November 30, 2011

VIA ELECTRONIC MAIL

Ms. Fran Kammerer
Office of Environmental Health Hazard Assessment
P.O. Box 4010
Sacramento, CA 95812-4010

Re: Proposed Amendment to Section 25707(b), Route of Exposure (Sept. 16, 2011)

Dear Ms. Kammerer:

The American Chemistry Council's Hexavalent Chromium [Cr(VI)] Panel (ACC) appreciates the opportunity to comment on OEHHA's Proposed Amendment to Section 25707(b) of the California Code of Regulations (Sept. 16, 2011) (Proposed Amendment). ACC strives to ensure appropriate product stewardship, and, as part of its mission address important science and public policy issues related to the chemical industry, including OEHHA's Proposed Amendment.

OEHHA is proposing to remove hexavalent chromium [Cr(VI)] from the list of five chemicals that present no significant risk of cancer by the route of ingestion. OEHHA's sole basis for this proposed rule change is the Public Health Goal (PHG), which was released on July 27, 2011. The PHG is significantly flawed and cannot be used to support a departure from the existing "no significant risk of cancer" exemption. The attached technical comments (Attachment A) show that OEHHA's assumption in the final PHG of a mutagenic mode of action (MOA) and use of linear extrapolation from the risk at high doses to a one-in-a-million risk level at a dose close to zero is not supported by the best available science. Importantly, new research sponsored by ACC supports a non-mutagenic MOA and indicates a threshold for effects.

OEHHA recognized the importance of these studies and the likelihood that these research findings would directly impact and change the final PHG in its response to comments:

> OEHHA acknowledges that new studies may alter a PHG, such as this statement 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."[1]

Because the MOA and pharmacokinetics (PK) data are likely to change the PHG, the PHG is not definitive support for removing Cr(VI) from the Section 25707(b) list.

Title 27 of the California Code of Regulations Sec. 25707(a) provides that "[w]here scientifically valid absorption studies conducted according to generally accepted standards demonstrate that absorption of a chemical through a specific route of exposure can be reasonably anticipated to present no significant risk of cancer at levels of exposure not in excess of current regulatory standards, the lead agency may identify the chemical as presenting no significant risk by that route of exposure."

OEHHA included Cr(VI) on the list of chemicals that "present no significant risk of cancer by the route of ingestion" in Section 25707(b) in 1990 after it determined that "the available data suggest that the cancer risk from ingestion of these listed substances is minimal, principally due to the poor absorption of these substances across the intestinal mucosa and into the blood stream of those who may ingest them."[2] In addition, the Agency concluded that "because many of these substances occur in nature, there is difficulty in identifying them, and there is difficulty in taking action to remove them, particularly when their presence may be widespread."[3] Finally, OEHHA determined that "current regulation of these substances, where it exists, together with the evidence of poor absorption, should adequately protect the public from any significant risk of cancer from such chemicals by the route of ingestion."[4]

A. The PHG is not based on the best available science and is not an adequate basis for the Proposed Amendment.

California Health and Safety Code Section 116365(c)(1) requires OEHHA to use the best available science in developing a PHG:

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[1] OEHHA, Responses to Major Comments on Technical Support Document, Public Health Goal for Hexavalent Chromium (Cr VI) in Drinking Water (July 2011), at 38. Available at http://oehha.ca.gov/water/phg/pdf/Cr6PHGresp072911.pdf.

[2] Final Statement of Reasons, 22 California Code of Regulations (Aug. 1990), at 3. Available at http://oehha.ca.gov/prop65/law/pdf_zip/12707%20Route%20of%20Exposure%20Ni%20FSOR%20August%201990.pdf.

[3] Id. at 4.

[4] Id.

> The Office of Environmental Health Hazard Assessment shall prepare and publish an assessment of the risks to public health posed by each contaminant for which the department proposes a primary drinking water standard. The risk assessment shall be prepared 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. (emphasis added)

As explained in detail in Attachment A, OEHHA did not use the "most current principles, practices, and methods" or best available science in developing the PHG. OEHHA's use of the linear low-dose extrapolation (no-threshold) method is based on a finding that Cr(VI) causes cancer in mice that a single review article speculated was due to a mutagenic mode of action (MOA).[5] Other review papers, also available to OEHHA, that offered alternative MOA action were not considered.[6,7] Additionally, in developing the PHG, OEHHA did not consider newly developed data presented to them directly by researchers that indicate that Cr(VI) does not act by a mutagenic MOA and shows a threshold for toxic effects in mice, which are precursor or sentinel effects for cancer as defined by USEPA.[8] The new research indicates that Cr(VI) carcinogenesis in mouse small intestine occurs by a non-mutagenic MOA that involves oxidative stress, tissue damage and compensatory growth. In its PHG and response to comments, OEHHA acknowledged the imminent release of these MOA and PK data and the statutory requirement under the California Health and Safety Code Section 116365(e) to consider new data.

Moreover, California Health and Safety Code Section 116365(c)(1)(D) provides that "if adequate scientific evidence demonstrates that a safe dose response threshold for a contaminant exists, then the public health goal should be set at that threshold." The MOA research studies demonstrate a threshold in mice between 100 ppb and 1000 ppb. At the current Federal maximum contaminant level (MCL) for total chromium in drinking water of 100

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[5] 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 Mol Mutagen* 51, 89-111, 10.1002/em.20525.

[6] 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. *Toxicol Sci* 119, 20-40, kfq320 [pii] 10.1093/toxsci/kfq320. Available at http://toxsci.oxfordjournals.org/content/119/1/20.full.pdf+html?sid=896b4c7e-b535-4884-aa79-261ecc512de8.

[7] Nickens, K. P., Patierno, S. R. and Ceryak, S. 2010. Chromium genotoxicity: A double-edged sword. *Chem Biol Interact* 188, 276-88, S0009-2797(10)00304-2 [pii], 10.1016/j.cbi.2010.04.018.

[8] U.S. EPA. 2005. *Guidelines for carcinogen risk assessment*, EPA/630/P-03/001F. Risk Assessment Forum: U.S. Environmental Protection Agency, Washington, D.C.

ppb, no effects are observed. Since humans appear to be less sensitive to Cr(VI) effects than mice, these new research findings suggest that a threshold exists.

B. The available evidence indicates that Cr(VI) is poorly absorbed across the intestinal mucosa and into the blood stream.

The PHG does not present any scientific evidence that Cr(VI) is better absorbed across the intestinal mucosa and into the blood stream than OEHHA determined in 1990. In fact, as explained in Section 4.3.5 of Attachment A, Cr(VI) is reduced to trivalent chromium [Cr(III)], which is poorly absorbed by the intestinal epithelium, in the stomach and gastrointestinal tract of rodents and humans. This chemical reduction limits the amount of Cr(VI) available for uptake.

In the PHG, OEHHA dismissed the experimental evidence for rapid reduction of Cr(VI) to [Cr(III)]. Moreover, OEHHA did not consider the results of the ACC-sponsored research studies. As part of these research studies, rates of reduction of Cr(VI) to Cr(III) are being measured in the stomach fluid of rats, mice and humans. The human stomach fluid includes samples from fed and fasted individuals as well as those taking proton pump inhibitors for acid control. Preliminary data from this study indicate that human stomach fluid reduces Cr(VI) at a much higher rate than does rodent stomach fluid. These data will be used to develop a physiologically-based pharmacokinetic (PBPK) model for Cr(VI) in both rodents and humans.

C. Cr(VI) is naturally-occurring and widespread in drinking water in California.

Low levels of Cr(VI) exist naturally in groundwater.[9,10,11] For example, in California, approximately one-third of drinking water supply sources contain Cr(VI) at low levels, with 65% of detectable concentrations ranging from 1 to 5 ppb.[12] These low concentrations occur naturally in groundwater related to certain geological deposits.[9, 10]

The presence of naturally occurring levels of Cr(VI) in California groundwater will result in significant and costly modifications by water purveyors to treat natural source waters used

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[9] Gonzalez, A.R., Ndundu, K., Flegal, A.R. 2005. Natural occurrence of hexavalent chromium in the Aromas Red Sands Aquifer, California. *Environ. Sci. Technol*. 39, 5505–11.

[10] Ball, J.W., Izbicki, J.A. 2004. Occurrence of hexavalent chromium in ground water in the western part of the Mojave Desert, California. *Appl. Geochem.* 19, 1123-35.

[11] Oze, C., Bird, D.K., Fendorf, S. 2007. Genesis of hexavalent chromium from natural sources in soil and groundwater. *Proc. Nat. Acad. Sci.* 104, 6544–49.

[12] California Department of Public Health. 2010. Chromium-6 in Drinking Water Sources: Sampling Results. Sacramento, CA. Available at: http://www.cdph.ca.gov/certlic/drinkingwater/Pages/Chromium6sampling.aspx

for drinking water. Costs will include research and development for new and innovative Cr(VI) treatment technologies, capital costs associated with new water treatment processes, and operations and maintenance associated with on-going water treatment operations. Some water purveyors in California have begun pilot projects for removing Cr(VI) from source water with costs already running several million dollars, just for pilot tests.[13]

D. Current regulation of Cr(VI) adequately protects the public from any significant risk of cancer by the route of ingestion.

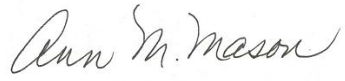
As discussed in Section 5 of Attachment A, the ACC-sponsored study indicates a threshold for effects in mice somewhere between 100 ppb and 1000 ppb. No effects were observed at the current Federal MCL of 100 ppb. The current California MCL for total chromium is 50 ppb. Since humans appear to be even less sensitive to Cr(VI) effects than mice, the current Federal and state MCLs adequately protect the public from any significant risk of cancer by the route of ingestion.

* * *

Based on the foregoing, the PHG is not an adequate basis for the proposed amendment to remove hexavalent chromium [Cr(VI)] from the list of five chemicals that present no significant risk of cancer by the route of ingestion. The best available science continues to support OEHHA's 1990 determination that Cr(VI) presents no significant risk of cancer by the route of ingestion.

ACC is available to meet with the appropriate OEHHA staff to discuss these comments in detail. If you have questions, please contact me at 202.249.6704 or at ann_mason@americanchemistry.com.

Sincerely,



Ann M. Mason
Senior Director
American Chemistry Council

[13] Long, J.T. 2010. State pushes costly new water standard. *CalWatchdog*. February. Available at: http://www.calwatchdog.com/2010/02/10/new-state-pushes-costly-new-water-standard/.

Attachments:

Attachment A Technical Comments In Support of the Comments of the American Chemistry Council Hexavalent Chromium Panel In Opposition to the Proposed Amendment to Proposition 65

Attachment B Timeline of the Development of the California Public Health Goal (PHG) for Hexavalent Chromium [Cr(VI)]

ATTACHMENT A

Technical Comments In Support of the Comments of the American Chemistry Council Hexavalent Chromium Panel In Opposition to the Proposed Amendment to Proposition 65

Ted Simon, Ph.D., DABT
Ted Simon LLC
http://www.tedsimon-toxicology.com
ted@tedsimon-toxicology.com

Executive Summary

These technical comments provide a critique of OEHHA's final public health goal (PHG) technical support document. OEHHA has used the PHG support document as the basis for a proposed rule change under Proposition 65. The proposed rule change would remove hexavalent chromium [Cr(VI)] from the list of five chemicals that present no significant risk of cancer by the route of ingestion.

These technical comments show that OEHHA's assumption in the final PHG of a mutagenic MOA and use of linear extrapolation from the risk at high doses to a one-in-a-million risk level at a dose close to zero is not supported by the best available science.

- OEHHA did not consider the best available science as the basis for the PHG.
    - OEHHA's use of the linear low-dose extrapolation (no-threshold) method is based on an incorrect assertion that Cr(VI) causes cancer in mice by a mutagenic mode of action (MOA) based on the suggested interpretation in a single review article (McCarroll et al., 2010). Other review papers (Thompson et al., 2011a; Nickens et al., 2010) suggesting alternative MOA were available to OEHHA, but either were not considered or ignored since these papers were not listed in the PHG or its reference list.
    - OEHHA dismissed the experimental evidence for rapid reduction of Cr(VI) to trivalent chromium [Cr(III)] in the stomach and gastrointestinal tract of rodents and humans. This reduction markedly reduces the amount of Cr(VI) available for absorption by animal or human cells. Reduction of Cr(VI) to Cr(III) results in a non-linear dose-response curve with a threshold.
    - OEHHA ignored newly developed data that researchers directly presented to key OEHHA scientists that indicate that Cr(VI) does not act by a mutagenic MOA and shows a threshold for toxic effects in mice, which are precursor or sentinel effects for cancer as defined by U.S. EPA (U.S. EPA, 2005a).
- The new research indicates that Cr(VI) carcinogenesis in mouse small intestine occurs by a non-mutagenic MOA that involves oxidative stress, tissue damage and compensatory growth (Thompson et al., 2011b).

- The research demonstrates a threshold in mice between 100 ppb and 1000 ppb. At the current Federal maximum contaminant level for total chromium in drinking water of 100 ppb, no effects are observed.
- Since humans appear to be less sensitive to Cr(VI) effects than mice, these findings suggest that a threshold exists.

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1 Introduction

These technical comments provide a critique of OEHHA's final public health goal (PHG) technical support document. OEHHA has used the PHG support document as the basis for a proposed rule change under Proposition 65. The proposed rule change would remove hexavalent chromium [Cr(VI)] from the list of five chemicals that present no significant risk of cancer by the route of ingestion.

These technical comments show that OEHHA's assumption in the final PHG of a mutagenic MOA and use of linear extrapolation from the risk at high doses to a one-in-a-million risk level at a dose close to zero is not supported by the best available science.

