February 14, 2011

Mr. Michael Baes
Pesticides and Environmental Toxics Branch
Office of Environmental Health Hazard Assessment
California Environmental Protection Agency
1515 Clay Street, 16th Floor
Oakland, CA 94612

Re: Hexavalent Chromium – Draft December 2010, Public Health Goal (PHG) for Hexavalent Chromium

Dear Dr. Baes:

Thank you for granting a 15-day extension to the comment period for the revised draft PHG for hexavalent chromium to February 15, 2011.

Attached to this letter please find the comments of the American Chemistry Council's Hexavalent Chromium Panel (ACC). In these comments, ACC focuses on the cancer endpoint because it is the driver for the revised draft PHG OEHHA should not interpret this focus to mean that ACC does not have comments on the noncancer endpoints; we do.

The most important point in the ACC comments is that OEHHA improperly applies age sensitivity factors, without consideration of mode of action (MOA), to reduce the PHG from 0.06 ppb to 0.02 ppb. OEHHA also fails to support significant changes from the 2009 draft PHG and provide the documentation and rationale for some of its calculations and assumptions used to support the revised draft PHG. Moreover, in many cases, OEHHA does not adequately consider the comments of peer reviewers in the revised draft PHG. Finally, OEHHA should use the best available science, including MOA data, fully present both linear and nonlinear approaches, present the rationale and justification for its calculations and assumptions, and fully address and incorporate the comments from its own invited experts who provided peer review comments.

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

cc: Joan Denton, Director, Office of Environmental Health Hazard Assessment
Allan Hirsch, Chief Deputy Director, Office of Environmental Health Hazard Assessment
Tim Shestek, Senior Director, American Chemistry Council
Michael J. Rogge, Policy Director, Environmental Quality, California Manufacturers and Technology

Technical Comments Regarding:

DRAFT Public Goal for Hexavalent Chromium in Drinking Water December 2010

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

Ann Mason
Senior Director
American Chemistry Council

Laura Brust
Assistant General Counsel
American Chemistry Council

February 15, 2011

Hexavalent Chromium Panel
American Chemistry Council
700 2nd St NE
Washington, DC 20002

Executive Summary

The American Chemistry Council's Hexavalent Chromium [Cr(VI)] Panel (ACC) appreciates the opportunity to comment on OEHHA's Draft Public Health Goal for Hexavalent Chromium (Dec. 2010) (Draft Dec. 2010 PHG document and January 25, 2011 corrections). 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 Draft Dec. 2010 PHG document.

OEHHA is proposing a Public Health Goal (PHG) for Cr(VI) of 0.02 parts per billion (ppb) in drinking water. OEHHA asserts that the proposed PHG level is "protective against all identified toxic effects from both oral and inhalation exposure to hexavalent chromium that may be present in drinking water," based on "the available data on the toxicity of hexavalent chromium" (p. 1). Moreover, OEHHA contends "there is now sufficient evidence that hexavalent chromium is also carcinogenic by the oral route of exposure, based on studies in rats and mice conducted by the National Toxicology Program (NTP, 2008)" (*Id.*). OEHHA also proposes a PHG of 2 ppb for non-cancer effects based on liver toxicity in female rats in the NTP study (2008).

The Draft Dec. 2010 PHG document is deficient in a number of aspects:

- While purporting to meet the requirement to use the best science in decisions that relate to protecting public health, OEHHA continues to use default assumptions rather than chemical-specific information and sound science to inform the risk assessment. Data about the mode of action (MOA) of Cr(VI) currently are being developed as part of a major research initiative that began in early 2009, and these data will be presented in March at the 2011 meeting of the Society of Toxicology.

- OEHHA fails to address comments from peer reviewers of the draft August 2009 PHG document and the Draft Dec. 2010 PHG document, and expert panel comments on the draft 1999 PHG document.

- OEHHA inadequately responds to public comments on earlier PHG documents, including:

    - Lack of any MOA consideration, especially when MOA forms the overarching conceptual framework for cancer risk assessment (EPA, 2005a).

    - Regarding the MOA, lack of consideration of interspecies differences in toxicokinetics of Cr(VI) and the failure to recognize that pathologies seen in rodents are likely portal-of-entry effects.

- Regarding the MOA, lack of consideration of nonlinear toxicodynamic effects of Cr(VI) that likely underlie the cancer response. These effects include reactions with DNA, oxidative stress, inflammation, and disruption of gene networks that regulate the cell cycle. Instead, the Draft Dec. 2010 PHG document correctly assumes that the metabolic products of Cr(VI) are DNA-reactive and wrongly assumes that DNA-reactivity equates to mutagenicity.

- Lack of consideration of nonlinearity and the presence of a threshold. Although Appendix A, titled "Carcinogenic Threshold?," (in the Draft Dec. 2010 PHG document) discusses the idea of a threshold, this appendix considers only reductive capacity and absorption, and because of the lack of any consideration of MOA, fails to take into account epigenetic changes (such as those mentioned in the previous bullet) that underlie the tumor responses and that likely do have thresholds. The lack of consideration of MOA also prevents exploration of the use of precursor effects as recommended in EPA's *Guidelines for Carcinogen Risk Assessment* (EPA, 2005a).

- Regarding the water consumption to calculate life-stage exposures, use of an atypical calculation method that expresses life-stage as a unit less fraction of a lifespan. In addition, the water consumption rates used in the non-substantive change document released by OEHHA on January 25, 2011 cannot be verified from the original sources and appear to be incorrect for some age groups (EPA, 2008; Kahn and Stralka, 2009).

- OEHHA inappropriately uses the age-sensitivity adjustment detailed in OEHHA (2009) because of lack of consideration of MOA. The age sensitivity adjustment was derived from data using solely statistical methods without consideration of biology or MOA other a single paragraph classifying the chemical as genotoxic or non-genotoxic (p.4 of OEHHA, 2009). In addition, it is difficult to validate the calculations that employ this adjustment because the necessary data are scattered throughout the document.

- OEHHA uses scientific literature in a biased or inappropriate manner, including:

  - The use of two highly flawed studies in mice and humans, respectively (Borneff *et al*., 1968; Zhang and Li, 1987), to attempt to establish a link between Cr(VI) exposure and gastrointestinal cancer in humans. The use of these studies is in direct contradiction of the advice of an expert panel convened by the University of California in 2001 to review the 1999 PHG document.

  - An attempt to impeach the results of the Gatto *et al*. (2010) meta-analysis that found no association between occupational exposure to Cr(VI) and gastrointestinal cancer in humans. Although the Draft Dec. 2010 PHG document makes several suggestions

to "improve" the Gatto *et al.,* meta-analysis, it is unlikely that any of these suggestions would alter the results published in Gatto *et al.,* 2010.

- OEHHA fails to explore the uncertainty associated with dose-response modeling. The narrative and tables describing the modeling are very brief and difficult to follow. The number of animals at risk for the various dose groups in NTP (2008) was changed from those in the Draft August 2009 PHG document without explanation, and neither set of values are the results of the commonly used poly-3 survival adjustment (Portier and Bailer, 1989). While the change in the number of animals at risk does not substantively change the risk assessment, this change, the difficulty of reproducing many of the calculations, and the 11th hour correction released on Jan. 25, all make the reader wonder what other flaws might exist in the Draft Dec 2010 PHG document.

These omissions and errors in the document are discussed in detail in these comments. The overall recommendation is that OEHHA examine the MOA and develop the risk assessment with consideration of the human relevance of the effects seen in rodents.

Table of Contents

Executive Summary i

Table of Contents v

Appendices vi

1 Introduction 1

2 OEHHA Improperly Considers Age Adjustment and Applies the Early Life Exposure Correction 2

2.1 OEHHA's Calculations Are Difficult to Reproduce. 3

3 OEHHA Has Not Properly Supported Changes from the 2009 Draft PHG 6

3.1 Dose Response Modeling 6

4 OEHHA Does Not Properly Respond to Previous Peer and Public Comments 10

5 OEHHA Continues to Fail to Consider Mode of Action. 11

5.1 Toxicokinetics 13

5.2 Mutagenicity or DNA-Reactivity 15

5.3 Consideration of Nonlinearity in the MOA: The Case for a Threshold 17

6 There Is Insufficient Evidence for an Association of Cr(VI) Exposure and Gastrointestinal Cancer in Humans 19

6.1 Occupational Exposure to Cr(VI) and Gastrointestinal Cancer 20

6.2 *Helicobacter pylori* Infection and the MOA of Human Gastric Cancer 22

7 The Inhalation Slope Factor Is Inappropriate. 22

8 OEHHA's Risk Characterization Is Inadequate. 23

9 OEHHA May Miss the Chance to be a Leader and Develop a Ground-breaking Risk Assessment 24

10 Conclusions 25

11 References 27

Appendices

Appendix A: Hexavalent Chromium [Cr(VI)] MOA Research Project To Inform EPA's IRIS Assessment.

