
**Comments on the California Office of Environmental
Health Hazard Assessment's Draft Public Health Goal
for Hexavalent Chromium in Drinking Water
(December 2010)**

Prepared on behalf of:

The California Manufacturers & Technology Association
1115 Eleventh Street
Sacramento, California 95814

Prepared by:

Janet E. Kester, Ph.D., D.A.B.T.
NewFields
155 Cedar Lake Drive
Wentzville, Missouri 63385

Submitted February 15, 2011

Contents

Introduction	1
Summary of CMTA Comments on the 2009 Draft Cr(VI) PHGs	1
Summary of CMTA Comments on the 2010 Draft Cr(VI) PHG for Carcinogenic Effects	3
Comments Regarding Changes in the Calculation of the 2010 Draft Cr(VI) PHG for Carcinogenic Effects.....	4
Drinking Water Intake Rates are Overly Conservative and Insufficiently Documented	4
Age Sensitivity Factors are Inappropriate for Cr(VI)and Insufficiently Documented	5
Comments Regarding the Carcinogenic Mode of Action of Cr(VI) in Drinking Water.....	6
Data Gaps Associated with the NTP Two-Year Bioassay for Cr(VI) in Drinking Water	6
OEHHA’s Evaluation of MOA and Human Relevance for Cr(VI) in Drinking Water is Incomplete and Inadequate	7
Summary of Peer Reviewers’ Comments on the Accuracy of Information Presented on the MOA in the 2009 Draft Carcinogenic PHG for Hexavalent Chromium	10
Overview of Proposed MOAs for the Oral Carcinogenicity of Cr(VI).....	11
Important New Information Pending from the Cr(VI) MOA Research Project.....	13
Saturation of Upper Gastrointestinal Reductive Capacity and Intestinal Cellular Uptake.....	13
Oxidative Stress and Inflammation.....	17
Cell Proliferation	18
DNA Damage.....	18
Mutagenesis.....	20
Conclusion.....	20
References	21

California Manufacturers & Technology Association's Comments on the *Draft Public Health Goal for Hexavalent Chromium in Drinking Water (December 2010)*

Introduction

The California Manufacturers & Technology Association (CMTA) has reviewed the California Environmental Protection Agency's Office of Environmental Health Hazard Assessment's (OEHHA's) document entitled *Draft Public Health Goal for Hexavalent Chromium in Drinking Water*, dated December 2010. Substantive technical comments on the draft 2009 public health goal (PHG) for hexavalent chromium (Cr(VI)) (OEHHA 2009a) were submitted previously by CMTA as well as by other members of the public, peer reviewers, and the California Department of Toxic Substances Control (DTSC). CMTA's major comments on the draft 2009 PHGs are also relevant and responsive to the draft 2010 PHGs, and as such are incorporated herein by reference.

CMTA's comments on the 2010 draft PHG document are focused on (1) the significant changes between the 2009 and 2010 draft PHGs for carcinogenic effects, and (2) application of data from the Cr(VI) Mode of Action (MOA) Research Project studies to critical questions and data gaps concerning the carcinogenic MOA(s) of Cr(VI) administered via drinking water to rats and mice, and its relevance to humans.

Summary of CMTA Comments on the 2009 Draft Cr(VI) PHGs

The CMTA's comments of the 2009 draft PHG for Cr(VI) can be summarized as follows:

- Current EPA and international guidance placing primary emphasis on MOA and human relevance is designed to assist risk assessors to meet the significant challenges associated with extrapolating among species and from high to low doses in order to establish scientifically sound and defensible dose-response relationships for risk assessment (*e.g.*, Sonich-Mullen *et al.* 2001; Cohen *et al.* 2004; EPA 2005a; Boobis *et al.* 2006; Meek 2008). As stated by EPA (2005a), "[t]he use of mode of action in the assessment of potential carcinogens is a main focus of these cancer guidelines" (page 1-10). OEHHA's approach to the critical evaluation of data for Cr(VI) does not follow current EPA and international guidelines for human cancer risk assessment. 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.

- 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. Sponsored by the Cr(VI) Panel of the American Chemistry Council, the protocols for this work were critically reviewed by an independent science advisory board (SAB) convened by Toxicology Excellence for Risk Assessment (TERA) to ensure that the studies were of the highest quality and appropriate for providing the data needed to understand the MOA. Without coherent evaluations of (1) animal MOA, and (2) human relevance to support its selection of a linear non-threshold (LNT) low-dose extrapolation method, the draft PHG does not comport with current guidance and is fatally flawed. 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.
- The non-cancer PHG for Cr(VI) was developed using methods that are inconsistent with current OEHHA guidance, which recommends using benchmark dose (BMD) modeling to quantify the dose-response relationship. 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). 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. The non-cancer risk assessment should also apply the refined PBPK model being developed in the Cr(VI) MOA Research Project in order to characterize interspecies differences in kinetics, and evaluate the human relevance of non-cancer effects in the NTP study (Seed *et al.* 2005; Boobis *et al.* 2008).
- 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. OEHHA should provide a holistic and balanced review of the experimental and epidemiological literature, focusing on the MOA and human relevance of the findings of the NTP study, and evaluating epidemiologic findings for exposures to Cr(VI) at levels that are relevant for the California drinking-water supply.

- 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.

Summary of CMTA Comments on the 2010 Draft Cr(VI) PHG for Carcinogenic Effects

The draft PHG for Cr(VI) based on carcinogenic effects was 0.06 micrograms per liter ($\mu\text{g/L}$) in 2009, and decreased three-fold to 0.02 $\mu\text{g/L}$ in 2010. Like the 2009 draft PHG, the 2010 draft PHG is overly-conservative and scientifically indefensible. CMTA's primary comments on the 2010 draft PHG are:

- 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 the 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.
- Application of generic age 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). This significant deviation from current EPA guidance and policy warrants careful examination by the scientific community before it is used in risk assessments. 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).
- 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. Conducted by a highly qualified team of experts, all of the results will be peer-reviewed by the independent SAB convened by TERA, and will be published in the peer-reviewed scientific literature by the summer of 2011. Clearly, such data are not only essential to extrapolating the results of high-dose animal studies like the two-year NTP bioassay to humans, but also consistent with national and international regulatory initiatives to improve

the predictive capability of toxicological testing. 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.

These comments are discussed in more detail in the following sections.

Comments Regarding Changes in the Calculation of the 2010 Draft Cr(VI) PHG for Carcinogenic Effects

OEHHA's draft PHG for Cr(VI) based on carcinogenic effects was 0.06 micrograms per liter ($\mu\text{g/L}$) in 2009, and decreased three-fold to 0.02 $\mu\text{g/L}$ in 2010. The two factors primarily responsible for this reduction are (1) increased lifetime drinking water rate, and (2) application of ASFs described in OEHHA's *Technical Support Document for Cancer Potency Factors: Methodologies for Derivation, Listing of Available Values, and Adjustments to Allow for Early Life Stage Exposures* (OEHHA 2009c).

