revised draft PHG.”

Response 3. The PHG document has been revised to address additional comments of the peer reviewers. Note that the peer reviewers do not always agree with each other so that a revision in response to comments from one reviewer may not appear to respond to comments from another peer reviewer. The critical point is that all comments have been considered by OEHHA.

Comment 4: “Finally, OEHHA should use the best available science, including MOA data, fully present both linear and nonlinear approaches, present the rationale and justification for its calculations and assumptions, and fully address and incorporate the comments from its own invited experts who provided peer review comments.”

Response 4. See the following sections of the PHG document for discussion of the MOA issues for which data are available: “Pharmacokinetics of Trivalent versus Hexavalent Chromium,” “Mechanism of Genotoxicity and Carcinogenicity,” Table 7 in the section entitled “Non-neoplastic findings – Possible relationship between tissue damage, inflammation, hyperplasia and tumors in rats and mice,” “Examination of Evidence for Chromium Carcinogenicity,” and “Risk Characterization.”

Executive Summary

Comment 1: “OEHHA also proposes a PHG of 2 ppb for non-cancer effects based on liver toxicity in female rats in the NTP study (2008).”

Response. The PHG document develops a single PHG value to protect against all toxic effects of Cr VI in drinking water: that of 0.02 ppb. The drinking water concentration of 2 ppb is protective from only non-cancer effects. It is not a PHG.

Comment 2: “While purporting to meet the requirements to use the best science in decisions that relate to protecting public health, OEHHA continues to use default assumptions rather than chemical-specific information and sound science to inform risk assessment.”

Response 2. OEHHA’s decision to perform linear extrapolation from the point of departure to the zero dose level was not a default decision. It was based on the extensive data base of studies covering the toxicity of Cr VI. That data base includes multiple studies indicating that Cr VI is both genotoxic and mutagenic (see “Genotoxicity” section of the PHG document). The data base also contains little support for an MOA other than genotoxicity/mutagenicity; for example, no cytotoxicity was observed at dose levels producing tumors in rodents (NTP, 2008). These were the primary considerations for choosing an MOA and analytical model. The U.S. EPA recently came to similar conclusions in their draft Toxicological Review of Hexavalent Chromium (2010), Presumably, this review was performed using “sound science.”

Comment 3: “Data about the mode of action (MOA) or Cr(VI) currently are being developed as part of a major research initiative that began in early 2009, and these data will be presented in March at the 2011 meeting of the Society of Toxicology.”

Response 3. OEHHA continues to use all available data collected through sound science in its development of the Cr VI PHG.

Comment 4: “OEHHA fails to address comments from peer reviewers of the draft August 2009 PHG document and the Draft Dec. 2010 PHG document, and expert panel comments on the draft 1999 PHG document.”

Response 4. The PHG document has been revised to address additional comments of peer reviewers. This document contains OEHHA’s responses to the 2008 and 2009 peer reviews (see Table of Contents). Note that the peer reviewers do not always agree with each other so that a revision in response to comments from one reviewer may not appear to respond to comments from another peer reviewer. The critical point is that all comments have been considered by OEHHA.

Comment 5: “OEHHA inadequately responds to public comments on earlier PHG document, including lack of any MOA consideration, especially when MOA forms the overarching conceptual framework for cancer risk assessment (EPA, 2005a).”

Response 5. See the following sections of the PHG document for discussion of the MOA issues for which data are available: “Pharmacokinetics of Trivalent versus Hexavalent Chromium,” “Mechanism of Genotoxicity and Carcinogenicity,” “Examination of Evidence for Chromium Carcinogenicity,” and “Risk Characterization.”

Comment 6: “Regarding the MOA, lack of consideration of interspecies differences in toxicokinetics of Cr(VI) and the failure to recognize that pathologies seen in rodents are likely portal-of-entry effects.”

Response 6. The rodent tumors observed in the two-year bioassay (NTP, 2008) may well be site-of-contact effects. However, given the available data, calculation of the cancer potency would be the same whether a site-of–contact effect or a systemic effect were assumed.

Comment 7: “Instead, the Draft Dec. 2010 PHG document correctly assumes that the metabolic products of Cr(VI) are DNA-reactive and wrongly assumes that DNA-reactivity equates to mutagenicity.”

Response 7. OEHHA does not equate DNA reactivity with mutagenicity. A specific citation within the PHG document would be most helpful. In cultured mammalian cells, there are a number of published studies which report robust (in excess of 3-fold increases) mutagenic responses to Cr VI (see Paschin et al. (1983) Mut Res103(3-6):345-347; Mitchell et al. (1988) Environ Mol Mutagen 12(Suppl 13):37-101; Myhr and Caspary (1988) Environ Mol Mutagen 13(12):103-194; McGregor et al. (1987) Environ Mutagen 9(2):143-160; Oberly et al (1982) J Toxicol Environ Health 9(3):367-376). Reviews discussing these studies are cited in the first paragraph of the “Genetic Toxicity” section of the PHG document. Cr VI also caused mutations in bacteria, yeast, D. melanogaster and mice (see reviews mentioned above and U.S. EPA, 2010).

Comment 8: “Lack of consideration of nonlinearity and the presence of a threshold. Although Appendix A, titled ‘Carcinogenic Threshold?,’ (in the Draft Dec. 2010 PHG document) discusses the idea of a threshold, this appendix considers only reductive capacity and absorption, and because of the lack of any consideration of MOA, fails to take into account epigenetic changes (such as those mentioned in the previous bullet) that underlie the tumor responses and that likely do have thresholds. The lack of

consideration of MOA also prevents exploration of the use of precursor effects as recommended in the EPA's *Guidelines for Carcinogen Risk Assessment* (EPA, 2005a)."

Response 8. The title of Appendix A has been revised to read, "Carcinogenic Threshold?: Was the reductive capacity of the rodent GI tract exceeded in the NTP (2008) bioassay?" Discussion has been added to the PHG document concerning the MOA data that support the use of a low dose linear extrapolation to calculate cancer potency. For example, see the "Mechanism of Genotoxicity and Carcinogenicity" section for studies demonstrating a genotoxic, and possibly mutagenic MOA for Cr VI. See also the discussion of Table 7 that presents tissue and cellular findings from NTP studies that do not support an MOA based on cytotoxicity and compensatory cellular proliferation.

Comment 9: "Regarding the water consumption to calculate life-stage exposures, use of an atypical calculation method that expresses life-stage as a unit less fraction of a lifespan. In addition, the water consumption rates used in the non-substantive change document released by OEHHA on January 25, 2011 cannot be verified from the original sources and appear to be incorrect for some age groups (EPA, 2008; Kahn and Stralka, 2009)."

Response 9. For the methodology used to correct for early-in-life exposures (life-stage calculations) to carcinogens, see OEHHA (2009). The methodology for calculating water consumption rates using the data from U.S. EPA (2008) and Kahn and Stralka (2009) is now presented in the footnote to Table 17 and in the discussion of Table 17.

Comment 10: "OEHHA inappropriately uses the age-sensitivity adjustment detailed in OEHHA (2009) because of lack of consideration of MOA. The age sensitivity adjustment was derived from data using solely statistical methods without consideration of biology or MOA other than a single paragraph classifying the chemical as genotoxic or non-genotoxic (p.4 of OEHHA, 2009). In addition, it is difficult to validate the calculations that employ this adjustment because the necessary data are scattered throughout the document."

Response 10. It is difficult to know if this comment refers to OEHHA (2009) or to the Cr VI PHG document. The "Calculation of The PHG," subheading "Carcinogenic Effects" section of the PHG document illustrates how the age sensitivity factors are incorporated into the PHG calculation.

Comment 11: "OEHHA uses scientific literature in a biased or inappropriate manner, including: The use of two highly flawed studies in mice and humans, respectively (Borneff et al., 1968; Zhang and Li, 1987), to attempt to establish a link between Cr(VI) exposure and gastrointestinal cancer in humans. The use of these studies is in direct contradiction of the advice of an expert panel convened by the University of California in 2001 to review the 1999 PHG document."

Response 11. The primary recommendation of the Chromate Toxicity Review Committee (CTRC, 2001) that a drinking water standard for Cr VI ***not*** be based on the study by Borneff et al. (1968) was followed by OEHHA. In 2005 UC reviewers Roberto Gwiazda, Leonard Bjeldanes and Michael Kelner provided comments on the draft PHG document which included the recommendation to move discussion of Borneff et al.

(1968) and the Helicobacter hypothesis to the Appendix. The PHG document was revised accordingly. With regard to the two studies by Zhang and Li (1987; 1997), the 1997 report was retracted by the journal in which it was published. Although the names of the second authors for the two studies appeared the same, they actually are two different individuals. The Zhang and Li (1987) study was thoroughly analyzed (see Beaumont et al., 2008). Although the magnitude of the exposure to Cr VI in drinking water is unclear, the population did appear to be exposed to high levels of chromium VI. A statistically significant increase in stomach tumors was detected when compared to a nearby unexposed population. The high levels of chromium VI in the wells is what triggered the study (lower levels probably would have gone undetected). Lower levels of chromium VI may still cause cancer but at a rate that would have been undetected in a population of several thousand. Another recent reevaluation of the Zhang and Li (1987) study by Kerger et al. (2009) has been added to the PHG document.

Comment 12: "OEHHA uses scientific literature in a biased or inappropriate manner, including: An attempt to impeach the results of the Gatto et al. (2010) meta-analysis that found no associations between occupational exposure to Cr(VI) and gastrointestinal cancer in humans. Although the Draft Dec. 2010 PHG document makes several suggestions to 'improve' the Gatto et al., meta-analysis, it is unlikely that any of these suggestions would alter the results published in Gatto et al., 2010."

Response 12. Our discussion of Gatto et al. (2010) is a straightforward identification of some possible limitations of the study.

Comment 13: "OEHHA fails to explore the uncertainty associated with dose-response modeling. The narrative and tables describing the modeling are very brief and difficult to follow. The number of animals at risk for the various dose groups in NTP (2008) was changed from those in the Draft August 2009 PHG document without explanation, and neither set of values are the results of the commonly used poly-3 survival adjustment (Portier and Bailer, 1989)."

Response 13. The numbers of animals at risk are shown in Table 5 and Table 6. As indicated in the footnotes to both tables, these are the mice alive at the time of the first occurrence of tumor (day 451 for males and day 625 for females) and if the tissue was available for analysis. This is a standard method for determining the number of animals at risk for tumors (U.S. EPA, 2005; OEHHA, 2009).

Specific Comments and Responses

Comment 1-1: "In early 2009, ACC's Cr(VI) Panel initiated the Cr(VI) MOA Framework Research Program (Appendix A) designed to elucidate details of the carcinogenic mode of action (MOA) of Cr(VI) in rodents from oral exposures…Hence, OEHHA personnel knew that the study was in progress but nonetheless released the Draft August 2009 PHG document and the Draft Dec. 2010 PHG document, neither of which considers the MOA."

Response 1-1. The carcinogenic mode of action (MOA) of Cr VI is discussed in detail in the PHG document (see especially sections "Pharmacokinetics of Trivalent versus

Hexavalent Chromium, Genetic Toxicity, Mechanism of Genotoxicity and Carcinogenicity, and Examination of Evidence for Carcinogenicity"). OEHHA will consider new data as they become final and available.

Health and Safety Code Section 116365.5 required the Department of Public Health to develop a primary drinking water standard for Cr VI by January 1, 2004. Health and Safety Code Section 116365 requires the development of a PHG by OEHHA before the department can adopt a primary drinking water standard. In light of this statutory mandate, it would be very difficult for OEHHA to justify further delay to finalizing the PHG in order to incorporate any appropriate findings from the Hamner Institutes research program.

Health and Safety Code Section 116365 contains an important provision that addresses new scientific research when it becomes available: "(e) (1) Public health goals established by the office shall be reviewed at least once every five years and revised, pursuant to the provisions of subdivision (c), as necessary based upon the availability of new scientific data". OEHHA acknowledges that new studies may alter a revised PHG. From the risk characterization section of the 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."

OEHHA will review papers and materials relating to the Hamner Institutes study when they are published. If the study produces compelling information that should be reflected in the PHG document, OEHHA will take appropriate action.

Comment 2-1: "Some of the bioassay data from which OEHHA (2009) developed these age sensitivity ratios was found to be flawed by the National Toxicology Program (NTP) and EPA…Given these problems with data from the Ramazzini Institute, codifying this idea in guidance such as OEHHA (2009) or EPA (2005b) may have been premature."

Response 2-1. OEHHA's derivation of Age Sensitivity Factors (AFSs) in OEHHA (2009) was based on data from 23 carcinogenic chemicals. One of these, vinyl chloride, is on the list of six chemicals to be re-reviewed by U.S. EPA. This re-review was deemed necessary to resolve possible differences of opinion between pathologists at NTP and at the Ramazzini Institute. The comment suggests that OEHHA's (2009) document on early-in-life susceptibility to carcinogens is unreliable due to its use of vinyl chloride data from the laboratory of Maltoni, who later founded Ramazzini. Should the Maltoni et al. (1981) data be found to be unreliable, OEHHA will revise its document (2009) accordingly. However, given that vinyl chloride is only one of twenty-three chemicals comprising the data base for OEHHA (2009), it is likely that the ASFs would change very little.

Comment 2-2: "The ASF values on page 86 of the Draft Dec. 2010 PHG document are:

Prenatal (in utero) ASF 10
Postnatal (Birth-2 yr.) ASF 10

Juvenile (2-16 yr.) ASF 3
Adult (>16 yr.) ASF 1

OEHHA does not explain why these [ASF] values are different from the values presented in OEHHA (2009)."

Response 2-2. The ASF values used in the Cr VI PHG are the same values presented in OEHHA (2009). See the Technical Support Document, page 3.

Comment 2-3: "In addition, the method of calculating drinking water intake *in utero* is not provided. We can only assume that OEHHA is referring to exposure *in utero* through maternal water consumption."

Response 2-3. The section "Correction for Early-in-Life Exposures" states that for the third trimester of pregnancy the drinking water consumption rate is assumed to be that of an adult (i.e., maternal consumption). We have added this information to the "Calculation Of The PHG," subheading "Carcinogenic Effects" section.

Comment 2-4: "The equation for calculating individual life-stage exposures for water consumption is $Exposure_j = ASF_j \times d_j \times cons^o_j$. However, life-stage exposure duration is not the appropriate multiplier."

Response 2-4. Because the four life-stages are of different durations, calculating the lifetime exposure requires a weighted mean calculation. This is achieved by including a duration adjustment for each life-stage.

Comment 2-5: "Because the slope factors are in units of $(mg/kg\text{-}d)^{-1}$, the exposure term should remain in units of L/kg-d. The unit kg-d will cancel to leave a water concentration in mg/L. OEHHA does not explain why it expresses life-stage as a unit less fraction of a 70-year lifespan in the Draft Dec. 2010 PHG document."

Response 2-5. The correct units have been added to the equation that immediately follows Table 17. See "Correction for Early-in-Life Exposures" section of the PHG document for a definition of the duration adjustment term.