Appendix B: ToxStrategies (2010) Technical Comments Regarding: EPA's Toxicological Review of Hexavalent Chromium In Support of Summary Information on the Integrated Risk Information System (IRIS).

Appendix C: Zacharewski, T. (2010) The U.S. EPA determined that the MOA for intestinal tumors involves a mutagenic MOA. Molecular data in this target tissue are lacking and are needed to discern whether the MOA is more likely related to mutagenicity or observed species differences in intestinal hyperplasia.

Appendix D: Summit Toxicology (2010) Comments on USEPA's Draft IRIS Report for Hexavalent Chromium. December 10, 2010.

Appendix E: De Flora, S. (2009) Comments on the draft document entitled "Public Health Goal for Hexavalent Chromium in Drinking Water", prepared by the California Environmental Protection Agency (Office of Environmental Health Assessment).

Appendix F. Texas Commission on Environmental Quality (TCEQ) (2010) Comments Regarding the U.S. Environmental Protection Agency Draft Toxicological Review of Hexavalent Chromium in Support of Summary Information on the Integrated Risk Information system (IRIS). Notice of Public Comment Period and Listening Session, 75 FR 60454, September 30, 2010, Docket ID No. EPA-HQ-ORD-2010-0540.

1 Introduction

The American Chemistry Council's Hexavalent Chromium [Cr(VI)] Panel (ACC) appreciates the opportunity to comment on OEHHA's Draft Public Health Goal for Hexavalent Chromium (Dec. 2010) (Draft Dec. 2010 PHG document). 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 Draft Dec. 2010 PHG document and the January 25, 2011, corrections.

The Draft Dec. 2010 PHG document presents risk values based on both carcinogenic and non-carcinogenic endpoints. These comments address the carcinogenic endpoint based on the oral slope factor and inhalation slope factor because the cancer endpoint forms the basis of the proposed PHG. It should be noted that the inhalation slope factor contributes only slightly to the overall risk that drives the PHG.

OEHHA received comments from the public and from peer reviewers that were highly critical of the technical aspects of the Draft August 2009 PHG document. OEHHA personnel did not adequately consider the comments. Instead, it lowered the PHG by 2/3 by applying a default adjustment based on increased early life-stage sensitivity (OEHHA, 2009).

In early 2009, ACC's Cr(VI) Panel initiated the Cr(VI) MOA Framework Research Program (Appendix A) designed to elucidate details of the carcinogenic mode of action (MOA) of Cr(VI) in rodents from oral exposures. This MOA research program includes measurements of gene expression and biochemical changes as well as traditional histopathology. An independent Science Advisory Board (SAB) was convened by Toxicology Excellence in Risk Assessment (TERA) to provide peer review and to guide this ongoing research.

OEHHA personnel have been aware of the is ongoing MOA research program and OEHHA staff attended a TERA SAB review of the Cr(VI) research plan prior to the start of the laboratory work for this research program. Hence, OEHHA personnel knew that the study was in progress but nonetheless released the Draft August 2009 PHG document and the Draft Dec. 2010 PHG document, neither of which considers the MOA. By not considering the results of the Cr(VI) MOA Framework Research Program, OEHHA has taken a position that is inconsistent with its own mission and its stated requirement to use the best available science in public health determinations.

Although there may be substantive scientific issues with the noncancer toxicity criteria derived in the Draft Dec. 2010 PHG document, these potential issues are not addressed here because of time constraints.

2 OEHHA Improperly Considers Age Adjustment and Applies the Early Life Exposure Correction

The Draft Dec. 2010 PHG document includes changes to the cancer potency slope factors derived for male and female mice; however, the adjustment for age sensitivity is the primary reason that the value of the PHG was reduced from 0.06 ppb in the Draft August 2009 PHG document to 0.02 ppb in the latest PHG document. The application of age sensitivity factors (ASFs) is based on Appendix J of *Air Toxics Hot Spots Risk Assessment Guidelines Part II: Technical Support Document for Cancer Potency Factors,* titled "In Utero and Early Life Susceptibility to Carcinogens: The Derivation of Age-at-Exposure Sensitivity Measures" (OEHHA, 2009). This appendix took a similar, but not identical, approach to that of Barton *et al.* (2005) and EPA (2005b). OEHHA developed frequency distributions of the ratios of cancer potency from exposure at early life-stages to cancer potency from exposure as an adult. In contrast, EPA (2005b) calculated point estimates not distributions.

Some of the bioassay data from which OEHHA (2009) developed these age sensitivity ratios was found to be flawed by the National Toxicology Program (NTP) and EPA.[1] After identifying chemicals for which dose response data were available to assess cancer risk from exposure at different life-stages, OEHHA (2009) then calculated distributions for the ratio between the cancer potency at an early life stage (prenatal, postnatal, juvenile) and the cancer potency during the adult life-stage for each chemical. Because exposure during an early life stage means a longer period of time in which to develop cancer compared to exposure as an adult, a time-of-dosing factor was developed that incorporates the life-stage duration and the fact that cancer risk increases by the third power of age.[2] These life-stage potency (LP) ratio distributions were then multiplied by a

---

[1] The chemical that initiated consideration of the possibility of increased susceptibility to cancer from early life exposure was vinyl chloride. The Ramazzini Institute, an animal testing facility in Italy, conducts cancer bioassays. Maltoni *et al*. (1981) and (1984) provided the data upon which the inhalation unit risk value for vinyl chloride in EPA's Integrated Risk Information System (IRIS) is based, and this work on juvenile animals provoked the idea of increased early life sensitivity.

Recently, EPA placed four IRIS assessments on hold and indicated it would review the risk assessments for vinyl chloride and 1,1-dichloroethylene because of problems with methodologies used and conclusions reached by the Ramazzini Institute that were identified by the NTP (EPA, 2010b). Hence, the idea of increased early life susceptibility was based on bioassays that may be methodologically flawed. Nonetheless, this idea of increased early life susceptibility has gained considerable traction within the risk assessment community. Given these problems with data from the Ramazzini Institute, codifying this idea in guidance such as OEHHA (2009) or EPA (2005b) may have been premature.

[2] The third power of age is also used in survival adjustment of the number of animals at risk in cancer bioassays, called poly-3 adjustment (Portier and Bailer, 1989).

time-of-dosing factor to yield the ASF. A Monte Carlo approach was used to develop the distributions of ASFs. The 50th percentiles of the ASF distributions are:

Prenatal (in utero) ASF	3
Postnatal (Birth – 2 yr.) ASF	13
Juvenile (2-16 yr.) ASF	5
Adult (>16 yr.) ASF	1

(OEHHA, 2009, App. J, Table 1, p. 7)

Similar to the Draft Dec. 2010 PHG document, the OEHHA (2009) document does not consider MOA. EPA's *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (EPA, 2005b) indicates that "default adjustment factors are meant to be used only when no chemical-specific data are available to assess directly cancer susceptibility form early-life exposure to a carcinogen acting through a mutagenic mode of action." The difference in application is that OEHHA (2009) indicates that life-stage adjustment should be applied for <u>all</u> carcinogens, whether acting by a mutagenic MOA or not. OEHHA (2009) combines all chemicals considered into a distribution of life-stage potency ratios regardless of the MOA of individual chemicals.

2.1 OEHHA's Calculations Are Difficult to Reproduce.

While the Draft Dec. 2010 PHG document provides sufficient detail to reproduce the calculations underlying the PHG value (page 93), the methodology and values used are not clear.

The ASF values on page 86 of the Draft Dec. 2010 PHG document are:

Prenatal (in utero) ASF	10
Postnatal (Birth – 2 yr.) ASF	10
Juvenile (2-16 yr.) ASF	3
Adult (>16 yr.) ASF	1

OEHHA does not explain why these values are different from the values presented in OEHHA (2009).

In addition, the method of calculating drinking water intake *in utero* is not provided. We can only assume that OEHHA is referring to exposure *in utero* through maternal water consumption. OEHHA should document its calculation method and clarify all assumptions.

The equation for calculating individual life-stage exposures for water consumption is

$$Exposure_j = ASF_j \, x \, d_j \, x \, cons_j^o$$

where ASF_j = Age sensitivity factor for the jth age group
d_j = exposure duration for the jth age group

$cons_j^o$ = water consumption for the jth age group

However, life-stage exposure duration is not the appropriate multiplier. Because the slope factors are in units of $(mg/kg\text{-}d)^{-1}$, the exposure term should remain in units of L/kg-d. The unit kg-d will cancel to leave a water concentration in mg/L. OEHHA does not explain why it expresses life-stage as a unit less fraction of a 70-year lifespan in the Draft Dec. 2010 PHG document.