Section 2 (The Role of Thresholds in Risk Assessment) summarizes the role of thresholds in risk assessment and the scientific origin of the linear no-threshold hypothesis and its application to environmental risk assessment. The outcome of a risk assessment using the linear no-threshold assumption will be contrasted to that based on a demonstrated threshold.

Section 3 (Mode of Action, Mutagenicity and Genotoxicity) considers the knowledge of the mode of action (MOA) by which a chemical may cause cancer and the use of this knowledge in risk assessment. Such consideration is the guiding principle of U.S. EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a). This section also discusses the difference between DNA damage and DNA mutation and outlines the steps used by U.S. EPA to establish a mutagenic MOA.

Section 4 (What is the MOA of Cr(VI) Carcinogenesis in Rodents?) describes the MOA based on the science available in late July 2011 (when OEHHA released the final PHG) and discusses the results of the MOA Research Program sponsored by the American Chemistry Council (ACC)

Section 5 (Does a Threshold Exist for the Carcinogenic Effects of Cr(VI) in Rodents?) discusses the data supporting a threshold for adverse effects of Cr(VI).

Finally, Section 6 (Is this MOA Relevant to Humans?) discusses the human relevance of the proposed MOA.

2 The Role of Thresholds in Risk Assessment

Since 1977, the practice of cancer risk assessment has been to use, as a default, linear low dose extrapolation to predict cancer risk at very low doses based on the observed cancer incidence at the often very high doses given to experimental

animals in cancer bioassays. Low dose extrapolation is necessitated by the limited number of animals in a standard cancer bioassay, typically 50 animals per dose group, which is not sufficient to detect risks at very low levels of risk such as one-in-a-million. The primary, and precautionary, assumption of linear low dose extrapolation is that even a single molecule, an amount too small to measure, could still pose a small but quantifiable risk of cancer. This assumption has become known as the linear no-threshold hypothesis.

2.1 The Low Dose Linear Assumption Ignores Biology

Although the low-dose linear approach has been used for regulation of carcinogenic chemicals for many years as a matter of precaution given the lack of understanding of the cancer pathobiology, this approach ignores the fact of biological thresholds and endogenous production. Continued existence for any organism is a matter of maintaining homeostasis in the face of the continuing stresses of life. Living organisms are routinely exposed to genotoxic or DNA-damaging chemicals from both endogenous and exogenous sources. DNA damage may be a potentially mutagenic effect. A major development of cancer research in the past 20 years has been the discovery of significant levels of DNA damage and subsequent DNA repair. Most of this DNA damage arises from endogenous cellular sources debunking the notion that the human genome is pristine in the absence of exposure to environmental carcinogens (Marnett, 2000; Sablina et al., 2005).

Evolutionarily successful organisms have developed redundant systems that provide both immediate capacity and fail-safe mechanisms to deal with many different stressors. DNA repair is just such a fail-safe mechanism (Swenberg et al., 1987). However, these capacities are finite, and when one or more of these capacities are exceeded, a departure from homeostasis, usually in the form of disease or death, occurs. The fact of biological thresholds is implicit in Paracelsus' dictum that "the dose makes the poison" (Amdur et al., 1991).

Specific aspects of an organism's biology determine these thresholds for adverse health effects. For example, humans have a finite capacity for the activity of various enzymes. Reduced glucuronidation capacity in some individuals may be expressed phenotypically as hyperbilirubinemia and jaundice or fatal kernicterus in some cases (Maruo et al., 2005). Polymorphisms in the various subtypes of glutathione-S-transferase (GST) convey increased susceptibility to the genotoxic effects of tobacco smoke (Norppa, 2004; Sato, 1988; Townsend and Kew, 2003).

Low dose linearity is based on the erroneous notion that normal physiological processes reflect a pathological continuum toward cancer or other

diseases and that exposure to a stressor will act in an additive fashion with these ongoing pathological processes (Crump et al., 1976). Guyton et al. (2008) foster this myth by stating there is a lack of broad consideration of the causes of human disease and the potential for chemical exposure to act additively with background exposures and endogenous disease processes. This thinking ignores the need of all organisms to maintain homeostasis and that their ability to do so was shaped by evolution. Holsapple and Wallace (2008) discuss the relationship of dose response to homeostasis and conclude that advances in the biological sciences, including systems biology, high-throughput screening and genomics will affect not only the understanding of biological thresholds but also the distinction between adaptive and adverse responses.

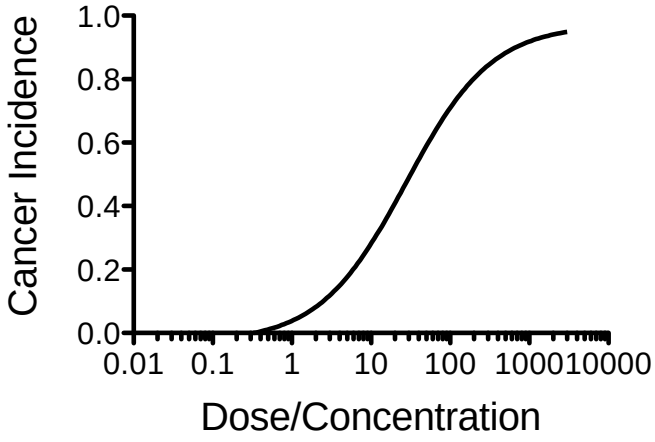
It is commonly believed that the dose response of a genotoxic carcinogen does not exhibit a threshold because many scientists incorrectly equate genotoxicity with mutagenicity. This incorrect assumption will be discussed in Section 3 of these technical comments. The absence of a threshold cannot be proven, as it is impossible to prove a negative. There is, however, ample experimental evidence that even carcinogens known to have mutagenic MOAs exhibit dose thresholds (Deal et al., 1989; Williams et al., 1996, 1999; Fukushima et al., 2002; Tsuda et al., 2003; Waddell et al., 2006). These observed thresholds are likely due to DNA repair and other compensatory processes.

2.2 Using the Linear No-Threshold Hypothesis Alters the Results of a Risk Assessment

An important perspective on the no-threshold hypothesis is revealed when comparing outcomes of risk assessments using linear low-dose extrapolation versus the threshold approach. Critical to this point is the importance of selection of the dose range used in a cancer study. The cancer bioassays are commonly conducted in either rats or mice by the National Toxicology Program (NTP). The study design uses about fifty animals per dose group, and the animals are dosed, maintained and observed for two years. These are extremely expensive studies and thus, the choice of the experimental dose range is critical in obtaining information from the study that will be useful in risk assessment.

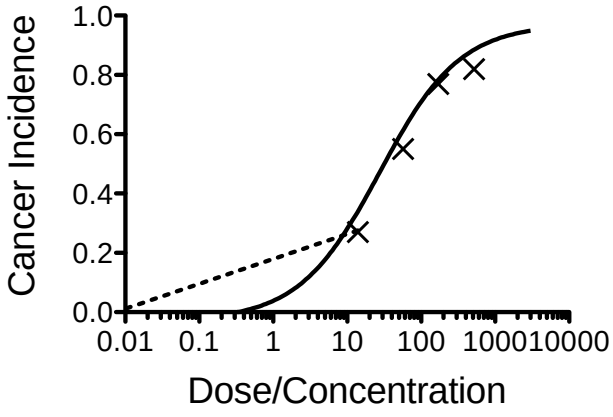
Figure 1 shows an idealized dose response curve using arbitrarily chosen units for dose. This idealized dose response curve is S-shaped or sigmoidal., the most common type of dose response curve that occurs when a chemical interacts with one or more biological molecules; this is the case with Cr(VI) (Davey et al., 1976; Amdur et al., 1991).

Figure 1. Idealized dose response curve



Assuming doses/concentrations for use in a cancer bioassay of 0, 14, 57, 170, and 520, Figure 2 shows hypothetical results of a cancer bioassay superimposed on the idealized dose response relationship knowledge. Note that with this set of concentrations, one does not observe a threshold dose, and the risk assessment would be forced to use linear low-dose extrapolation from an observed cancer response back through zero, as shown by the dotted line in Figure 2, below.

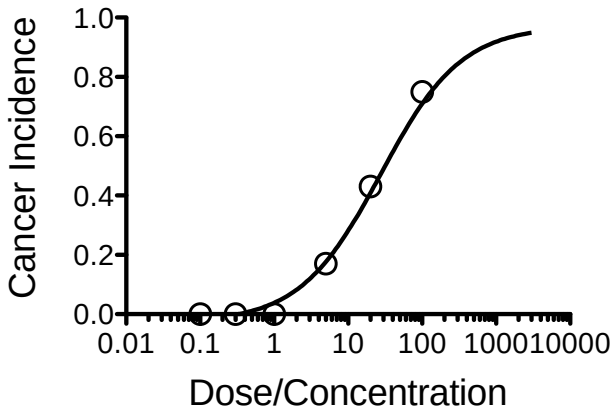
Figure 2. Idealized dose response curve and a high dose range



Now, assuming doses/concentrations of 0, 0.1, 0.3, 1, 5, 20 and 100, the doses and the responses are shown in Figure 3 also superimposed on the idealized dose

response relationship. Note that this second set of doses includes the low dose region, three doses show a zero response and there is clear indication of a threshold.

Figure 3. Idealized dose response curve and a lower dose range



Based on the foregoing, it is clear that both the biology of a carcinogenic chemical <u>and</u> the design of the experiment or bioassay used to assess its carcinogenicity can alter the understanding of the MOA and the choice of whether to use linear low-dose extrapolation.

3 Mode of Action, Mutagenicity and Genotoxicity

Mode of action (MOA) provides the link between biology and risk assessment. By definition, the mode of action of a carcinogen is "a sequence of key events and processes, starting with interaction of the agent with a cell, proceeding through operational and anatomical changes, and resulting in cancer formation" (U.S. EPA, 2005a).

In 1965, Sir Austin Bradford Hill delivered a landmark lecture to Britain's Royal Society of Medicine titled *The Environment and Disease: Association of Causation?* (Hill, 1965). Hill listed nine considerations or viewpoints for causation:

1) Strength of Association;
   The disease is associated with the exposure to a significant extent, as measured by valid statistical tests.
2) Consistency;
   If a relationship is causal, we would expect to find it consistently in different studies, in different populations, and in a range of circumstances.

3) Specificity;
   Associations between a specific disease and a specific exposure may provide evidence for causation.
4) Temporality;
   The exposure must always precede the disease.
5) Dose-Response or Biological Gradient;
   If a dose-response relationship is present, it is strong evidence for a causal relationship.
6) Biological Plausibility;
   The putative agent produces effects in a manner that is plausible, given the currently accepted understanding of biological processes, and a theoretical basis exists for making an association between an agent and a disease.
7) Coherence;
   The association should be compatible with existing theory and knowledge.
8) Experiment; and
   Can the condition be altered or prevented by an appropriate experimental regimen?
9) Alternate Explanations
   What else could cause the disease?

The Hill considerations for causation are used not only to judge epidemiological evidence but also to consider the MOA of various carcinogens and to assess the human relevance of effects seen in animals based on the MOA (U.S. EPA, 2005a, 2005b, 2007; Meek et al., 2003; Sonich-Mullin et al., 2001; Seed et al., 2005; Cohen et al., 2004; Boobis et al., 2008).

3.1 Use of Mode of Action in Risk Assessment

When there is <u>no</u> knowledge about the MOA, the default choice of most environmental regulators is to use linear low-dose extrapolation through zero dose based on the linear no-threshold hypothesis. In the case of chemicals for which there is limited or no information on MOA, the use of the linear no-threshold hypothesis may be the most defensible choice for regulation. However, the lack of information and the precautionary position of regulators may serve to spur the collection of data to reduce uncertainty about the MOA. In the case of hexavalent chromium, this focused data collection effort is exactly what has occurred.

U.S. EPA's *Guidelines for Carcinogen Risk Assessment* is a landmark document that suggests the use of the threshold assumption and non-linear extrapolation as follows:

> *A nonlinear approach should be selected when there are sufficient data to ascertain the mode of action and conclude that it is not linear at low doses <u>and</u> the agent does not demonstrate mutagenic or other activity*

consistent with linearity at low doses. 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. (U.S. EPA, 2005a, p. 3-22 (emphasis in original))

The use of data on biological mechanisms to inform risk assessment both increases biological plausibility and diminishes the need to use default assumptions (Haber et al., 2001; Holsapple and Wallace, 2008). Gori (1992) laments that linear modeling of cancer incidence has become the regulatory norm because the reductionist hypotheses inherent in the models render them scientifically inadequate given the fact of individual differences in phenotype, exposure history, and defense or repair capacities. Conolly et al. (2005) suggest that a stronger experimental and regulatory focus on biological mechanisms would enable greater flexibility in the regulation of carcinogens without compromising human health.

In summary, the problems inherent in both interspecies extrapolation and low dose extrapolation will not be solved by biological knowledge alone, by epidemiology alone, or by statistics alone. The best risk assessments are those that manage to incorporate the most relevant knowledge. U.S. EPA's *Guidelines for Carcinogen Risk Assessment* strongly emphasize the importance of understanding MOA in terms of basic biological processes (U.S. EPA, 2005a).