Comment 2-6: "The tap water ingestion rates used to derive the water consumption rates are hidden…OEHHA should use the correct values for adults' and children's drinking water ingestion rates when revising this Draft PHG document."

Response 2-6. The **per capita** drinking water values from Kahn and Stralka (2009) were mistakenly used in the previous draft PHG document. Instead, the **consumers only** drinking water values from the same publication have been used to revise the PHG document. This resulted in the same final PHG value of 0.02 ppb (rounded). The protocol OEHHA followed to calculate the time-weighted mean drinking water ingestion rates using the data from Kahn and Stralka (2009) and U.S. EPA (2008) is now given in the "Calculation Of The PHG" section of the document.

Comment 2-7: "In the Draft Dec. 2010 PHG document, there is absolutely no discussion about the form in which this tap water is consumed. This is an important issue for Cr(VI), as it is well known to be rapidly reduced to Cr(III) in some beverages that are made from tap water in the home (e.g., orange juice, lemonade, coffee, tea). It appears that OEHHA assumes that all water consumed by the adult is from the same source, despite the fact that most people go to work or school and move several times in a 70-year lifetime. Such compounded conservative assumptions in exposure assessment overestimate the true risk."

Response 2-7. We have added to the "Calculation of The PHG" section of the document that the water intake data cover pure water consumed as a beverage or used in the home or local establishments to prepare food or drink (Kahn and Stralka, 2009). The PHG document does not attempt to determine how often the average American adult relocates during a lifetime. The document makes the health-protective assumption that some adults will live most of their lives in the region they were born. We believe this is a reasonable assumption for the purposes of this risk assessment.

Comment 3-1: "Neither the Draft Dec. 2010 PHG document nor the Draft August 2009 PHG document provides the information needed for dose-response modeling in a single place."

Response 3-1. The "Dose-Response Modeling" section of the PHG document now states the places in the document where the tumor incidence data and the lifetime time-weighted average doses in rodents are located.

Comment 3-2: "Why did the number of animals at risk increase from the 2009 document to the 2010 document?"

Response 3-2. In the 2009 draft an animal was excluded from the analysis if it died more than 40 days prior to the appearance of the first tumor of the small intestine. In the 2010 document an animal was excluded from the analysis if it died at any time prior to the appearance of the first tumor of the small intestine. The latter methodology is in keeping with OEHHA (2009) and U.S. EPA (2005) guidance.

Comment 3-3: "By using only a single model in the Draft Dec. 2010 PHG document, and by not exploring a range of PODs, OEHHA thwarts the intent of U.S. EPA's Cancer Guidelines (EPA, 2005a)…As pointed out by Dr. Michael Kelner, the cancer potency slope can be highly dependent on which POD (10%, 5%, or 1%) is selected for its determination. Prof. Mitchell Cohen, in his 2010 comments, points out that the set of values of 1%, 5%, and 10% excess cancer risk could be used as points of departure. Dr. Cohen reminds OEHHA of U.S. EPA's recommendation to "routinely calculate and present the point estimate of the ED_x (a central tendency estimate) and the corresponding upper and lower 95% statistical bounds." It is not clear why OEHHA disregards these peer reviewers' comments."

Response 3-3. Current standard methodology followed by both the U.S. EPA (2005; Davis et al., 2010) and OEHHA (2009) is to use the LED_{10} to derive the cancer slope factor.

Contrary to what is stated in this comment from the American Chemistry Council, OEHHA has not disregarded the comments of the two peer reviewers mentioned here. We are aware of Dr. Kelner's point that the cancer slope factor depends on which point of departure is selected. See OEHHA's responses 5 and 6 to Dr. Kelner's comments on this particular point (see Table of Contents, this document). While it is true that Dr. Cohen discussed using the 1, 5 and 10 percent effect levels as points of departure, after further consideration he went on to conclude that, "the use of the LED_{10} was appropriate for generating the PHG for human exposures to Cr^{6+} in drinking water." Both the ED_{10} values and the LED_{10} values for tumor incidence in male and female mice are presented in Tables 10 and 11 of the PHG document.

Comment 3-4: "As currently written, the Draft Dec. 2010 PHG document suggests there is only one possible value for the PHG when, in fact, consideration of uncertainty suggests a range of values and that the value depends on the choices made in the course of data evaluation, calculations and modeling…An example of the type of modeling that is indicated in EPA (2005a) is shown in the tables below…OEHHA did not choose the best-fitting model based on Chi-square or the model that provides the best fit and most parsimony based on the Akaike information criterion, as is indicated in EPA's *Benchmark dose Technical Guidance Document* (EPA, 2000)…The empirical fits discussed here were conducted using the number of animals at risk in the Draft Dec. 2010 PHG document and thus, OEHHA should have conducted a full exploration of the uncertainty in empirical dose response modeling. Had OEHHA done so, it is likely that the probit model rather than the multistage model would have been chosen."

Response 3-4. The PHG document develops two health-protective concentrations for Cr VI in drinking water; one to protect against non-carcinogenic effects and one to protect against carcinogenic effects. The lower concentration is designated the PHG to be protective against all adverse health effects. OEHHA does not develop a range of PHG values. Such an approach was considered when the PHG program was established and judged not to be helpful, given the role of PHGs as non-regulatory guidelines that the Department of Public Health uses in developing regulatory drinking water standards. In addition, the California Safe Drinking Water Act of 1996 (Health and Safety Code, Section 116365) requires OEHHA to develop a Public Health Goal (PHG) for environmental contaminants in drinking water, not a range of PHGs for each contaminant.

Modeling the dose-response data using a variety of different models, as suggested in the comment, is not an uncertainty analysis. As stated in the "Dose-Response Assessment," subheading "Carcinogenic Effects," subheading "Dose-Response Modeling" section of the PHG document, the multistage model was used to model the tumor incidence data because this is the model preferred by OEHHA (2009) and U.S. EPA (2010) for conducting cancer dose-response assessments. This is primarily due to

the multistage model's generally good fit of the data in the relatively high dose range used in rodent bioassays (Armitage and Doll, 1961). With regard to the criteria U.S. EPA recommends for choosing between models when using its benchmark dose software, when the benchmark dose 95% lower bound confidence limit (BMDL) is different for models with adequate fits, the model yielding the lowest BMDL should be selected (Davis et al., 2010).

Comment 3-5: "Hence, the 2% POD is within the range of observation for male mice and the 1% POD is within the range of observation for female mice and, according to EPA (2005a), should be the preferred value from which to extrapolate to lower doses."

Response 3-5. Recent guidance from U.S. EPA (Davis et al., 2010) recommends using a benchmark response of 10 percent when dealing with dichotomous data from cancer bioassays performed with 50 animals per dose group.

Comment 3-6: "To calculate a slope factor, one would need a means for species extrapolation. EPA's Cancer Guidelines and the Agency guidance on the use of physiologically-based pharmacokinetic (PBPK) modeling in risk assessment indicate that a PBPK model is the preferred means of species extrapolation (EPA, 2005a, 2006). However, because the effects observed in mice are likely portal-of-entry effects (as discussed below), the current generation PBPK model for Cr(VI) developed by O'Flaherty et al. (2001) cannot be used because it does not include intestinal segmentation and therefore is structurally unable to address portal-of-entry effects in the intestinal epithelium. Instead, a next-generation PBPK model that extends the model of O'Flaherty et al. (2001) and incorporates the toxicokinetic features of polarity along the small intestine and partial reduction of ingested Cr(VI) while in the stomach is under development (Summit Toxicology, 2010). This report on this next-generation model is provided as Appendix D."

Response 3-6. The Cr VI cancer slope factor in the PHG document was calculated using the best data and methods available at this time, in accordance with the cancer risk assessment guidelines of the U.S. EPA (2005) and OEHHA (2009). OEHHA will consider the completed, next-generation PBPK model when it is published in final form in a peer-reviewed journal.

Comment 4-1: "As discussed in detail below, the Draft Dec. 2010 document relies on a single analysis of very uncertain epidemiological data from China (Zhang and Li, 1987, 1997; Beaumont et al., 2008) and supports this reliance with an equally uncertain animal study (Borneff et al., 1968). The use of these data was criticized by an expert panel convened by the University of California under contract to the California EPA (OEHHA, 2001b)."

Response 4-1. The PHG for Cr VI in drinking water is developed using a two-year bioassay performed in rats and mice (NTP, 2008), not the human study cited in this comment. The study by Borneff et al. (1968) is only discussed in the Appendix of the PHG document, and is not used to develop the PHG.

With regard to the two studies by Zhang and Li (1987; 1997), the 1997 report was retracted by the journal in which it was published. The Zhang and Li (1987) study was thoroughly analyzed (see Beaumont et al., 2008). Although the magnitude of the exposure to Cr VI in drinking water is unclear, the population did appear to be exposed to high levels of chromium VI. A statistically significant increase in stomach tumors was detected when compared to a nearby unexposed population. The high levels of chromium VI in the wells is what triggered the study (lower levels probably would have gone undetected). Lower levels of chromium VI may still cause cancer but at a rate that would have been undetected in a population of several thousand. Another recent reevaluation of the Zhang and Li (1987) study by Kerger et al. (2009) has been added to the PHG document.

Comment 4-2: "Five peer reviewers designated by the University of California, several public water agencies, other governmental agencies, and non-governmental institutions provided extensive comments on the Draft August 2009 PHG document. These comments have been largely unaddressed in the current document."

Response 4-2. All substantive comments by all these reviewers have been addressed in our "Responses to Major Comments" and the PHG document revised accordingly.

Comment 5-1: "Despite comments submitted by ACC and others in response to the Draft August 2009 PHG document, OEHHA does not consider MOA in the Draft Dec. 2010 PHG document."

Response 5-1 (repeat of Response 5). See the following sections of the PHG document for discussion of the MOA issues for which data are available: "Pharmacokinetics of Trivalent versus Hexavalent Chromium," "Mechanism of Genotoxicity and Carcinogenicity," "Examination of Evidence for Chromium Carcinogenicity," and "Risk Characterization."

Comment 5-2: "With regard to the MOA, OEHHA did not consider several key questions about the NTP study results, including:

1. Why did mice get tumors in the small intestine, but rats did not?
2. Why do fewer tumors occur in mice in distal parts of the small intestine (jejeunum, ileum) than in the duodenum as a function of dose?
3. If Cr(VI) were acting as a mutagen, then why were no tumors present in the stomach or forestomach of either mice or rats; why not in multiple tissues?
4. Why were intestinal tumors only observed in animals experiencing prolonged hyperplasia of the intestinal epithelium?
5. Is there a no effect level (NOEL) for intestinal hyperplasia in the mouse?
6. Is there a dose at which Cr(VI) reduction in the stomach is sufficient to lower the dose to the intestinal epithelium such that key events in the carcinogenic MOA do not occur?
7. Are cancer observations in mice relevant to humans who are exposed at much lower levels?
8. And finally, what is the MOA in mice and is it relevant to humans?"

Response 5-2.

1. Interspecies differences in tumor location are not uncommon.
2. This finding is stated in the "Toxicological Effects in Animals," subheading "Carcinogenicity," subheading "Mouse," subheading "Neoplasms" section of the PHG document. The biological basis for this finding is not known.
3. Ingested Cr VI is a multi-site carcinogen in rodents (NTP, 2008).
4. In discussing hyperplasia in NTP (2008), one should also address why there were tumors of the oral cavity in rats in the absence of hyperplasia. Both of these issues are discussed in the "Toxicological Effects in Humans," subheading "Carcinogenicity," subheading "Non-neoplastic findings – Possible relationship between tissue damage, inflammation, hyperplasia and tumors in rats and mice" section of the PHG document. The intestinal epithelial hyperplasia observed in NTP (2008) was **not** cellular proliferation in response to tissue damage, as discussed in this section of the PHG document.
5. We do not know the answer to this question.
6. We do not know the answer to this question.
7. Observations in mice are relevant to humans and this is one of the basic principles of animal testing. OEHHA and other regulatory agencies have established a method for low dose extrapolation. Experiments at lower doses in animals are not feasible due to the much larger numbers of animals needed to detect an effect.
8. See the section of the PHG document entitled "Examination of Evidence for Chromium Carcinogenicity" for a discussion of the evidence for Cr VI carcinogenicity in animals and humans, including aspects of its MOA.

Comment 5-3: "The results of the research will be reported at the March 2011 Society of Toxicology Conference, and manuscripts for peer review and publications are expected to be complete by June 2011. By not considering the results of the Cr(VI) MOA Framework Research Program, OEHHA has taken a position that is inconsistent with its own mission and stated requirements to use the best available science in public health determinations."

Response 5-3. Health and Safety Code Section 116365.5 required the Department of Public Health to develop a primary drinking water standard for Cr VI by January 1, 2004. Health and Safety Code Section 116365 requires the development of a PHG by OEHHA before the department can adopt a primary drinking water standard. In light of this statutory mandate, it would be very difficult for OEHHA to justify further delay to finalizing the PHG in order to incorporate any appropriate findings from the Hamner Institutes research program.

Health and Safety Code Section 116365 contains an important provision that addresses new scientific research when it becomes available: "(e) (1) Public health goals established by the office shall be reviewed at least once every five years and revised, pursuant to the provisions of subdivision (c), as necessary based upon the availability of new scientific data". OEHHA acknowledges that new studies may alter a revised PHG.

From the risk characterization section of the 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.”

OEHHA will review papers and materials relating to the Hamner Institutes study when they are published. If the study produces compelling information that should be reflected in the PHG document, OEHHA will take appropriate action.

Comment 5-4: “The risk analyses conducted in the Draft Dec. 2010 PHG document assume that humans and mice are equally susceptible, even though mice and rats are not equally susceptible. The findings in NTP (2008) indicate the need for a careful deliberative consideration of MOA rather than simply choosing the most sensitive response upon which to base a toxicity criterion.”

Response 5-4. The PHG document does not assume that humans and mice are equally susceptible to ingested Cr VI. Choosing data from the most sensitive species for dose-response modeling when adequate human data are not available does not imply that the two species are equally sensitive. We assume that humans are at least as sensitive as the most sensitive species to be health protective. This conforms to standard risk assessment practice used by both the U.S. EPA (2005) and OEHHA (2009). Further, the inhalation risk estimates in humans versus the oral potency in animals indicates that Cr VI was approximately 1000 times more potent in humans by the inhalation route compared to the animal potency from the drinking water studies.

Comment 5-5: “Because the effects of ingested Cr(VI) observed in rats and mice in NTP (2008) are portal-of-entry effects, the use of allometric scaling (e.g., BW^0.25) is not an appropriate method for species extrapolation.”

Response 5-5. Given the inadequate toxicokinetic data for ingested Cr VI in mice and humans, allometric scaling has been judged to be an appropriate method for extrapolating dose between the two species (Stern, 2010; U.S. EPA, 2010; OEHHA, this PHG document).