The tap water ingestion rates used to derive the water consumption rates are hidden. For example, in the Dec. 31 release on page 93, a value of 0.045 L/kg-d is cited as the adult rate. What is not readily apparent, however, is that this number corresponds to a per-person adult consumption rate of 3.15 L/d for a 70 kg individual. This value of 3.15 L/d is higher than the 90% upper confidence interval on the 95th percentile of adults, which is 2.883 L/d (EPA, 2000). OEHHA did not calculate the water consumption rates for the other groups, and these rates may also be unrealistically high. Moreover, no body weights for the sensitive age groups are presented in the December draft (p. 93). OEHHA should provide documentation for its calculations. OEHHA should present point estimates of body weights and water consumption rates in L/d for the various age groups so the values could be more readily evaluated because these units are more familiar to most risk assessors than the water consumption expressed in L/kg-d.

The Draft Dec. 2010 PHG document cites OEHHA (2010) as the reference for water intake. OEHHA (2010) could not be found on the website; however, OEHHA (2000) is an earlier version of the *Air Toxics "Hot Spots" Program Risk Assessment Guidelines Part IV Exposure Assessment And Stochastic Analysis Technical Support Document*. The recommended values for water intake from OEHHA (2000) are 0.024 L/kg-d as the central estimate and 0.054 L/kg-d as the high-end estimate and are applicable to all age groups. These recommendations can be found on p. 8-11 of OEHHA (2000). This is another instance of the Draft Dec. 2010 PHG document contradicting OEHHA's own guidance.

Regarding tap water ingestion rates, on January 25, 2011, OEHHA released "Corrected portions of draft PHG document for hexavalent chromium." The source of the tap water ingestion rates was changed to EPA (2008) and Kahn and Stralka (2009). The tap water ingestion rates derived from these sources were 0.114, 0.041 and 0.038 L/kg-d for infants, children, and adults respectively. An attempt to derive these values is shown in the table below using the community tap water ingestion rates from Table 4 in Kahn and Stralka (2009) (Third column of that table) or from Table 3-9 in EPA (2008) (Ninth column of that table, identical values).

Age	95th %ile ingestion rate (L/kg-d)	Age Group Duration	% of total age range	Duration adjusted ingestion rate (L/kg-d)
<1 month	0.232	1 mo.	4.17%	0.00967
1 to <3 mo.	0.205	2 mo.	8.33%	0.0171
3 to <6 mo.	0.159	3 mo.	12.50%	0.0199
6 to <12 mo.	0.126	6 mo.	25.00%	0.0315
1 to <2 yr.	0.071	12 mo.	50.00%	0.0355
			Infant (0 to <2 yr.) Ingestion rate =	**0.114**
2 to <3 yr.	0.06	1 yr.	7.14%	0.00429
3 to <6 yr.	0.061	3 yr.	21.43%	0.0131
6 to <11 yr.	0.043	5 yr.	35.71%	0.0154
11 to <16 yr.	0.034	5 yr.	35.71%	0.0121
			Childhood (2 to <16 yr.) Ingestion Rate =	**0.045**
16 to <18 yr.	0.031	2 yr.	3.70%	0.00115
18 to <21 yr.	0.027	3 yr.	5.56%	0.0015
21 to <70 yr.	0.031	49 yr.	90.74%	0.0281
			Adult (16 to <70 yr.) Ingestion Rate =	**0.031**

Hence, the value for infants appears correct, but not the values for children and adults. OEHHA should show the derivation of these tap water ingestion rates. The value of 0.038 L/kg-d corresponds to 2.66 L/d in a 70 kg adult. This value, though not as high as that of 3.15 L/d in the Draft Dec. 2010 PHG document, is still much higher than the value of 2 L/d, recommended in EPA's *Exposure Factors Handbook* (1997) and familiar to risk assessors.[3] Assuming that OEHHA's calculations of the tap water ingestion rates were incorrect and those shown above are correct, the adult tap water ingestion rate of 0.031 L/kg-d would correspond to a value of 2.17 L/d, much closer to the EPA default value. OEHHA should use the correct values for adults' and children's drinking water ingestion rates when revising this Draft PHG document.

In the Draft Dec. 2010 PHG document, there is absolutely no discussion about the form in which this tap water is consumed. This is an important issue for Cr(VI), as it is well known to be rapidly reduced to Cr(III) in some beverages that are made from tap water in the home (*e.g.*, orange juice, lemonade, coffee, tea). It appears that OEHHA assumes that all water consumed by the adult is from the same source, despite the fact that most people go

[3] It should be noted that in 2009 EPA released and external review draft of an update to the *Exposure Factors Handbook* (EPA, 2009). This update recommends the use of the data from Kahn and Stralka (2009).

to work or school and move several times in a 70-year lifetime. Such compounded conservative assumptions in exposure assessment overestimate the true risk.

In addition, the definitions following the equation on page 93 of the Draft Dec. 2010 PHG document indicate that an ASF for the combined life-stages of the third trimester + infancy was used. This is not consistent with Table 17 on page 94 of the Draft Dec 2010 PHG document that shows separate groups for the third trimester and infancy.

In summary, the early-in-life adjustment should not be used because there is no evidence of increased early life stage susceptibility. If OEHHA had considered MOA data, this would be clear. Also, the Draft Dec. 2010 PHG document should provide readers sufficient detail to repeat the calculations therein and adequate citations for government source documents including hyperlinks and page numbers.

3 OEHHA Has Not Properly Supported Changes from the 2009 Draft PHG

3.1 Dose-Response Modeling

Dose-response modeling for the oral slope factor was based on the results from NTP (2008). Neither the Draft Dec. 2010 PHG document nor the Draft August 2009 PHG document provides the information needed for dose-response modeling in a single place. Instead, the doses, the number of animals with cancer, and the number of animals at risk are scattered throughout the documents. In the Draft Dec. 2010 PHG document, the number of mice with cancer and number at risk are shown in Tables 5 and 6 on page 49 and the doses are buried in the narrative on page 74. Not presenting this information in a single place hinders the investigation of the differences and reconstruction of the modeling so the results can be verified.

Moreover, the dose-response curves for male and female mice that illustrate these data are shown in Figure 13 on page 53 of the August 2009 draft document. Figure 13 was deleted from the Draft Dec. 2010 PHG document, which is notable because Figure 13 showed that the dose-response was not linear at the doses used in the 2008 NTP report.

One possible reason why the ED_{10} and LED_{10} values have changed is the use of different numbers of animals at risk, as shown in Tables 5 and 6 in both draft documents (pp. 51 and 52 of the 2009 draft and p. 49 of the 2010 draft). The rationale for choosing the number of animals at risk is given in a footnote below each table. The rationale in both documents seems quite similar and it is not clear why there is a discrepancy in the values. Why did the number of animals at risk increase from the 2009 document to the 2010 document?

The purpose of dose-response modeling is to explore various model choices, and then use the model to determine the doses associated with a range of point of departure (POD) values in order to provide an estimate of the range of uncertainty in the selected cancer potency slope (EPA, 2005a). In Tables 9 and 10 in the 2009 draft document, OEHHA

presented the ED_{10} and LED_{10} values for eight different cancer models with a single POD – 10%. In the Draft Dec. 2010 PHG document, Tables 9 and 10 now only show the results of a single model, the Multistage (MS) model, at the same 10% POD.

By using only a single model in the Draft Dec. 2010 PHG document, and by not exploring a range of PODs, OEHHA thwarts the intent of U.S. EPA's Cancer Guidelines (EPA, 2005a). Moreover, the Draft Dec. 2010 PHG document lacks transparency by not showing the uncertainties in risk assessment and not providing the basis for the choices of various numbers.

The lack of consideration of uncertainty also runs counter to comments made by peer reviewers. As pointed out by Dr. Michael Kelner, the cancer potency slope can be highly dependent on which POD (10%, 5%, or 1%) is selected for its determination. Prof. Mitchell Cohen, in his 2010 comments, points out that the set of values of 1%, 5%, and 10% excess cancer risk could be used as points of departure. Dr. Cohen reminds OEHHA of U.S. EPA's recommendation to "routinely calculate and present the point estimate of the ED_x [a central tendency estimate] and the corresponding upper and lower 95% statistical bounds." It is not clear why OEHHA disregards these peer reviewers' comments. OEHHA should revise its Draft PHG document to present these data prior to issuing a final PHG.

A risk assessment should be sufficiently robust in its calculations and explanations of choices or decisions made to withstand healthy skepticism. Equally important, OEHHA should heed the U.S. EPA Science Advisory Board's recommendations cited by Dr. Cohen so that its risk assessment is written in a manner that would better inform decision makers, primarily about the uncertainty that is inherent but unknown when only a single point estimate of risk is presented. As currently written, the Draft Dec. 2010 PHG document suggests there is only one possible value for the PHG when, in fact, consideration of uncertainty suggests a range of values and that the value depends on the choices made in the course of data evaluation, calculations and modeling.