3.2 Determination of a Mutagenic Mode of Action

In the *Guidelines for Carcinogen Risk Assessment*, U.S. EPA states:

Furthermore, carcinogenesis involves a complex series and interplay of events that alter the signals a cell receives from its extracellular environment, thereby promoting uncontrolled growth. Many, but not all, mutagens are carcinogens, and some, but not all, agents that induce cell proliferation lead to tumor development. Thus, understanding the range of key steps in the carcinogenic process upon which an agent might act is essential for evaluating its mode of action. Determination of carcinogens that are operating by a mutagenic mode of action, for example, entails evaluation of in vivo or in vitro short-term testing results for genetic endpoints, metabolic profiles, physicochemical properties, and structure-activity relationship (SAR) analyses in a weight-of-evidence approach (Dearfield et al., 1991; U.S. EPA, 1986b;

> *Waters et al., 1999). Key data for a mutagenic mode of action may be evidence that the carcinogen or a metabolite is DNA-reactive and/or has the ability to bind to DNA. Also, mutagenic carcinogens usually produce positive effects in multiple test systems for different genetic endpoints, particularly gene mutations and structural chromosome aberrations, and in tests performed in vivo which generally are supported by positive tests in vitro.* (U.S. EPA 2005a, pp. 2-30 to 2-31)

In the *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens*, U.S. EPA states:

> *Key data for a mutagenic mode of action may be evidence that the carcinogen or a metabolite is DNA reactive and/or has the ability to bind to DNA.* (U.S. EPA 2005b, p. 8)

and highlights the importance of integrating newer methods including genomics and in vitro testing to inform the MOA:

> *Determination of chemicals that are operating by a mutagenic mode of action entails evaluation of test results for genetic endpoints, metabolic profiles, physicochemical properties, and structure-activity analyses in a weight-of-evidence approach (Waters et al., 1999). Established protocols are used to generate the data (Cimino, 2001; OECD, 1998; U.S. EPA, 2002b); however, it is recognized that newer methods and technologies such as those arising from genomics can provide useful data and insights to a mutagenic mode of action. Carcinogens acting through a mutagenic mode of action generally interact with DNA and can produce such effects as DNA adducts and/or breakage. Carcinogens with a mutagenic mode of action often produce positive effects in multiple test systems for different genetic endpoints, particularly gene mutations and structural chromosome aberrations, and in tests performed in vivo, which generally are supported by those performed in vitro. This mode of action is addressed in more detail in Section 2.3.5 of EPA's cancer guidelines (U.S. EPA, 2005).* (U.S. EPA, 2005b. p. 31 n.5)

In 2007, U.S. EPA released the *Draft Framework for Determining a Mutagenic Mode of Action for Carcinogenicity* (U.S. EPA, 2007), which provides a method for deciding whether a chemical carcinogen acted by a mutagenic MOA. The primary issue addressed in this document is that DNA damage and mutation <u>are not the same thing</u>. This document provides a framework and a systematic weight-of-

evidence approach for deciding whether a chemical causes cancer via a mutagenic mode of action. U.S. EPA provides the hallmark of a mutagenic MOA:

> *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****.* (U.S. EPA 2007, p. 8 (emphasis added))

U.S. EPA states that there is no "default MOA" and that understanding the MOA of any carcinogen using weight-of evidence approach is a necessary part of any risk assessment:

> ***The determination that a chemical carcinogen can induce mutation in one of a number of mutation assays is not sufficient to conclude that it causes specific tumors by a mutagenic MOA or that mutation is the only key event in the pathway to tumor induction.*** *It is important to use the tools of weight of evidence and the Cancer Guidelines MOA framework in the determination of a mutagenic MOA. It should also be noted that there is no "default MOA."* (U.S. EPA 2007, p. 8, (emphasis in original))

Each day, naturally occurring oxidants in the body modify about 20,000 bases of DNA in each cell in the human body (Sablina et al., 2005). Consequently, DNA repair enzymes have evolved to ameliorate this potentially large amount of DNA damage. DNA damage is recognized by repair enzymes and repaired. If the damage is not repaired, the cell will be targeted for programmed cell death, a process that removes damaged cells. Programmed cell death, also called apoptosis, is controlled by the action of a number of genes (Tomko et al., 2006).

A mutation can occur when DNA is incorrectly repaired, resulting in a change in the DNA sequence on both DNA strands. DNA repair is necessary for mutation. In contrast to DNA damage (e.g., adducts and strand breaks), a mutation is a change in the DNA sequence that cannot be recognized by the DNA repair enzymes. Recent information about DNA repair, the production of reactive oxygen species (ROS), control of the cell cycle, and apoptosis suggests that these factors, rather than DNA damage, play a much greater role in carcinogenicity (Hanahan and Weinberg, 2011).

Consequently, mechanisms have evolved to ameliorate this potentially large amount of DNA damage.

4 What is the MOA of Cr(VI) Carcinogenesis in Rodents?

4.1 The NTP Bioassay

4.1.1 Results of the NTP Bioassay

NTP generally collects data on gross and microscopic histopathological changes. What this means is that a team of laboratory pathologists examines the tissue with the naked eye immediately following sacrifice and also looks at thinly sliced tissue sections that have been fixed and stained on a glass slide for observation through a microscope. It should be noted that these visual determinations are subjective, and often, expert pathology working groups are convened to sort out any inconsistencies in determinations (e.g., Maronpot et al., 1986; Goodman and Sauer, 1992; Romo et al., 2011). In addition, NTP collects data on easily measured clinical parameters in blood and, if possible, tissue concentrations of the chemical under study or its metabolites in selected tissues. Measurements of biochemical changes, DNA modifications, or alterations in the activity of genes are generally not conducted by NTP, even though these data would provide helpful information on the MOA.

Epithelial Hyperplasia: a form of regenerative growth of the cells lining the gut or epithelium in response to injury. Epithelial cells are organized for absorption or secretion and fit tightly against one another like "bricks and mortar". Hyperplasia may be localized, called "focal," or wide-spread, called "diffuse."

Histiocytic cellular infiltration: the migration into a tissue of white blood cells, generally with immune function. Histiocytic cellular infiltration may occur in organs, such as the liver or intestine, or in lymph nodes.

In the two-year Cr(VI) drinking water bioassay, groups of 50 male and female rats were exposed to 0, 14.3, 57.3, 172 and 516 mg/L SDD, equivalent to 0, 5, 20, 60 and 180 mg/L Cr(VI), in drinking water for two years. Five groups of 50 male mice each were exposed for two years to 0, 14.3, 28.6, 85.7 and 257.4 mg/L SDD, equivalent to 0, 5, 10, 30 and 90 mg/L of Cr(VI) for two years. Five groups of 50 female mice each were exposed for two years to 0, 14.3, 57.3, 172 and 516 mg/L SDD, equivalent to 0, 5, 20, 60, and 180 mg/L Cr(VI).

In rats, NTP observed dose-dependent increases in oral tumors, histiocytic infiltration in the small intestine, liver and abdominal lymph nodes. The incidence of oral tumors in both male and female rats exposed to 516 mg/L was significantly

greater than in control animals. Histiocytic cellular infiltration occurred in the small intestine, and abdominal lymph nodes of male and female rats exposed to 57.3 mg/L SDD and greater. Diffuse epithelial hyperplasia was not observed in the mouth, stomach or intestines of rats.

In mice, NTP observed dose-dependent occurrences of small intestinal tumors, diffuse epithelial hyperplasia in the small intestine, and histiocytic cellular infiltration in the small intestine, liver and abdominal lymph nodes. Tumor incidence in the small intestine was significantly increased in males exposed to 85.7 and 257.4 mg/L and in females exposed to 172 and 516 mg/L.

4.1.2 Use of the NTP Bioassay in Risk Assessment

Section 2 highlights the importance of the shape of the dose response curve in linear extrapolation because the slope of the line drawn from a chosen level of response, also called a point of departure or POD, on the dose-response curve back to zero dose determines the cancer potency for the chemical. In fact, the cancer "slope" factor is the slope of that line and is expressed in units of risk per unit dose.

However, dose-response relationships are not likely to be linear. As noted above, biological processes such as DNA repair and both biochemical and metabolic detoxification of chemicals are also responsible for non-linear dose responses and observed thresholds.

OEHHA's policy is to find the best fitting empirical model to the dose-response data, use a point of departure of 10%, and draw a straight line from the lower 95% confidence limit on the dose producing a 10% response, known as the LED10, and 10% response back to the origin at zero dose and zero or background response. The plot below shows this procedure graphically using the hypothetical high dose data discussed in Section 2.

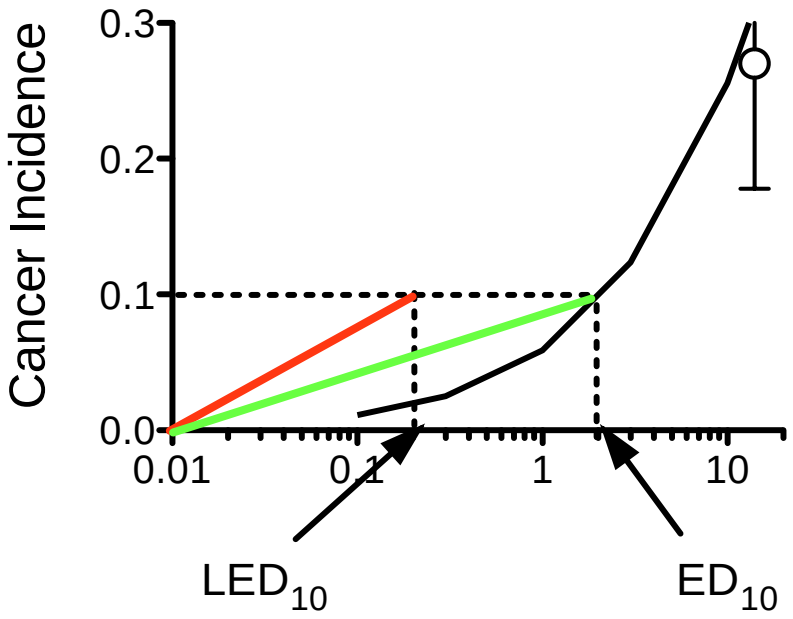


Figure 4. Understanding how linear low dose extrapolation works

In Figure 4 above, only the lower portion of the dose response curve is shown. The lowest dose chosen for the NTP study is shown along with an empirical dose-response curve fit to the data. The point marked "ED10" corresponds to the dose level associated with a 10% cancer response. The point marked "LED10" corresponds to the lower 95% confidence limit on this dose producing a 10% response. Three points are evident from this figure.

- The low dose extrapolation goes from a POD of 10% all the way down to 1-in-a-million risk. Hence, 1-in-a-million risk would correspond to a very, very low dose.

- From this graph, it is clear that linear extrapolation from a chosen point of departure to zero dose (green line) overestimates the slope and thus the risk in the low dose region.

- Using the 95% lower confidence level on the ED10 as the POD for linear extrapolation (red line) overestimates the risk to an even greater extent. Dr. Michael Kelner in his peer review comments on the 2009 draft PHG points out how the selection of the LED10 could overestimate risk (Kelner, 2009).

Initially, the NTP scientists chose water concentrations of 0, 62.5, 125 and 250 mg/L or ppm. Hence, the lowest dose initially proposed was over 1,000 times greater than the California MCL for total chromium of 0.05 mg/L. As noted in Attachment B, scientists at the 2002 NTP external review committee meeting urged NTP to add lower concentration groups in the range of 1-5 or 1-10 mg/L (1 mg/L is 20 times the California MCL value). The lowest concentration that was used in the NTP study was 14 mg/L, almost 300 times greater than the California MCL (Johnson, 2002).

The findings of the NTP bioassay raised many questions. Notably:

- Why did mice get tumors in the small intestine, but rats did not?
- Why do fewer tumors occur in mice in distal parts of the small intestine (jejeunum, ileum) than in the proximal part of the intestine (duodenum) as a function of dose?
- If Cr(VI) were acting via a mutagenic MOA, then why were no tumors present in the stomach or forestomach of either mice or rats; and why not in multiple tissues?
- Why were intestinal tumors only observed in animals experiencing prolonged hyperplasia of the intestinal epithelium?
- Is there a no effect level (NOEL) for intestinal hyperplasia in the mouse?
- 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?
- Are cancer observations in mice relevant to humans who are exposed at much lower levels?
- And finally, **what is the mode of action in mice and is it relevant to humans?**

In mice, diffuse epithelial hyperplasia appeared to be a precursor event to small intestinal tumor formation in mice. In rats, however, the oral cavity tumors were not preceded by any obvious pathology (Thompson et al., 2011a). In both species, the tumors occurred relatively late in the two year bioassay period.

4.2 Hypothesized MOA for Cr(VI) Carcinogenesis in Mice

Using the data from NTP (2008) and the general scientific literature, Thompson et al. (2011a) present a hypothesized mode of action shown below.

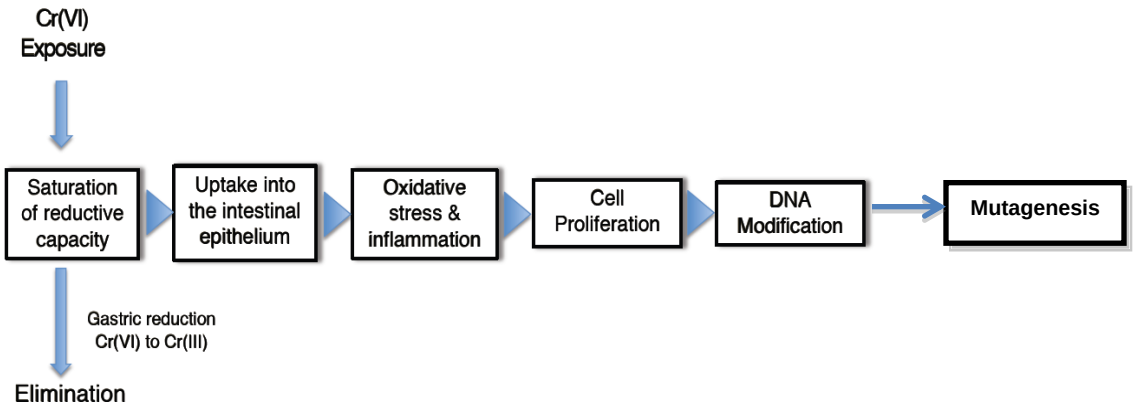


Figure 5. Mode of Action for Cr(VI) carcinogenesis is mouse small intestine proposed by Thompson et al. (2011a)

In the final PHG, OEHHA makes a determination that Cr(VI) acts via a mutagenic MOA; however, no weight of evidence approach was conducted and only a single literature review/interpretation paper (McCarroll et al., 2010) was used to support OEHHA's decision.