Drinking Water Intake Rates are Overly Conservative and Insufficiently Documented

OEHHA (2009a) assumed a single default lifetime drinking water intake rate (2 liters per day for a 70-kilogram (kg) person). The age groups represented by these values were not specified. However, assuming that both represent lifetime average values, the lifetime body weight-normalized drinking water intake rate would thus be $2\text{L}/70\text{ kg} = 0.029\text{ L/kg-day}$. According to data presented in EPA's most recent *Exposure Factors Handbook* (EPA 2009, Table 3-22), this intake rate corresponds to approximately the 85th percentile of "all ages" intake by the U.S. population. 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. Because this document is still in draft and not publicly available, OEHHA "...determined it would be more appropriate to cite comparable published studies as a basis for calculation of...the draft PHG," and recently issued a minor modification of the drinking water intakes based on EPA's *Child-Specific*

Exposure Factors Handbook (EPA 2008) and Kahn and Stralka (2009) (<http://www.oehha.ca.gov/water/phg/chrom012511.html>). The revised (lower) intake rates for the infancy and childhood age groups (0.114 and 0.041 L/kg-day) could not be verified based on these references.

It should also be noted that the revised intake rates are uncertain for two important reasons: (1) the body weight-normalized water intake rates are based on self-reported rather than measured body weights, and (2) the sample sizes for infants aged 0 to 1, 2 to 3, and 3 to 6 months, who have the highest estimated daily water intake rates of all age groups, did not meet minimum reporting requirements (EPA 2008, 2009; Kahn and Stralka 2009). These limitations would increase the variability due to sampling rather than variability in the population, making the 95th percentile artificially high. 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.

Age Sensitivity Factors are Inappropriate for Cr(VI) and Insufficiently Documented

Another new OEHHA policy implemented in the 2010 draft PHG is application of generic ASFs to the four age groups identified above in order to address the potentially greater susceptibility of fetuses and children to chemical carcinogenesis. In guidance that has undergone public review, EPA determined that “age-dependent adjustment factors” (ADAFs) may be suitable for risk assessment of carcinogens known to act via a mutagenic MOA (EPA 2005b), noting, “[i]n general, the Agency prefers to rely on analyses of data, rather than general defaults. When data are available for a sensitive lifestage, they should be used directly to evaluate risks for that chemical and that lifestage on a case-by-case basis” (EPA 2004). EPA’s rationale for applying ADAFs in its *Draft Toxicological Review of Hexavalent Chromium* (EPA 2010) is that it has explicitly designated Cr(VI)’s carcinogenic MOA as mutagenic. A measure of the scientific uncertainty surrounding this issue is the fact that the New Jersey Department of Environmental Protection (NJDEP), which developed the oral slope factor for Cr(VI) adopted in EPA’s draft *Toxicological Review*, did not consider the evidence that Cr(VI) is carcinogenic via a mutagenic MOA to be definitive, and hence did not apply ADAFs (Stern 2009).

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. 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). Under certain circumstances this additional exposure might be expected to result in an increased response. With an increased focus on assessing impacts of toxicants on children (U.S. Congress, 1996), the U.S. EPA explored the use of protocols similar to that employed by Borneff et al., which included perinatal exposure of animals (U.S. EPA, 1996). They concluded, "quantitatively, perinatal carcinogenicity dosing may or may not result in higher tumor incidence than standard dosing." For 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.

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).

Comments Regarding the Carcinogenic Mode of Action of Cr(VI) in Drinking Water

Data Gaps Associated with the NTP Two-Year Bioassay for Cr(VI) in Drinking Water

Both the 2009 and 2010 draft PHGs for carcinogenic effects were based on the incidence of adenomas + carcinomas in the small intestine of male mice (driven by increased incidence of adenomas in the duodenum) in a two-year bioassay (NTP 2008). No increased tumor incidence was observed at the lowest dose administered in this study, 5 mg Cr(VI)/L, which is nearly 200 times higher than the 95th percentile concentration of Cr(VI) detected in California drinking water (excluding non-detects) (26 µg/L) (Thompson *et al.* 2011), 83,333 times higher than the

2009 draft PHG, and 250,000 times higher than the 2010 draft PHG. Tumors occurred only at dose levels that (1) are extremely high compared to conceivable human exposure levels, and (2) probably chronically overwhelmed most if not all physiological protective mechanisms.

Although the NTP bioassay was “well conducted,” it is widely recognized that such high-dose protocols leave important data gaps regarding MOA and dose extrapolation (*e.g.*, Meijers *et al.* 1997; Gold *et al.* 1998; Ennever and Lave 2003; Gaylor 2005; Knight *et al.* 2006a&b; NAS 2007). 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 concentration of Cr(VI) does not constitute proof that humans exposed to much lower concentrations are at increased risk.

As a result, protocols like the two-year bioassay are being supplemented by and/or replaced by a new generation of toxicity testing strategies designed to be enlightening with regard to MOA and more directly relevant to human biology and human exposures (*e.g.*, NAS 2007; Collins *et al.* 2008; Hartung 2009). Regarding current initiatives in toxicity testing, the NTP states (<http://ntp.niehs.nih.gov/?objectid=720163BA-BDB7-CEBA-F282B5977D9A571E>),

The last decade of the 20th century and the turn of the 21st century have produced dramatic technological advances in molecular biology and computer science. During this period, scientists have increasingly identified critical cellular and molecular events (mechanisms) that lead to adverse responses to toxicants. The NTP recognizes that over the next decade the expanding knowledge of the physiological, biochemical, and molecular basis of disease will lead to improvements in our ability to predict the toxicological impact of environmental agents. As a focal point within the federal government for providing information about potentially hazardous agents, the NTP seeks to take advantage of these advances and identify and incorporate more mechanistic approaches into its toxicology assessments.

The Cr(VI) MOA Research Project was designed to use such testing strategies to fill data gaps and support regulatory decision making. Lines of investigation and preliminary results are discussed in more detail below.

OEHHA’s Evaluation of MOA and Human Relevance for Cr(VI) in Drinking Water is Incomplete and Inadequate

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. There is also no reference to EPA’s draft *Framework for Determining a Mutagenic Mode of Action for Carcinogenicity* (EPA 2007).

In the section entitled “Mechanism of Genotoxicity and Carcinogenicity,” which changed little between OEHHA’s 2009 and 2010 drafts, it is correctly acknowledged that “[a]lthough 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” (OEHHA 2010, page 38). Further, OEHHA acknowledges that while “...there is substantial evidence of DNA [deoxyribonucleic acid] damage following oral exposure to Cr VI..., it is not known if this would occur at environmental exposure levels” (OEHHA 2010, page 73). Although OEHHA’s rationale for using the LNT was not explicitly stated, some insight is provided by the response to a comment by Dr. Leonard Bjeldanes of the University of California at Berkeley regarding an earlier (and higher) proposed Cr(VI) PHG. Dr. Bjeldanes commented,

[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.

OEHHA’s response to Dr. Bjeldanes was, “[f]or 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. The fallacy of uncritically assuming that positive results 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):

- Self-sufficiency in growth signals
- Insensitivity to growth-inhibitory signals

- Evasion of apoptosis
- Unlimited replicative potential
- Sustained angiogenesis
- Tissue invasion and metastasis

Each of the hallmarks represents “the successful breaching of an anticancer defense mechanism hardwired into cells and tissues” (Hanahan and Weinberg 2000). As noted by Jarabek *et al.* (2009), these characteristics can be acquired through either genetic changes (mutations) or epigenetic changes (transcriptional or translational changes at the level of DNA, ribonucleic acid, or protein), or both. Based on this new understanding, many scientists have concluded that the LNT model of chemical carcinogenesis is not valid for non-genotoxic carcinogens, and also for some known genotoxic chemicals (*e.g.*, Wilson 1997; Parry *et al.* 2000; Bolt 2003; Bolt *et al.* 2004a&b; Slikker *et al.* 2004; Rietjens and Alink 2006; Holsapple and Wallace 2008; Swenberg *et al.* 2008; Williams 2008; Bailey *et al.* 2009; Calabrese 2009; Jarabek *et al.* 2009; Johnson *et al.* 2009; Kirsch-Volders *et al.* 2009; Pottenger and Gollapudi 2009).