An example of the type of modeling that is indicated in EPA (2005a) is shown in the tables below. This example is not comprehensive nor is it intended to be; rather, the modeling below attempts to explore some of the choices for quantitative empirical dose-response modeling. The two tables below show the fit of the various models including the quadratic multistage model (MS2) and cubic multistage model (MS3) for male and female mice using different choices for the number of animals at risk. The doses, cancer incidence and animals at risk are shown in the column headings. The best fitting model is shown in bold face and shaded gray.

Model	Male Mice (doses = 0, 0.45, 0.9, 2.4, 5.7 mg/kg-d)								
	# at Risk from Dec 2010 draft (1/49, 3/49, 2/49, 7/50, 20/48)			# at Risk from Aug 2009 draft (1/39, 3/43, 2/44, 7/48, 20/39)			Poly-3 # at Risk (1/37, 3/38, 2/40, 7/44, 20/39		
	AIC	Chi-sq	p-value	AIC	Chi-sq	P-value	AIC	Chi-sq	p-value
Gamma	161.773	1.09	0.5812	148.184	0.95	0.6219	145.728	1.10	0.5768
MS2	161.6	0.9	0.6376	146.072	0.83	0.8434	145.63	1.04	0.5959
MS3	163.558	0.82	0.3642	148.174	0.94	0.6237	145.51	0.88	0.6449
Logistic	161.762	1.07	0.5851	148.009	0.78	0.6778	143.625	0.96	0.8105
Log-Logistic	161762	1.07	0.5851	148.16	0.96	0.6179	145.716	1.09	0.5798
Probit	**159.707**	**0.96**	**0.8116**	**146.044**	**0.84**	**0.8409**	**143.546**	**0.92**	**0.8194**
Log-Probit	161.848	1.11	0.5728	148.182	0.92	0.6308	145.739	1.08	0.5838
Weibull	161.725	1.04	0.5953	148.165	0.95	0.6228	145.695	1.08	0.5814

Model	Female Mice (doses: 0, 0.3, 1.2, 3.2, 8.8 mg/kg-d)								
	# at Risk from Dec 2010 draft (1/44, 1/45, 4/47, 17/45, 22/49)			# at Risk from Aug 2009 draft (1/40, 1/40, 4/47, 17/41, 22/47)			Poly-3 # at Risk (1/45, 1/46, 4/48, 17/47, 22/48)		
	AIC	Chi-sq	p-value	AIC	Chi-sq	P-value	AIC	Chi-sq	p-value
Gamma	183.454	6.19	0.1028	177.505	7.24	0.0646	183.443	5.16	0.1604
MS-quadratic	183.454	6.19	0.1028	177.505	7.24	0.0646	183.443	5.15	0.1604
MS-cubic	183.454	6.19	0.1028	177.505	7.24	0.0646	183.443	5.15	0.1604
Logistic	194.687	18.16	0.0004	188.569	19.25	0.0002	194.532	16.88	0.0007
Log-Logistic	184.222	4.73	0.0941	178.174	5.63	0.0598	184.391	3.96	0.1378
Probit	193.275	16.87	0.0008	187.219	18.01	0.0004	193.072	15.54	0.0014
Log-Probit	**183.394**	**3.91**	**0.1415**	**177.371**	**4.83**	**0.0894**	**183.600**	**3.18**	**0.2038**
Weibull	185.130	5.65	0.0594	179.178	6.67	0.0356	185.271	4.85	0.0885

OEHHA did not choose the best-fitting model based on Chi-square or the model that provides the best fit and most parsimony based on the Akaike information criterion, as is indicated in EPA's *Benchmark Dose Technical Guidance Document* (EPA, 2000). As seen in the tables above, the models meeting these criteria are the probit model for male mice and the log-probit model for female mice.

EPA indicates that the highest dose is often omitted to improve the fit to empirical models, especially when competing toxicities at high doses or several MOAs that occur at different dose ranges (EPA, 2000, 2005a). When the highest dose was excluded, the fit of the probit model for male mice was worse (p-value = 0.6442). However, when the highest dose was excluded, the fit of all models was better for female mice. The best fitting model was the log-probit model (p-value = 0.9685). The best-fitting and most parsimonious was the probit model (p-value = 0.9467). Hence, the probit model was chosen in the example here for both male and female mice. The empirical fits discussed here were conducted using the number of animals at risk in the Draft Dec. 2010 PHG document and thus, OEHHA should conducted a full exploration of the uncertainty in empirical dose response modeling. Had OEHHA done so, it is likely that the probit model rather than the multistage model would have been chosen.

EPA's Cancer Guidelines (2005a) indicate that the point of departure should be chosen as the lower 95% confidence limit on the lowest dose level than can be supported by modeling the data. This means that the central value of the POD (the benchmark dose or BMD) should fall above the lowest dose used in the bioassay. The lowest dose for males was 0.45 mg/kg-d and for females 0.3 mg/kg-d. The BMD and its lower confidence limit (BMDL) were calculated for the best fitting models for males and females and are shown in the table below. Hence, the 2% POD is within the range of observation for male mice and the 1% POD is within the range of observation for female mice and, according to EPA (2005a), should be the preferred value from which to extrapolate to lower doses. The difference in the ED_{10} and LED_{10} values from the Draft Dec. 2010 PHG document is that a different model was used.

Male Mice # at Risk from Dec 2010 draft (doses = 0, 0.45, 0.9, 2.4, 5.7 mg/kg-d) (incidence = 1/49, 3/49, 2/49, 7/50, 20/48) Probit Model		
	BMD/ED (mg/kg-d)	**BMDL/LED (mg/kg-d)**
10% POD	2.47	2.06
5% POD	1.54	1.24
2% POD	0.755	0.472
1% POD	0.413	0.304

Female Mice # at Risk from Dec 2010 draft (doses: 0, 0.3, 1.2, 3.2) (incidence: 1/44, 1/45, 4/47, 17/45) Probit Model		
	BMD/ED (mg/kg-d)	**BMDL/LED (mg/kg-d)**
10% POD	1.60	1.30
5% POD	1.05	0.80
2% POD	0.55	0.38
1% POD	0.31	0.21

To calculate a slope factor, one would need a means for species extrapolation. EPA's Cancer Guidelines and the Agency guidance on the use of physiologically-based pharmacokinetic (PBPK) modeling in risk assessment indicate that a PBPK model is the preferred means of species extrapolation (EPA, 2005a, 2006). However, because the effects observed in mice are likely portal-of-entry effects (as discussed below), the current generation PBPK model for Cr(VI) developed by O'Flaherty *et al.* (2001) cannot be used because it does not include intestinal segmentation and therefore is structurally unable to address portal-of-entry effects in the intestinal epithelium. Instead, a next-generation PBPK model that extends the model of O'Flaherty *et al*. (2001) and incorporates the toxicokinetic features of polarity along the small intestine and partial reduction of ingested Cr(VI) while in the stomach is under development (Summit Toxicology, 2010). This report on this next-generation model is provided as Appendix D.

4 OEHHA Does Not Properly Respond to Previous Peer and Public Comments

As discussed in detail below, the Draft Dec. 2010 PHG document relies on a single analysis of very uncertain epidemiological data from China (Zhang and Li, 1987, 1997; Beaumont *et al*., 2008) and supports this reliance with an equally uncertain animal study (Borneff *et al*., 1968). The use of these data was criticized by an expert panel convened by the University of California under contract to the California EPA (OEHHA, 2001b). The panel consulted additional experts in laboratory animal medicine and veterinary pathology, and in the opinion of these experts, the lesions observed in the stomach were not cancer but rather "highly proliferative inflammatory lesions" resembling fibrosarcomas and were caused by the mouse pox epidemic in the F0 generation of the mice used by Borneff *et al*. (1968). It is noteworthy that, although the F1 and F2 generations were also exposed to Cr(VI), no excess tumors were found. The expert panel states:

> "We also conclude that the OEHHA risk assessment was not in concert with the statutory language in SB 635. SB 635 requires that '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.'" [OEHHA, 2001b, mentioning California Health & Safety Code Sec. 116365(c)(1)]

Contemporary practice in risk assessment would necessitate eschewing the use of 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.

Hence, in using Borneff *et al*. (1968) as support for the PHG value in the Draft Dec. 2010 PHG document, OEHHA is ignoring the recommendations of the University of California's expert panel.

Five peer reviewers designated by the University of California, several public water agencies, other governmental agencies, and non-governmental institutions provided extensive comments on the Draft August 2009 PHG document. These comments have been largely unaddressed in the current document. OEHHA should revisit these comments and attempt to address them in a meaningful manner. A list of the commenters/reviewers, their affiliations and the review dates is provided below. The reviews listed are for a 2008 "peer-review-only" draft and the revised Draft August 2009 PHG document. This list is comprehensive but may not be complete.