Comment 5-6: “The Draft Dec. 2010 PHG document provides a lengthy discussion of reduction by saliva and gastric fluids and the effect of this reduction on absorption and subsequent tissue concentrations of chromium (pp. 9-12). However, OEHHA has not carefully examined tissue levels in the study used as the basis of OEHHA’s cancer slope factor. NTP (2008) provides tissue chromium concentrations in both male rats and female mice in the forestomach, glandular stomach and liver (Appendix J in NTP, 2008).”

Response 5-6. The chromium tissue levels from NTP (2008) are presented in Figures A3 to A6 and discussed in Appendix A of the PHG document.

Comment 5-7: "On page 72 of the Draft Dec. 2010 PHG document, OEHHA states that Cr(VI) is genotoxic both in vivo and in vitro. It would be more correct, however, to state that Cr(VI) is DNA-reactive both in vivo and in vitro."

Response 5-7. The "Genetic Toxicity" section of the PHG document discusses a number of studies where Cr VI was genotoxic following administration to animals (Table 2). The first paragraph of that section cites review articles that themselves cite a large number of studies showing that Cr VI is genotoxic in cultured mammalian cells and bacteria.

Comment 5-8: "The papers cited above show evidence of DNA-reactivity but not necessarily genotoxicity and definitely not mutagenicity. Neither DNA-reactivity nor genotoxicity can be equated with mutagenicity. As peer reviewer Prof. Toby Rossman comments on page 2 of his 2009 review, 'DNA damage per se does not inform us about eventual heritable change (i.e., a mutation), which is the true issue.' Prof. Rossman went on to say 'the description of an agent as 'genotoxic carcinogen' is out of date. What we really need to know is whether an agent has a mutagenic mode of action (MOA).' 'Genotoxicity' is not a specific finding, and the term 'DNA-reactivity' should be used instead. More importantly, OEHHA must make a determination that Cr(VI) has a mutagenic MOA to justify the use of linear extrapolation from the point of departure to zero."

Response 5-8. Genotoxicity has been and continues to be an important concept in toxicology and risk assessment. As illustrated in Table 2 of the PHG document, ingested Cr VI was genotoxic in short-term tests performed in vivo. As demonstrated by the review articles cited in the first paragraph of the "Genetic Toxicity" section of the PHG document, Cr VI was also mutagenic in bacteria, cultured mammalian cells, D. melanogaster and mice.

Comment 5-9: "Assuming that Cr(VI) acts by a mutagenic MOA ignores the existence of repair mechanisms, the production of reactive oxygen species (ROS) from reduction of Cr(VI), and resulting alterations in control of the cell cycle and apoptosis. Peer reviewer Prof. Elizabeth Snow in her 2009 comments remarks: "a low dose, linear response (based on mutagenicity) also assumes a lack of DNA repair and other protective mechanisms with an expected maximum protective effect at low dose (cf. comment #4 on p. 3)."

Response 5-9. A linear cancer response at low dose levels is consistent with DNA repair. Consider radiation induced carcinogenesis, the best data set we have covering cancer induction by low dose levels of any genotoxic carcinogen (radiation-induced cancer in human A-bomb survivors). The cancer incidence responds linearly at low doses of radiation despite the well-characterized ability of mammalian cells to repair potentially lethal DNA damage (PLD repair).

Comment 5-10: "It is clear the tumor development [in NTP (2008)] is related to local inflammation and hyperplasia in the target tissue…All of these features point to the conclusion that ingested doses of Cr+6 that are insufficient to produce local irritation,

tissue damage, inflammation and regenerative hyperplasia are also without additional carcinogenic risk."

Response 5-10. The non-neoplastic findings of NTP (2008) are presented in Table 7 of the PHG document. The discussion accompanying Table 7 stresses that tissue damage was not observed. Also, inflammation was not observed in the tissue where tumors developed. Thus, it is incorrect to conclude that the cellular changes listed in Comment 5-10 are required for tumor induction.

Comment 5-11: "The NTP drinking water studies provide strong evidence that epithelial proliferation is likely to be an early and necessary key event underlying Cr(VI)-induced carcinogenesis of the mouse small intestine (NTP, 2007, 2008). These bioassay results also provide evidence for the temporal sequencing of subsequent key events. If Cr(VI) were acting by a mutagenic MOA, the early hyperplasia, evident by 90 days, should result in a short time-to-tumor. However, the time-to-tumor formation was extended (>451 days), and treatment did not affect survival (i.e., animals were not dying as would be expected if tumors developed early in life) in the NTP drinking water study."

Response 5-11. Hyperplasia was not observed in the rat oral cavity (NTP, 2008) suggesting that a non-genotoxic MOA is unlikely. Given the propensity of some carcinogens to act as both initiators and promoters, we believe it is premature to conclude that the diffuse hyperplasia observed in the duodenum of mice in NTP (2008) is the initiating event in the MOA of Cr VI, rather than a promoting event for cells already initiated via a genotoxic alteration.

Comment 5-12: "The temporal progression of responses observed in the NTP bioassays indicates that histiocytic infiltration occurs in mice by 90 days; hyperplasia occurs in mice at both non-tumorigenic and tumorigenic doses by 90 days; and tumors occur at two years at doses above 1 mg/kg/d, corresponding to a concentration of 28.6 mg/L in drinking water (NTP, 2007, 2008). These data indicate a multi-step progression that is more consistent with the rarity of these tumors and their long latency. Hence, the implicit assumption of a mutagenic MOA is unfounded. Indeed, data from the same study upon which the draft PHG is based contradict this assumption. For these reasons, the choice of linear low dose extrapolation cannot be supported."

Response 5-12. The PHG document's section "Non-neoplastic findings – Possible relationship between tissue damage, inflammation, hyperplasia and tumors in rats and mice" presents the inconsistencies that accompany the hypothesis that histiocytic infiltration and hyperplasia are required for tumor induction by Cr VI. The finding that these two endpoints were observed at 90 days in some tissue is not sufficient for a determination that a threshold MOA is operative. OEHHA did not assume a genotoxic MOA. Rather, the data presented in the PHG are consistent with a genotoxic or mutagenic MOA and do not support an alternative MOA. See also Response 5-10 for a discussion of why inflammation and tissue damage are unlikely to be key events in tumor induction by Cr VI, and Response 5-11 for a discussion of why the observed hyperplasia is also not likely to be an initiating event.

Comment 5-13: "Had OEHHA considered MOA, or had MOA been the overarching principle of the Cr(VI) PHG risk assessment, as suggested in EPA's Cancer Guidelines (EPA, 2005a), then the idea of nonlinearity and the possibility of a threshold might have received proper consideration. OEHHA should fully discuss its rationale for choosing a linear approach over a non-linear approach by fully demonstrating both to just its choice."

Response 5-13. The MOA for tumor induction by ingested Cr VI is discussed in detail in the following sections of the PHG document: "Pharmacokinetics of Trivalent versus Hexavalent Chromium," "Mechanism of Genotoxicity and Carcinogenicity," Table 7 in the section entitled "Non-neoplastic findings – Possible relationship between tissue damage, inflammation, hyperplasia and tumors in rats and mice," "Examination of Evidence for Chromium Carcinogenicity," and "Risk Characterization." To more fully respond to Comment 5-13 we have added the following statement in two of these sections, "Therefore, an MOA other than that of genotoxicity or mutagenicity is not supported by these findings. The standard approach for carcinogens operating via a genotoxic or mutagenic MOA is to apply a linearized multistage model to calculate the cancer potency (U.S. EPA, 2005, 2010; OEHHA, 2009)."

Comment 5-14: "The default assumption of linearity has also been questioned in reviews of the Draft August 2009 PHG for Cr(VI)."

Response 5-14. OEHHA did not assume linearity. The linear approach was chosen based on the positive findings for genotoxicity by Cr VI and the inconsistent data in support of any alternative mechanism.

Comment 5-15: "In this regard, it is standard OEHHA practice to assume the animal data can be described by a linear dose-response relationship (LMS), but no data (other than reference to the results of standard short term tests for genotoxicity) to support that assumption were provided. As written, there is no a priori reason to accept the OEHHA assumption that Cr+6-induced tumors of the gastrointestinal tract in rodents can be described most accurately with a statistical model that is linear at low-dose."

Response 5-15. See Response 5-14 above.

Comment 5-16: "The Draft Dec. 2010 PHG document dismisses Bednar and Kies (1991) because the analysis was for total chromium and the sampling occurred for two years only. Nonetheless, Dr. Bjeldanes is correct that Bednar and Kies (1991) could provide a rough estimate of a no effect level in humans as a means to 'groundtruth' the PHG value."

Response 5-16. The PHG document discusses a number of limitations of the Bednar and Kies (1991) study including that sampling in the study occurred for only two years. Unlike the Zhang and Li (1987) study, no specific source of Cr VI exposure was

identified. Also, while overall cancer mortality rates were evaluated, tumors at specific sites were not.

Comment 5-17: "Prof. Elizabeth Snow comments on the use of the 2007 NTP data saying that 'a linear fit to the NTP data is the default protocol as defined by the U.S. EPA and OEHHA and that the data could equally well be fitted to a nonlinear, supralinear (concave) or 'hockey stick' response model (cf. p. 3).' She further states that 'based on this study (NTP, 2007), along with very limited evidence for tumor response at the lower levels of Cr6, there is very limited evidence for a linear dose response (cf. p. 3).'"

Response 5-17. This is the case with most carcinogens. Dose-response data are not available in the low dose region where human exposures are expected. Under these circumstances, where data support a genotoxic/mutagenic MOA but do not provide substantial support for an alternative MOA, use of a linear model is recommended by both U.S. EPA (2005) and OEHHA (2009).

Comment 5-18: "The 2009 peer review comments of Prof. Mitchell Cohen are even more explicit 'it is clear that the data presented in the Draft (PHG) document shows that the tumor formation in the mice (NTP data) as a function of Cr^{6+} [Cr(VI)] level in drinking water is not linear (cf. p. 6). Unfortunately, OEHHA decided to remove Figure 13 from the 2010 revised PHG. This figure would have allowed the reader to visualize the actual shape of the dose-response curves for both male and female mice in the NTP studies.'"

Response 5-18. The absence of statistically significant increases in tumors at the two lowest drinking water concentrations should not be interpreted as a threshold (i.e., nonlinear response) for tumorigenicity, since the number of animals may have been too low to detect tumors at the two lowest drinking water concentrations. The use of high doses in cancer bioassays is by design to offset the statistical limitations of using small numbers of animals (50 /sex/dose level) to measure a relatively rare event (tumors). The mouse tumor incidence data for NTP (2008) are presented in Tables 5 and 6 of the PHG document. Therefore, the Figure 13 cited in this comment was thought to be unnecessary and was not included in the final PHG document.

Comment 5-19: "One study that was not considered in the Draft August 2010 PHG document was Armienta-Hernandez and Rodriguez-Castillo (1995)."

Response 5-19. This study is not included in Table 8 of the PHG document because it is not an epidemiological study of cancer. However, a paragraph has been added to the PHG document about the 1995 study. Please see Response 6-1 below.

Comment 5-20: "OEHHA also did not consider the population of Hinkley, California, a small desert town in San Bernadino county."

Response 5-20. A paragraph describing the March 2011 report on rates of all cancers combined in Hinkley, California (Morgan 2011) along with the paper by Fryzek et al. (2001) are discussed in the "Toxicological Effects in Humans," subheading "Carcinogenicity" section of the PHG document.

Comment 5-21: "Consideration of the MOA of Cr(VI) tumorigenesis using mechanistic knowledge of H. pylori infection in humans would likely enable the understanding of why Armienta-Hernandez and Rodriguez-Castillo (1995) observed a NOEL orders of magnitude higher than the proposed PHG value."

Response 5-21. The cited study did not report data for cancers of the GI tract.

Comment 6-1. "One study that was not considered in the Draft August 2010 {sic} PHG document was Armienta-Hernandez and Rodriguez-Castillo (1995) … {in which} no adverse health effects, including cancer, were observed."

Response 6-1. Armienta-Hernandez and Rodriguez-Castillo (1995) was not an epidemiologic study of cancer. For example, the Methods section of the article contains no methods related to health effects. The study was helpful, however, with respect to the possibility of self-limited exposure at high concentrations. The investigators reported that residents near a chromate production facility did not want to consume water with Cr VI concentrations above 0.5 mg/L because of its yellowish color. OEHHA has added a paragraph to the PHG document about this study.

Comment 6-2. "OEHHA also did not consider the population of Hinkley, California… Dr. John Morgan examined cancer rates…The rate for all cancers was not elevated in Hinkley."

Response 6-2. OEHHA has added a paragraph to the PHG about the Morgan (2011) cancer incidence rate study. The paragraph notes that fewer cases of all types of cancer occurred (196) than were expected (224.2) and that results were not presented for specific types of cancer.

Comment 7-1: "With regard to inhalation exposure from showering, there is a mismatch between the exposures used to develop the inhalation slope factor and showering. The inhalation slope factor was derived for a chromate processing facility. A domestic shower with a temperature of 38°C is not a reasonable target of extrapolation from metal fumes generated at temperatures over 1000°C. This issue was also raised by Dr. David Berry in his comments on an early draft of the Draft August 2009 PHG document. OEHHA should respond to Dr. Berry's comments and provide the justification for its application of an industrial inhalation slope factor to residential exposures."

Response 7-1. Classification of Cr VI by OEHHA, U.S. EPA, ATSDR and IARC as an inhalation carcinogen does not specify nor differentiate between the possible airborne forms (particulate, mist or vapor). The inhalation cancer potency estimate is based on measured levels of chromium in air in the relevant studies. The OEHHA estimate of

potential exposure to Cr VI in air from domestic tap water was based on a study by Keating and McKone (1993) that showed that droplet production in showering was highly variable, depending on the shower head type; the shower head used by Paustenbach et al. (2003) was apparently one of the lower droplet-production types. This has no effect on the proposed PHG because the inhalation exposure from showering is so small (less than one percent of the total).

Comment 8-1: "In the very last paragraph on page 96 of the August 2009 draft PHG document, under the subheading 'Risk Characterization,' OEHHA states '[t]here are many sources of uncertainty in the calculation of the proposed PHG.' This statement is the only discussion of uncertainty. No further discussion of the sources of uncertainty and how they might impact the calculation of the PHG is provided. Risk managers cannot determine the level of uncertainty in the PHG. The sentence that follows the one quoted above reads: 'The NTP carcinogenicity studies provide robust data for the assessment of oral cancer risk attributed to Cr VI (cf. p. 96).' This is an accurate statement; yet, OEHHA deleted seven of the eight dose-response analyses it conducted using the NTP data set, the results of which were shown in Tables 10 and 11 of the Draft August 2009 PHG. In addition, OEHHA did not choose the most appropriate model based on EPA guidance (EPA, 2000, 2005a). OEHHA should fully document and provide the justification for deleting data and selecting a model that does not provide the best fit the data and violates the EPA guidance."