The new understanding has also been incorporated in regulatory guidance. As explained by the EPA (2005a),

Special attention is important when the data support a nonlinear mode of action but there is also a suggestion of mutagenicity. 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.

This concept is further clarified in EPA’s draft *Framework for Determining a Mutagenic Mode of Action for Carcinogenicity* (EPA 2007):

*...[I]n assessing evidence for a mutagenic MOA for cancer, there are a couple of important considerations: (1) when (in relationship to other key events) the mutation occurs among the events that lead to cancer; and (2) whether the action of the carcinogen as a mutagen is a key event in its carcinogenic process. For a mutagenic MOA for cancer, mutagenicity is an obligatory early action, i.e., generally a very early key event for the MOA, of the chemical (or its metabolite). This is contrasted with other MOAs wherein mutations are acquired subsequent to other key events (*e.g.*, cytotoxicity, regenerative proliferation). Consequently, for a mutagenic MOA for carcinogenesis, the chemical is expected to*

interact with DNA early in the process and produce changes in the DNA that are heritable (page 8).

It is noted that not all carcinogenic chemicals that are capable of interacting with DNA will have a mutagenic MOA for cancer (emphasis in original) (page 9).

Summary of Peer Reviewers' Comments on the Accuracy of Information Presented on the MOA in the 2009 Draft Carcinogenic PHG for Hexavalent Chromium

The first charge question to the five peer reviewers of the 2009 draft (in which the proposed PHG was 0.06 µg/L) solicited their comment on the “accuracy of the information presented on...mode(s) of action...” (<http://www.oehha.ca.gov/water/phg/chrom092010.html>). Dr. William Shotyk, who is not a toxicologist, did not address this question, but pointed out that,

...[T]he PHG value (60 parts per trillion) is below the values we have found for contemporary snow in southern Ontario (an important source of water to our streams and lakes) and generally below the values for total dissolved Cr in the streams and lakes of the Kawagama Lake watershed from rural Ontario (ca. 3 h driving N of Toronto).

...[T]he PHG value (60 parts per trillion) is significantly below the value I have found (200 ppt) for tap water from the city of London, ONT.

...[T]he PHG value presented here is low, relative to surface water and tap water, even when those samples are collected, handled, and measured using clean lab methods.

The three-fold reduction in the 2010 draft PHG renders it lower still compared to natural waters, and therefore less scientifically credible and defensible.

Dr. Mitchell Cohen noted that “...no determination as to the accuracy of an MOA ‘section’ can be rendered (as there is no formal MOA portion in the document)...” (page 2). Dr. Sharada Balakrishnan did not directly address the question, but assumed, like OEHHA but contrary to current EPA and international guidance, that the existing evidence of Cr(VI) genotoxicity suffices to demonstrate a mutagenic MOA for carcinogenesis. Dr. Elizabeth Snow also did not directly address the question, but observed that although Cr(VI) is genotoxic, evidence for epigenetic modes of action exist as well.

The only peer reviewer who critically addressed the charge question regarding MOA was Dr. Toby Rossman, who stated,

“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).

The assumption is that Cr(VI) in drinking water has a mutagenic MOA with no threshold. This is not valid for the following reasons:

- 1. 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 dose range (i.e. it has a threshold).*
- 2. 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.*

Dr. Rossman’s comments are consistent with those previously made by other peer reviewers, DTSC, and by CMTA and other public commenters. The peer (and public) review process is critical to insure sound scientific basis for public policy decisions. Thus, to develop a credible PHG, OEHHA must perform a complete and balanced review of the literature, and address the substantive issues raised by reviewers.

Overview of Proposed MOAs for the Oral Carcinogenicity of Cr(VI)

Although OEHHA did not provide an MOA analysis in the 2010 PHG document, three hypothesized MOAs for Cr(VI) carcinogenicity via the oral exposure route have been published (McCarroll *et al.* 2010; EPA 2010; Thompson *et al.* 2011). As indicated in Figure 1, they differ in the number of proposed key events, and, more importantly, in their sequence. The processes proposed by the EPA scientists McCarroll *et al.* (2010) and in EPA’s draft *Toxicological Review of Hexavalent Chromium* (EPA 2010) are (not surprisingly) essentially the same, positing that Cr(VI)-induced mutagenesis precedes cell proliferation and tumor formation. In contrast, the MOA proposed by Thompson *et al.* (2011) posits that cell proliferation is initiated in response to chronic oxidative stress and inflammation, and that tumor development evolves as a result of chronic irritation and regenerative hyperplasia that occur when physiological defenses are overwhelmed at portals of entry. This MOA would exhibit a non-linear dose-response because below a critical amount of Cr(VI) exposure, cellular damage and the resultant proliferative response with increased of tumor initiation would not occur.

This contrast between the two proposed MOAs highlights a critical question: is tumorigenesis driven by (1) mutagenesis, which theoretically lacks a threshold but may exhibit a practical threshold imposed by extra-cellular reduction processes as well as intracellular detoxification mechanisms, (2) regenerative hyperplasia and inflammation of chronically injured tissue, which occurs only above a threshold, or (3) some combination of these effects?