- Dr. Sharada Balakrishnan, Toxicologist, DABT, Practical Innovators, Inc. 8/25/2010
- Danielle Blacet, Regulatory Advocate, Association of California Water Agencies, 8/26/2009

- Mark S. Rentz, Director of Regulatory Affairs, Association of California Water Agencies, 8/26/2009
- Mitchell D. Cohen, PhD, Associate Professor, New York University, 8/23/2010
- David L. Berry, PhD, DABT, California Department of Toxic Substances Control, 10/23/2008
- Leonard Bjeldanes, PhD, Professor, University of California, Los Angeles, 2/13/2008
- Michael J. Rogge, California Manufacturers and Technology Association, 11/2/2009
- Robert Gwiazda, PhD, University of California, Santa Cruz, 9/09/2009
- Michael Kelner, M.D., University of California, San Diego, 2/14/2008
- Robert Gastelum, Southern California Water Committee, 10/29/2009
- Toby Rossman, PhD, Professor, New York University, 8/19/2010
- Silvio De Flora, PhD, Professor, Univeristy of Genoa, 10/19/2009
- William Shotyk, PhD, Professor, University of Heidelberg, 9/11/2010
- Elizabeth Snow, PhD, Associate Professor, University of Tasmania, 9/16/2010

5 OEHHA Continues to Fail to Consider Mode of Action.

Despite comments submitted by ACC and others in response to the Draft August 2009 PHG document, OEHHA does not consider MOA in the Draft Dec. 2010 PHG document. The term is never mentioned in the document despite the fact that MOA is the overarching concept of both the EPA (2005a) *Guidelines for Carcinogen Risk Assessment* and the current state of practice in risk assessment (Meek *et al*., 2003; Seed *et al*., 2005; Boobis *et al*., 2006; Meek, 2008; Boobis *et al*., 2009; Julien *et al*., 2009).

Chemicals may act through multiple MOAs (OEHHA, 2009; EPA, 2005a, NRC, 2009). OEHHA's own guidance indicates that when information is available about the carcinogenic MOA, this information should be used in developing toxicity criteria (OEHHA, 2009). In the case of Cr(VI), OEHHA uses default approaches for carcinogens, including linear low dose extrapolation, allometric scaling for interspecies extrapolation, and the application of age sensitivity factors. The use of default approaches is in direct contradiction with OEHHA's guidance.

With regard to the MOA, OEHAA did not consider several key questions about the NTP study results, including:

- 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 duodenum as a function of dose?
- If Cr(VI) were acting as a mutagen, then why were no tumors present in the stomach or forestomach of either mice or rats; why not in multiple tissues?
- 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 MOA in mice and is it relevant to humans?**

ACC's Cr(VI) MOA Framework Research Program (Appendix A) was developed to answer these questions. Female B6C3F1 mice approximately 5-7 weeks of age on the first day of dosing have received drinking water containing sodium dichromate dihydrate (SDD) at concentrations of 0, 0.3, 4, 14, 60, 170, or 520 mg/L (equivalent to 0, 0.1, 1.4, 4.9, 20.9, 59.3, and 181.4 mg Cr/L, respectively). These concentrations are similar to those used in the NTP studies with the exception of the 0.3 and 4 mg/L dose levels. The latter two concentrations were included in the current study to evaluate the MOA at more relevant environmental exposure levels. 0.3 mg/L SDD is equivalent to the EPA drinking water MCL of 0.1 mg/L for total chromium.

One cohort of 25 mice/group was removed from the study after 7 days of dosing (*i.e.,* on Day 8) and the remaining mice were removed on Day 91 or 92. Microscopic lesions were observed in the duodenum and jejeunum at 170 mg/L and 520 mg/L at 8 days. Similar lesions were observed at 60 mg/L, 170 mg/L and 520 mg/L after 90 days. A dose-dependent increase in 8-isoprostane and a dose-dependent decrease in the reduced-to-oxidized glutathione ration (GSH/GSSG) were observed at both 8 and 90 days, indicating an oxidative stress response. However, no increase in oxidative DNA damage measured by 8-hydroxydeoxyguanine (8-OHdG) was observed at any dose. Preliminary evaluation of toxicogenomic responses are consistent with increased oxidative stress. Finally, exposure

to mice of 0.1 mg/L Cr(VI), corresponding to the MCL for total chromium levels, did not result in an increase in chromium levels in any tissue, including the duodenum.

The results of the research will be reported at the March 2011 Society of Toxicology Conference, and manuscripts for peer review and publication are expected to be complete by June 2011.

By not considering the results of the Cr(VI) MOA Framework Research Program, OEHHA has taken a position that is inconsistent with its own mission and stated requirement to use the best available science in public health determinations.

For additional information, OEHHA should refer to ACC's comments on U.S. EPA's Integrated Risk Information System (IRIS) Review of Hexavalent Chromium, including the following documents attached as appendices.

- ToxStrategies (2010) Hexavalent Chromium [Cr(VI)] MOA Research Project To Inform EPA's IRIS Assessment (Appendix A);
- ToxStrategies (2010) Technical Comments Regarding: U.S. EPA's Toxicological Review of Hexavalent Chromium In Support of Summary Information on the Integrated Risk Information System. (Appendix B);
- Zacharewski, T. (2010) The U.S. EPA determined that the MOA for intestinal tumors involves a mutagenic MOA. Molecular data in this target tissue are lacking and are needed to discern whether the MOA is more likely related to mutagenicity or observed species differences in intestinal hyperplasia. (Appendix C);
- Summit Toxicology (2010) Comments on USEPA's Draft IRIS Report for Hexavalent Chromium. December 10, 2010. (Appendix D).

5.1 Toxicokinetics

NTP (2008) observed that most small intestinal tumors observed in mice were located in the duodenum, proximal to the stomach. Dose-related chromium levels were measured in the glandular stomach and livers of female mice and were higher than dose-related levels in these same tissues in rats (NTP, 2008). This suggests that the first susceptible tissue in mice encountered by ingested Cr(VI) is the <u>small intestinal</u> mucosa. In contrast, the tumors of the <u>oral</u> mucosa observed in rats are likely a portal-of-entry effect. These species differences in tumor site concordance and tissue susceptibility raise the question of human relevance. The risk analyses conducted in the Draft Dec. 2010 PHG document assume that humans and mice are equally susceptible, even though mice and rats are not equally susceptible. The findings in NTP (2008) indicate the need for a careful deliberative consideration of MOA rather than simply choosing the most sensitive response upon which to base a toxicity criterion.

Also supporting a portal-of-entry effect is the observation that tumor frequency was reduced in the jejeuna of the mice and tumors were absent from the ilea in both male and female mice. Hence, the tumors in the small intestine of mice are related to direct contact of Cr(VI) with the small intestinal epithelium. The oral tumors observed in rats are very likely a direct-contact effect as well. These observations highlight the fact that consideration of variability in species-specific gastrointestinal (GI) tract anatomy and physiology is critical to understanding the relevance of the mouse intestinal tumors to low-concentration oral exposures to Cr(VI) in humans.

Because the effects of ingested Cr(VI) observed in rats and mice in NTP (2008) are portal-of-entry effects, the use of allometric scaling (*e.g.,* BW^0.25) is not an appropriate method for species extrapolation. Additional discussion of this point is provided in the comments of ToxStrategies (2010), Appendix B, and Summit Toxicology (2010), Appendix D.

Bioavailability of Cr(VI) and the role of the reductive capacity of the stomach are important considerations for toxicokinetics. The Draft Dec. 2010 PHG document provides a lengthy discussion of reduction by saliva and gastric fluids and the effect of this reduction on absorption and subsequent tissue concentrations of chromium (pp. 9-12). However, OEHHA has not carefully examined tissue levels in the study used as the basis of OEHHA's cancer slope factor. NTP (2008) provides tissue chromium concentrations in both male rats and female mice in the forestomach, glandular stomach and liver (Appendix J in NTP, 2008). From these tissue concentrations, it is evident that tissues of female mice absorb considerably more chromium than male rats. It cannot be determined from these data whether the increased absorption in mice is due to a <u>lower reductive capacity</u> of gastric fluid in mice than that observed in rats or <u>greater absorption</u> of Cr(VI) in mice than that in rats. In either case, these toxicokinetic differences raise the question of the extent of reductive capacity and actual absorption that would occur in humans and whether the tumors observed in mice are relevant to humans.