Response 8-1. As stated in the "Dose-Response Assessment," subheading "Carcinogenic Effects," subheading "Dose-Response Modeling" section of the PHG document, the multistage model was used to model the tumor incidence data because this is the model preferred by OEHHA (2009) and U.S. EPA (2010) for conducting cancer dose-response assessments unless the data suggest otherwise. This is primarily due to the multistage model's generally good fit of the data in the relatively high dose range used in rodent bioassays (Armitage and Doll, 1961). This is the reason the other seven models are no longer presented in the PHG document. Modeling the data according to eight different formulas is not an uncertainty analysis. It should be noted that recent EPA guidance, for situations in which different BMD models of adequate fit yield different BMDLs, is to choose the lowest BMDL, not necessarily the model that provides the best fit (Davis et al., 2010).

Comment 8-2: "There is no discussion of the uncertainty in water consumption rates or that these revised water consumption rates would likely produce an overly health-protective PHG value even if the ASFs were not used. In addition, the calculation of age-specific drinking water rates presented in the Jan. 25, 2011, corrections appear incorrect. OEHHA should clarify the rationale for this choice of drinking water rates and provide details of how they were calculated…The Draft Dec. 2010 PHG document does not make clear whether the water ingested consists of all water or tap water."

Response 8-2. The **per capita** drinking water values from Kahn and Stralka (2009) were mistakenly used in the previous draft PHG document. Instead, the **consumers only** drinking water values from the same publication have been used to revise the

PHG document. This resulted in the same final PHG value of 0.02 ppb (rounded). The protocol OEHHA followed to calculate the time-weighted mean drinking water ingestion rates using the data from Kahn and Stralka (2009) and U.S. EPA (2008) is now given in the "Calculation Of The PHG" section of the document. We have added to the "Calculation Of The PHG" section of the document that the water intake data cover pure water consumed as a beverage or used in the home or local establishments to prepare food or drink (Kahn and Stralka, 2009).

Comment 8-3: "Several peer reviewers commented on the absence of analyses of the data that would show a range of results that better reflect the uncertainty and inconsistency in the shape of the dose-response curves in rats and mice. OEHHA has not responded to these comments and should do so prior to making any final decision related to its draft PHG."

Response 8-3 (repeat of Response 3-4). The PHG document develops two health-protective concentrations for Cr VI in drinking water; one to protect against non-carcinogenic effects and one to protect against carcinogenic effects. The lower concentration is designated the PHG to be protective against all adverse health effects. OEHHA does not develop a range of PHG values. Such an approach was considered when the PHG program was established and judged not to be helpful, given that the purpose of the PHG is to provide guidance to the Department of Public Health in setting regulatory drinking water standards. Modeling the dose-response data using a variety of different models, as suggested in the comment, is not an uncertainty analysis. As stated in the "Dose-Response Assessment," subheading "Carcinogenic Effects," subheading "Dose-Response Modeling" section of the PHG document, the multistage model was used to model the tumor incidence data because this is the model preferred by OEHHA (2009) and U.S. EPA (2010) for conducting cancer dose-response assessments. This is primarily due to the multistage model's generally good fit of the data in the relatively high dose range used in rodent bioassays (Armitage and Doll, 1961).

Conclusions: All conclusions, except the one shown below, are recapitulations of points raised earlier.

Comment 10-2: "The documents lacks consideration of the ubiquitous and widespread presence of Cr(VI) in both groundwater and drinking water supplies. To date, no human cancers or other adverse health effects have been attributed to natural background levels of Cr(VI) in drinking water."

Response 10-2. It is doubtful that any study has ever attempted to measure the fraction of human cancers attributable to background levels of Cr VI, since this is probably logistically impossible.

Comments from Michael Rogge and Janet Kester, Ph.D, D.A.B.T., California Manufacturers & Technology Association

Transmittal Letter

Comment TL-1: "Because the vast majority of chrome 6 in groundwater in California is naturally occurring, adoption of the proposed PHG at 0.02 ppb will likely compel drinking water rate payers to fund the high costs of construction and operation of a new treatment technology and the purchase of expensive alternative drinking water supplies…However, when one considers the cumulative effect of the various PHGs recently adopted or proposed by OEHHA, including the proposed PHG for chrome 6, as well as a number of additional pending PHGs, it is reasonable to expect substantial additional future reductions in water allocations to agricultural operations, new residential and business development projects, and potentially, to future environmental restoration and management projects."

Response TL-1. The PHG is a non-regulatory guideline that the Department of Public Health (DPH) uses to set regulatory drinking water standards. DPH can consider the kinds of economic impacts cited by the commenter when setting drinking water standards. State law prohibits OEHHA from considering economic factors when developing PHGs.

Comment TL-2: "Numerous external scientific peer reviewers, including Cal/EPA's Department of Toxic Substances Control and members of the public, have criticized OEHHA for its failure to comprehensively evaluate the applicability of alternative MOAs as a basis for identification of the MOA most pertinent to test animals and its extrapolation to humans at relevant doses."

Response TL-2. The mechanism by which ingested Cr VI causes cancer in animals and humans is discussed in a number of places in the PHG document. The acronym MOA has been added to the document to help readers locate these discussions. The sections with concentrated discussions of mechanisms are: "Pharmacokinetics of Trivalent versus Hexavalent Chromium," "Mechanism of Genotoxicity and Carcinogenicity," Table 7 and the section entitled "Non-neoplastic findings – Possible relationship between tissue damage, inflammation, hyperplasia and tumors in rats and mice," "Examination of Evidence for Chromium Carcinogenicity," and "Risk Characterization." The DTSC comments were contained in an internal memorandum, not a formal scientific peer review.

Comment TL-3: "This issue is the subject of the Hexavalent Chromium Mode of Action Research Project (the Research Project), a multi-year research project being undertaken by a select group of scientists with substantial expertise in risk assessment, toxicology and other appropriate scientific specialties…The overall goal of the Research Project is to provide critical information to address gaps inherent in the scientific database used to support the assessment of human health risks posed by oral exposures to chrome 6. Although highly germane to the proposed PHG for chrome 6 in California, OEHHA's current schedule for adoption of a final PHG does not appear to allow the time necessary for consideration of the imminent release of the scientific data that will be generated in the MOA study noted above."

Response TL-3. Health and Safety Code Section 116365.5 required the Department of Public Health to develop a primary drinking water standard for Cr VI by January 1, 2004. Health and Safety Code Section 116365 requires the development of a PHG by OEHHA before the department can adopt a primary drinking water standard. In light of this statutory mandate, it would be very difficult for OEHHA to justify further delay to finalizing the PHG in order to incorporate any appropriate findings from the Hamner Institutes research program.

Health and Safety Code Section 116365 contains an important provision that addresses new scientific research when it becomes available: "(e) (1) Public health goals established by the office shall be reviewed at least once every five years and revised, pursuant to the provisions of subdivision (c), as necessary based upon the availability of new scientific data". OEHHA acknowledges that new studies may alter a revised PHG. From the risk characterization section of the 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."

OEHHA will review papers and materials relating to the Hamner Institutes study when they are published. If the study produces compelling information that should be reflected in the PHG document, OEHHA will take appropriate action.

Specific Comments

Comment 1: "In particular, OEHHA should provide a detailed evaluation of the oral carcinogenic MOA for Cr(VI) and human relevance of the National Toxicology Program (NTP) two-year bioassay data (NTP 2008) used as the basis for oral cancer potency factor development."

Response 1. The mechanism by which ingested Cr VI causes cancer in animals and humans is discussed in a number of places in the PHG document. The acronym MOA has been added to the document to help readers locate these discussions. The sections with concentrated discussions of mechanisms are: "Pharmacokinetics of Trivalent versus Hexavalent Chromium," "Mechanism of Genotoxicity and Carcinogenicity," Table 7 and the section entitled "Non-neoplastic findings – Possible relationship between tissue damage, inflammation, hyperplasia and tumors in rats and mice," "Examination of Evidence for Chromium Carcinogenicity," and "Risk Characterization."

Comment 2: "A Cr(VI) MOA Research Project designed in accordance with current EPA guidance to elucidate critical questions and data gaps inherent in the existing data base concerning the nature and sequence of key events in oral Cr(VI) carcinogenesis is currently underway…OEHHA should use the soon-to-be published results of the Cr(VI) MOA Research Project to fill data gaps in the MOA and inform extrapolation across doses and species using refined physiologically-based pharmacokinetic (PBPK) models for mice, rats, and humans."

Response 2. OEHHA will review papers and material relating to this study when they are published. If the study produces compelling information that should be reflected in the PHG document, OEHHA will take appropriate action.

Comment 3: "Using BMD analysis of the same NTP data set (chronic inflammation of the liver in female rats), the Agency for Toxic Substances and Disease Control calculated a Minimal Risk Level for Cr(VI) (0.001 mg/kg-day) that is five times higher than the Health Protective Dose (HPD) developed by OEHHA for the non-cancer PHG (0.0002 mg/kg-day) (ATSDR 2008)."

Response 3. At present the Cr VI PHG based on cancer effects is 100-fold lower than if it were based on non-cancer effects (see "Calculation Of The PHG" section of the document). OEHHA will be applying the BMD approach in future analyses of the non-cancer data. Our preliminary analysis applying the BMD approach to the non-cancer data followed by an uncertainty factor of 100 yields a final value that is more than 100-fold ***higher*** than the proposed PHG based on cancer effects. Thus, the proposed PHG (0.02 ppb) for protecting against both cancer and non-cancer effects would not change.

Comment 4: "Further, the derivation of the non-cancer PHG did not adequately consider questions, highlighted by the NTP, about the biological significance of non-neoplastic liver effects at low doses, particularly in light of the high background levels of these effects in control animals, and potential gender and species differences in Cr(VI) pharmacokinetics and pharmacodynamics suggested by the NTP (2008) study results."

Response 4. The PHG document now contains additional discussion of the liver changes observed in female rats in NTP (2008). See section "Toxicological Effects in Animals," subheading "Chronic Toxicity," subheading NTP, 2008. It is true that liver changes were observed in treated females and in aged, control animals. However, when a concurrent control population is available, as was the case here, OEHHA would not discount an effect that was significant relative to the concurrent control, even if the values fell within the historical control range.

Comment 5: "The literature review performed by OEHHA is incomplete, and in some cases misquotes or misrepresents the results of key studies. Of particular concern is the discussion of epidemiological evidence for cancer of the gastrointestinal (GI) tract."

Response 5. OEHHA has attempted to include all studies in the PHG document containing data that are important for development of the PHG for Cr VI. Additional epidemiological studies have been added since the December 2010 draft PHG document. Misquotes and misrepresentations should be specified so that they can be corrected.

Comment 6: "OEHHA should include a quantitative uncertainty analysis along with an expanded qualitative uncertainty analysis. This should include OEHHA's rationale for making specific science policy choices, including the scientific support for alternatives, and evaluation of their quantitative impact on the PHG calculation."

Response 6. OEHHA is not aware of an established methodology for quantifying the uncertainty associated with cancer risk extrapolation.

Comment 7: "95th percentile drinking water intake rates are overly conservative and insufficiently documented. These intake rates are based on self-reported rather than measured body weights (Kahn and Stralka 2009), and the sample sizes for young

infants, who have the highest estimated daily water intake rates of all age groups, did not meet minimum reporting requirements, rendering the 95th percentile artificially high. Moreover, the intake rates for infancy and childhood age groups (0.114 and 0.041 L/kg-day) could not be verified based on the references provided. Based on these shortcomings, OEHHA should replace the water consumption values used in the 2010 draft with more appropriate (and transparently derived) values."

Response 7. The methodology for calculating water consumption rates using the data from U.S. EPA (2008) and Kahn and Stralka (2009) is now presented in the footnote to Table 17 and in the discussion of Table 18 in the PHG document. In the previous draft of the PHG document the **per capita** water consumption rates were mistakenly used. The revised PHG document uses the **consumers only** rates. The final PHG value of 0.02 ppb (rounded) was unaffected. The drinking water consumption rates reported by Kahn and Stralka (2009) are the same values recommended by U.S. EPA (2008) for use in human health risk assessments. They came out of the largest survey of its kind: the United States Department of Agriculture's (USDA's) 1994-1996 and 1998 Continuing Survey of Food Intake by Individuals (CSFII). With regard to the 95th percentile drinking water rate being overly conservative, OEHHA has traditionally sought to protect this large fraction of the at risk population.

Comment 8: "Application of generic sensitivity factors (ASFs) is inappropriate for Cr(VI) and insufficiently documented. Whereas EPA has determined that children may be more susceptible than adults to carcinogens known to act via a mutagenic MOA (EPA 2005b), OEHHA's new policy will be applied to all carcinogens, regardless of the theorized MOA (OEHHA 2009c)."

Response 8. The Age Sensitivity Factor (ASF) for modifying cancer potency was used as described (OEHHA, 2009). This approach applies to all carcinogens, regardless of purported mechanism of action, unless chemical-specific data exist that could be used to make more specific adjustments to risk. Such chemical-specific data are not available for Cr VI.

Comment 9: "Regardless of MOA, there is no basis for applying ASFs in the particular case of oral exposure to Cr(VI), because it causes tumors only at the portal of entry at extremely high doses, and as noted by OEHHA, 'little…would be expected to get to the conceptus because of all the reduction in the intervening maternal organs' (OEHHA 2010, page 128)."

Response 9. First, application of an ASF to infants and children is not counter-indicated by a "portal of entry" MOA (OEHHA, 2009). Second, chromium accumulation in a number of tissues indicates that the hexavalent form enters the body and becomes distributed systemically (see "Distribution" section and Appendix A of the PHG document). Thus, it is premature to conclude that Cr VI does not reach the conceptus. The quoted text from Appendix B of the PHG document has been revised accordingly.

Comment 10: "The multifaceted Cr(VI) MOA Research Project is designed to directly address critical questions and data gaps concerning the MOA of Cr(VI) administered via drinking water…Considering the imminent availability and direct relevance of Cr(VI) MOA Research Project studies for elucidating the carcinogenic MOA of orally administered Cr(VI), CMTA emphatically reiterates the opinion, also expressed by

DTSC in its review of a previous draft, that OEHHA should suspend finalization of the Cr(VI) PHGs for both carcinogenic and non-carcinogenic effects until it has thoroughly reviewed these data and incorporated them into its quantitative analyses."

Response 10. OEHHA acknowledges that new research is on-going and looks forward to the new data when available for consideration. The Safe Drinking Water Act of 1996, amended 1999 (Health and Safety Code [H&SC], Section 116365) contains an important provision that addresses new scientific research when it becomes available: "(e) (1) Public health goals established by the office shall be reviewed at least once every five years and revised, pursuant to the provisions of subdivision (c), as necessary based upon the availability of new scientific data". When new research data become available, OEHHA will consider them in the development of a revised PHG for hexavalent chromium. OEHHA acknowledges that new studies may alter a revised PHG. From the risk characterization section of the 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."

Health and Safety Code Section 116365.5 required the Department of Public Health to develop a primary drinking water standard for Cr VI by January 1, 2004. Health and Safety Code Section 116365 requires the development of a PHG by OEHHA before the department can adopt a primary drinking water standard. In light of this statutory mandate, it would be very difficult for OEHHA to justify further delay to finalizing the PHG in order to incorporate any appropriate findings from the Hamner Institutes research program.