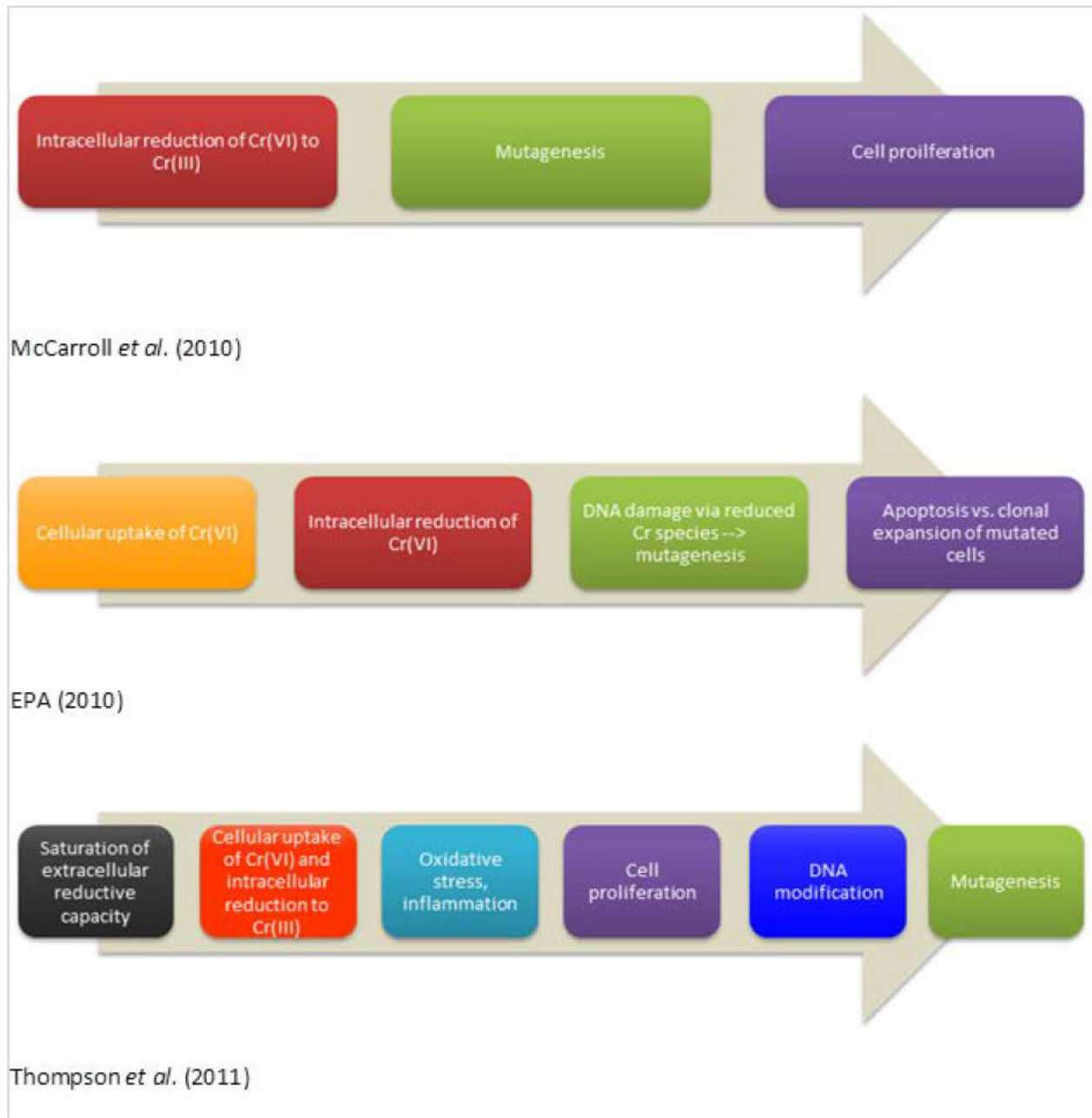


Figure 1. Proposed Oral Carcinogenic Modes of Action for Cr(VI)

Important New Information Pending from the Cr(VI) MOA Research Project

As indicated in Table 1, the Cr(VI) MOA Research Project comprises four multifaceted studies: (1) a comprehensive examination of the genomic changes that precede tumor formation; (2) biochemical investigations to evaluate mutations, genotoxicity and other potential key events in the MOA; (3) *in vitro* high-content imaging studies to characterize the potential species differences in tumor formation between rodents and humans; and (4) pharmacokinetic studies to determine the comparative gastrointestinal reductive capacity in mice, rats, and humans, and develop the data supporting a PBPK model, the approach that EPA prefers for extrapolating cancer findings between species and from the high doses administered to experimental rodents to the much lower levels relevant for humans exposure. The studies are being conducted by a highly qualified team of experts, including the Southern Research Institute (which conducted the NTP study), academic experts from Michigan State University, George Washington University Medical Center, and University of Cincinnati Medical Center, and experts in risk assessment and pharmacokinetic modeling (ToxStrategies, Inc. and Summit Toxicology, Inc.).

The overall goal of this research is to support scientifically defensible assessment of human health risks posed by oral exposures to Cr(VI). All of the results will be peer-reviewed by the independent SAB convened by TERA, and will be published in the peer-reviewed scientific literature. Preliminary results will be presented at the 2011 Society of Toxicology meeting in March, with publication of all results in the scientific literature anticipated by the summer of 2011. Clearly, such data are not only essential to extrapolating the results of high-dose animal studies like the two-year NTP bioassay to humans, but also consistent with national and international regulatory initiatives to improve the predictive capability of toxicological testing.

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.

Saturation of Upper Gastrointestinal Reductive Capacity and Intestinal Cellular Uptake

The well-recognized capability of the gastrointestinal tract to reduce Cr(VI) to the relatively non-toxic trivalent form has important implications for the carcinogenicity of Cr(VI), as it necessarily implies a dispositional threshold for adverse effects. OEHHA stated that "...exhaustion of the capacity of saliva and gastric fluids to reduce Cr VI appears unlikely" at doses likely to be ingested by humans (page 10).

Table 1. Summary of Critical Questions/Data Gaps Regarding Proposed Key Events in Oral Carcinogenesis of Cr(VI), Targeted MOA Research Project Studies, and Preliminary Results

Proposed Key Event in GI Carcinogenesis	Critical Questions/Data Gaps	Cr(VI) MOA Research Project Studies	Preliminary Results
Saturation of upper GI extracellular reductive capacity	<ul style="list-style-type: none"> • What is the reductive capacity of the upper GI tract? • Is there a dose-dependent transition in kinetic behavior? • Are there meaningful species differences in extracellular reduction, cellular uptake, etc.? 	Develop PBPK model to describe Cr(VI) transport, reduction, and absorption in the GI tract: <ul style="list-style-type: none"> • Determine species-specific reductive capacities of stomach fluid <i>ex vivo</i> • Examine degree of passage to small intestine • Model Cr(VI) disposition in tissues • Extrapolate among species • Extrapolate from high to low doses 	Cr(VI) exposure alters solute carrier transporter gene expression differentially in rats and mice (Kim <i>et al.</i> 2011)
Cellular uptake of Cr(VI) by GI cells			
Oxidative stress and inflammation	<ul style="list-style-type: none"> • What are the temporal and biochemical characteristics of Cr(VI)-induced oxidative stress and inflammation in the GI tract? • What is the dose-response? 	<ul style="list-style-type: none"> • Biochemical analyses <ul style="list-style-type: none"> ○ Oxidative stress ○ Lipid peroxidation • Cytokine assays • Gene expression in GI epithelia 	Dose-dependent biochemical and histopathological changes in mice (Proctor <i>et al.</i> 2011)
Cell proliferation	<ul style="list-style-type: none"> • What is the mechanism of hyperplasia? • Are there species differences? • Can a NOEL be identified? 	Toxicogenomic analysis: <ul style="list-style-type: none"> • Histopathologic dose-response • Gene expression changes anchored to histopathology • Correlate data to determine cause of proliferation 	Differential expression of genes associated with cell cycle, lipid metabolism, immune response, and cancer correlate with histopathology in mouse duodenum > jejunum > oral mucosa (Zacharewski <i>et al.</i> 2011)

Proposed Key Event in GI Carcinogenesis	Critical Questions/Data Gaps	Cr(VI) MOA Research Project Studies	Preliminary Results
<p>DNA damage via reduced Cr species (direct and/or epigenetic)</p>	<ul style="list-style-type: none"> • What forms of DNA damage occur in target tissue? • Do tumors result from direct DNA damage, indirect DNA damage, proliferation pressure, or some combination of these actions? • Are there epigenetic changes? 	<p>Measurement of:</p> <ul style="list-style-type: none"> • Oxidative DNA damage • Cr bound to DNA • Changes in DNA and histone methylation status in target tissues • Changes in expression of mismatch repair (MMR) genes 	<ul style="list-style-type: none"> • No dose-dependent increase in 8-OH-dG in duodenum • No change in DNA-protein crosslinks • Dose-dependent increase in Cr-DNA binding in upper GI, with an apparent threshold in all tissues examined (O'Brien <i>et al.</i> 2011)
<p>Mutagenesis</p>	<ul style="list-style-type: none"> • Measures of DNA mutations in target tissue • Are there hot spots for Cr(VI)-induced mutation? • Do mutations occur early in the carcinogenic process, or only after sustained tissue injury? 	<p>Measurement of:</p> <ul style="list-style-type: none"> • <i>In vivo</i> mutation analysis of selected codons or exons in frequently mutated targets 	<ul style="list-style-type: none"> • Results pending

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. 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. However, it is important to note that although chromium concentrations were increased in non-target tissues at the high exposure levels used in the NTP bioassay, lower oral exposures did not result in measurable transport beyond the gastrointestinal tract (Sutherland *et al.* 2000; NTP 2007).