The consideration of toxicokinetics in the previous section also bears on the analysis and determination of the MOA. With a mutagenic MOA, as presented in McCarroll *et al*. (2010) (referenced on page 35 of the Draft Dec. 2010 PHG document), one would expect neoplasms at sites of contact with the highest Cr(VI) concentrations (*e.g.*, the glandular stomach and forestomach). The logical explanation for the absence of tumors in these tissues, and duodenal-to-ileal polarity of the tumors that were observed, is that Cr(VI) reduction in the mouth and stomach lowered the effective dose to the stomach. There are two conclusions one can reach, either there was greater uptake in the small intestine epithelium, or this tissue possessed greater sensitivity to the effects of Cr(VI) than did the epithelium of the glandular stomach or forestomach. Additionally, there is no evidence of systemic carcinogenesis despite the accumulation of Cr in tissues such as the liver and kidney.

For additional discussion of the ramifications of reductive capacity, bioavailability, absorption and toxicokinetics, please refer to the comments of ToxStrategies (2010), Appendix B, and Summit Toxicology (2010), Appendix D.

5.2 Mutagenicity or DNA-Reactivity

Currently, data that would enable a determination of the MOA for tumor development in the small intestines of mice are not available. However, several recent papers provide additional information on the MOA of tumor formation by Cr(VI) (Holmes *et al*., 2008; Thompson *et al*., 2011; Nickens *et al*., 2010; Chiu *et al*., 2010). On page 72 of the Draft Dec. 2010 PHG document, OEHHA states that Cr(VI) is genotoxic both *in vivo* and *in vitro*. It would be more to correct, however, to state that Cr(VI) is DNA-reactive both *in vivo* and *in vitro*.

The papers cited above show evidence of DNA-reactivity but not necessarily genotoxicity and definitely not mutagenicity. Neither DNA-reactivity nor genotoxicity can be equated with mutagenicity. As peer reviewer Prof. Toby Rossman comments on page 2 of his 2009 review, "DNA damage *per se* does not inform us about eventual heritable change [*i.e.,* a mutation], which is the true issue." Prof. Rossman went on to say "[t]he description of an agent as 'genotoxic carcinogen' is out of date. What we really need to know is whether an agent has a mutagenic mode of action (MOA)." "Genotoxicity" is not a specific finding, and the term "DNA-reactivity" should be used instead. More importantly, OEHHA must make a determination that Cr(VI) has a mutagenic MOA to justify the use of linear extrapolation from the point of departure to zero.

McCarroll *et al*. (2010) (referenced on page 35 of the Draft Dec. 2010 PHG document) suggest that Cr(VI) acts by a mutagenic MOA. In EPA's recent Toxicological Review of Cr(VI), this conclusion drove the decision to use linear extrapolation and to apply an age-dependent adjustment factor (EPA, 2005a, 2005b, 2010). However, this conclusion is incorrect. Assuming that Cr(VI) acts by a mutagenic MOA ignores the existence of DNA repair mechanisms, the production of reactive oxygen species (ROS) from reduction of Cr(VI), and resulting alterations in control of the cell cycle and apoptosis. Peer reviewer Prof. Elizabeth Snow in her 2009 comments remarks: "[a] low dose, linear response [based on a mutagenicitiy] also assumes a lack of DNA repair and other protective mechanisms with an expected maximum protective effect at low dose (cf. comment #4 on p. 3)." Each day, ROS occurring from naturally occurring substances in the body modify 20,000 bases of DNA in each cell (Sablina *et al*., 2005). Consequently, mechanisms have evolved to ameliorate this potentially large amount of DNA damage. Cr(VI) and some of its metabolic products [*e.g.,* Cr(V) and Cr(IV)] are DNA-reactive and may produce DNA damage, but DNA damage does not necessarily result in mutagenesis. 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. Repair enzymes cannot recognize a mutation once the base change is present in both DNA strands, and thus a mutation cannot be repaired. Mutations should not be equated

with genotoxicity or DNA damage. (see also peer reviewer Prof. Rossman's comment discussed above.)

In fact, DNA repair in response to damage induced by hexavalent chromium appears necessary for mutagenesis. 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 produced by Cr(VI) leads to genomic instability and 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). DNA damage may lead to genomic instability, or to mutagenesis. It is important to recognize that both these effects of DNA damage may produce cancer, but do so by very different modes of action.

Oxidative stress and ROS produce epigenetic adaptive changes in cell function that may lead to cancer. Anti-oxidant enzymes increase in tissues in rat small intestine and mouse liver in response to hexavalent chromium (Arivarasu *et al*., 2008; Wang *et al*., 2006). The p53 protein is expressed in a dose-dependent fashion in mouse liver in response to hexavalent chromium (Wang *et al*., 2010). p53 has long been known to control the cell cycle by either mitotic arrest or apoptosis, and oxidative stress by itself is a strong inducer of p53 (Tomko *et al*., 2006). Chromium forms adducts preferentially in coding regions of the p53 gene (Arakawa *et al*., 2006). In several human cell lines, hexavalent chromium induces an increase in p53 that in turn leads to an apoptotic response (Hill *et al*., 2008). The oxidative stress caused by Cr(VI) has also been shown to activate a variety of transcription factors, NF-κB, AP-1 and HIF-1 as well as p53. These factors regulate the cell cycle and are very likely involved in chromium carcinogenesis. Examination of the MOA may be able to identify some key events, such as activation of transcription factors, and whether the low dose extrapolation should be linear or nonlinear.

Holmes *et al*. (2008) suggest that the DNA damage leads to cell cycle arrest in the G2 phase of mitosis. Prolonged G2 arrest leads to chromosomal instability and ultimately to neoplastic transformation and cancer. Nickens *et al*. (2010) indicate that Cr(VI)-induced DNA damage can lead to dysfunctional DNA replication and transcription, aberrant cell cycle checkpoints, dysregulated DNA repair mechanisms, microsatelite instability, inflammatory responses, and the disruption of key regulatory gene networks. The upshot of these changes is to confer a survival-advantage to cells undergoing neoplastic transformation. Chiu *et al*. (2010) also indicate that Cr(VI)-induced cancer likely results from failure of pathways involved in cell cycle arrest and apoptosis. Thompson *et al*. (2011) focus more directly on the mouse intestinal tumors in NTP (2008) and postulate that oxidative stress and inflammation are proximal key events in the tumor MOA. In a number of studies of cancer, both inflammation and oxidative stress lead to changes in the regulation of the cell cycle and apoptosis (Fingelton *et al*., 2007; Komarova *et al*., 2005; Bucala *et al*., 2007; Valko *et al*., 2006). While it is true that DNA damage can lead to

mutations via DNA repair (Zhitkovich, 2005), it is more likely that the MOA for the late-occurring (>451 days) tumors in the small intestines of mice are fostered by epigenetic changes resulting from the cellular response to DNA damage as opposed to mutagenesis (Yao *et al*., 2008).

Dr. David Berry, then senior toxicologist for the Human and Ecological Risk Division of the California Department of Toxic Substance Control, in a memo dated October 23, 2008, echoed the conclusions of Thompson *et al*. (2011) and Nickens *et al*. (2010) indicating that mutagenesis likely does not play a role in the carcinogenic MOA of Cr(VI) (Berry, 2008). 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.

5.3 Consideration of Nonlinearity in the MOA: The Case for a Threshold

The NTP drinking water studies provide strong evidence that epithelial proliferation is likely to be an early and necessary key event underlying Cr(VI)-induced carcinogenesis of the mouse small intestine (NTP, 2007, 2008). These bioassay results also provide evidence for the temporal sequencing of subsequent key events. If Cr(VI) were acting by a mutagenic MOA, the early hyperplasia, evident by 90 days, should result in a short time-to-tumor. However, the time-to-tumor formation was extended (>451 days), and treatment did not affect survival (*i.e.,* animals were not dying early as would be expected if tumors developed early in life) in the NTP drinking water study.

Hyperplasia could easily be used as a precursor effect to inform dose-response modeling, as it is a key event in the MOA. Figure 6 in Thompson *et al*. (2011) shows that the dose response for hyperplasia is supralinear and occurs at lower doses than does the tumor response. Hence, this noncancer event could be used to develop a reference dose that is protective of the cancer endpoint. Such a method is discussed in EPA's Cancer Guidelines (EPA, 2005a, pp. 3-17 to 3-18). Certainly, the NTP bioassay results for epithelial hyperplasia in mice represent "good precursor data" and could be used to derive a point of departure for nonlinear extrapolation. For a number of reasons, including the long time-to-tumor formation (>451 days), it is highly likely that Cr(VI) produces tumors by a nonlinear MOA.