McCarroll et al. (2010) fail to consider much of the information about the MOA. In fact, the data from the MOA research program was not available when McCarroll et al. (2010) was published. Thompson et al. (2011a) was first available online without cost on October 14, 2010, well before the publication of the either the draft or final PHG. No references to either Thompson et al. (2011a) or Thompson et al. (2011b) can be found in either the draft or final PHG, indicating that the ACC-sponsored MOA research program was not considered.

In addition, OEHHA did not consider the work of Nickens et al. who state:

> *The transcriptional regulation of survival genes and the signaling pathways they control have proven to be critical in the survival of Cr(VI)-exposed cells. Taken together, survivors of Cr(VI) exposure harboring altered repair and survival signaling mechanisms may form the basis for the development of a population of neoplastic precursor cells, which may lead to tumor cell formation.* (Nickens et al., 2010)

Both the MOA presented by McCarroll et al. (2010) and Thompson et al. (2011a) involve mutagenesis. The difference is that McCarroll et al. (2010) assume the mutagenesis occurs as an early key event in the MOA whereas Thompson et al. (2010a) conclude that mutagenesis occurs late in the progression to cancer and is unlikely to be a pivotal key event.

4.3 Mutagenesis versus Genotoxicity

It is known that Cr(VI) can produce DNA damage, but DNA damage does not equate to mutagenesis. Damages are physical abnormalities in the DNA, such as single and double strand breaks, 8-hydroxydeoxyguanosine residues and chemical adducts. DNA damage can be recognized by repair enzymes and correctly repaired if redundant information, such as the undamaged sequence in the complementary DNA strand or in a homologous chromosome, is available for copying. In contrast to DNA damage, a mutation is a change in the base sequence of the DNA. A mutation cannot be recognized by enzymes once the base change is present in both DNA strands, and thus a mutation cannot be repaired. McCarroll et al. (2010) equate DNA damage with mutation – they are not the same thing.

In fact, DNA repair in response to damage induced by Cr(VI) appears necessary for mutagenesis. Dr. Anatoly Zhitkovich is a full professor in the Department of Molecular Pharmacology, Physiology and Biotechnology at the Brown University Medical School. He also was chair of the external peer review committee for U.S. EPA's Toxicological Review of Hexavalent Chromium in support of the IRIS risk assessment. Zhitkovich et al. (2005) state that "the spectrum of mutations observed in chromium-induced human lung tumors is more consistent with the mutator phenotype of cancer cells rather than reflecting the direct mutagenic activity of Cr(VI)." The DNA damage that McCarroll et al. (2010) equate with mutation is, in fact, genomic instability produced by Cr(VI). Disruption of the control of genomic stability results in a cascade of changes in the entire genome. Genomic instability manifests as microsatellite instability and chromosomal instability and leads to cancer in humans (Lengauer et al., 1998). Genomic instability has been observed in lung cancers of chromate workers and is produced by Cr(VI) in many systems (Hirose et al., 2002; Holmes et al., 2008). Table II in McCarroll et al., 2010, and the accompanying discussion provide many examples of genomic instability, but not mutation, produced by Cr(VI).

Dr. Toby Rossman, Director of Molecular Toxicology and Carcinogenesis at the NYU School of Medicine, served as a peer reviewer for the risk assessments supporting both the OEHHA PHG and U.S. EPA's ongoing Cr(VI) risk assessment. Her area of expertise is the mutagenic and non-mutagenic (also called epigenetic)

mechanisms of action of carcinogens. Dr. Rossman points out repeatedly that genotoxicity, or the propensity of a substance to produce DNA damage, is not the same as mutagenicity, the propensity of a substance to produce heritable mutations in mammalian cells:

> *Genotoxic is not the same as mutagenic, and sections 4.5 and 4.73 [of EPA's draft assessment] must be completely rewritten, as they consistently confuse these terms. Standard genotoxicity assays were not designed to inform specific modes of tumor induction. With the exception of mutagenesis, these other assays (non-mutagenic assays) do not measure heritable events, but rather measure evidence of DNA damage or its repair. Non-mutagenic assays include chromosome aberrations, micronuclei, comet assays, DNA lesion measurements, and DNA repair assays. These assays are useful for hazard identification or as biomarkers of exposure. They provide only supportive evidence that mutagenesis might be a MOA. DNA damage per se does not inform us about eventual heritable change, which is the true issue. Most (but not all) mutagens cause heritable changes in DNA sequences by causing damage to DNA (pre-mutagenic lesions) that is converted to mutation after cell division.* (Rossman, 2011, p. A-55)

Dr. Rossman also opines similarly in her peer review of OEHHA's 2009 draft PHG for Cr(VI):

> *There are a number of issues here, especially in the areas of genotoxicity and mode of action (MOA). These sections are very much out of date and inaccurate.*
>
> *The concept of "genotoxicity": This represents positive results in a group of assays that measure a number of very different things. On page 41, lumped together under "a wide range of DNA damage" are <u>DNA adducts, DNA-protein crosslinks, DNA-DNA crosslinks, DNA strand breaks, oxidized DNA bases</u>, chromosome aberrations, mutations, sister chromatid exchanges and micronuclei. Only some of these represent actual DNA damage (underlined). 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.*

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).

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., Chemico-Biological Interactions in press: doi:10.1016/j.cbi.2010.04.018; see also other references below). 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. These are important considerations for Cr(VI), since cells grown in the presence of Cr(VI) show selection for mismatch repair-deficient cells that are Cr(VI)-resistant (reviewed in Salnikow and Zhitkovich, Chem. Rev. Toxicol. 21:28-44, 2008). These cells are mutators (having a high spontaneous mutation rate) and show microsatellite instability, as do chromium-induced lung cancer cells (Takahashi et al., Mol. Carcinog. 281:150-158, 2005). 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. (Rossman, 2010, p. 2 (emphasis in original))

With regard to Cr(VI), Dr. David Berry, then senior toxicologist for the Human and Ecological Risk Division of the California Department of Toxic Substance Control (part of the California EPA), now with U.S. EPA Region 8 in Denver, writes in a memo dated October 23, 2008, that mutagenesis was unlikely to play a role in the carcinogenic MOA of Cr(VI). Dr. Berry writes:

It is clear the tumor development [in NTP (2008)] is related to local inflammation and hyperplasia in the target tissue. One candidate MOA concerns the chronic local inflammation induced by the chronic tissue damage inflicted by high-dose chromate and the role of reactive oxygen species. Since the NTP concluded that the lesions in the duodenum in mice were seen in concert with local regenerative hyperplasia, it appears that the highest dose induced overt tissue damage (in addition to the presence of chronic inflammation) and that the tumors arose as a

> *result of that damage. Given that the subchronic investigations revealed hyperplasia in the rat oral mucosa and in the mouse small intestine, the tumor response is very similar to the promotional response in epithelial cells induced by phorbol diesters. 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.* (Berry, 2008, pp. 5-6)

Dr. Berry also questions the default assumption of linearity:

> *Most regulatory guidance is based on 'scientific principles' that provide the foundation for that guidance. Situations can occur where strict adherence to default regulatory guidance may violate (or significantly depart from) the basic principle(s) that the guidance was supposed to support. 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.* (Berry, 2008, p. 2)

4.4 The MOA Research Studies Sponsored by the American Chemistry Council Cr(VI) Panel

Once the draft NTP bioassay report was released in May 2007, ToxStrategies, Inc., a scientific consulting company with headquarters in Texas, proposed a 90 day study in both mice and rats with collection of histopathological data, biochemical data and genomic data from affected tissues in both rats and mice. The study design proposed using the dose range used by NTP plus two lower doses. The ToxStrategies study was specifically intended to address the deficiencies in the NTP bioassay and to provide information needed to understand the MOA in terms of genomic and biochemical changes as well as tissue pathology.

To ensure the high quality of these studies, Toxicology Excellence in Risk Assessment (TERA) convened an expert panel to peer review the study protocol. Comments from these experts resulted in a revised protocol that was funded by the ACC. TERA continues to peer review the draft manuscripts from these research studies. All of the TERA comments from the initial protocol review and each

manuscript review are available at http://www.tera.org/Peer/Chromium/Chromium.htm.

The study of the MOA of Cr(VI) carcinogenesis in rodents begun in the first half of 2009. In this study, mice and rats were administered the same drinking water concentrations as in the NTP bioassay as well as two lower doses – 0.1 mg/L and 1.3 mg/L of Cr(VI) - for a period of 90 days. The purpose of using these lower doses was to obtain data in a concentration range that was more relevant to environmental exposures experienced by humans. The lowest dose of 0.1 mg/L is the same as current federal maximum contaminant level for total chromium, except that all chromium in the water consumed by the rats and mice was Cr(VI).

In addition to the use of environmentally relevant doses, the MOA study obtained data on biochemical and microscopic changes in the target tissues, data on DNA alterations, mutations, levels of chromium in tissues, and finally, data on changes in the activity of genes to assess whether the response of the tissues to Cr(VI) was similar in terms of gene expression to responses known to be associated with cancer formation. Initial results were presented in a symposium at the 2011 Society of Toxicology meeting in March. Results from the mice in the study have recently been published in the scientific literature and personnel at both U.S. EPA and OEHHA have been briefed on this material on several occasions during 2010 and 2011 (Thompson et al., 2011a, 2011b, 2011c).

Measurements of Key Events in the MOA

1) Oxidative Stress: Oxidative stress may be measured by the ratio of reduced to oxidized glutathione, a sulfur-containing molecule that combats oxidative stress. It may also be measured by the formation of oxidation products of proteins, called protein carbonyls, or of fats, called isoprostanes.

2) Inflammation: Inflammation may be measured by the occurrence of signaling molecules called cytokines in blood or tissue. These cytokines are the chemical signal by which a damaged tissue communicates with the immune systems. Interleukins are the best-known type of cytokine. Interleukin-1β and Tumor Necrosis Factor-α are two well-known pro-inflammatory cytokines.

3) Cell Proliferation: In the Cr(VI) study, cell proliferation was determined by histopathology, that is, examining the tissue through a microscope.

4) DNA Modification: DNA modification is measured by the presence of 8-hydroxydeoxyguanine (8-OHdG). Guanine is one of the building blocks of DNA and 8-OHdG is its oxidation product.

Richard Atwater, the Executive Director of the Southern California Water Committee, and other commenters noted the absence of consideration of MOA in the

draft PHG and chided OEHHA for not using the results of the ACC-sponsored MOA study:

> *While purporting to meet the requirement 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 the risk assessment. Data about the mode of action of Cr(VI) has been recently obtained 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.* (Atwater, 2011, p. 1)

As noted in Attachment B, in their peer review comments, the U.S. EPA peer reviewers also urged U.S. EPA to wait until the results of the MOA research program were available before finalizing the Toxicological Review (Consolidated Comments from the External Peer Review are available at http://cfpub.epa.gov/ncea/iris_drafts/recordisplay.cfm?deid=221433).

4.4.1 Biochemical and Inflammatory Changes in Mice and Rats

The results from the MOA research studies are published in Thompson, et al., 2011b and Thompson et al., 2011c. Other manuscripts are either in the peer review process or in development. All data are expected to be published in the peer review literature by early 2012. Below is a summary of the published results.

In female mice, a dose-dependent decrease in the reduced-to-oxidized glutathione ratio (GSH/GSSG) was observed at 60 mg/L or greater in the small intestine after 8 days. A similar decrease was observed after 90 days at 14 mg/L or greater. These changes indicate an oxidative stress response. In the oral mucosa, a decrease in this ratio was observed at 8 days at 170 mg/L or greater. This decrease was not observed at 90 days.

8-isoprostane was significantly increased in the duodenums of female mice, but only at the highest dose of 520 mg/L. No dose-dependent changes were observed in 8-isoprostane in the oral cavity.

In mice, protein carbonyls were generally elevated in higher dose groups but the values were not significantly different than controls.

In rats, no changes were observed in 8-isoprostane at any dose in either the duodenum or the oral cavity. Although GSH/GSSG was measured in rats, the data are not yet available.

Interleukin-1β (IL-1β) and tumor necrosis factor-α (TNFα) are cytokines that are involved in inflammation, cell proliferation and apoptosis. In mice, both IL-1β and TNFα both decrease in the duodenal epithelium with increasing Cr(VI) concentrations in drinking water. In rats, there did not appear to be any dose related changes in these cytokines.

4.4.2 DNA Modification and Gene Expression Changes in Mice and Rats Exposed to Cr(VI)

In mice, no increase in oxidative DNA damage measured by 8-hydroxydeoxyguanine (8-OHdG) was observed at any dose. This finding is consistent with that of De Flora et al. (2008). One possible reason for the inability to find oxidative DNA damage is that DNA repair processes may "ramp up" as part of an adaptation to chronic Cr(VI) administration. In rats, no dose-related changes in 8-OHdG were observed.

In mice, preliminary evaluation of toxicogenomic responses in the small intestine are consistent with increased oxidative stress. The genomic studies in rats are still ongoing. There are data that strongly suggest that Cr(VI) does not act by a mutagenic MOA. Using the considerations detailed on page 23 and 24 of U.S. EPA's 2007 *Framework for Determining a Mutagenic Mode of Action for Carcinogenicity*, a preliminary evaluation can be made. These considerations are restated below in italicized type-face with the results and observations about Cr(VI) in the mouse small intestine immediately following.