Comment 11: "EPA currently uses a body weight-normalized drinking water intake rate of 0.032 L/kg-day for calculating Regional Screening Levels for tap water based on carcinogenic effects, assuming residential exposure from birth through age 30 (EPA 2010). This value corresponds to approximately the 88th percentile of 'all ages' intake by the U.S. population. In the 2010 draft PHG, OEHHA departed from this current regulatory practice by using 95th percentile age-specific body weight-normalized drinking water intake rates for (1) the third trimester of pregnancy, (2) infancy (0-2 years), childhood (2-16 years), and adulthood (16-70 years), based on an unpublished 2010 OEHHA guidance document…The values used by OEHHA are therefore not only overly conservative (as a result of using the 95th percentile versus the already conservative 85th – 88th percentiles), but their validity is also questionable. Based on these shortcomings, the water consumption values used in the 2010 draft should be replaced with more appropriate (and transparently derived) values."

Response 11 (repeat of Response 7). The methodology for calculating water consumption rates using the data from U.S. EPA (2008) and Kahn and Stralka (2009) is now presented in the footnote to Table 17 and in the discussion of Table 18 in the PHG document. In the Announcement of Non-substantive Reference Change for the December 31, 2010 Draft Chromium VI PHG Document the **per capita** water consumption rates were mistakenly used. The final PHG document uses the **consumers only** rates. The final PHG value of 0.02 ppb (rounded) was unaffected. The drinking water consumption rates (Kahn and Stralka, 2009) utilized in the PHG

document are the same values recommended by U.S. EPA (2008) for use in human health risk assessments. They came out of the largest survey of its kind: the United States Department of Agriculture's (USDA's) 1994-1996 and 1998 Continuing Survey of Food Intake by Individuals (CSFII). With regard to the 95th percentile drinking water rate being overly conservative, OEHHA has traditionally sought to protect this large fraction of the at risk population.

Comment 12: "In contrast with EPA guidance, OEHHA's new policy '…will be applied to all carcinogens, regardless of the theorized mode of action' (OEHHA 2009, page 51). Also unlike EPA, OEHHA included the third trimester of pregnancy as a 10-fold more sensitive life stage. This significant deviation from current EPA guidance and policy, and the significant extrapolation beyond the existing database that it constitutes, warrants careful examination by the scientific community. Although OEHHA's document provides general information about the methodology used, detail is insufficient to allow thorough review. None of the studies examined involved Cr(VI), nor did they include carcinogenesis occurring at the portal of entry."

Response 12. Both OEHHA and U.S. EPA have found it to be scientifically necessary to apply age susceptibility factors (ASFs) to account for potential early-in-life increased susceptibility to Cr VI (OEHHA, "Correction for Early-in-Life Exposures" section of this PHG document; U.S. EPA, 2010). It is correct that the PHG document for Cr VI, unlike U.S. EPA (2010), includes a ten-fold ASF for calculating the fraction of the lifetime cancer risk due to exposure to Cr VI during the third trimester. The detailed rationale for correcting for exposure during the third trimester is presented in OEHHA (2008), available as Appendix J online at http://www.oehha.ca.gov/air/hot_spots/tsd052909.html Note that in the final PHG document, exposure to Cr VI during the third trimester contributed only one percent of the total lifetime risk of cancer due to Cr VI in drinking water. Thus, if the third trimester were excluded from the lifetime cancer calculation, the final PHG value of 0.02 ppb (rounded) would not change. It is also correct that Cr VI was not one of the carcinogens analyzed for age-related increased susceptibility (OEHHA, 2008). The reason was the insufficiency of the database for Cr VI. Lastly, a "portal of entry" mode of action does not preclude increased susceptibility in infants and children.

Comment 13: "It is especially noteworthy that OEHHA's application of ASFs to Cr(VI) is not supported by the only relevant data currently available, the Borneff et al. (1968) multigenerational study, and conflicts with its own discussion of this issue in Appendix B of the 2010 draft PHG document (OEHHA 2010, page 128):

'The Borneff study used a multigenerational protocol, which resulted in two generations exposed in utero and during weaning (F1 and F2) and one generation that was not (F0)…For Cr VI, 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 Cr VI would be expected to get to the conceptus because of all the reduction in the intervening maternal organs.'"

Response 13. Chromium accumulation in a number of tissues indicates that the hexavalent form enters the body and becomes distributed systemically (see "Distribution" section and Appendix A of the PHG document). Thus, it is premature to

conclude that Cr VI does not reach the conceptus. The quoted text from Appendix B of the PHG document has been revised accordingly.

Comment 14: "OEHHA's new policy of applying ASFs to all carcinogens regardless of MOA should be thoroughly explicated and peer-reviewed before it is used in risk assessments. Because OEHHA has not identified a mutagenic (or any) MOA for Cr(VI), its application of ASFs to Cr(VI) is inconsistent with current EPA guidance. Regardless of MOA, there is no basis for applying ASFs in the particular case of oral exposure to Cr(VI), because it causes tumors only at the portal of entry at extremely high doses, and "little… would be expected to get to the conceptus because of all the reduction in the intervening maternal organs" (OEHHA 2010)."

Response 14. The Age Sensitivity Factor (ASF) for modifying cancer potency was used as described (OEHHA, 2009). This approach applies to all carcinogens, regardless of purported mechanism of action, unless chemical-specific data exist that could be used to make more specific adjustments to risk. Such chemical-specific data are not available for Cr VI. Application of an ASF to infants and children is not counter-indicated by a "portal of entry" MOA (OEHHA, 2009). In addition, chromium accumulation in a number of tissues indicates that the hexavalent form enters the body and becomes distributed systemically (see "Distribution" section and Appendix A of the PHG document). Thus, it is premature to conclude that Cr VI does not reach the conceptus. The text that was quoted in Comment 13 above has been revised accordingly.

Both OEHHA and U.S. EPA have found it to be scientifically necessary to apply age susceptibility factors (ASFs) to account for potential early-in-life increased susceptibility to Cr VI (OEHHA, "Correction for Early-in-Life Exposures" section of this PHG document; U.S. EPA, 2010). It is correct that the PHG document for Cr VI, unlike U.S. EPA (2010), includes a ten-fold ASF for calculating the fraction of the lifetime cancer risk due to exposure to Cr VI during the third trimester. The detailed rationale for correcting for exposure during the third trimester is presented in OEHHA (2008), available as Appendix J online at http://www.oehha.ca.gov/air/hot_spots/tsd052909.html Note that in the final PHG document, exposure to Cr VI during the third trimester contributed only one percent of the total lifetime risk of cancer due to Cr VI in drinking water. Thus, if the third trimester were excluded from the lifetime cancer calculation, the final PHG value of 0.02 ppb (rounded) would not change. It is also correct that Cr VI was not one of the carcinogens analyzed for age-related increased susceptibility (OEHHA, 2008). The reason was the insufficiency of the database for Cr VI.

Comment 15: "In view of the inherent shortcomings of the NTP two-year bioassay protocol, it must be recognized that "clear evidence of carcinogenicity" from long-term exposure to extremely high concentrations of Cr(VI) does not constitute proof that humans exposed to much lower concentrations are at increased risk."

Response 15. Two-year bioassays in rodents, traditionally performed at high dose levels, have been used for many years to estimate cancer risks to humans. The scientific justification for this has been discussed previously (U.S. EPA, 2005; OEHHA, 2009).

Comment 16: "As mentioned previously, OEHHA did not provide coherent evaluations of (1) animal MOA, and (2) human relevance to support its selection of an LNT low-dose extrapolation method in the 2009 or 2010 drafts, notwithstanding extensive criticism of previous drafts by DTSC, peer reviewers, and members of the public. Indeed, the term "mode of action" does not appear anywhere in the text, and EPA's 2005 Carcinogen Risk Assessment Guidance was not cited in the context of MOA, although OEHHA purportedly adhered to this guidance."

Response 16. Discussion has been added to the following sections of the PHG document concerning the MOA data that support the use of a low dose linear extrapolation to calculate cancer potency: "Mechanism of Genotoxicity and Carcinogenicity" (large number of studies demonstrating a genotoxic, and possibly mutagenic, MOA for Cr VI); "Toxicological Effects in Animals," subheading "Carcinogenicity," subheading "Non-neoplastic findings – Possible relationships between tissue damage, inflammation, hyperplasia and tumors in rats and mice" (that the tissue and cellular findings shown in Table 7 do not support an alternative MOA for tumor induction by Cr VI). The acronym MOA has been added to the document to help readers locate these discussions. The sections with concentrated discussions of mechanisms are: "Pharmacokinetics of Trivalent versus Hexavalent Chromium," "Mechanism of Genotoxicity and Carcinogenicity," Table 7 and the section entitled "Non-neoplastic findings – Possible relationship between tissue damage, inflammation, hyperplasia and tumors in rats and mice," "Examination of Evidence for Chromium Carcinogenicity," and "Risk Characterization."

Comment 17: "Dr. Bjeldanes commented,

'The proposed PHG for Cr(VI), which is fully six orders of magnitude lower than the active concentrations in mice , is well below current safety standards, appears to be lower than levels in uncontaminated waters, is near the limits of detection with currently available analytical methods, and apparently does not consider the likelihood of a threshold for Cr(VI) biological activity, requires further justification.'

OEHHA's response to Dr. Bjeldanes was, 'for this risk assessment, OEHHA has followed the most recent carcinogen guidelines of the U.S. EPA (2005) and OEHHA's own principles (OEHHA, 2005). Basically, if there is evidence that an agent acts through a genotoxic mechanism (as there is for Cr VI), no threshold for effect is assumed' (OEHHA 2009b, page 9). This interpretation of current scientific thought and EPA and international guidance is clearly out of date and incorrect."

Response 17. The OEHHA quotation cited here by the CMTA is incomplete. OEHHA's response to Dr. Bjeldanes went on two sentences later to say, 'An inability to absorb Cr VI could be considered a pharmacokinetic threshold (independent of genotoxicity considerations). However, all the available pharmacokinetic studies indicate that a portion of the Cr VI is orally absorbed, at the doses studied, with results far too variable to indicate or estimate a threshold.' Dr. Bjeldanes' comment, along with those of other reviewers, has prompted OEHHA to expand its discussion of MOA in the final PHG document. The PHG document now emphasizes that the two primary considerations driving the decision to perform a linear low-dose extrapolation are the genotoxicity of Cr VI and insufficient support for an alternative threshold MOA. Text covering these

issues has been added at two places in the PHG document: “Mechanism of Genotoxicity and Carcinogenicity” section and “Toxicological Effects in Animals,” subheading “Carcinogenicity,” subheading “Non-neoplastic findings – Possible relationships between tissue damage, inflammation, hyperplasia and tumors in rats and mice.”

Comment 18: “The fallacy of uncritically assuming that positive tests in genotoxicity tests necessarily imply a mutagenic MOA is evidenced by (1) the high incidence of positive results in genotoxicity testing with many common chemicals (including sugar and salt) that do not appear to pose a carcinogenic risk under conceivable human exposure conditions (e.g., Dearfield and Moore, 2005; Pottenger et al., 2007); and (2) the now well-established fact that cancer is the end result of a multi-step process by which a normal cell is transformed into a cancerous one exhibiting the six “hallmarks” of cancer (Hanahan and Weinberg, 2000)…”

Response 18. Data presented or cited in the PHG document demonstrate that Cr VI is both genotoxic and mutagenic. There is no assumption of a mutagenic MOA.

Comment 19: “The only peer reviewer who critically addressed the charge question regarding MOA was Dr. Toby Rossman, who stated,…’These events generally show thresholds.’”

Response 19. See Responses to Dr. Rossman’s comments.

Comment 20: “OEHHA should use the soon-to-be published results of the Cr(VI) MOA Research Project to support a robust evaluation of carcinogenic MOA in animals, and use the refined PBPK model to inform extrapolation across doses and species for development of Cr(VI) PHGs for both carcinogenic and non-carcinogenic effects.”

Response 20. OEHHA will review papers and materials relating to the Hamner Institutes study when they are published. If the study produces compelling information that should be reflected in the PHG document, OEHHA will take appropriate action.

Comment 21: “Yet, as addressed in detail in previous comments, OEHHA’s conclusion that reductive capacity is exceeded was based primarily on high-dose studies involving non-oral routes of exposure that are not relevant to potential human exposure conditions.”

Response 21. The “Metabolism and Pharmacokinetics” section of the PHG document contains extensive discussion of Cr VI reduction to Cr III. The general conclusion is that the reductive capacity of the GI tract of rodents and humans was rarely if ever exceeded.

Comment 22: “As discussed by Thompson et al. (2011) and illustrated in Figure 2 taken from that publication, depicting toxicokinetic data collected by the NTP (2007), gastrointestinal reduction of Cr(VI) undergoes a transition in mice at concentrations above 3 to 10 mg/L in drinking water. Such data clearly indicate that (1) a dispositional threshold exists for systemic Cr(VI) uptake, and (2) even the lowest concentration of Cr(VI) in the NTP bioassay probably exceeded the animals’ gastrointestinal reductive capacity, resulting in systemic uptake and increased chromium concentrations in liver and kidney.”

Response 22. With regard to (1), the methodology used in NTP (2007) to measure chromium accumulation in tissue and blood may have lacked the sensitivity to measure the small increases that may occur at the lower drinking water concentrations. This same reservation applies to the data in Sutherland et al. (2000). Note that ingestion studies with radioactive Cr VI have reported absorption at dose levels below those shown in Figure 2 from Thompson et al. (2011). With regard to (2), Collins et al. (2010) analyzed chromium accumulation in tissue of mice and rats given drinking water containing from 5 to 180 mg/L of Cr VI for two years. These data were collected during the NTP bioassay, performed at the same drinking water concentrations tested for tumor induction. The chromium accumulation data indicated that the gastric reduction capacity was not saturated in these animals. Issue (1) is discussed in the "Metabolism and Pharmacokinetics" section of the PHG document while issue (2) is discussed in Appendix A of the PHG document.

Comment 23: "The target tissues in the NTP bioassay demonstrated a readily apparent dose-response gradient (duodenum>jejunum>ileum), both anatomical and temporal. Dose-related increases in lesions associated with tissue damage (degeneration, edema, inflammation, hemorrhage, erosion, ulceration, infiltration, and hyperplasia), observed after 90 days of treatment, occurred along this gradient. These observations are consistent with tumorigenesis secondary to cellular injury, oxidative stress, inflammation, and necrosis due to direct contact of Cr(VI) with the small intestine epithelium, followed by cell regeneration and inhibition of apoptosis."

Response 23. The PHG document contains a section entitled "Non-neoplastic findings – Possible relationships between tissue damage, inflammation, hyperplasia and tumors in rats and mice." This section presents data from the two-year bioassay (NTP, 2008) in Table 7 of the PHG document. This section also discusses data from the 90 day study by NTP (2007). These data do ***not*** support an MOA of tumorigenesis secondary to tissue damage.

Comment 24: "OEHHA's approach to developing Cr(VI) PHGs for carcinogenic and non-carcinogenic effects does not comport with current EPA and international guidelines for human cancer risk assessment."