Environmentally-relevant doses of Cr(VI), such as those occurring naturally in California drinking water supplies, likely would not provide a sufficient dose of Cr(VI) to the small intestine to induce hyperplasia and, thus, carcinogenesis would not occur. The temporal progression of responses observed in the NTP bioassays indicates that histiocytic infiltration occurs in mice by 90 days; hyperplasia occurs in mice at both non-tumorigenic and tumorigenic doses by 90 days; and tumors occur at two years at doses above 1 mg/kg/d, corresponding to a concentration of 28.6 mg/L in drinking water (NTP, 2007, 2008). These data indicate a multi-step progression that is more consistent with the rarity of these tumors and their long latency. Hence, the implicit assumption of a mutagenic MOA is unfounded. Indeed, data from the same study upon which the draft PHG is based contradict this assumption. For these reasons, the choice of linear low dose extrapolation cannot be supported.

Appendix A of the Draft Dec. 2010 PHG document is titled "Carcinogenic Threshold?" but presents only data related to absorption and reductive capacity. Examination of the cancer MOA of Cr(VI), as was conducted in Thompson *et al*. (2011) and Nickens *et al*. (2010), would have provided a much stronger basis for assessing whether the observed rodent tumor response was linear or not. Had OEHHA considered MOA, or had MOA been the overarching principle of the Cr(VI) PHG risk assessment, as suggested in EPA's Cancer Guidelines (EPA, 2005a), then the idea of nonlinearity and the possibility of a threshold might have received proper consideration. OEHHA should fully discuss its rationale for choosing a linear approach over a non-linear approach by fully demonstrating both to justify its choice.

The default assumption of linearity has also been questioned in reviews of the Draft August 2009 PHG for Cr(VI). Dr. David Berry (already mentioned) wrote:

> 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.

Regarding the choice of linearity, Dr. Leonard Bjeldanes, a peer reviewer of the Draft August 2009 PHG documents, suggested that data from Bednar and Kies (1991) showed no association between cancer mortality and background levels of Cr(VI) in drinking water. Although Cr(VI) concentrations in water are not provided in Bednar and Kies (1991), it might be possible to estimate these concentrations from the total chromium concentrations.

The Draft Dec. 2010 PHG document dismisses Bednar and Kies (1991) because the analysis was for total chromium and the sampling occurred for two years only. Nonetheless, Dr. Bjeldanes is correct that Bednar and Kies (1991) could provide a rough estimate of a no effect level in humans as a means to "groundtruth" the PHG value. It should be noted that the PHG value of 0.02 ppb is five-fold lower than the detection limit and 50-fold lower than the highest concentration in Bednar and Kies (1991). Dr. Bjeldanes concludes in his comments that the Draft August 2009 PHG value is near the limits of detection and that OEHHA should justify the assumption of no-threshold. ACC agrees with this request for justification.

Further support for a nonlinear dose response is provided in the comments of Dr. Silvio De Flora of the University of Genoa in Italy. He notes that a statistically significant increase in tumors only occurred at concentrations of 172 mg/L or higher in NTP (2008). Dr. De Flora also provides extensive commentary on thresholds in his comment #9. Dr. De Flora's comments are attached in their entirety as Appendix E.

Two academic peer reviewers of the 2009 draft PHG also questioned OEHHA's assumption of a linear dose-response. Prof. Elizabeth Snow comments on the use of the 2007 NTP data saying that "a linear fit to the NTP data is the default protocol as defined by the U.S. EPA and OEHHA and that the data could equally well be fitted to a nonlinear, supralinear (concave) or 'hockey stick' response model (cf. p. 3)." She further states that "based on this study [NTP, 2007], along with very limited evidence for tumor response at the lower levels of Cr6, there is very limited evidence for a linear dose response (cf. p. 3)." Nevertheless, OEHHA continually fails to present an analysis of a nonlinear dose response as a possible alternative to its default linear extrapolation model. The 2009 peer review comments of Prof. Mitchell Cohen are even more explicit "it is clear that the data presented in the Draft [PHG] document shows that the tumor formation in the mice [NTP data] as a function of Cr^{6+} [Cr(VI)] level in drinking water is not linear (cf. p. 6)." Unfortunately, OEHHA decided to remove Figure 13 from the 2010 revised PHG. This figure would have allowed the reader to visualize the actual shape of the dose-response curves for both male and female mice in the NTP studies. These are the same data to which Professors Snow and Cohen refer.

6 There Is Insufficient Evidence for an Association of Cr(VI) Exposure and Gastrointestinal Cancer in Humans

The draft PHG document puts great weight on the data of Zhang and Li (1987) and the reanalysis of these data by Beaumont *et al*. (2008). In fact, OEHHA conducted an additional analysis and observed a statistically significant increase in the rate ratio for stomach cancer in the five villages near the Lianoning Province chromium plant using the entire province as a comparison group. The Draft Dec. 2010 PHG document points out limitations in the studies of Bednar and Kies (1991) in Nebraska and Fryzek *et al*. (2001) in California.

One study that was not considered in the Draft August 2010 PHG document was Armienta-Hernandez and Rodriguez-Castillo (1995). In contrast to Bednar and Kies (1991), analysis for Cr(VI) was conducted and a chromite refinery and tannery in town provided a continuing source of Cr(VI). Groundwater concentrations were between 0.5 and 10 mg/L. Analysis of urine of residents living near the contaminated groundwater found increased total chromium in comparison to a reference group. The authors estimated that some of the population living near the facility had been consuming water with Cr(VI) concentrations as high as 0.5 mg/L for 5 to 7 years. Yet, no adverse health effects, including cancer, were observed. These data provide evidence for a threshold for adverse effects for Cr(VI) exposure from drinking water above 0.5 mg/L. It should be noted that this observed human No-Effect-Level (NOEL) is 25,000 times greater than the PHG value in the Draft Dec. 2010 PHG document.

OEHHA also did not consider the population of Hinkley, California, a small desert town in San Bernadino county. The residents of Hinkley were potentially exposed to Cr(VI) in groundwater. 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).

The Draft Dec. 2010 PHG document points out a number of limitations with the use of the Zhang and Li data, but nonetheless uses these data to support the implicit claim of human relevance of small intestinal cancer in mice. Additional discussion of studies of human exposure to Cr(VI) in drinking water can be found in ToxStrategies (2010), included as Appendix B.

6.1 Occupational Exposure to Cr(VI) and Gastrointestinal Cancer

As a means of providing information on the possible association of ingested Cr(VI) and gastrointestinal cancer in humans, Gatto *et al*. (2010) conducted a meta-analysis of 32 studies of cancer in populations with occupational exposure to Cr(VI). The meta-analysis did not find a statistically significant effect between exposure and death from oral, esophageal, gastric or small intestinal cancer. The number of deaths from esophageal cancer appears slightly elevated when four studies of US populations were considered.

The OEHHA suggests that some populations of leather tanning workers that were included in the meta-analysis may not have had exposure due to historical changes in the leather tanning process. In addition, OEHHA indicates that Gatto *et al*. (2010) excluded a number of studies of cement workers, welders and chromate pigment production workers and provides citations (Danielsen *et al*. 1996; Enterline, 1974; Langard and Norseth, 1979) (incorrectly cited in the Draft Dec. 2010 PHG document as Enterline *et al*., 1974 and Langard *et al*., 1979).

If the statements in the Draft Dec. 2010 PHG document about historical changes in the leather tanning process are correct and had indeed occurred at the facilities studied in Iaia *et al.* (2006) and Montanero *et al*. (1997), then the inclusion criteria of Gatto *et al*. (2010), which are clearly stated in the Methods section, may need amending. It should be noted that industrial processes change slowly, with changes occurring at different times in different facilities.

The studies that OEHHA suggests adding to the meta-analysis are unlikely to change the results. The rate ratios for gastrointestinal cancers shown in Table 8 of the Draft Dec. 2010 PHG document from Danielson *et al*. (1997) (Stomach: 1.03 [0.26-2.82]) and Enterline (1974) (All digestive: 1.53 [0.91-2.45]) were not significantly elevated and thus their inclusion would be unlikely to change the meta-analysis results.

The rate ratio from Langard and Norseth (1979), provided in Table 8 of the Draft Dec. 2010 PHG document, was significantly elevated. Langard and Norseth (1979) found standardized incidence ratios of 6.38 (95% CI = 1.63 – 17.37). However, Langard and Norseth (1979) was likely not included because it was not listed in PubMed. This paper was published in *Arh Hig Rada Toksikol* (Archives of Industrial Hygiene and Toxicology), the headquarters of which are in Zagreb, Croatia. The journal has been in existence since 1950 and a PubMed search of "Arh Hig Rada Toksikol"[Journal] turned up 1694 articles. None of these articles has S. Langard as the first author.

From 1998 forward, the publications in *Arh Hig Rada Toksikol* are available online without cost at http://hrcak.srce.hr/index.php?lang=en&show=casopis&id_casopis=7. A search of Google Scholar for "Langard" and "chromate" did turn up the article listed in the Proquest database. The abstract is shown below:

> Three cases of gastrointestinal cancer are reported in a group of 24 chromate pigment workers with more than three years of chromate exposure. The expected number of gastrointestinal cancers (I.C. D. nos 150-159) in the group was estimated to be 0.47. The results indicate an increased risk of gastrointestinal cancer in the chromate pigment industry.