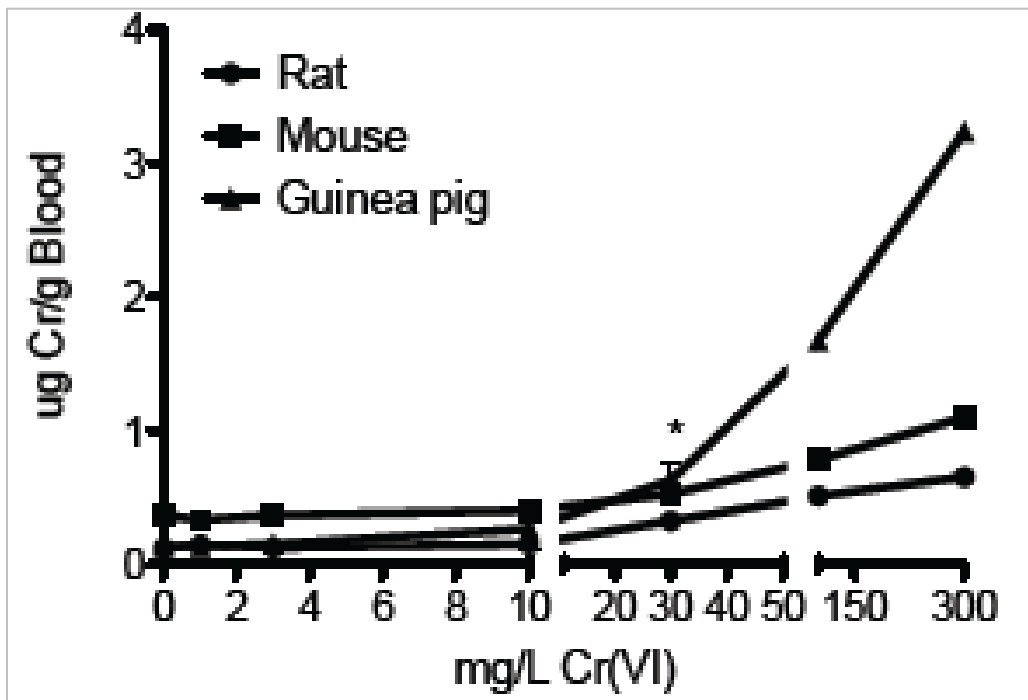


Figure 2. Concentration of Chromium in Blood of Rats, Mice, and Guinea Pigs Following 21 Days of Exposure to Cr(VI) in Drinking Water (data from NTP [2007]; figure from Thompson *et al.* [2011])

In response to the previously cited comment by Dr. Bjeldanes that a previous draft PHG "...does not consider the likelihood of a threshold for Cr(VI) biological activity...", OEHHA acknowledged "...the possibility of a dispositional threshold, [but] we have no quantitative basis for the extrapolation, and have felt constrained to utilize the standard cancer risk assessment methodology in this case" (OEHHA 2009b, page 9). The MOA Research Project PBPK studies will fill this critical data gap, providing the needed quantitative basis for extrapolation from high to low exposure levels, as well as characterizing important species differences in reductive capacity, and Cr(VI) dose and absorption in the small intestine at environmentally relevant concentrations. Preliminary results from one of these studies has shown that Cr(VI) exposure alters solute carrier transporter gene expression differentially in rats and mice (Kim *et al.* 2011).

Oxidative Stress and Inflammation

Cr(VI) is well known to be corrosive at high concentrations, and intracellular reduction of Cr(VI) results in generation of a variety of free radicals and reactive oxygen species that could cause oxidative stress and inflammation. In a recent review, Nickens *et al.* (2010) observed that "[t]he damage induced by Cr(VI) can lead to dysfunctional DNA replication and transcription, aberrant cell cycle checkpoints, dysregulated DNA repair mechanisms, microsatellite instability, inflammatory responses, and the disruption of key regulatory gene networks responsible for the balance of cell survival and cell death, which may all play an important role in Cr(VI) carcinogenesis."

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. Such an MOA would be expected to exhibit a threshold (*e.g.*, Cohen and Ellwein 1990; Clewell *et al.* 1995; Schulte-Hermann *et al.* 2000; Cohen *et al.* 2004; EPA 2005a). The fact that tumors were not observed in tissues outside the digestive tract, despite accumulating increased levels of chromium, reinforces the notion that repeated direct contact at high concentrations is a necessary condition of tumor formation. It is noteworthy in this regard that the incidence of liver adenomas in both male and female mice in the NTP study was statistically significantly reduced, despite the presence of elevated levels of chromium in this organ (NTP 2008).

The MOA Research Project studies are examining the dose-response and anatomical specificity of biochemical markers of oxidative stress and changes in gene expression anchored to histopathology. Preliminary results are indicative of graded, dose-dependent changes in cell physiology beginning with mild oxidation, followed by oxidative stress, cytoplasmic toxicity, villous blunting and regenerative cell proliferation (Proctor *et al.* 2011).

Cell Proliferation

Regarding the small intestine tumors in mice, the NTP (2008) stated,

Collectively, these lesions are considered consistent with regenerative hyperplasia secondary to previous epithelial cell injury.

While diffuse hyperplasia was significantly increased at all dose levels in male mouse duodenum (Figure 3; data from Stout *et al.* 2009), tumors were only increased (not significantly) at the two higher doses. In contrast, hyperplasia was not observed in rats at any doses. On this basis, Thompson *et al.* (2011) have suggested that the presence of hyperplasia both with and without tumor formation suggests that Cr(VI) induced cell proliferation independent of mutagenesis, and that cell proliferation is a necessary but not sufficient precursor for the development of intestinal neoplasms.

A primary objective of the MOA Research Project studies is to identify a no-observed-adverse-effects-level (NOAEL) for hyperplasia based on histopathologic and genomic data. Preliminary results indicate tissue-specific differential expression of genes associated with the cell cycle, lipid metabolism, immune response, and cancer (Zacharewski *et al.* 2011). O'Brien *et al.* (2011) observed histopathologic evidence of villous cytotoxicity, suggesting that cytotoxicity in the duodenum villi may contribute to Cr(VI) carcinogenicity through a wound-rehealing mechanism involving the crypt cells.

DNA Damage

The ability of Cr(VI) to damage DNA both directly and indirectly is well documented (*e.g.*, O'Brien *et al.* 2003; Salnikow and Zhitkovich 2008; McCarroll *et al.* 2010; Nickens *et al.* 2010; Thompson *et al.* 2011). Epigenetic effects relevant to carcinogenesis are also being actively investigated (*e.g.*, Salnikow and Zhitkovich 2008; Dai *et al.* 2009; Nickens *et al.* 2010; Thompson *et al.* 2011).