Response 24. The PHG document develops a ***single*** PHG value that is protective of both carcinogenic and non-carcinogenic effects. The PHG document follows U.S. EPA (2005) risk assessment guidelines and OEHHA guidelines (2009). Both entities utilized linear extrapolation to develop essentially identical cancer slope factors for oral exposure to Cr VI (U.S. EPA, 2010; final PHG for Cr VI in drinking water). It is not clear what "international guidelines" are being referenced here.

Comment 25: "Based on overly conservative and insufficiently documented exposure and toxicity assumptions, and lacking coherent evaluations of (1) animal MOA, and (2) human relevance, both the 2009 and 2010 draft PHGs are fatally flawed. The weight of experimental and epidemiological evidence and exercise of best risk assessment practices under current regulatory guidance support development of a health-protective PHG that is orders of magnitude higher. "

Response 25. OEHHA disagrees with this comment for a number of reasons:

- The human relevance of exposure to Cr VI in drinking water is demonstrated by the only two epidemiology studies to measure organ-specific cancer in exposed human populations: Zhang and Li (1987) and Linos et al. (2011). See the section in the final PHG document entitled "Toxicological Effects in Humans" for data demonstrating a statistically significant increase in stomach cancer (Zhang and Li, 1987) and liver cancer (Linos et al., 2011) in exposed populations.
- Note also that the Peer Reviewers of the August 2009 draft PHG document were generally supportive of OEHHA's approach. See the General Comments and Responses provided at the beginning of each 2009 Peer Reviewer's Comments in this document (see Table of Contents).
- U.S. EPA (2010) and the New Jersey Department of Environmental Protection (2009) took similar approaches to that taken by OEHHA in developing essentially identical cancer slope factors for Cr VI in drinking water. The commonality of the approach suggests it is not fatally flawed.
- The mechanism by which ingested Cr VI causes cancer in animals and humans is discussed in a number of places in the PHG document. The acronym MOA has been added to the document to help readers locate these discussions. The sections with concentrated discussions of mechanisms are: "Pharmacokinetics of Trivalent versus Hexavalent Chromium," "Mechanism of Genotoxicity and Carcinogenicity," Table 7 and the section entitled "Non-neoplastic findings – Possible relationship between tissue damage, inflammation, hyperplasia and tumors in rats and mice," "Examination of Evidence for Chromium Carcinogenicity," and "Risk Characterization."

Comments from Timothy Quinn, Association of California Water Agencies

Comment 1: "ACWA understands other studies exist and are referenced in the document providing evidence that complete reduction may not always occur, but we believe the administered doses in the NTP study are so large they easily overwhelmed the reductive capacity of both the oral cavity and the stomach in the rodents. This is especially significant as the NTP study did not find excess cancers at the lowered studied doses in both rats and mice."

Response 1. See Appendix A in the PHG document for a discussion of the data showing that the Cr VI reducing capacity of the rodent GI tract was not saturated over the dose range tested in the NTP (2008) two-year study. The absence of excess tumors at the lower dose levels may have been due to the use of a small number of animals to detect a relatively rare event (tumor formation).

Comment 2: "Equally as important, the stomach composition of humans and rodents is very different, with humans having a much more sophisticated and higher level of gastric juices than rodents."

Response 2. Cr VI is reduced to Cr III in both the rodent and human stomach. This is discussed in detail in the PHG document in the sections "Hexavalent Chromium Reduction by Saliva and Gastric Fluids", "Absorption" and "Pharmacokinetics of

Trivalent versus Hexavalent Chromium." See also Appendix A. While Cr VI reduction in the GI tract of rodents compared to humans has not been fully described, the U.S. EPA (2010), the New Jersey Department of Environmental Protection (NJDEP, 2009) and OEHHA (this PHG) have all found that they are similar enough to allow calculation of a human cancer slope factor for Cr VI based on the NTP two-year bioassay.

Comment 3: "The Borneff et al study is seriously flawed due to the fact there was only a single-dose level examined and an ectromelia epidemic affected both control and treated groups with significant loss of mice. ACWA still feels this study should not be considered in the development of the PHG."

Response 4. The Borneff et al. (1968) study is not a key study in the derivation of the PHG for Chromium VI and was moved to the Appendix for that reason. OEHHA provided extensive analysis of its findings in its endeavor to consider all available scientific data when evaluating chemicals and developing PHGs aimed at protecting public health. The weight of evidence approach taken by OEHHA necessitated a discussion of Borneff et al. (1968).

Comment 5: "The organs exposed to the largest concentrations of chromium and that were in most immediate contact with the chromium were the forestomach, glandular stomach, serum, and red blood cells. In examining the results provided it is clear that after a year of exposure to 5 mg/L of hexavalent chromium, none of the mice or rats showed any higher concentrations of chromium in these four tissues than did the mice or rats in the control population exposed to no hexavalent chromium. Further, none of the rodents exposed to 5 mg/L hexavalent chromium for two years in the histopathology showed any excess cancers. The NTP data supports the well-established observation that the reductive capacity of the mammalian stomach can convert hexavalent chromium to the non-toxic reduced chromium at even very high concentrations."

Response 5. Tables 2 and 3 from Collins et al. (2010) show that statistically significant increases in chromium occurred in a number of tissues from both mice and rats receiving 5 mg/L of Cr VI in their drinking water during the two-year bioassay. The absence of excess tumors at 5 mg/L Cr VI may have been due to the use of a small number of animals to detect a relatively rare event (tumor formation).

Comment 6: "In addition, we believe this point would be made clearer if the public had access to the results of the full study…The complete set of 10 results per organ would have been very helpful to ACWA in its effort to assess OEHHA's draft PHG document."

Response 6. These data have been published in a peer-reviewed scientific journal by Collins et al. (2010). Our understanding from reading this paper is that while up to ten animals per exposure group were put into individual metabolism cages for collection of urine and feces, measurements of tissue chromium were performed on three animals per exposure group. Those are the values presented in Tables 2 and 3 of that paper and in Tables J1 and J2 of the original NTP study report.

Comment 7: "Dr Cohen states, 'It is clear the data presented in the Draft document (c.f. Figure 13; Editorial note: abscissa needs the addition of units as the values shown do not correspond to any of the reported doses in Tables 5 and 6) shows that tumor formation in the mice as a function of Cr6+ level in drinking water is not linear.'"

Response 7. In the 2008 NTP study statistically significant increases in tumors of the small intestine were observed for both male and female mice at the two highest drinking water concentrations. Exact trend tests were positive for both sexes. The absence of statistically significant increases in tumors at the two lowest drinking water concentrations should not be interpreted as a threshold for tumorigenicity (i.e., should not be construed as a nonlinear dose-response curve), since the number of animals may have been too low to detect tumors at the two lowest drinking water concentrations.

Comment 8: "Dr. Rossman provides several reasons objecting to the use of a linear dose response model for the draft PHG and supporting his statement, 'The assumption is that Cr(VI) in drinking water has a mutagenic MOA with no threshold. This is not valid for the following reasons.'"

Response 8. OEHHA does not know the mechanism by which Cr VI causes cancer in humans or animals. It is both genotoxic and mutagenic as described in the PHG document. Since there are insufficient data to support an MOA other than that via genotoxicity/mutagenicity, the PHG document models the tumor data according to a linear multistage model as recommended (U.S. EPA, 2005; OEHHA, 2009; McCarroll et al., 2010).

Comment 9: "Dr. Snow states, "Based on this study, along with very limited evidence of tumor response at lower levels of Cr6, there is very limited evidence for a linear dose response. It is more likely, due to the high probability of extracellular conversion of the Cr6 to the much less toxic Cr3, that uptake and bioavailability of the Cr6, in itself, will exhibit a non-linear (threshold) dose response."

Response 9. It is the case with most carcinogens that dose-response data are not available in the low dose region where human exposures are expected. With regard to a high probability that extracellular Cr VI will be converted to Cr III, this may or may not be true. The PHG document contains examples of Cr VI absorption at dose levels that are far below the calculated capacity of the GI tract of humans and rodents to reduce all ingested Cr VI to Cr III. The PHG document also discusses examples where Cr VI absorption was not concentration dependent.

Comment 10: "By using a default linear dose response model, when the data supports a non-linear dose response, OEHHA is justifying an overly conservative PHG based on an assumption that represents the most critical driver for the PHG calculation."

Response 10. The linear dose response model was chosen for two reasons. First, Cr VI is genotoxic and mutagenic (see "Genetic Toxicity" section of the PHG document). Second, there are insufficient data to support a threshold MOA in which tissue damage (or other type of cellular effect) is the primary carcinogenic event (see "Toxicological Effects in Animals," subheading "Carcinogenicity," subheading "Non-neoplastic findings – Possible relationship between tissue damage, inflammation, hyperplasia and tumors in rats and mice" section of the PHG document).

Comment 11: "We are aware of some significant new studies addressing the health effects of hexavalent chromium…ACWA urges OEHHA to follow the progress of this work and consider the results of this study and others that might emerge as soon as

they are completed in order to ensure the subsequent hexavalent chromium MCL is based on the best available science."

Response 11. OEHHA will review papers and materials relating to the Hamner Institutes study when they are published. If the study produces compelling information that should be reflected in the PHG document, OEHHA will take appropriate action.

Comments from David Chang, California-Nevada Section, American Water Works Association

Comment 1: "In the calculation of the PHG for hexavalent chromium, an aggregate uncertainty factor of 3000 is applied, the maximum recommended by the California's Risk Assessment Advisory Committee and the U.S. Environmental Protection Agency."

Response. The proposed PHG for Cr VI in drinking water is 0.02 ppb. No uncertainty factor was used in its calculation. Ingesting drinking water containing Cr VI at this concentration for seventy years is associated with a one in one million extra risk of developing cancer.

Comments from Elliott Rothman, City of Pomona

Comment 1: "As indicated in the draft PHG document, several studies previously estimated that saliva and stomach fluids have the capacity to reduce hexavalent chromium to trivalent chromium in amounts much larger than the "maximum plausible levels of hexavalent chromium in water that would likely be ingested by humans…" The document further asserts that "…exhaustion of the capacity of saliva and gastric fluids to reduce hexavalent chromium appears unlikely." We understand that other studies exist and are referenced in the document providing evidence that complete reduction may not always occur, but we believe the administered doses in the National Toxicology Program (NTP) study are so large they easily overwhelmed the reductive capacity of both the oral cavity and the stomach in the rodents. This is especially significant as the NTP study did not find excess cancers at the lowered studied doses in both rats and mice. Equally as important, the stomach composition of humans and rodents is very different, with humans having a much more sophisticated and higher level of gastric juices than rodents."

Response 1. See Appendix A in the PHG document for a discussion of the data showing that the Cr VI reducing capacity of the rodent GI tract was not saturated over the dose range tested in the NTP (2008) two-year study. The absence of excess tumors at the lower dose levels may have been due to the use of too few animals to detect a relatively rare event (tumor formation). Cr VI is reduced to Cr III in both the rodent and human stomach. This is discussed in detail in the PHG document in the sections "Hexavalent Chromium Reduction by Saliva and Gastric Fluids", "Absorption" and "Pharmacokinetics of Trivalent versus Hexavalent Chromium." See also Appendix A. While Cr VI reduction in the GI tract of rodents compared to humans has not been fully described, the U.S. EPA (2010), the New Jersey Department of Environmental Protection (NJDEP, 2009) and OEHHA (this PHG) have all found that they are similar enough to allow calculation of a human cancer slope factor for Cr VI based on the NTP two-year bioassay.

Comment 2: "The Borneff et al study is seriously flawed due to the fact there was only a single-dose level examined and an ectromelia epidemic affected both control and treated groups with significant loss of mice. The City of Pomona feels this study should not be considered in the development of the PHG."

Response 2. The Borneff et al. (1968) study is not a key study in the derivation of the PHG for Chromium VI and was moved to the Appendix for that reason. OEHHA provided extensive analysis of its findings in its endeavor to consider all available scientific data when evaluating chemicals and developing PHGs aimed at protecting public health. The weight of evidence approach taken by OEHHA necessitated a discussion of Borneff et al. (1968).

Comment 3: "However, in examining the results of the tissue distribution study as presented in Tables J1 and J2 of the above mentioned study, only three results are presented for each exposure group per sample period instead of ten…The complete set of 10 results per organ would have been very helpful to Pomona in our effort to assess OEHHA's draft PHG document."

Response 3. These data have been published in a peer-reviewed scientific journal by Collins et al. (2010). Our understanding from reading this paper is that while up to ten animals per exposure group were put into individual metabolism cages for collection of urine and feces, measurements of tissue chromium were performed on three animals per exposure group. Those are the values presented in Tables 2 and 3 of that paper and in Tables J1 and J2 of the original NTP study report.

Comment 4: "We are aware of some significant new studies addressing the health effects of hexavalent chromium. These studies are nearing completion and could potentially provide a more thorough understanding of hexavalent chromium's mode of action and other critical issues that should be included in a risk assessment. The City of Pomona urges OEHHA to follow the progress of this work and consider the results of this study and others that might emerge as staff must review and revise, if appropriate, all public health goals at least once every five years "…based upon the availability of new scientific data." [Health and Safety Code §116365(E)(e)(1)]."

Response 4. OEHHA acknowledges that new research is on-going and looks forward to the new data when available for consideration. The Safe Drinking Water Act of 1996, amended 1999 (Health and Safety Code [H&SC], Section 116365) contains an important provision that addresses new scientific research when it becomes available: "(e) (1) Public health goals established by the office shall be reviewed at least once every five years and revised, pursuant to the provisions of subdivision (c), as necessary based upon the availability of new scientific data". When new research data become available, OEHHA will consider them in the development of a revised PHG for hexavalent chromium. OEHHA acknowledges that new studies may alter a revised PHG. From the risk characterization section of the 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."

Comments from Kevin Milligan, City of Riverside

Comment 1: "OEHHA ultimately issued its current revised draft PHG of 0.02 ppb, citing updated information regarding sensitive sub-populations. Riverside requests clarification on whether the revision was based in response to the peer review and public comments, or if OEHHA was concurrently considering their own 2009 report regarding effects of early in life exposures to hexavalent chromium."

Response 1. The part of the PHG document concerning sensitive sub-populations was revised in response to comments from both the public and peer reviewers. The revisions are in accordance with the OEHHA (2009) report on early-in-life susceptibility to carcinogens.

Comment 2: "Moreover, Riverside understands that there are studies currently in progress, (and scheduled to be completed in the summer of 2011), that may provide critical information on the mode of action and carcinogenicity of orally ingested hexavalent chromium. Accordingly, Riverside requests that OEHHA thoroughly evaluate the findings of these studies before establishing a final PHG that will be used by the California Department of Public Health to set its MCL."

Response 2. Health and Safety Code Section 116365.5 required the Department of Public Health to develop a primary drinking water standard for Cr VI by January 1, 2004. Health and Safety Code Section 116365 requires the development of a PHG by OEHHA before the department can adopt a primary drinking water standard. In light of this statutory mandate, it would be very difficult for OEHHA to justify further delay to finalizing the PHG in order to incorporate any appropriate findings from the Hamner Institutes research program.