- *There is direct DNA reactivity.*
  Cr(VI) itself does not react directly with DNA; it is the short-lived intermediate valence species, Cr(V) and Cr(IV) that bind to DNA (Chiu et al., 2010);

- *DNA of the target cell or tissue is damaged.*
  No, oxidative DNA damage was not increased in any treatment group (Thompson et al., 2011b).

- *Mutation is an early event in the carcinogenesis process, rather than a by-product or observation at later stages.*
  Cancer did not occur in small intestines of mice until 450 days or later (NTP, 2008). If mutation was an early event in the carcinogenic process, the tumors would have occurred earlier.

- *The target cell/tissue is exposed to the ultimate DNA-reactive chemical (parent chemical or metabolite). A demonstrated pathway exists for the chemical to reach the target cell (or surrogate tissue) or, if the DNA reactive chemical is a*

metabolite, it has been observed to be produced in or distributed to the target or surrogate tissue.
At high doses, chromium accumulates in mouse small intestine in a dose-dependent fashion (Thompson et al., 2011b). However, no chromium accumulation was observed at the federal MCL of 100 ppb.

- *Termination of treatment does not reverse the carcinogenic effect; for example, in a recovery experiment in which the post-exposure observation time is purposely shorter than the normal expression time. In the parlance of the initiation-promotion testing protocols, the chemical is an initiator.*
  Data to respond to this factor are unavailable at this time.

- *Tumors are observed in multiple sites, in multiple species, and from multiple routes of exposure*.
  Tumors are observed in different sites in mice and rats from oral exposures (NTP, 2008). In humans, Cr(VI) is a known carcinogen by inhalation.

- *An increase in tumor-bearing animals, an increase in tumor multiplicity, or a decrease in time to tumors is observed after a short-term exposure to the chemical followed by exposure to tumor promoters.*
  Unknown. An initiation-promotion protocol has not been used with Cr(VI).

- *Tumor responses generally occur early in chronic studies (e.g., within 52 weeks).*
  Tumors did not occur until after 450 days (NTP, 2008).

- *Mutations by the chemical (or its metabolite) observed in genes that affect carcinogenesis (e.g., tumor suppressor p53, Rb) increase the weight of evidence. This does not refer to the general characterization of mutations found in tumors but rather to mutations that can be specifically associated with exposure to the chemical being assessed. At the time of this writing the ability to detect these rare mutational events is limited.*
  Data are unavailable at this time.

4.4.3 Chromium Levels in Tissues in Mice and Rats

Exposure to mice of 0.1 mg/L Cr(VI), corresponding to the federal standard for total chromium levels in water, did not result in an increase in chromium levels in any tissue, including the duodenum (Figure 8, next page). Cr levels in the duodenum were not significantly greater than control until the mice received 5 mg/L Cr(VI) (fifty-fold greater) in drinking water.

One finding of the NTP bioassay is lack of concordance of tumor sites between rats and mice. Thompson et al. (2011d) indicate that after 90 days of exposure, total chromium concentrations in the rat and mouse oral mucosae were

comparable, yet significant dose-dependent decreases in reduced-to-oxidized glutathione ratio (GSH/GSSG), a measure of oxidative stress, were observed only in rats. In the duodenum, changes in GSH/GSSG were only observed in mice. Serum and bone marrow levels of iron were reduced to a greater extent in rats than in mice. This suggests that anemia may possibly be a factor in the oral cancers observed in rats (Prime et al., 1983).

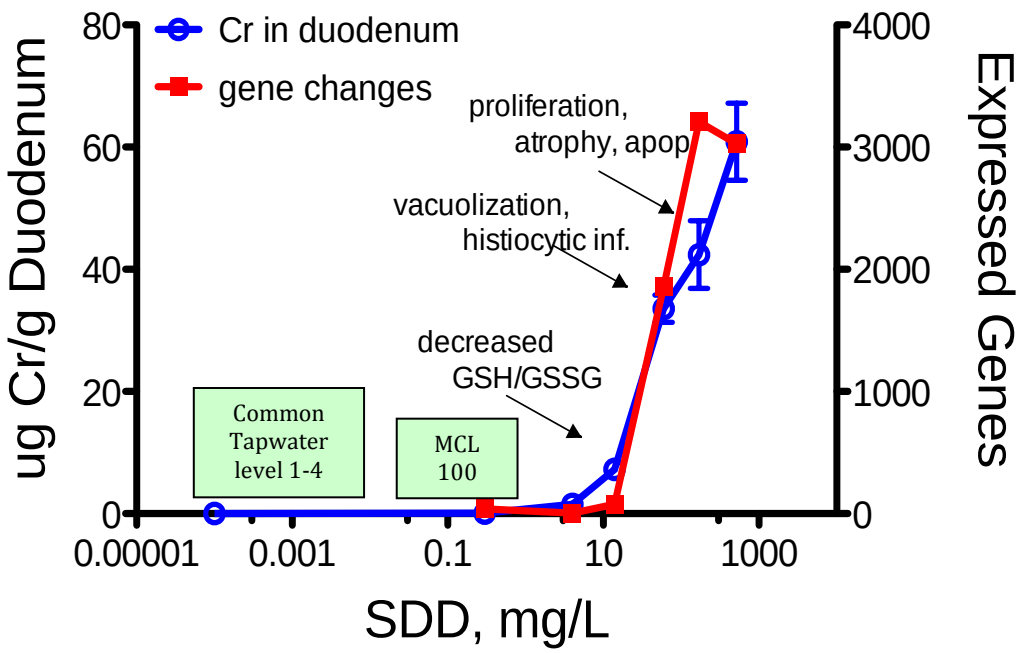


Figure 8. Cr accumulation in the duodenum, the part of the small intestine closest to the stomach, as well as the biochemical and pathological changes that occur at various doses. [The green boxes indicate the current federal MCL of 100 ppb and common U.S. tap water levels.

4.4.4 Toxicogenomic Data from Mice

U.S. EPA's *Framework for Determining a Mutagenic Mode of Action for Carcinogenicity* discusses the use of toxicogenomics for determination of a mutagenic MOA (U.S. EPA, 2007). Briefly, toxicogenomics explores changes in the patterns of gene expression as result of a dose of a toxic agent. These patterns of expression are good indicators of an animal's early response to chemical insults. In such an experiment, animals are treated with doses of an agent and then the messenger-RNA that is transcribed from activated genes is isolated from these

animals. The mRNA is then applied to a commercially available gene microarrays containing single stranded DNA from known genes. When the m-RNA binds to complimentary single-stranded DNA on the microarray, one knows which genes were activated. These microarrays may contain up to 30,000 different genes. When mRNA isolated from an animal receiving the dose of an agent is compared to that of a control animal, one can determine which genes are differentially expressed due to the agent.

Without consideration of which genes were differentially expressed, Figure 8 above shows that changes in gene expression in the mouse small intestine do not occur above 10 mg/L, 100 times higher than the current federal MCL.

Toxicogenomics has been used recently to discern characteristic gene expression profiles for genotoxic and non-genotoxic carcinogens (Ellinger-Ziegelbauer et al., 2004; van Delft et al., 2004). In mice exposed to Cr(VI) as part of the ACC study, the gene expression profile matched that of known genotoxic carcinogens (Thompson et al., 2011c). In addition, a comparison of gene expression profiles in mice and rats indicated that intestinal tissue from mice was over twice as responsive to Cr(VI) in terms of level of gene expression than was intestinal tissue from rats. This result provides a possible explanation for the difference in intestinal tumor outcome in the two species (Kopec et al., 2011).

4.4.5 Reduction of Cr(VI) in the Stomach

Once Cr(VI) is ingested, it is chemically reduced to trivalent chromium [Cr(III)] in the stomach (De Flora, 2000; Febel et al., 2001; Kerger et al., 1996). In general, reduction causes chemicals to be less chemically and biologically active. Cr(III) is poorly absorbed by the intestinal epithelium, so chemical reduction results in a protective mechanism because less Cr(VI) is then available for uptake.

It is important to understand that reduction of Cr(VI) is a competing reaction with transport of Cr(VI) into a cell. It is reduction of Cr(VI) <u>inside</u> a cell that can lead to cellular damage and DNA damage. Chemical reduction in the stomach reduces the dose of Cr(VI) to epithelial cells in the gastrointestinal tract. The percentage of Cr(VI) reduced in the stomach is a function of the amount of Cr(VI) ingested. Less and less Cr(VI) is reduced as the amount ingested increases, primarily because the amount of reductants in the stomach becomes depleted. Thus the dose of Cr(VI) available for transport into a cell is not a constant percentage of the ingested dose. This fact alone causes a Cr(VI) dose-response curve for cancer to be nonlinear over a large range of doses. (Proctor et al. in press)

In the intestine, which has a much less acidic environment than the stomach, Cr(VI) exists as chromate ion and is readily taken up into the epithelial cells lining the intestine (De Flora, 2000; Chiu et al., 2010). If sufficient chromate ion enters the intestinal epithelial cells, it creates oxidative stress that can damage the cells (O'Brien et al., 2003; Yao et al., 2008). If sufficient cell damage accumulates, the cell will die, and cell proliferation will occur to replace dead or irretrievably damaged cells (Potten, 1991). In some cells, oxidative damage to DNA may occur. DNA damage does not correspond to mutation because of ongoing DNA repair processes. In addition, cells with a sufficient amount of DNA damage may undergo a genetically programmed cell death known as apoptosis so that cells with damaged DNA are removed. In some cases, DNA repair is faulty and the coding in the DNA base sequence is altered; this faulty repair is a form of mutagenesis.

Reduction of Cr(VI) to Cr(III) in the stomach occurs to a different extent in rats, mice and humans. Because humans have much more stomach acid than rodents, most, if not all, of ingested Cr(VI) will be reduced to Cr(III) in the gastrointestinal tract (Donaldson and Barreras, 1996; De Flora, 2000; Proctor et al., 2002). The GI region where reduction to Cr(III) is the stomach with its acidic environment. Because NTP (2008) observed a dose-dependent increase in chromium in the liver of mice and Thompson et al. (2011b) observed a dose-dependent increase of chromium in the small intestine, it is clear that reduction is not complete, especially when natural protective mechanisms are overwhelmed by unusually high doses.

As part of the ACC-sponsored study, rates of reduction of Cr(VI) to Cr(III) are being measured in the stomach fluid of rats, mice and humans. The human stomach fluid includes samples from fed and fasted individuals as well as those taking proton pump inhibitors for acid control. Preliminary data indicates that human stomach fluid reduces Cr(VI) at a much higher rate than does rodent stomach fluid.

These data will be used to develop a physiologically-based pharmacokinetic (PBPK) model for Cr(VI) in both rodents and humans to enable quantitative interspecies extrapolation. Cr(VI) does accumulate in rodent tissues in a dose-dependent manner. The PBPK model will allow scientists to determine the administered dose necessary to achieve a given tissue concentration in humans.

The initial biochemical changes are not observed until around 10 ug/g Cr in mouse small intestinal tissue achieved with a dose of about 5 mg/L or 5000 ppb. There is a clear threshold at around 0.1 mg/L. The PBPK model will allow scientists to determine the margin of safety for accumulation of chromium in human tissue.

4.4.6 Diffuse Epithelial Hyperplasia – a Key Event in the Mode of Action

Using the results of the NTP bioassay in mice, both U.S. EPA and Cal-EPA developed linear cancer slope factors for oral exposure (e.g., drinking water) to Cr(VI). Diffuse epithelial hyperplasia could conceivably serve as a precursor event for tumors as described in U.S. EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a). Thompson et al. (2011a) hypothesize that diffuse epithelial hyperplasia represents regenerative proliferation in response to Cr(VI)-induced tissue injury.

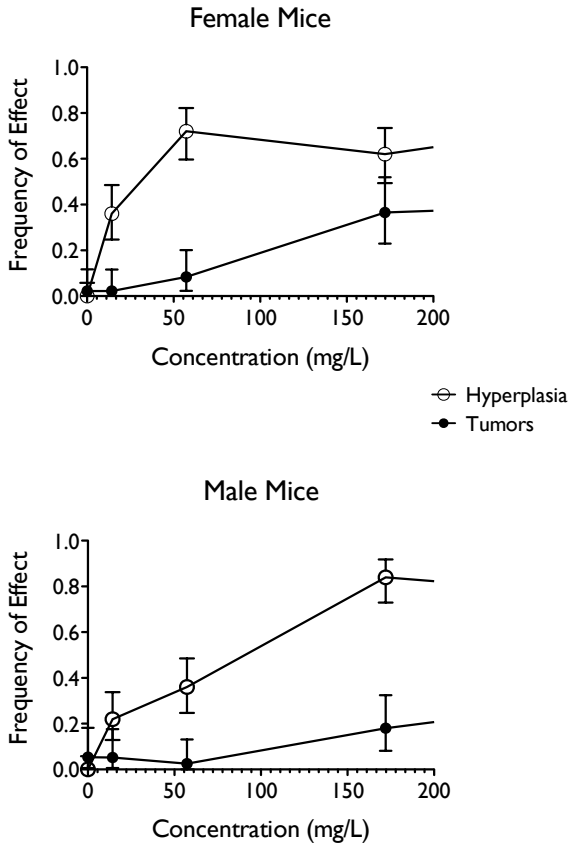


Figure 6. Comparison of Concentration-Response Curves for Diffuse Epithelial Hyperplasia and Small Intestinal Tumors in Mice from the two year Cr(VI) bioassay (NTP, 2008). Note that hyperplasia occurs at lower concentrations than tumors.

Diffuse epithelial hyperplasia occurs earlier and at lower doses than do intestinal tumors (Figure 6, above). In addition, animals with diffuse epithelial hyperplasia of the small intestine tend to have a higher frequency of tumors (Figure 7, next page).