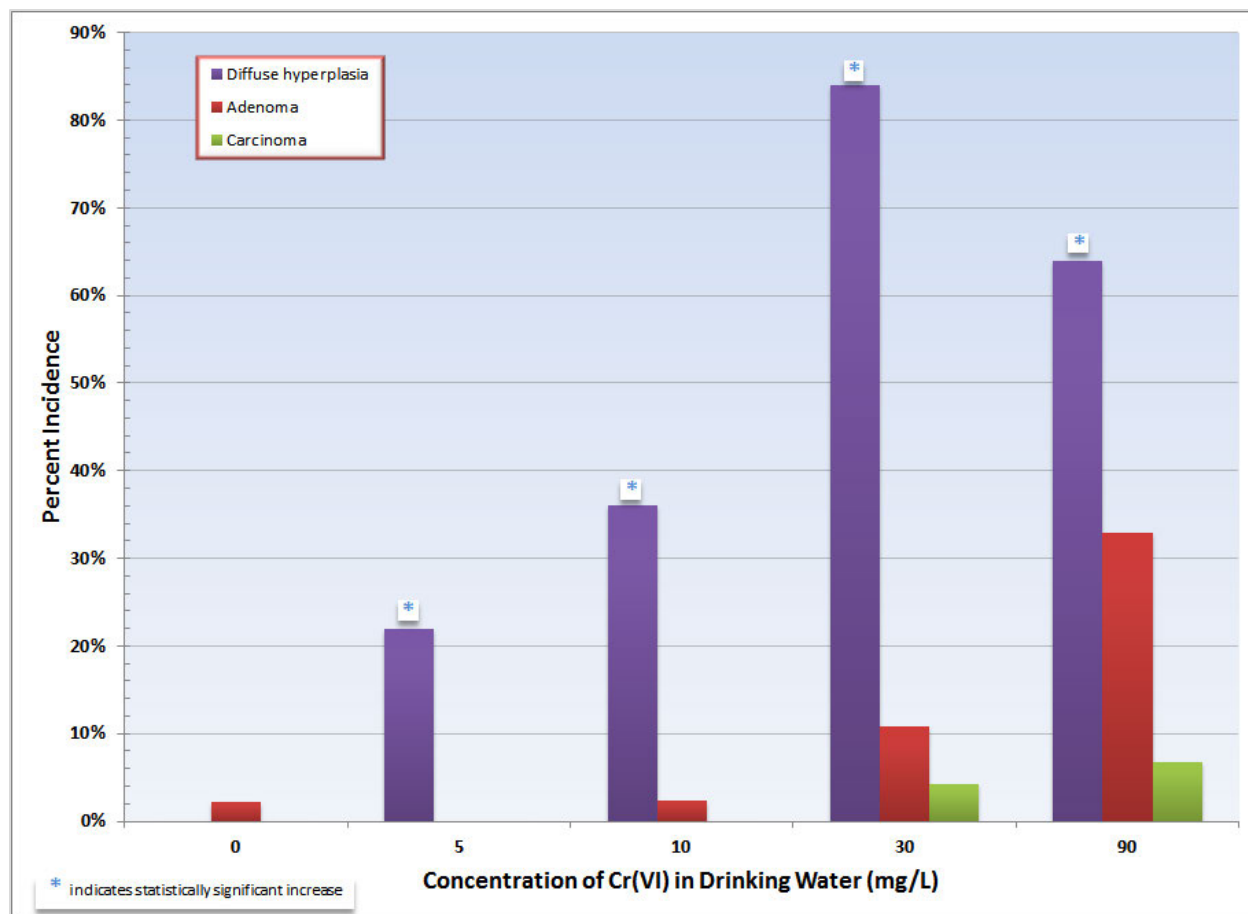


Figure 3. Dose-Response Gradients of Diffuse Hyperplasia, Adenoma, and Carcinoma in the Duodenum of Male Mice in the NTP Two-Year Cr(VI) Bioassay

In the only study to date that has examined oral Cr(VI) genotoxicity in a target tissue, De Flora *et al.* (2008) observed no significant changes in either oxidative DNA damage (8-hydroxy-2'-deoxyguanosine, 8-OH-dG) or DNA-protein cross-links in the forestomach, glandular stomach, or duodenum after nine months of exposure to drinking water containing 5 and 20 mg/L Cr(VI). The fact that direct exposure of mucosal scrapings from these tissues to Cr(VI) *in vitro* did exhibit significant increases in these parameters suggests that gastrointestinal reduction was sufficient to limit exposures *in vivo*.

The primary objectives of the MOA Research Project genotoxicity studies are to (1) characterize the nature and dose-response of DNA damage in target tissues, and (2) determine whether the tumors observed in the NTP study resulted from DNA damage, proliferative pressure, or some combination of these factors. Similar to the report by De Flora *et al.* (2008), preliminary data indicate no dose-dependent increase in oxidative DNA damage or DNA-protein cross-links in

mouse duodenum (O'Brien *et al.* 2011). An apparent threshold exists for chromium-DNA binding in all tissues examined (O'Brien *et al.* 2011). Studies in progress are examining chromosomal aberrations in crypt cells and immunostaining for key DNA damage/repair/cell cycle control proteins.

Mutagenesis

A critical unresolved question is when mutagenesis occurs in the sequence of events leading to tumor formation in target tissues. The MOA Research Project studies seek to determine whether mutagenesis or chronic injury and regenerative cell proliferation is the driving force in carcinogenesis. *In vivo* mutation analysis of selected codons in target tissues is being conducted, including hot spots for colon cancer and mutations in MMR genes.

Conclusion

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. 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. 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 scientifically defensible Cr(VI) PHGs for both carcinogenic and non-carcinogenic effects.