Health and Safety Code Section 116365 contains an important provision that addresses new scientific research when it becomes available: "(e) (1) Public health goals established by the office shall be reviewed at least once every five years and revised, pursuant to the provisions of subdivision (c), as necessary based upon the availability of new scientific data". OEHHA acknowledges that new studies may alter a revised PHG. From the risk characterization section of the 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."

OEHHA will review papers and materials relating to the Hamner Institutes study when they are published. If the study produces compelling information that should be reflected in the PHG document, OEHHA will take appropriate action.

Comment 3: "To our knowledge the PHG does not include a risk characterization of the microbial risk related with current disinfection practices compared to the health risk associated with the conversion of trivalent chromium to hexavalent chromium. Has OEHHA considered this risk assessment for the proposed hexavalent chromium PHG? If not, how will these challenges be addressed in the current regulatory framework?"

Response 3. The PHG document addresses the risk of health effects due to Cr VI in drinking water. Related risks due to microbial contamination and the compounds used to treat such contamination are outside the scope of the PHG.

Comments from Steve Bigley, Coachella Valley Water District

Comment 1: "The Office of Environmental Health Hazard Assessment (OEHHA) draft Cr6 PHG of 0.02 parts per billion (ppb) is calculated from cancer observed in the National Toxicology Program rodent study completed in 2007. Specifically, this calculation is based on cancer found in the small intestines of 5 of the 50 male mice exposed to drinking water containing 90,000 ppb of Cr6 for 2-years or the typical life span of a mouse. The male mice in this study that received doses of 5,000 ppb, 10,000 ppb and 30,000 ppb showed no statistically significant increase in cancer when compared to cancer observed in control mice receiving no Cr6 in their drinking water."

Response 1. The data in Table 5 of the PHG document show that male mice given drinking water containing 30,000 or 90,000 ppb of Cr VI had incidences of intestinal tumors (adenomas or carcinomas) that were significantly greater than controls. In addition, there was a positive trend for increasing intestinal tumors with increasing concentration of Cr VI.

Comment 2: "While this data clearly shows a threshold below which no increased cancer was observed in the rodents, OEHHA is allowed to use a default linear dose response model when there is insufficient data to explain the mode of action by which the 5 male mice developed cancer in their intestines."

Response 2. There were seven male mice at 30,000 ppb of Cr VI and 20 male mice at 90,000 ppb that developed intestinal tumors (adenomas or carcinomas), compared to one control mouse (both pair-wise comparisons were statistically significant). As mentioned above, there was also a positive trend for increasing intestinal tumors with increasing concentration of Cr VI. At the two lowest dose levels the increases in tumor incidence were not significant compared to the control incidence. This should not be interpreted as a threshold for tumor induction, since it may be due to the use of too few animals to detect a relatively rare event (tumorigenesis).

Comment 3: "Dr. Cohen states, 'It is clear that the data presented in the Draft document (c.f. Figure 13; Editorial note: abscissa needs the addition of units as the values shown do not correspond to any of the reported doses in Tables 5 and 6) shows that tumor formation in the mice as a function of Cr6+ level in drinking water is not linear.'"

Response 3. In the 2008 NTP study statistically significant increases in tumors of the small intestine were observed for both male and female mice at the two highest drinking water concentrations. Exact trend tests were positive for both sexes. The absence of statistically significant increases in tumors at the two lowest drinking water concentrations should not be interpreted as a threshold for tumorigenicity (i.e., should not be construed as a nonlinear dose-response curve), since the number of animals may have been too low to detect tumors at the two lowest drinking water concentrations. The use of high doses in cancer bioassays is designed to offset the

statistical limitations of using small numbers of animals (50 /sex/dose level) to measure a relatively rare event (tumors).

Comment 4: "Dr. Rossman provides several reasons objecting to the use of a linear dose response model for the draft PHG and supporting his statement, 'The assumption is that Cr(VI) in drinking water has a mutagenic MOA with no threshold. This is not valid for the following reasons.'"

Response 4. OEHHA does not know the mechanism by which Cr VI causes cancer in humans or animals. It is both genotoxic and mutagenic as described in the PHG document. Since there are insufficient data to support an MOA other than that via genotoxicity/mutagenicity, the PHG document models the tumor data according to a linear multistage model as recommended (U.S. EPA, 2005; OEHHA, 2009; McCarroll et al., 2010).

Comment 5: "Dr. Snow states, "Based on this study, along with very limited evidence of tumor response at lower levels of Cr6, there is very limited evidence for a linear dose response. It is more likely, due to the high probability of extracellular conversion of the Cr6 to the much less toxic Cr3, that uptake and bioavailability of the Cr6, in itself, will exhibit a non-linear (threshold) dose response."

Response 5. It is the case with most carcinogens that dose-response data are not available in the low dose region where human exposures are expected. With regard to a high probability that extracellular Cr VI will be converted to Cr III, this may or may not be true. The PHG document contains examples of Cr VI absorption at dose levels that are far below the calculated capacity of the GI tract of humans and rodents to reduce all ingested Cr VI to Cr III. The PHG document also discusses examples where Cr VI absorption was not concentration dependent.

Comment 6: "OEHHA has also disregarded and twisted the scientific opinion of one of the most highly respected toxicologists on the subject of Cr6 toxicology. Dr. Silvio De Flora has studied Cr6 toxicity for over 30 years and many of his studies are referenced in the draft PHG…While the study results include statistically significant decreases in certain tumors in the Cr6 exposed rodent test groups, these findings do not support a health benefit from ingestion Cr6 just as the statistically significant increases in cancer observed in male mice at the highest Cr6 dose is not biologically significant and does not bear relevance to human exposures."

Response 6. First, OEHHA agrees that Cr VI is not likely to protect against cancer. Second, biological significance does not follow from occasional increases or decreases in tumors. Rather, OEHHA looks for a dose-responsive change exhibiting statistical significance. In mice such a pattern was observed for intestinal tumors.

Comment 7: "The NTP study report is actually based on three distinct studies: a clinical study, a histopathology study, and a tissue distribution study. While the clinical study is used to support the PHG, the histopathology and tissue distribution studies are given little consideration by OEHHA. The tissue distribution study clearly showed no increase in Cr6 levels in the tissues studied when rodents ingested 5,000 ppb of Cr6 for one year. Likewise, rodents exposed to 5,000 ppb of Cr6 for 2-years in the histopathology study showed no excess cancers."

Response 7. See Appendix A of the PHG document for presentation of the tissue chromium levels in the animals comprising the two-year bioassay. As discussed in that section, increases in tissue chromium were detected at all dose levels, including 5,000 ppb. The histopathological findings of the two-year bioassay are discussed in the section entitled "Non-neoplastic findings – Possible relationships between tissue damage, inflammation, hyperplasia and tumors in rats and mice," located in the "Carcinogenicity" section of the PHG document.

Comment 8: "This NTP data supports the well-established observation that the reductive capacity of the mammalian stomach can convert Cr6 to the non-toxic reduced form of chromium even at levels 100 times greater than the current California drinking water MCL for chromium."

Response 8. See the "Metabolism and Pharmacokinetics" section of the PHG document for examples of Cr VI absorption occurring at concentrations estimated to be lower than the reductive capacity of the mammalian stomach. This would be possible if Cr VI absorption and reduction were competing processes occurring in the GI tract, as discussed in the PHG document.

Comment 9: "OEHHA and the peer reviewers also failed to identify some important information missing from the tissue distribution study. The scope of this study included the collection of samples of 4 specific tissues from each of the 10 animals selected from each test group. However, the summary tables (Table J1 and J2) for this study only include results for 3 to 6 animals depending on the tissue. No explanation has been provided for why the additional tissue data has not been made available to the public."

Response 9. These data have been published in a peer-reviewed scientific journal by Collins et al. (2010). Our understanding from reading this paper is that while up to ten animals per exposure group were put into individual metabolism cages for collection of urine and feces, measurements of tissue chromium were performed on three animals per exposure group. Those are the values presented in Tables 2 and 3 of that paper and in Tables J1 and J2 of the original NTP study report.

Comment 10: "OEHHA takes the position that using a more precautionary linear model assumption when there is a gap in available science is justified and the best way to reduce health risks."

Response 10. As discussed in the PHG document, the data on hand for Cr VI suggest it acts via a genotoxic mode of action. Carcinogens with genotoxic MOAs are modeled using a linear model by both U.S. EPA (2005) and OEHHA (2009), based in part on the linear dose-response relationship observed for radiation-induced human cancer; the data set covering the lowest dose levels and cancer incidences so far measured (Brenner et al., 2003).

Comment 11: "Studies that focus on exposing rodents to unrealistic levels of an element to illicit an adverse response do not provide the good science needed to properly predict potential health risks at realistic low levels of exposure."

Response 11. Two-year bioassays with rodents are traditionally performed at high dose levels in order to offset the statistical limitations of using 50 animals per sex per dose level to detect a relatively rare event (tumor formation).

Comment 12: "Studies designed to properly evaluate the mode of action and provide sufficient information to determine if a threshold dose response exists for the subject element is critical to completing an accurate risk assessment."

Response 12. See the following sections of the PHG document for discussion of the MOA issues for which data are available: "Pharmacokinetics of Trivalent versus Hexavalent Chromium," "Mechanism of Genotoxicity and Carcinogenicity," Table 7 in the section entitled "Non-neoplastic findings – Possible relationship between tissue damage, inflammation, hyperplasia and tumors in rats and mice," "Examination of Evidence for Chromium Carcinogenicity," and "Risk Characterization."

Comment 13: "The obvious gap in science used to support the draft Cr6 PHG has already been identified and studies are ongoing to help determine the mode of action for Cr6 toxicity observed in rodents. These studies are nearing completion and are designed to provide a more thorough understanding of the mode of action and other critical issues that should be included in a Cr6 risk assessment for drinking water."

Response 13. OEHHA acknowledges that new research is on-going and looks forward to the new data when available for consideration. The Safe Drinking Water Act of 1996, amended 1999 (Health and Safety Code [H&SC], Section 116365) contains an important provision that addresses new scientific research when it becomes available: "(e) (1) Public health goals established by the office shall be reviewed at least once every five years and revised, pursuant to the provisions of subdivision (c), as necessary based upon the availability of new scientific data". When new research data become available, OEHHA will consider them in the development of a revised PHG for hexavalent chromium. OEHHA acknowledges that new studies may alter a revised PHG. From the risk characterization section of the 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."

Comments from David Luker, Desert Water Agency

Comment 1: "Specifically, Desert Water Agency awaits epidemiology study results that demonstrate the affect of hexavalent chromium in humans, as the difference in indigestion processes, stomach composition, and levels of gastric juices between rodents and humans were not taken into account in the referenced study. We are also hopeful that such a study will be based upon realistic concentrations of water with hexavalent chromium present."

Response 1. See the "Toxicological Effects in Humans" section of the PHG document, subheading "Carcinogenicity," for a detailed discussion of the study by Zhang and Li (1987). This study detected a statistically significant increase in stomach cancer mortality in persons drinking water contaminated with Cr VI. Also see Beaumont et al. (2008; Cancer mortality in five villages in China with hexavalent chromium-contaminated drinking water. Epidemiology 19:12-23).

Comments from Ron Hunsinger, East Bay Municipal Utility District

Comment 1: "The risk characterization section in the draft PHG report should include an assessment of the risk associated with the formation of Chromium 6 following exposure to a disinfectant such as ozone, chlorine, or chloramines. In order to fully characterize the risk posed by Chromium 6, OEHHA should acknowledge this source of Chromium 6 and incorporate a microbiological risk component into the discussion."

And,

"The draft PHG report does not identify the conversion of Chromium 3 to Chromium 6 following exposure to a disinfectant such as ozone, chlorine, or chloramine as a source of Chromium 6. Pathogenic inactivation and Chromium 6 production occur simultaneously and present different public health threats. However, the draft PHG report addresses only the chemical threat."

And,

"Risk characterization should enumerate the microbiological risks (USEPA 2009) associated with the current disinfection practices and compare them to the health risk associated with the conversion of Chromium 3 to Chromium 6 via the interaction of a disinfectant and Chromium 3."

Response 1. While oxidation of Cr III to Cr VI by drinking water disinfectants such as ozone, chlorine or chloramines is theoretically possible, we have been unable to locate any data relating to this specific topic. Were such data to be located, they would be included in the "Environmental Occurrence and Human Exposure" section of the PHG document. However, calculation of the PHG value would not be affected by such data. Consideration of microbial risk is outside the scope of the PHG document.

Comment 2: "We recommend the following references be incorporated into the report to ensure the toxicological literature review is exhaustive…"

Response 2. There is a very large body of published literature on chromium covering its chemistry, environmental occurrence, toxicity, possible human dietary requirement and other characteristics. We have attempted to include in the PHG document those papers most relevant for developing a drinking water value that protects human health. Two of the three papers cited in this comment come from the laboratory of Professor De Flora. The final PHG document discusses five other papers from this laboratory, indicating that this group's research on chromium has been adequately considered.

Comments from Rebecca Sutton, Environmental Working Group

Comment 1: "EWG and NRDC urged OEHHA to ensure adequate protection of another sensitive population, those with medical conditions or on medications that reduce stomach acidity. Conversion of hexavalent to trivalent chromium can be impaired in individuals with low-acid stomachs, a condition brought about by several widely-used medications, including antacids and proton pump inhibitors, prescribed for gastroesophageal reflux disease, peptic ulcer disease, and chronic gastritis. Other health conditions that can result in reduced stomach acid production include pernicious

anemia, pancreatic tumors, infection with *Helicobacter pylori*, mucolipidosis type IV, and some autoimmune diseases.

A susceptible subpopulation united by a variety of common to rare medical conditions faces an elevated risk from oral exposure to hexavalent chromium. We hope the revised public health goal of 0.02 ppb will protect such individuals from the effects of hexavalent chromium in tap water. We suggest that OEHHA examine this issue further during its periodic review of public health goals."

Response 1. Some of these potentially sensitive subpopulations are discussed in the "Sensitive Subpopulations" section of the PHG document. For calculation of the acceptable daily dose (ADD) for noncarcinogenic effects ("Calculation OF The PHG, Noncarcinogenic Effects" section of the PHG), an uncertainty factor of 10 was judged sufficient for protecting potentially sensitive human subpopulations, such as antacid users. Methodology does not currently exist for incorporating the potentially heightened sensitivity of these subpopulations into the calculation of the protective dose for carcinogenic effects.

Comments from Dan Askenaizer, Glendale Water and Power

Comment 1: "When OEHHA published the revised draft PHG of 0.02 ppb, the PHG document cited updated information regarding sensitive sub-populations. In the press release for the revised PHG, OEHHA states 'new research has documented that young children and other sensitive populations are more susceptible than the general population to health risks from exposure to carcinogens. The changes were recommended by the peer review and reflect OEHHA's new guidelines for early-in-life exposures, which acknowledge this susceptibility.' The need to incorporate OEHHA's policy on sensitive subpopulations was clearly stated by one of the peer reviewers of the 2009 draft PHG. However, these statements by OEHHA seem to imply that new research involving CrVI and sensitive sub-populations became available to OEHHA. If there is additional new information regarding CrVI and protecting the health of sub-populations, it would be helpful for OEHHA to make that information public."