4.4.7 Oral Tumors in Rats But Not in Mice

A possible explanation for the lack of intestinal tumors in rats may be the occurrence of anemia in both rats and mice; rats begin recovering from anemia after about 22 days of exposure, whereas mice exhibit continued anemia. It is not known

exactly how Cr(VI) produces anemia. One effect of anemia is the up-regulation of intestinal transport protein for trivalent iron (Sharp and Srai, 2007). This transport protein may also take up Cr(III), although this has not been demonstrated. Although unproven, the up-regulation of the iron transport protein and consequent increased chromium uptake provides an explanation of why mice likely absorb more chromium into the lining of the small intestine over time than do rats. This explanation is consistent with the observation that at all times and at all doses, the levels of chromium in the livers of female mice were higher than those in the livers of male rats administered the same chromium concentration in drinking water.

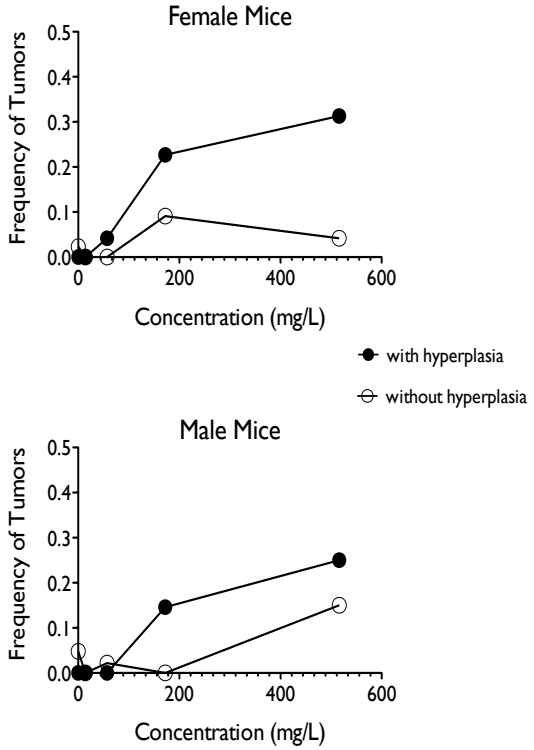


Figure 7. Comparison of Tumor Frequency in the Duodenums of Female and Male Mice with and without Diffuse Epithelial Hyperplasia. Cancer frequency was much greater in animals with hyperplasia that those without hyperplasia. These plots are based on individual animal data available at http://ntp.niehs.nih.gov/index.cfm?objectid=5FE88732-F1F6-975E-70FA764DD21980C

What the anemia fails to explain is the occurrence of oral tumors in rats. However, iron deficiency in rats has been shown to reduce the time to oral tumor occurrence in response to a known carcinogen (Prime et al., 1983). Inflammation of the lining of the mouth may occur in rats by impingement of hair shafts or large food particles (Garant and Cho, 1979a, 1979b). Oral tumors have been observed in rats in twelve bioassays conducted by NTP (Yoshizawa et al., 2005). There are no reports of similar effects in mice. Hence, the oral tumors in rats may possibly be due to mechanical irritation, subsequent inflammation and tumor promotion due to anemia.

5 Does a Threshold Exist for the Carcinogenic Effects of Cr(VI) in Rodents?

In mice, no treatment related lesions were observed in the oral cavity after either 8 or 90 days of dosing. In the small intestine, some cellular changes were observed at 170 mg/L SDD in the small intestine. Villous atrophy and crypt cell hyperplasia, suggesting tissue injury and compensatory regrowth, were observed after 8 days at a dose of 520 mg/L SDD. Histocytic cellular infiltration was not observed at any dose after 8 days.

After 90 days, early cellular changes were observed at 60 mg/L in half the mice. Villous atrophy, apoptosis and crypt cell hyperplasia were observed in mice receiving 170 mg/L SDD or greater. Histiocytic cellular infiltration was observed in the small intestines of at least 1 mouse at 60 mg/L or greater. These results are similar to those noted in NTP (2007).

In rats, no treatment related lesions were observed in the oral cavity after either 8 or 90 days of dosing. In the small intestine, cell death and hyperplasia were observed at 60 mg/L SDD or greater after 8 days. Also in the small intestine, apoptosis was observed at 60 mg/L or greater after 90 days, and villous atrophy and crypt cell hyperplasia were observed at 170 mg/L and 520 mg/L. Histiocytic cellular infiltration was observed in the small intestine in 90% or more of the rats after 90 days at 60 mg/L or greater. The results in rats are roughly similar to those in NTP (2007), but in the ACC MOA study, additional histopathological changes were noted.

6 Is this MOA Relevant to Humans?

With regard to human relevance, McCarroll et al. (2010) cite Zhang and Li (1987). The expert panel requested by OEHAA and convened by the University of California in 2001 criticized the use of Zhang and Li (1987) to support human relevance of cancers observed in rodents in response to drinking water exposure to Cr(VI). The data in the controversial work was re-examined by the same authors ten years later (Zhang and Li, 1997) and they concluded that chromium was not associated with stomach cancer mortality. These data have since been reanalyzed by two different groups of scientists and the demonstration of an increased rate of gastric cancer in those villages closest to the source of the chromium depends on whether the entire province or a local village without Cr(VI) water contamination is used as a control group (Beaumont et al., 2008; Kerger et al., 2009).

McCarroll et al (2010) also cite Beaumont et al (2008) to support their claim of human relevance. Beaumont et al (2008) qualify their conclusions by indicating that *Helicobacter pylori* infection is a significant risk factor for gastric cancer in China and that this factor affected their results. In 2009, Dr. Li and others wrote a letter to the journal disputing the findings of Beaumont et al. (2008) (Kerger et al., 2009). In addition, McCarroll et al. (2010) do not consider the negative findings of Fryzek et al. (2001) in California populations exposed to Cr(VI) through drinking water or the findings of Amienta-Herrnandez and Rodriguez-Castillo (1995), Rosas et al., (1989) or Neri et al., (1982) in Mexican populations also exposed through drinking water. These are examples of OEHHA's selective use of data to support its findings and conclusions. An objective approach would have used the weight of the evidence, where negative results are considered along with positive results. Use of the weight of evidence approach is consistent with the U.S. EPA Cancer Risk Guidelines and the U.S. EPA Framework for Determining a Mutagenic Mode of Action.

6.1.1 The Use of Linos et al. (2011) to Support Human Relevance of the Results in Mice is Incorrect and Inappropriate.

OEHHA cites a very recent ecologic epidemiology study from Greece, Linos et al. (2011) that suggests that increases in primary liver cancer, lung cancer, kidney cancer and genitourinary cancers in women observed in the Oinofita municipality in Greece, 50 km north of Athens, may be due to the disposal of wastes containing Cr(VI) into the Asopos River during a period of rapid industrialization from 1969 until 2007. Like the aforementioned Chinese study, this study is also highly flawed. Although the authors selected to match the control population obtained from the entire Voiotia prefecture (that contains the Oinofita municipality) because of similar geographical, population density, socioeconomic and ethnic origin of the people, they did not account for confounders such as alcoholism and viral hepatitis, both of which are associated with primary liver cancer, or with smoking that is known to be associated with lung cancer. Stomach cancer in Linos et al. (2011) was not elevated, in contrast to Zhang and Li (1987).

Linos et al. (2011) did not account for possible confounders, such as alcohol consumption. Schutze et al. (2011) showed that 28% of liver cancer in Greek men and 24% of liver cancer in Greek women can be attributed to alcohol consumption. In addition, viral hepatitis is a risk factor for liver cancer as are the use of oral contraceptives in women. The rate of occurrence of anti-hepatitis C virus (HCV) antibodies in Greek patients observed at health centers throughout the country varies from 0 to almost 13% (Lionis et al., 2000). This is a large variation and it is

not known whether Oinofita had high or low rates of being seropositive for HCV. Additionally, NTP demonstrated that the liver is not a target for Cr(VI) carcinogenicity as is the GI tract (NTP, 2007, 2008).

Finally, the final PHG dismisses epidemiological studies that do not show a relation between Cr(VI) exposure and health effects. OEHHA's own peer reviewers mentioned these studies in the August 2001 University of California peer review comments. Direct quotes from these peer review comments are provided in Attachment B.

6.1.2 OEHHA Ignored Studies of Residents Living near Chromate Plants in Mexico

Finally, the final PHG dismisses epidemiological studies that do not show a relation between Cr(VI) exposure and health effects. These studies are also mentioned in the August 2001 University of California peer review comments and direct quotes are provided in Attachment B.

Armienta-Hernandez and Rodriguez-Castillo (1995) provide results to suggest that Cr(VI) does not pose a risk to human health at current regulatory levels. Inadequate solid waste disposal practices at a chromate plant in southwest Leon, Mexico, in operation since 1970, resulted in groundwater concentrations of Cr(VI) of up to 50 mg/L over about a 5 km^2 area. The source of contamination was an alumina waste pile containing on average 6.12% by weight of Cr(VI). Groundwater sampling was carried out in 30 wells and 5 temporary sample points over an area of 10 km^2. Both Cr(VI) and total chromium analyses were conducted. Analyses of 24 hour urine collection samples for chromium were conducted in four groups: (1) 45 chromate factory workers, (2) nine residents living with 0.5 km of the plant, (3) six workers at a tannery 3.5 km from the chromate plant who were presumable exposed by Cr(III) only, and (4) seven reference individuals from Leon and Mexico City.

A single well near the plant had a concentration of 50 mg/L. Another well slightly farther from the plant had a concentration of 10 mg/L. This second well supplied a great deal of the town's water and extraction from this well affected the shape of the Cr(VI) plume in groundwater. Five other wells had concentrations between 0.05 and 2.3 mg/L. The authors conclude that the population living near the plant likely consumed water containing 0.5 mg/L Cr(VI) for periods of 5 to 7 years. Higher concentrations of Cr(VI) resulted in yellow-colored water and the authors conclude it was unlikely that such water would be consumed.

Chromium can be measured in urine and its occurrence in the urine is generally indicative of recent exposure, within the previous several days. In urine, chromium concentration is measured in units of nanograms per milliliter (ng/ml). In the urinary measurements, the average concentration of chromium in urine from the nearby residents was 26.25 ng/ml and the average in the reference population was 20.03 ng/ml. 34 of the factory workers worked with chromite ore and had an average urinary concentration of 46.8 ng/ml. The average concentration in the tannery workers was 19.2 ng/ml.

It should be noted that for chromium to appear in urine, it would have to be absorbed from the GI tract or lungs and then excreted by the kidneys. Hence, these urinary measurements are reliable indicators of recent exposure to Cr(VI) by ingestion or inhalation. The authors suggest that the residents received most of their exposure via groundwater consumption whereas the factory workers received most of their exposure from chromate dust in the air.

More importantly, the authors were unable to find any health effects in any of the exposed populations. This resident population studied was albeit quite small; however, the lack of health effects at 0.5 mg/L suggests this level as a no-effect concentration or NOEC. Application of the default uncertainty or safety factor of 10 for human variability would result in a value of 0.05 mg/L, the same as the current California MCL of 50 parts per billion.

In Lecheria, a town in southern Mexico, 3000 residents were exposed to Cr(VI) in drinking water from wells at an average concentration of 0.9 mg/L due to a nearby chromate production plant. Chromium concentrations in the urine of 182 residents and the hair of 156 residents were also measured. Chromium in hair would be representative of long-term exposure. Chromium concentrations in both the hair and urine of residents were elevated in comparison to a control population. However, there was no increase in the death rate from all cancers measured over a period of 24 years when compared to the control population (Neri et al., 1980, 1982).

6.1.3 Studies of California Residents Exposed to Cr(VI) near PG&E Facilities Did Not Find Elevated Cancer

Fryzek et al (2001) compared the death rates for lung cancer, all cancers combined and all causes of death for residents in Kettleman City, Hinkley, and Topock, California with those in the rest of California. These towns were located near PG & E facilities that used Cr(VI). These authors did not find elevated cancer or death rates in these areas.

Dr. John Morgan, an epidemiologist working for the California Cancer Registry examined cancer rates in Hinkley, California, in response to concerns about a potential excess in the number of new cancer cases. Drinking water exposure to Cr(VI) was not measured or considered by Dr. Morgan. He examined registry data from 1988 to 1993, from 1993 to 1996, and from 1996 to 2008. The rate for all cancers was not elevated in Hinkley during any of these time periods (Morgan and Prendergast, 2000; Dr. John W. Morgan, personal communication to T. Simon).

6.1.4 Studies from Occupational Inhalation Exposure with Assumed Oral Co-Exposure Do Not Support an Association With Cancer

Gatto et al. (2010) conducted a meta-analysis of 32 studies of workers occupationally exposed to Cr(VI). Although the primary route of exposure was inhalation, Cr(VI) occurs as particulates and redistribution may occur to the stomach and gastrointestinal tract due to oral respiration. The meta-standardized mortality ratio (SMR) was the statistic used for comparison. This statistic is the ratio of the cancer death rate in the exposed group to that in a control population. None of the meta-SMRs were elevated for any gastrointestinal cancer when all available studies were considered. For a group of four U.S. studies, esophageal cancer was slightly elevated. The authors concluded that the meta-analysis did not support an association between occupational exposure to Cr(VI) and mortality from cancer of the gastrointestinal tract.

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Attachment B

Timeline of the Development of the California Public Health Goal (PHG) for Hexavalent Chromium [Cr(VI)]

Feb. 1999 OEHHA publishes a PHG for "total" chromium [Cr(VI) and Cr(III)] of 2.5 ug/L. The total chromium PHG assessment is based on a German study by Borneff et al. (1968), a two-generation study of the carcinogenicity of Cr(VI) and benzo(a)pyrene in mice exposed to only a single dose. During the 8th month of the experiment, 512 animals died of mouse pox. Mouse pox may produce stomach lesions that resemble cancer. This 1999 PHG technical support document and related cancer potency for Cr(VI) is available at http://oehha.ca.gov/water/phg/pdf/chrom_f.pdf.