References

- Agency for Toxic Substances and Disease Registry (ATSDR) (1996). *Draft Toxicological Profile for Chromium*. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service.
- Bailey, G.S., Reddy, A.P., Pereira, C.B., Harttig, U., Baird, W., Spitsbergen, J.M., Hendricks, J.D., Orner, G.A., Williams, D.E., and Swenberg, J.A. (2009). Nonlinear cancer response at ultralow dose: A 40800-animal ED001 tumor and biomarker Study. *Chem Res Toxicol* **22**:1264-1276.
- Bolt, H.M. (2003). Genotoxicity -- threshold or not? Introduction of cases of industrial chemicals. *Toxicol Lett* **140-141**:43-51.
- Bolt, H.M., and Degen, G.H. (2004a). Human carcinogenic risk evaluation, Part II: Contributions of the EUROTOX Specialty Section for Carcinogenesis. *Toxicol Sci* **81**:3-6.
- Bolt, H.M., Foth, H., Hengstler, J. G., and Degen, G.H. (2004b). Carcinogenicity categorization of chemicals-new aspects to be considered in a European perspective. *Toxicol Lett* **151**:29-41.
- Boobis, A.R., Cohen, S.M., Dellarco, V., McGregor, D., Meek, M.E., Vickers, C., Willcocks, D., and Farland, W. (2006). IPCS framework for analyzing the relevance of a cancer mode of action for humans. *Crit Rev Toxicol* **36**:781-792.
- Boobis, A.R., Doe, J.E., Heinrich-Hirsch, B., Meek, M.E., Munn, S., Ruchirawat, M., Schlatter, J., Seed, J. and Vickers, C. (2008). IPCS framework for analyzing the relevance of a noncancer mode of action for humans. *Crit Rev Toxicol* **38**: 7-96.
- Calabrese, E.J. (2009). The road to linearity: why linearity at low doses became the basis for carcinogen risk assessment. *Arch Toxicol* **83**:203-225.
- California Environmental Protection Agency, Office of Environmental Health Hazard Assessment (OEHHA) (2009a). *Draft Public Health Goal for Hexavalent Chromium in Drinking Water*. Pesticide and Environmental Toxicology Branch. August 2009.
- ___ (2009b). *Draft Responses to Major Comments on Technical Support Document Public Health Goal for Hexavalent Chromium in Drinking Water*. Pesticide and Environmental Toxicology Branch. September 2009.
- ___ (2009c). *Technical Support Document for Cancer Potency Factors: Methodologies for Derivation, Listing of Available Values, and Adjustments to Allow for Early Life Stage Exposures*. Air Toxicology and Epidemiology Branch. May 2009.
- ___ (2010). *Draft Public Health Goal for Hexavalent Chromium in Drinking Water*. Pesticide and Environmental Toxicology Branch. December 2010.
- Clewell, J.H, Gentry, P.R., Gearhart, J.M., Allen, B.C., Andersen, M.E. (1995). Considering pharmacokinetic and mechanistic information in cancer risk assessments for environmental contaminants: Examples with vinyl chloride and trichloroethylene. *Chemosphere* **31**:2561-2578.

- Cohen, S.M. and Ellwein, L.B. (1990). Cell proliferation in carcinogenesis. *Science* **249**, 1007-1011.
- Cohen, S.M., Klaunig, J., Meek, M.E., Hill, R.N., Pastoor, T., Lehman-McKeeman, L., Bucher, J., Longfellow, D. G., Seed, J., Dellarco, V., Fenner-Crisp, P., and Patton, D. (2004). Evaluating the human relevance of chemically induced animal tumors. *Toxicol Sci* **78**:181-186.
- Collins, F.S., Gray, G.M. and Bucher, J.R. (2008). Toxicology. Transforming environmental health protection. *Science* **319**:906-907.
- Dai, H., Liu, J., Malkas, L. H., Catalano, J., Alagharu, S. and Hickey, R. J. (2009). Chromium reduces the in vitro activity and fidelity of DNA replication mediated by the human cell DNA synthesome. *Toxicol Appl Pharmacol* **236**:154-165.
- Dearfield, K. L., and Moore, M. M. (2005). Use of genetic toxicology information for risk assessment. *Environ Mol Mutagen* **46**:236-245.
- De Flora, S., D'Agostini, F., Balansky, R., Micale, R., Baluce, B. and Izzotti, A. (2008). Lack of genotoxic effects in hematopoietic and gastrointestinal cells of mice receiving chromium(VI) with the drinking water. *Mutat Res* **659**:60-67.
- Ennever, F.K. and Lave, L.B. (2003). Implications of the lack of accuracy of the lifetime rodent bioassay for predicting human carcinogenicity. *Regul Toxicol Pharmacol* **38**:52-57.
- Gaylor, D.W. (2005). Are tumor incidence rates from chronic bioassays telling us what we need to know about carcinogens? *Regul Toxicol Pharmacol* **41**:128-33.
- Gold, L.S., Slone, T.H. and Ames, B.N. (1998). What do animal cancer tests tell us about human cancer risk?: Overview of analyses of the carcinogenic potency database. *Drug Metab Rev* **30**:359-404.
- Hanahan, D. and Weinberg, R. A. (2000). The hallmarks of cancer. *Cell* **100**:57-70.
- Hartung, T. (2009). A toxicology for the 21st century--mapping the road ahead. *Toxicol Sci* **109**:18-23.
- Holsapple, M.P., and Wallace, K.B. (2008). Dose response considerations in risk assessment--An overview of recent ILSI activities. *Toxicol Lett* **180**:85-92.
- Jarabek, A.M., Pottenger, L.H., Andrews, L.S., Casciano, D., Embry, M.R., Kim, J.H., Preston, R.J., Reddy, M.V., Schoeny, R., Shuker, D., Skare, J., Swenberg, J., Williams, G.M., and Zeiger, E. (2009). Creating context for the use of DNA adduct data in cancer risk assessment: I. Data organization. *Crit Rev Toxicol* **39**:659-678.
- Johnson, G.E., Doak, S.H., Griffiths, S.M., Quick, E.L., Skibinski, D.O., Zair, Z.M., and Jenkins, G. J. (2009). Non-linear dose-response of DNA-reactive genotoxins: Recommendations for data analysis. *Mutat Res* **678**:95-100.
- Kahn, H.D. and Stralka, K. (2009). Estimated daily average per capita water ingestion by child and adult age categories based on USDA's 1994-1996 and 1998 continuing survey of food intakes by individuals. *J Expo Sci Environ Epidemiol* **19**:396-404.

- Kim, S., Thompson, C.M., Kopec, A.K., Harris, M.A., and Zacharewski, T.R. (2011). Comparison of basal and CrVI-mediated solute carrier gene expression in rodent duodenal epithelium. Society of Toxicology 2011 Meeting abstract #1597.
- Kirsch-Volders, M., Gonzalez, L., Carmichael, P., and Kirkland, D. (2009). Risk assessment of genotoxic mutagens with thresholds: A brief introduction. *Mutat Res* **678**:72-75.
- Knight, A., Bailey, J. and Balcombe, J. (2006a). Animal carcinogenicity studies: 1. Poor human predictivity. *Altern Lab Anim* **34**:19-27.
- ___ (2006b). Animal carcinogenicity studies: 2. Obstacles to extrapolation of data to humans. *Altern Lab Anim* **34**:29-38.
- McCarroll, N., Keshava, N., Chen, J., Akerman, G., Kligerman, A. and Rinde, E. (2010). An evaluation of the mode of action framework for mutagenic carcinogens case study II: chromium (VI). *Environ Molec Mutagen* **51**:89-111.
- Meek, M.E., Bucher, J.R., Cohen, S.M., Dellarco, V., Hill, R.N., Lehman-MnKeeman, L.D., Longfellow, D.G., Pastoor, T., Seed, J., and Patton, D.E. (2003). A framework for human relevance analysis of information on carcinogenic modes of action. *Crit Rev Toxicol* **33**:591-653.
- Meijers, J.M. M., Swaen, G.M. H. and Bloemen, L.J.N. (1997). The predictive value of animal data in human cancer risk assessment. *Regul Toxicol Pharmacol* **25**:94-102.
- National Academies of Science (NAS) (2007). *Toxicity Testing in the 21st Century*. The National Academies Press.
- National Toxicology Program (NTP) (2007). NTP Technical Report on the Toxicity studies of Sodium Dichromate Dihydrate (CAS No. 7789-12-0) Administered in Drinking Water to Male and Female F34/N Rats and B6C3F1 Mice and Male BALB/c and am3-C57BL/6 Mice. *Toxic Rep Ser***72**:1-74. NIH Publication No. 07-5964.
- ___ (2008). NTP Technical Report on the Toxicology and Carcinogenesis Studies of Sodium Dichromate Dihydrate (CAS No. 7789-12-0) in F344/N Rats and B6C3F1 Mice (Drinking Water Studies). *Tox Rep Ser***546**:1-192. NIH Publication No. 08-5887.
- Nickens, K.P., Patierno, S.R. and Ceryak, S. (2010). Chromium genotoxicity: A double-edged sword. *Chem-Biol Interac* **188**:276-88.
- O'Brien, T.J., Ceryak, S. and Patierno, S.T. (2003). Complexities of chromium carcinogenesis: Role of cellular response, repair and recovery mechanisms. *Mutat Res* **533**:3-36.
- O'Brien, T.J., Ding, H., Hébert, C.D., May, R.D., Patierno, S.R. (2011). Genetic toxicity of hexavalent chromium in the gastrointestinal tract following oral exposure. Society of Toxicology 2011 Meeting abstract #918.
- Parry, J. M., Jenkins, G. J., Haddad, F., Bourner, R., and Parry, E. M. (2000). In vitro and in vivo extrapolations of genotoxin exposures: consideration of factors which influence dose-response thresholds. *Mutat Res* **464**:53-63.