Response 1. The "new research" quoted above refers to the new OEHHA guidelines for early-in-life exposures to carcinogens and the data that are the basis for those guidelines (OEHHA, 2009). No new data on Cr VI were received.

Comment 2: "While Appendix A in the December 31, 2010 draft PHG presents a discussion of the issue of a carcinogenic threshold, the information presented does not appear to directly address the question of a threshold as raised by several of the peer review comments. "

Response 2. We agree with this comment. The title to Appendix A has been modified to the following, "Carcinogenic Threshold: Was the Reductive Capacity of the Rodent GI Tract Exceeded in the NTP (2008) Bioassay?"

Comments from David Chang, Golden State Water Company

Comment 1: "In the calculation of the PHG for hexavalent chromium, an aggregate uncertainty factor of 3000 is applied, the maximum recommended by the California Risk Assessment Advisory Committee and the U.S. Environmental Protection Agency."

Response 1. The proposed PHG for Cr VI in drinking water is 0.02 ppb. No uncertainty factor was used in its calculation (see "Calculation of the PHG," subheading "Carcinogenic Effects" section of the PHG document). Ingesting drinking water containing Cr VI at this concentration for seventy years is associated with a one in one million extra risk of developing cancer.

Comments from Mic Steward, The Metropolitan Water District of Southern California

Comment 1: "OEHHA ultimately issued its current revised draft PHG of 0.02 ppb, citing updated information regarding sensitive sub-populations. Metropolitan requests clarification on whether the revision was based in response to the peer review and public comments, or if OEHHA was concurrently considering their own 2009 report regarding effects of early in life exposures to chromium 6."

Response 1. The part of the PHG document concerning sensitive sub-populations was revised in response to comments from both the public and from peer reviewers. The revisions were in accordance with the OEHHA (2009) report on early-in-life susceptibility to carcinogens.

Comment 2: "Moreover, Metropolitan understands that there are studies currently in progress (and scheduled to be completed in the summer of 2011) that may provide critical information on the mode of action and carcinogenicity of orally ingested chromium 6. Accordingly, Metropolitan requests that OEHHA thoroughly evaluate the findings of these studies as part of establishing a final PHG that will be used by the California Department of Public Health to set its MCL."

Response 2. OEHHA will review papers and materials relating to the Hamner Institutes study when they are published. If the study produces compelling information that should be reflected in the PHG document, OEHHA will take appropriate action.

Comments from Richard Atwater, Southern California Water Committee

Comment 1: "While purporting to meet the requirements to use the best science in decisions that relate to protecting public health, OEHHA continues to follow the practice of using default assumptions rather than chemical-specific information and sound science to inform risk assessment."

Response 1. The PHG document finds that Cr VI is genotoxic and mutagenic (see "Genetic Toxicity" section of the PHG document). It also finds that the available data do ***not*** support a threshold MOA of tissue damage followed by regenerative cell proliferation (see "Toxicological Effects in Animals," subheading "Carcinogenicity," subheading "Non-neoplastic findings – Possible relationships between tissue damage,

inflammation, hyperplasia and tumors in rats and mice" section of the PHG document). Unless there are data suggesting a different approach, both U.S. EPA (2005) and OEHHA (2009) model tumor data for genotoxic/mutagenic carcinogens by linear extrapolation. This is not a default assumption, but a methodological choice driven by the data.

Comment 2: "The Draft December 2010 Public Health Goal document contains the following deficiencies: failure to address comments from peer reviewers of the August 2009 PHG document, the draft December 2010 PHG document and expert panel comments on the draft 1999 PHG document."

Response 2. This document responds to comments received in response to the August 2009 draft and the December 2010 draft. The findings of the expert panel were disavowed by the California Environmental Protection Agency following legislative hearings on allegations that some panel members had not properly disclosed their economic interests.

Comment 3: "Inadequate response to public comments on earlier PHG documents, including: Lack of any mode of action (MOA) consideration, especially when MOA forms the overarching conceptual framework for cancer risk assessment (EPA, 2005a)."

Response 3. See the following sections of the PHG document for discussion of the MOA issues for which data are available: "Pharmacokinetics of Trivalent versus Hexavalent Chromium," "Mechanism of Genotoxicity and Carcinogenicity," "Examination of Evidence for Chromium Carcinogenicity," and "Risk Characterization." The mutagenic mode of action described by McCarroll et al. (2010) has been added to the document.

Comment 4: "Inadequate response to public comments on earlier PHG documents, including: Regarding the MOA, lack of consideration of interspecies differences in toxicokinetics of Cr(VI) and the failure to recognize that pathologies seen in rodents are likely portal-of-entry effects."

Response 4. The rodent tumors observed in the two-year bioassay (NTP, 2008) may well be site-of-contact effects. However, given the available data, calculation of the cancer potency would be the same whether a site-of–contact effect or a systemic effect were assumed.

Comment 5: "Inadequate response to public comments on earlier PHG documents, including: Regarding the MOA, lack of consideration of nonlinear toxicodynamic effects of Cr(VI) that likely underlie the cancer response. These effects include reactions with DNA, oxidative stress, inflammation and disruption of gene networks that regulate the cell cycle. Instead, the draft December 2010 PHG document correctly assumes that its metabolic products of Cr(VI) are DNA-reactive and wrongly assumes that DNA-reactivity equates to mutagenicity."

Response 5. Potentially "nonlinear toxicodynamic effects of Cr(VI)" are discussed in the PHG document. For reactions with DNA and oxidative stress see the "Genetic Toxicity" section of the document. For discussion of the inflammation caused by Cr VI see the "Toxicological Effects in Animals," subheading "Carcinogenicity," subheading "Non-neoplastic findings – Possible relationships between tissue damage, inflammation,

hyperplasia and tumors in rats and mice" section of the PHG document. Disruption of gene networks was not discussed because few data were located on this topic. OEHHA does not equate DNA reactivity with mutagenicity and has not done so in any of the previous drafts or in the final PHG document. There are many ways to damage DNA that do not lead to mutations. Having said that, there are numerous published studies indicating that Cr VI is mutagenic. In cultured mammalian cells, there are a number of published studies which report robust (in excess of 3-fold increases) mutagenic responses to Cr VI (see Paschin et al. (1983) Mut Res103(3-6):345-347; Mitchell et al. (1988) Environ Mol Mutagen 12(Suppl 13):37-101; Myhr and Caspary (1988) Environ Mol Mutagen 13(12):103-194; McGregor et al. (1987) Environ Mutagen 9(2):143-160; Oberly et al (1982) J Toxicol Environ Health 9(3):367-376). Reviews discussing these studies are cited in the first paragraph of the "Genetic Toxicity" section of the PHG document. Cr VI also caused mutations in bacteria, yeast, D. melanogaster and mice (see reviews mentioned above and U.S. EPA, 2010).

Comment 6: "Inadequate response to public comments on earlier PHG documents, including: Lack of consideration of nonlinearity and the presence of a threshold. Although Appendix A, titled 'Carcinogenic Threshold?' gives lip service to the idea of a threshold, this appendix considers only reductive capacity and absorption, and because of the lack of any consideration of MOA, fails to take into account epigenetic changes that underlie the tumor response that likely do have thresholds. The lack of consideration of MOA also prevented exploration of the use of precursor effects as recommended in EPA's Guidelines for Carcinogen Risk Assessment (EPA, 2005a)."

Response 6. The title of Appendix A has been revised to read, "Carcinogenic Threshold?: Was the reductive capacity of the rodent GI tract exceeded in the NTP (2008) bioassay?" Discussion has been added to the following sections of the PHG document concerning the MOA data that support the use of a low dose linear extrapolation to calculate cancer potency: "Mechanism of Genotoxicity and Carcinogenicity" (large number of studies demonstrating a genotoxic, and possibly mutagenic, MOA for Cr VI); "Toxicological Effects in Animals," subheading "Carcinogenicity," subheading "Non-neoplastic findings – Possible relationships between tissue damage, inflammation, hyperplasia and tumors in rats and mice" (that the tissue and cellular findings shown in Table 7 do not support an alternative MOA for tumor induction by Cr VI).

Comment 7: "Use of deficient scientific literature, including: The use of two highly flawed studies in mice and humans respectively (Borneff et al., 1968; Zhang and Li, 1987) to attempt to establish a link between Cr(VI) exposure and gastrointestinal cancer in humans. The use of these studies is in direct contradiction of the advice of an expert panel convened at the University of California in 2001 to review the 1999 PHG document."

Response 7. The findings of the expert panel on the draft 1999 PHG document were disavowed by the California Environmental Protection Agency following legislative hearings on allegations that several members had not properly disclosed their economic interests. The study by Zhang and Li (1987) and its limitations are thoroughly discussed in the final PHG document. The section of the final PHG document entitled "Examination of the Evidence for Chromium Carcinogenicity" emphasizes the

importance of this study's finding of a statistically significant increase in stomach cancer mortality in the exposed population. This is an important finding that must be part of any serious discussion of whether Cr VI is carcinogenic in humans. The study by Borneff et al. (1987) is discussed in the Appendix. While it was not used to develop the PHG, its inclusion serves as a scientific resource and as a record of the issues that have been addressed in the research for and preparation of this PHG document.

Comment 8: "Use of deficient scientific literature, including: Attempt to impeach the results of the Gatto et al. (2010) meta-analysis that found no association between occupational exposure to Cr(VI) and gastrointestinal cancer in humans."

And,

"Although the draft December 2010 PHG document made several suggestions to "improve" the meta-analysis, it is unlikely that any of these suggestions would alter the results."

Response 8. Discussion of Gatto et al. (2010) is a straightforward identification of some possible limitations of the study.

Comment 9: "The Draft December 2010 Public Health Goal document contains the following deficiencies: Inappropriate use of the age-sensitivity adjustment detailed in OEHHA (2009) because of lack of consideration of MOA. In addition, it was difficult to validate the calculations that employed this adjustment because the necessary data were scattered throughout the document."

Response 9. See OEHHA (2008) for a detailed discussion of the relationship between MOA and early-in-life susceptibility to carcinogens. The "Calculation Of The PHG," subheading "Carcinogenic Effects" section of the PHG document illustrates how the age sensitivity factors are incorporated into the PHG calculation.

Comment 10: "The Draft December 2010 Public Health Goal document contains the following deficiencies: Failure to explore the uncertainty associated with dose-response modeling. The narrative and tables describing the modeling were very brief and difficult to follow. The number of animals at risk for the various dose groups in NTP (2008) was changed from those in the draft August 2009 PHG document without explanation, and neither set of values were the results of the commonly used poly-3 survival adjustment (Portier and Bailer, 1989)."

Response 10. The numbers of animals at risk are shown in Table 5 and Table 6. As indicated in the footnotes to both tables, these are the animals alive at the time of the first occurrence of tumor (day 451 for males and day 625 for females) and if the tissue was available for analysis. This is a standard method for determining the number of animals at risk for tumors (U.S. EPA, 2005; OEHHA, 2009).

Comment 11: "The revised drinking water consumption rates have decreased, even though they are said to be 'upper 95th percentile values estimated by OEHHA,' as were the original consumption rates. For example, the original drinking water rate for a 70 kg adult was 3.15 liters/day and it is now 2.66 liters/day. The question arises, will OEHHA go back to the 3.15 liter/day value when the Director adopts their draft document?"

Response 11. The methodology for calculating water consumption rates using the data from U.S. EPA (2008) and Kahn and Stralka (2009) is now presented in the footnote to Table 17 and in the discussion of Table 18 in the PHG document. In the previous draft of the PHG document the **per capita** water consumption rates were mistakenly used. The revised PHG document uses the **consumers only** rates. The final PHG value of 0.02 ppb (rounded) was unaffected. The drinking water consumption rates reported by Kahn and Stralka (2009) are the same values recommended by U.S. EPA (2008) for use in human health risk assessments. They came out of the largest survey of its kind: the United States Department of Agriculture's (USDA's) 1994-1996 and 1998 Continuing Survey of Food Intake by Individuals (CSFII).

Comment 12: "U.S. EPA used the 90th percentile for the drinking water rate in determining an acceptable concentration for fluoride. The 90th percentile is closer to the traditional 2 liters/day drinking water consumption rate."

Response 12. With regard to the 95th percentile drinking water rate being overly conservative, OEHHA has traditionally sought to protect this large fraction of the population.

Comment 13: "OEHHA does not distinguish between direct and indirect consumption of tap water…Yet there is no adjustment for this in OEHHA's tap water consumption rates."

Response 13. We have added to the "Calculation of The PHG" section of the document that the water intake data cover pure water consumed as a beverage or used in the home or local establishments to prepare food or drink (Kahn and Stralka, 2009). The data are not available for correcting for the amount of Cr VI that is reduced to Cr III when tap water is used to prepare beverages such as juice and coffee, just as there are inadequate data for calculating the amount of Cr III that is oxidized to Cr VI when tap water is handled in various other ways.

Comments from Michael Sovich, Three Valleys Municipal Water District

Comment 1: "OEHHA ultimately issued its current revised draft PHG of 0.02 ppb, citing updated information regarding sensitive sub-populations. TVMWD requests clarification on whether the revision was based in response to the peer review and public comments, or if OEHHA was concurrently considering their own 2009 report regarding effects of early in life exposures to hexavalent chromium."

Response 1. The part of the PHG document concerning sensitive sub-populations was revised in response to comments from both the public and from peer reviewers. The revisions were in accordance with the OEHHA (2009) report on early-in-life susceptibility to carcinogens.

Comment 2: "Moreover, TVMWD understands that there are studies currently in progress (and scheduled to be completed in the summer of 2011) that may provide critical information on the mode of action and carcinogenicity of orally ingested hexavalent chromium. Accordingly, TVMWD requests that OEHHA thoroughly evaluate the findings of these studies before establishing a final PHG that will be used by the California Department of Public Health to set its MCL."

Response 2. OEHHA will review papers and materials relating to the Hamner Institutes study when they are published. If the study produces compelling information that should be reflected in the PHG document, OEHHA will take appropriate action.

Comment 3: "To our knowledge, the PHG does not include a risk characterization of the microbial risk related with current disinfection practices compared to the health risk associated with the conversion of trivalent chromium to hexavalent chromium. Has OEHHA considered this risk assessment for the proposed hexavalent chromium PHG? If not, how will these challenges be addressed in the current regulatory framework?"

Response 3. While oxidation of Cr III to Cr VI by drinking water disinfectants such as ozone, chlorine or chloramines is theoretically possible, we have been unable to locate any data relating to this specific topic. Were such data to be located, they would be included in the "Environmental Occurrence and Human Exposure" section of the PHG document. However, calculation of the PHG value would not be affected by such data. Consideration of the microbial risk is outside the scope of the PHG document.

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