Aug. 2001 OEHHA requested the University of California to form an expert scientific panel to advise OEHHA on the development of a PHG for Cr(VI). The expert panel released a report on their review of the February 1999 PHG. Concerning OEHHA's reliance on Borneff et al. (1968), the expert panel wrote:

Several additional experts in laboratory animal medicine and veterinary pathology were consulted about mouse pox by the panel. … In the healing phase there can be exuberant proliferation of squamous epithelium, which may mimic that of a squamous papilloma, not dissimilar to what was described in the forestomach in the Borneff study. Also, importantly, mouse pox is caused by a polytropic virus; thus, there are lesions in multiple tissues/organs including the oral cavity and intestinal tract in addition to the cutaneous ones. … Whatever the correct interpretation, the ambiguity introduced by the mouse pox epidemic in the Borneff et al. study is adequate and sufficient to render these data unsuitable for use in a carcinogenesis risk assessment. These concerns underlie the rationale for the National Toxicology Program (NTP) and Environmental Protection Agency (EPA) guidelines that prohibit use of data from infected animals for such a risk assessment. … Contemporary practice in risk assessment would immediately discard data from any study where the animal health status was as compromised as in the Borneff study by an intercurrent outbreak of a highly lethal systemic disease such as mouse pox. (p. 7)

The expert panel continued:

We found no basis in either the epidemiological or animal data published in the literature for concluding that orally ingested Cr(VI) is a carcinogen, and a relatively large number of negative studies by the oral route of exposure, even at concentrations in excess of current MCLs. Definitive data on the potential carcinogenicity of orally ingested Cr(VI) should be provided by a planned NTP

study, but these results will not be available for several years. (p. 3)

The panel also reviewed the epidemiological data on the relationship of Cr(VI) exposure to cancer. The panel discussed the set of epidemiological studies by Zhang and Li (1987, 1997) conducted in five Cr(VI)-contaminated villages in China, and reported:

These papers thus provide no support for the hypothesis that high levels of Cr(VI) in drinking water are associated with an increase in stomach (or overall) cancer mortality. (p. 18)

and:

Taken together, the epidemiologic data on Cr(VI) exposure from environmental sources (as opposed to generally much higher occupational exposures) provide no support for a causal association of exposure to Cr(VI) and overall or site-specific cancer mortality for the general public. (pp. 19-20)

The full text of the expert panel's report is available at http://oehha.ca.gov/public_info/facts/pdf/crpanelrptfinal901.pdf.

Nov. 9, 2001 OEHHA withdraws the February 1999 PHG due to the findings of the expert panel. (http://oehha.ca.gov/public_info/press/nochromphg.html)

May 2002 OEHHA announces its intention to initiate an assessment for Cr(VI). (http://oehha.ca.gov/water/phg/pdf/Public%20Announcement%20for%20PHGs.pdf)

July 2002 In response to a nomination of Cr(VI) for inclusion in a rodent cancer bioassay by the National Toxicology Program (NTP), a public meeting of an external review committee was held at the NTP offices in Research Triangle Park, NC. (http://www.safedrinkingwater.com/community/Cr(VI)_Public_Meeting_Report1.pdf) Preliminary results from a 13 week study in mice and rats conducted by NTP are available at this time. (http://ntp.niehs.nih.gov/ntp/htdocs/Studies/HexChromium/CrVI13_wk_rpt.pdf)

The NTP external review committee members included Dr. Steven Patierno, the executive director of the George Washington University Cancer Institute and a well-known researcher in chromium carcinogenesis, and Dr. Ernest "Gene" McConnell, a veterinary pathologist, who has been active since 1961, a member of the expert scientific panel convened by the University of California to review the February 1999 PHG and one of the pioneers of the rodent cancer bioassay.

(http://www.katzencancer.org/index.php/about-us/board-of-directors/steven-r-patierno-phd/), (http://www.ierfinc.org/psa_eemc.html)

At this meeting, Dr. Robert Howd, an OEHHA toxicologist, indicated that OEHHA was concerned with carcinogenic risk from inhalation of Cr(VI) in water aerosols during showering. Dr. Patierno objected to the blanket statement made by Dr. Howd that "Cr(VI) is carcinogenic." Dr. Patierno stated that inhalation of relatively insoluble forms of Cr(VI) in particulates is known to be carcinogenic, but only at the point of contact (*i.e.*, only certain forms of Cr(VI) would be carcinogenic). He criticized Dr. Howd's concerns about inhaled aerosols during showering on these grounds and noted that all of the studies using soluble Cr(VI) were negative, and that inhaled soluble Cr(VI) would move away quickly from the point of contact but inhaled particulate Cr(VI) would slowly dissolve into the surrounding tissues.

Regarding the choice of doses in the planned NTP cancer bioassay, the lowest dose proposed by NTP for mice was 62.5 mg/L and the highest dose was 250 mg/L. With the exception of Dr. Howd, all the committee members asked that a dose within the range of 1-5 or 1-10 mg/L be included. Dr. Howd asked that the dose range be extended upwards to 500 mg/L to match the single dose used by Borneff et al. (1968). Ultimately, the lowest dose used in the mouse cancer bioassay performed by NTP is 14 mg/L.

June 2005 OEHHA announces that a draft PHG document for Cr(VI) is nearing completion and would be released as soon as possible. (http://oehha.ca.gov/water/phg/pdf/PHGinitiation062005.pdf)

Aug. 2005 OEHHA releases the report of peer reviewers Dr. Roberto Gwiazeda, Dr. Leonard Bjeldanes and Dr. Michael Kelner commenting on a pre-release draft of the Cr(VI) PHG risk assessment. The value of the draft PHG in the pre-release draft is 0.2 parts per billion (ppb) or 200 parts per trillion (ppt). These comments can be found at http://oehha.ca.gov/water/phg/pdf/ThreeCr6review.pdf.

Dr. Bjeldanes stated on the first page of his review:

However, in key areas of CrVI activities the document is selective in its use of certain published findings. For example, the Report reinterprets important published findings and comes to conclusions that are different from the conclusions drawn by the original authors. Furthermore, the Report has ignored

major concerns stated in other published reviews about the value of certain studies in projecting cancer effects in humans. (at p. 2)

Dr. Gwiazda indicates on the first page of his review that the Borneff et al. (1968) mouse study and Zhang and Li (1997) epidemiological study provide only weak support for the link between Cr(VI) exposure and stomach cancer, but the *"…precautionary principle would dictate that this finding of a relationship between oral uptake of Cr(VI) and stomach cancer incidence be accepted..."* (at p. 6)

Dr. Kelner points out that the PHG of 0.2 ppb relies on only two studies – Borneff et al. (1968) and Zhang and Li (1987) – and both of these contained numerous flaws. Dr. Kelner writes on pages 4 and 5 of his review:

So in summary, the sole primary supporting evidence for defining very strict guidelines for oral exposure to hexavalent chromium consists of the following <u>two</u> studies:

1) *A very flawed mouse study in which the majority of mice died from a virus …*
2) *A single report of a flawed human assessment study, which even one of the <u>original</u> authors subsequently concluded may not be correct.* (emphasis in original letter Kelner to C. Caraway dated April 12, 2005)

Jan. 2007 NTP publishes its 13-week bioassay of sodium dichromate dehydrate (SDD). (http://ntp.niehs.nih.gov/ntp/htdocs/ST_rpts/TOX072.pdf)

May 2007 NTP releases its draft report on the 2-year study on the toxicology of carcinogenesis of SDD in drinking water. The concentrations used in mice were 0, 14.3, 28.6, 85.7 and 257.4 mg/L (ppm) in males and 0, 14.3, 57.3 172, and 516 mg/L (ppm) in females. The concentrations used in rats were 0, 14.3, 57.3, 172 and 516 mg/L (ppm) in both males and females. In mice, NTP reported a dose-dependent increase in cancer of the small intestine was observed whereas in rats, it reported a dose-dependent increase in cancer of the mouth was observed.

Sept. 2007 OEHHA announces that a draft PHG document for Cr(VI) is in progress. (http://oehha.ca.gov/water/phg/phginit092007.html)

July 2008 NTP publishes its 2-year cancer bioassay of SDD in mice and rats and concludes there is clear evidence of carcinogenicity in rats based on the occurrence of oral

tumors and clear evidence of carcinogenicity in mice based on small intestinal tumors. (NTP. 2008. NTP Technical Report on the Toxiciology and Carcinogenesis Studies of Sodium Dichromate Hihydrate in F344/N rats and B6C3F1 Mice., pp. 8-9. Available at http://ntp.niehs.nih.gov/ntp/htdocs/LT_rpts/tr546.pdf)

July 2009 McCarroll et al. manuscript accepted for publication in *Environmental and Molecular Mutagenesis* (McCarroll et.al. 2010. An Evaluation of the Mode of Action Framework for Mutagenic Carcinogens Case Study II: Chromium(VI). Environ Mol Mutagen. 51(2):89-111).

Aug. 2009 OEHHA releases a draft PHG of 0.06 ppb or 60 ppt for Cr(VI) in drinking water, relying on the 2007 draft NTP rodent cancer bioassay of Cr(VI) in drinking water. (NTP. 2007). The draft PHG is available at http://oehha.ca.gov/water/phg/pdf/Cr6PHGdraft082009.pdf.

Sept. 2009 Drs. Gwiazda, Bjeldanes and Kelner provide peer review comments on the August 2009 draft PHG. (http://oehha.ca.gov/water/phg/chrom091009.html#comments)

Dr. Gwiazda points out there is considerable uncertainty in the PHG due to lack of information on the extent to which Cr(VI) is transformed into a non-toxic form trivalent chromium [Cr(III]) in both the mouse and human GI tracts. (pp. 1-3 in http://oehha.ca.gov/water/phg/pdf/Cr_Gwiazda090909.pdf)

Dr. Bjeldanes writes on p. 3 of his comments (http://oehha.ca.gov/water/phg/pdf/Cr_BJeldanes090909.pdf):

In summary, 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.

Dr. Kelner points out that OEHHA chose the most sensitive strain of mouse from the NTP bioassays upon which to base the risk assessment and that sufficient detail is not provided in the report. (http://oehha.ca.gov/water/phg/pdf/CrKelner090909.pdf)

Oct. 2009 OEHHA grants a request for additional external peer review of the August 2009 PHG pursuant to California Health and Safety Code Section 116365(c)(3)(D).

Nov. 2009 Stakeholders submit comments on the draft 0.06 ppb PHG. Comments are available at http://oehha.ca.gov/water/phg/cr6com112409.html.

Feb. 2010 ACC Mode of Action (MOA) study commences with dosing of mice.

Mar. 2010 McCarroll *et al.* (2010) final manuscript appears online as "An evaluation of the mode of action framework for mutagenic carcinogens case study II: chromium (VI)." Environ Mol Mutation. (See July 2009 above).

Apr. 2010 Nickens, Patierno et al. manuscript on Cr(VI) genotoxicity appears online at *Chemico-Biological Interactions* as "Chromium genotoxicity: A double-edged sword." (Nickens, Patierno, et al. (2010). Chromium genotoxicity: A double-edged sword. *Chemico-Biological Interactions*. 188(2):276-88).

July 2010 ACC MOA mouse study continues and dosing of rats commences.

Sep. 20, 2010 OEHHA releases the results of the additional external peer review of the August 2009 PHG. (http://oehha.ca.gov/water/phg/chrom092010.html)

The five peer reviewers were:

Dr. William Shotyk, Professor, Environmental Geochemistry, University of Heidelberg

Dr. Sharada Balakrishnan, Board-certified Toxicologist, Practical Innovators, Inc.

Mitchell Cohen, Ph.D., Professor, Environmental Medicine, NYU School of Medicine

Toby Rossman, Ph.D., Professor and Director, Molecular Toxicology and Carcinogenesis, NYU School of Medicine

Elizabeth Snow, Ph.D., Professor, Human Life Sciences, University of Tasmania

The comments of Dr. Rossman clearly address OEHHA's confusion between genotoxicity and mutagenicity. Dr. Rossman indicates that the sections on genotoxicity and mode of action were inaccurate and out of date. She also points out that the "assumption that Cr(VI) in drinking water has a mutagenic mode of action with no threshold" is invalid. These OEHHA assumptions result in an "unrealistically low" PHG of 0.06 ppb. (at p. 4 of Rossman comments)

Sep. 30, 2010 U.S. EPA's Integrated Risk Information System (IRIS) program releases a Draft Toxicological Review for Cr(VI) containing a risk assessment based on the same NTP mouse data used by OEHHA in its February 2009 PHG. These two assessments make almost identical recommendations regarding the carcinogenic potency of Cr(VI).

Oct. 2010 Thompson et al. MOA manuscript accepted and published in the online version of *Toxicological Sciences* (Thompson et al. 2011. Application of the US EPA Mode of Action Framework for Purposes of Guiding Future Research: A Case Study Involving the Oral Carcinogenicity of Hexavalent Chromium. Toxicol. Sci. 119(1). 20-40).

Dec. 2010 OEHHA releases a second revised draft PHG for Cr(VI) of 0.02 ppb or 20 ppt, three fold lower than the August 2009 value. This lower value is based on the determination of a mutagenic MOA based on McCarroll et al. (2010) and the use of an early life-stage adjustment factor to account for cancer occurring later in life from early life-stage exposure (USEPA, 2005b; OEHHA, 2009).

Jan. 2011 Thompson et al. (2011) MOA paper is published in the journal version of Toxicological Sciences.

Feb. 2011 Stakeholders submit comments to OEHHA on the draft 0.02 ppb PHG. These are available at http://oehha.ca.gov/water/phg/chrom123110.html#comments.