- Pottenger, L. H., Bus, J. S., and Gollapudi, B. B. (2007). Genetic toxicity assessment: employing the best science for human safety evaluation part VI: when salt and sugar and vegetables are positive, how can genotoxicity data serve to inform risk assessment? *Toxicol Sci* 98:327-331.
- Pottenger, L.H., and Gollapudi, B.B. (2009). A case for a new paradigm in genetic toxicology testing. *Mutat Res* 678:148-151.
- Proctor, D., Haws, L., Thompson, C. and Harris, M. (2011). Use of mode of action and pharmacokinetics to inform the cancer risk assessment of ingested Cr(VI): A case study. Society of Toxicology 2011 Meeting abstract #921.
- Rietjens, I. M. C. M., and Alink, G. M. (2006). Future of toxicology -- Low-dose toxicology and risk-benefit analysis. *Chem Res Toxicol* 19:977-981.
- Salnikow, K. and Zhitkovich, A. (2008). Genetic and epigenetic mechanisms in metal carcinogenesis and cocarcinogenesis: nickel, arsenic, and chromium. *Chem Res Toxicol* 21:28-44.
- Schulte-Hermann, R., Grasl-Kraupp, B., Bursch, W. (2000). Dose-response and threshold effects in cytotoxicity and apoptosis. *Mutat Res* 464:13-18.
- Seed, J., Carney, E.W., Corley, R.A., Crofton, K.M., DeSesso, J.M., Foster, P.M., Kavlock, R., Kimmel, G., Klaunig, J., Meek, M.E., Preston, R.J., Slikker, W., Jr., Tabacova, S., Williams, G.M., Wiltse, J., Zoeller, R.T., Fenner-Crisp, P. and Patton, D.E. (2005). Overview: Using mode of action and life stage information to evaluate the human relevance of animal toxicity data. *Crit Rev Toxicol* 35:664-72.
- Slikker, W., Jr., Andersen, M.E., Bogdanffy, M.S., Bus, J. S., Cohen, S.D., Conolly, R.B., David, R.M., Doerrler, N.G., Dorman, D.C., Gaylor, D.W., Hattis, D., Rogers, J.M., Woodrow Setzer, R., Swenberg, J.A., and Wallace, K. (2004). Dose-dependent transitions in mechanisms of toxicity. *Toxicol Appl Pharmacol* 201:203-225.
- Sonich-Mullin, C., Fielder, R., Wiltse, J., Baetcke, K., Dempsey, J., Fenner-Crisp, P., Grant, D., Hartley, M., Knaap, A., Kroese, D., Mangelsdorf, I., Meek, E., Rice, J.M., and Younes, M. (2001). IPCS conceptual framework for evaluating a MOA for chemical carcinogenesis. *Reg Toxicol Pharmacol* 34:146–152.
- Stern, A. (2009). *Derivation of Ingestion-Based Soil Remediation Criterion for Cr⁺⁶ Based on the NTP Chronic Bioassay Data for Sodium Dichromate Dihydrate*. Prepared for the Risk Assessment Subgroup of the NJDEP Chromium Workgroup.
- Stout, M.D., Herbert, R.A., Kissling, G.E., Collins, B.J., Travlos, G.S., Witt, K.L., Melnick, R.L., Abdo, K.M., Malarkey, D.E. and Hooth, M.J. (2009). Hexavalent chromium is carcinogenic to F344/N rats and B6C3F1 mice after chronic oral exposure. *Environ Health Perspect* 117:716-2
- Sutherland, J., Zhitkovich, A., Kluz, T. and Costa, M. (2000). Rats retain chromium in tissues following chronic ingestion of drinking water containing hexavalent chromium. *Biol Trace Element Res* 74:41-53.

- Swenberg, J.A., Fryar-Tita, E., Jeong, Y.C., Boysen, G., Starr, T., Walker, V.E., and Albertini, R.J. (2008). Biomarkers in toxicology and risk assessment: informing critical dose-response relationships. *Chem Res Toxicol* **21**:253-265.
- Thompson, C.M., Haws, L.C., Harris, M.A., Gatto, N.M. and Proctor, D.M. (2011). Application of the U.S. EPA mode of action Framework for purposes of guiding future research: a case study involving the oral carcinogenicity of hexavalent chromium. *Tox Sci* **119**:20-40.
- U.S. Environmental Protection Agency (EPA) (2004). EPA Response to the SAB Review Panel's Recommendations on the *Draft Supplemental Guidance for Assessing Cancer Susceptibility from Early-Life Exposure to Carcinogens*.
- ___ (2005a). *Guidelines for Carcinogen Risk Assessment*. EPA/630/P-03/001F. Risk Assessment Forum.
- ___ (2005b). *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens*. EPA/630/R-03/003F. Risk Assessment Forum.
- ___ (2007). *Framework for Determining a Mutagenic Mode of Action for Carcinogenicity*. External peer review draft, September 2007. EPA/R-07/002-A. Risk Assessment Forum Technical Panel on Mutagenic Mode of Action.
- ___ (2008). *Child-Specific Exposure Factors Handbook*. EPA/600/R-06/096F. Office of Research and Development, National Center for Environmental Assessment. Washington, D.C.
- ___ (2009). *Exposure Factors Handbook*. 2009 Update. External Review Draft. EPA/600/R-09/052A. Office of Research and Development, National Center for Environmental Assessment. Washington, D.C.
- ___ (2010). *Draft Toxicological Review of Hexavalent Chromium (CAS No. 18540-29-9)*. EPA/635/R-10/004A.
- ___ Regions 3, 6, and 9. (November 2010 update). Regional Screening Levels for Chemical Contaminants at Superfund Sites. <http://www.epa.gov/reg3hwmd/risk/human/rb-concentration-table/index.htm>.
- Williams, G.M. (2008). Application of mode-of-action considerations in human cancer risk assessment. *Toxicol Lett* **180**:75-80.
- Wilson, J. D. (1997). So carcinogens have thresholds: How do we decide what exposure levels should be considered safe? *Risk Anal* **17**:1-3.
- Zacharewski, T.R., Kopec, A.K., Forgacs, A.L., Kim, S., Grimes, S.D., Hébert, C. D. (2011). Toxicogenomic analysis of Cr(VI) effects on intestinal and oral epithelium. Society of Toxicology 2011 Meeting abstract #917.