May 2011 EPA's contractor, Eastern Research Group, convenes a public peer review meeting on U.S. EPA's draft IRIS Toxicological Review for oral hexavalent chromium. The EPA's draft Toxicological Review and final peer review comments are available at http://cfpub.epa.gov/ncea/iris_drafts/recordisplay.cfm?deid=221433.

June 2011 Thompson et al. Mouse Base paper is accepted and published. (Thompson et al. 2011. Investigation of the mode of action underlying the tumorigenic response induced in B6C3F1 mice exposed orally to hexavalent chromium. Toxicol. Sci. 123(1):58-70).

July 6, 2011 EPA releases the peer reviewers' post-meeting comments on the 2010 draft IRIS Toxicological Review (available from the EPA website at http://oaspub.epa.gov/eims/eimscomm.getfile?p_download_id=502646). One of the charge questions to the peer reviewers of the EPA's IRIS document was whether the scientific evidence supported a mutagenic mode of action. The reviewers were uniformly critical of this assumption, pointing out that while Cr(VI) could be shown to be mutagenic in some in vitro systems, evidence for its mutagenicity in vivo was lacking. The majority of the reviewers indicated that using the assumption of a mutagenic MOA as a justification for linear low-dose extrapolation is not supported by the evidence:

The positive laboratory results of mutagenicity tests do not prove genotoxicity

and are not necessarily biologically relevant to humans exposed in vivo to the environmentally relevant concentrations of Cr^{+6}. (Janusz Z. Byczkowski, Consultant, Fairborn, OH, at p. 59 and A-7).

There is considerable evidence that Cr(VI) is genotoxic in cell culture and in vitro, and under certain extreme conditions it can also be shown to be mutagenic. However, there is far less support that Cr(VI) is genotoxic or mutagenic in vivo by the oral route of exposure at doses of relevance to humans; conversely, there is considerable evidence that there are protective threshold mechanisms that significantly impact the ability of Cr(VI) to reach target tissues and cause DNA damage under physiological conditions. (Joshua Hamilton, Ph.D., Senior Scientist, Marine Biological Laboratory, Woods Hole, MA, at p. 59 and A-27).

However, it is not clear whether epigenetic changes produced by chromium exposure are acting alone or linked to chromium genotoxic effects and that both genetic and epigenetic changes are essential for tumor appearance and evolution. (Konstanin Salnikow, Ph.D., Program Director, Division of Cancer Biology, NIH, Bethesda, MD, at p. 67 and A-67).

Nearly all indices (NTP studies, inhalation studies, mammalian cell mutagenesis studies etc.) indicate that carcinogenicity of CrVI is only observed under exposure conditions that evoke cellular toxicity, inflammatory tissue damage, and tissue regeneration. The DNA damage and presumed mutagenicity (actually epigenetic or stochastic selection of cells that survived toxicity) of CrVI is only observed at doses that also cause cell death and tissue damage. … It is much more likely that the chronic tissue damage, with accompanying inflammation and subsequent proliferative regeneration, possibly in the presence of unrepaired DNA damage, all of which are only observed at the highest doses, is the principle mode of action. (Steven R. Patierno, Ph.D., Exec. Dir., GW Cancer Center, George Washington University, Washington, DC, at p. 60 and A-49).

The evidence for a mutagenic MOI is weak. Mutations can result from DNA damage, but can also be a secondary effect of the loss of mismatch repair, aneuploidy, and other types of genomic instability (in other words, it is a later effect). With the exception of the mouse lymphoma system, Cr(6) is only weakly mutagenic in mammalian cells… (Toby G. Rossman, Ph.D., Professor, Environmental Medicine, NYU School of Medicine, Tuxedo, NY, at p. 63 and A-57).

The frank reality is that no mode of action for Cr(VI) has been demonstrated,

even the mutagenic mode of action is only supported and not demonstrated. There should not be a double standard here where the mutagenic mode of action needs to only be supported, while other modes must be demonstrate. (John P. Wise Sr., Ph.D., Professor, Toxicology and Molecular Epidemiology, University of Southern Maine, Portland, ME, at p. 68 and A-96).

The other observation leading to the discussion of nonmutagenic or indirectly mutagenic mechanisms of carcinogenicity was the presence of diffuse epithelial hyperplasia in the NTP bioassay. Although the NTP study has not found significant necrosis in the small intestine of exposed mice, it is quite possible that the observed hyperplasia was a typical manifestation of regenerative responses. A combination of increased proliferation and inflammation could be presented as an alternative mechanism for indirect induction of mutations due to higher rates of cell division and by reactive oxygen species released by the recruited inflammatory cells. This carcinogenic pathway would exhibit a strongly sublinear, threshold-type dose dependence, as it relies on the induction of cell death and small doses would not kill cells, (Anatoly Zhitkovich, Ph.D., Professor, Brown University Medical School, Providence, RI, at p. 71 and A-107).

A mutagenic mode of action was proposed as the primary mode of action. On the one hand, EPA discussed data gap and uncertainties about the mutagenic MOA and other possible MOAs. On the other hand, EPA defended the mutagenic MOA despite the lack of data evidence. (Yiliang Zhu, Ph.D., Professor, Biostatistics, University of South Florida, Tampa, FL, at p. 71 and A-118).

July 25, 2011 MOA researchers, Dr. Mark Harris and Dr. Sean Hays, briefed OEHHA personnel on the progress of the ACC funded MOA study. Dr. Harris is a principal of ToxStrategies, Inc., the company directing the study, and Dr. Hays of Summit Toxicology is the principle scientist conducting physiological modeling of Cr(VI) as part of the MOA research program.

OEHHA toxicologists present at the briefing included Drs. George Alexeeff, Anna Fan, Charles Vidair, Melanie Marty, John Budroe and John Faust. On July 20, 2011, Dr. Harris sent copies of a mode-of-action paper and the paper on the mouse results (Thompson et al., 2011a, 2011b) to Janet Rennert and Dr. Alexeeff of OEHHA. During the meeting, Dr. Vidair, one of the co-authors of the final PHG document, indicated he had read these papers already. Dr. Vidair also had a copy of the EPA responses to peer review comments and indicated that he was reviewing these as well.

The meeting began at 9:30 AM and at 10:30, Dr. Alexeeff, Acting Director of OEHHA, was called away to Sacramento. The briefing continued. Near the end of the meeting at 11:00, Dr. Harris asked about the status of the OEHHA PHG document. Dr. Fan and Dr. Vidair indicated to Dr. Harris that OEHHA would be addressing comments from the "workshop" for the remainder of the summer.

July 27, 2011 OEHHA releases final PHG of 0.02 ppb.

Aug. 10, 2011 Dr. Harris e-mails Dr. Alexeeff:

Dr. Hays and I appreciated the opportunity to meet with you and your staff on Monday, July 25 to discuss the preliminary results of the Cr(VI) Mode of Action (MOA) research program. As we indicated in our meeting with you, results to date appear to be supportive of a non-mutagenic MOA, driven by cytotoxicity and oxidative stress which occurs at high doses, and is similar to the MOA for captan and folpet, which EPA deemed to be non-mutagenic (see attached paper) and not carcinogenic at relevant human exposures. In addition, the gastric fluid reduction rate and capacity data generated to date demonstrate that reduction is second order, varies by dose with strong pH dependence, and is extremely rapid at low doses at the acidic conditions of the human stomach. Additionally, within months, the study will provide the only in vivo target tissue DNA damage and mutagenicity data for Cr(VI) and should provide considerable insight into the MOA underlying intestinal tumors in rodents. In addition, these studies will provide critical toxicokinetic data allowing for a more informed risk assessment as a result of being able to extrapolate between species (rodents and humans) as well as from the high doses that induced tumors in rodents to environmentally relevant concentrations.

We were surprised that OEHHA finalized the PHG for Cr(VI) on Wednesday, July 27 in light of the current study results, the recent review by an expert panel of the EPA Cr(VI) risk assessment (which generally supported a non-linear dose response-assessment), and statements by the OEHHA staff during our meeting that OEHHA would be addressing comments from the workshop for some time.

I have attached the slides we presented. Given that OEHHA has already finalized the PHG, should we continue to provide you results from the studies?

Aug. 10, 2011 Dr. Alexeeff responds to Dr. Harris in an e-mail:

Thank you for the information you sent. We would appreciate seeing results you obtain from future studies. We would also appreciate a briefing/meeting in the

future as you obtain and synthesize more of the information.

Aug. 17, 2011 Dr. Harris responds to Dr. Alexeeff's e-mail:

Again, I would like to express my appreciation for the opportunity to meet with you , Anna Fan, Charles Vidair, Melanie Martin [sic], John Boudrou [sic] and John Foust [sic] on Monday, July 25 to discuss the preliminary results of our Cr(VI) Mode of Action (MOA) research program. We will definitely keep you and your staff in the loop with regard to additional results from the MOA and PK studies – we seem to be receiving new data almost daily recently.

As we presented at our meeting, the results to date appear to be supportive of a non-mutagenic threshold MOA, driven by cytotoxicity and oxidative stress which occurs at high doses. Notably, such an MOA was determined for captan and folpet, which EPA deemed to be non-mutagenic and not carcinogenic at relevant human exposures.

We are currently reviewing results received from EPL since our meeting. EPL is looking for micronuclei (MN) in crypt cells of mouse duodenum tissues following 7 days of Cr(VI) exposure at all exposure concentrations (0, 0.1, 1, 5, 20, 60 and 180 mg CrVI/L). We are seeing no evidence of MN in the crypt cells in the EPL data received so far. I have attached some papers that we have been reviewing on the subject of MN and thought you and your staff might find them useful. Dr. Toby Rossman offered relevant comments related to MN as a peer reviewer at the May 12, 2011 USEPA Peer Review Workshop of the draft IRIS risk assessment document. She noted in her May 5th pre-meeting and July 6th post-meeting comments:

"In the case of Cr(6), at lower concentrations, most of the micronuclei are kinetecore [sic]-positive, meaning that they arise from malsegregation and not DNA strand breaks (Seoane and Delout, Mutat. Res. 490:99-106, 2001; Figgitt et al., Mutat. Res. 688:53-61, 2010). Those that are kinetecore [sic]-negative (arising from chromosome breaks) occurred only at the highest concentrations. Thus, Cr(6) induces aneuploidy rather than DNA damage at lower concentrations (Holmes et al., 2010; Figgitt et al., Mutat. Res. 688(1-2):53-61, 2010). Aneugenesis is caused by alterations in proteins, not DNA, and has thresholds."

Further, EPL also measured crypt and villous surface areas in the mouse duodenal tissues. The villous/crypt ratio is significantly decreased at the two highest exposure concentrations at day 8 due to significant damage to the villous. At day

91 villous/crypt ratio is significantly decreased at 180 mg CrVI/L due mostly to crypt cell hyperplasia (the other day 91 doses are currently being analyzed). These structural changes in the tissue, which are clearly high dose effects, are believed to be due to cytotoxic effects of Cr(VI) on villi, causing pressure on the crypts to regenerate new cells. We believe that this change in tissue structure also is likely to render the tissues more sensitive to Cr(VI) exposure, clearly suggesting that the rates of tumor formation observed at the higher doses cannot be extrapolated in a linear model to lower doses which are not cytotoxic. These results, coupled with the other data and in particular the reduction kinetics, clearly lead us to the preliminary conclusion that there is sub-linearity in the dose-response, and likely a threshold for carcinogenicity in the mouse duodenum, as noted by several of the ERG panel reviewers.

Once we have received all of the remaining data and have had a chance to synthesize it, I will contact you regarding another meeting.

Sep. 8, 2011 Ms. Ann Mason of the American Chemistry Council writes to Dr. Alexeeff expressing disappointment that the PHG was released so hastily but also noting a willingness to engage in further discussions with the goal of a science-based PHG for Cr(VI). She writes:

The public interest is best served when the weight of scientific evidence is used to inform regulatory decisions.

Sep. 23, 2011 Dr. Alexeeff responds to Ms. Mason. He indicates the public interest would not be served by further delay. He also notes that OEHHA scientists were aware of the ACC study and the results in Thompson et al. (2011a). He also comments on the comments of the EPA peer reviewers:

While a majority of reviewers did not support a mutational basis for Cr VI carcinogenicity, there was substantially more support for a genotoxic mechanism of action. The opinions on mechanism of action were quite diverse, indicating that much remains to be learned about this topic.

Oct. 2011 MOA rat study is published. (Thompson et al. 2011. Comparison of the effects on hexavalent chromium in the alimentary canal of F344 rats and B6C3F1 mice following exposure in drinking water: Implications for carcinogenic modes of action. *Toxicological Sciences*, online access Oct 19, 2011. Available at: http://toxsci.oxfordjournals.org/content/early/recent).

The three MOA papers are sent to OEHHA, (Letter from A. Mason to G. Alexeeff, Oct. 20, 2011).

Nov. 2011 MOA mouse genomics study results are available (paper has been accepted in Toxicology and Applied Pharmacology and will appear in the online version of this journal soon as Kopec, AK., CM. Thompson, S Kim, AL Forgacs, TR Zacharewski. Comparative Toxicogenomic Analysis of Oral Cr(VI) Exposure Effects in Rat and Mouse Small Intestinal Epithelium. Accepted in Toxicology and Applied Pharmacology).

Dec. 2011 The first of the genomics papers was submitted to the peer review journal as Thompson, C.M., Kopec, A.K., Winkelman, W.A., Wolf, J.C., Hixon, J.G., Carakostas, M.C., Proctor, D.M., Haws, L.C., Suh, M., and Harris, M.A. (2011d). Assessment of the Genotoxic Potential of Cr(VI) in the B6C3F1 Mouse Duodenum Based on In Vivo Intestinal Micronucleus Data and Toxicogenomic Profiling. The researchers expect to hear from the journal in early Dec.