Given that there only 24 individuals in the group and that Gatto *et al.* (2010) used inverse variance weighting, which takes into account the size of the study population, it is unlikely that inclusion of Langard and Norseth (1979) would have affected the results of the meta-analysis of Gatto *et al.* (2010).

The Draft Dec. 2010 PHG document did not conduct a meta-analysis; instead, the results of individual studies were reported. In their Table 1, Gatto *et al.* (2010) provided the size of the study group so that readers could judge the relative power of the study. In contrast, Table 8 of the Draft Dec. 2010 PHG document does not provide the study size and no other indication of study power is provided. This is a serious omission, especially when conclusions are drawn about epidemiologic relationships, as on the bottom of page 60.

6.2 *Helicobacter pylori* Infection and the MOA of Human Gastric Cancer

Appendix B of the Draft Dec. 2010 PHG document attempts to create a link between infection with *Helicobacter pylori* and susceptibility to cancer in humans. On page 139, OEHHA states:

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

OEHHA is correct about the association of *H. pylori* infection and gastric cancer in humans. The narrative continues on page 140:

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

The association between *H. pylori* infection and gastric cancer in humans has been the subject of much research (Farinati *et al*., 2008). For example, genomic instability appears to be a hallmark of gastric cancer in humans (Rugge *et al*., 2005; Chung *et al*., 2010). Genomic instability results from DNA damage following inflammation and oxidative stress (Gonda *et al*., 2009; Hou *et al*., 2009). All of these epigenetic precancerous changes are hallmarks of both *H. pylori* infection in humans, and Cr(VI) tumorigenesis in mice (Thompson *et al*., 2011; Nickens *et al*., 2010; Holmes *et al*., 2008). Consideration of the MOA of Cr(VI) tumorigenesis using mechanistic knowledge of *H. pylori* infection in humans would likely enable the understanding of why Armienta-Hernandez and Rodriguez-Castillo (1995) observed a NOEL orders of magnitude higher than the proposed PHG value.

7 The Inhalation Slope Factor Is Inappropriate.

With regard to inhalation exposure from showering, there is a mismatch between the exposures used to develop the inhalation slope factor and showering. The inhalation slope factor was derived for a chromate processing facility. A domestic shower with a temperature of 38°C is not a reasonable target of extrapolation from metal fumes generated at temperatures over 1000°C. This issue was also raised by Dr. David Berry in his comments on an early draft of the Draft August 2009 PHG document. OEHHA should respond to Dr. Berry's comments and provide the justification for its application of an industrial inhalation slope factor to residential exposures.

8 OEHHA's Risk Characterization Is Inadequate.

On pages 94 to 96, OEHHA presents its formal risk characterization of the PHG for Cr(VI). Risk characterization is one of four major components of a health risk assessment; the other three components are hazard identification, dose-response evaluation, and exposure assessment. OEHHA's risk characterization is a qualitative summary of the steps taken to determine the PHG. In the very last paragraph on page 96, under the subheading "Risk Characterization," OEHHA states "[t]here are many sources of uncertainty in the calculation of the proposed PHG." This statement is the only discussion of uncertainty. No further discussion of the sources of uncertainty and how they might impact the calculation of the PHG is provided.

Risk managers cannot determine the level of uncertainty in the PHG. The sentence that follows the one quoted above reads: "The NTP carcinogenicity studies provide robust data for the assessment of oral cancer risk attributed to Cr IV (cf. p. 96)." This is an accurate statement; yet OEHHA deleted seven of the eight dose-response analyses it conducted using the NTP data set, the results of which were shown in Tables 10 and 11 of the Draft August 2009 PHG. In addition, OEHHA did not choose the most appropriate model based on EPA guidance (EPA, 2000, 2005a). OEHHA should fully document and provide the justification for deleting data and selecting a model that does not provide the best fit the data and violates the EPA guidance.

In the Draft Dec. 2010 PHG document, OEHHA introduces the application of age adjustment factors for the cancer potency slope of carcinogens to account for differences in susceptibility at different life stages. Application of these adjustment factors brought with them adjustment of the exposure doses from drinking water based on the 95th percentile of drinking water consumption rates at different ages. For an adult aged 17 to 70, this new consumption rate is more than 50% greater than in 2009, increasing from 2 L/day to almost 3 L/day. This new higher consumption rate would lower the numerical value of the PHG by 1/3, if the age sensitivity factors were not used. However, because of the ASFs, there is only a slight change in the value of the proposed PHG. There is no discussion of the uncertainty in water consumption rates or that these revised water consumption rates would likely produce an overly health-protective PHG value even if the ASFs were not used. In addition, the calculation of age-specific drinking water rates presented in the Jan. 25, 2011, corrections appears incorrect. OEHHA should clarify the rationale for this choice of drinking water rates and provide details of how they were calculated.

The Draft Dec. 2010 PHG document does not make clear whether the water ingested consists of all water or tap water. Kahn and Stralka (2009) indicate this was "community" water, representing tap water from a public supply. However, individuals consume tap water from a variety of sources, since both adults and children spend much of their time away from home (*e.g.*, work, school, etc.). In addition, water (from any source) may be used in commonly consumed beverages such as coffee, tea, or orange juice, and Cr(VI) would likely be reduced to Cr(III) in these beverages (Kerger *et al*., 1996).

OEHHA acknowledges other significant uncertainties in the body of the document that were not carried forward to the Risk Characterization section of the Draft Dec. 2010 PHG document. For example, on page 38, OEHHA states "[h]owever, due to the reductive capacities of the lung for inhalation exposures or the stomach for oral exposures (De Flora, 2000), it is unclear whether significant DNA damage is likely to result from low environmental exposures to Cr VI." This is critical to understanding the risk from very low environmental exposures, especially drinking water. Also on page 38, OEHHA states "currently, it is uncertain whether significant portions of lower oral doses of Cr VI evade *in situ* reduction and cause DNA damage in the oral cavity and gastrointestinal tract." Yet, there is no discussion of the possibility of a nonlinear dose-response and how this could affect the value of the PHG. In the Draft August 2009 PHG document on page 98, OEHHA clearly states there is "large uncertainty" in calculation of the PHG. This uncertainty is not discussed in the Draft Dec. 2010 PHG document. Several peer reviewers commented on the absence of analyses of the data that would show a range of results that better reflect the uncertainty and inconsistency in the shape of the dose-response curves in rats and mice. OEHHA has not responded to these comments and should do so prior to making any final decision related to its draft PHG.

9 OEHHA May Miss the Chance to be a Leader and Develop a Ground-breaking Risk Assessment

In 2009, the National Research Council (NRC) published a report entitled *Science and Decisions: Advancing Risk Assessment* (NRC, 2009). Dr. Lauren Zeise of OEHHA was one of the authors of this NRC report and an internal reviewer of the Draft Dec. 2010 PHG document. NRC (2009) indicated that dose response assessment should incorporate considerations of whether background disease processes or ongoing exposures could "linearize" the dose response. Thompson *et al.* (2011) and Nickens *et al.* (2010) provide descriptions of the possible events in the MOA of Cr(VI)-induced carcinogenesis. As indicated, these events are very similar to those associated with *H. pylori*-associated gastric cancer. OEHHA has missed an opportunity to conduct a risk assessment based on best-available science and explore in sufficient detail the quantitative relationships between key events and the apical event of cancer with consideration of background processes and exposures.

In addition, a large literature has developed regarding the genomics of gastric cancer in humans. A PubMed search for "gene expression gastric cancer" produced 4857 articles. Genomic and epigenetic profiles are being used for diagnosis and therapeutics of gastric cancer (Yamashita *et al.*, 2011). Increased expression of the MYC gene is associated with gastric cancer and measurement of MYC expression is being used a part of a predictor array for clinical outcome of chemotherapy for gastric cancer patients (Zhang *et al.*, 2010; Kim *et al.*, 2010). In recent work, Dr. Tim Zacharewski of Michigan State University investigated Cr(VI)-induced dose-dependent changes in gene expression in mouse duodenum using both microarray methods and RT-PCR. Dr. Zacharewski observed a

significant increase in MYC expression in the duodenal epithelia of mice, but only at a drinking water concentration of 520 mg/L. The observance of this threshold is also support for a nonlinear MOA. Dr. Zacharewski's preliminary report is included as Appendix C.

The use of data on the association of MYC gene expression in humans and the dose response of MYC in mouse duodenum to inform risk assessment is consistent with another NRC report, *Toxicity Testing in the 21st Century: A Vision and A Strategy* (NRC, 2007). Once the planned publications on the Cr(VI) MOA are completed, data will be available to enable the application of new and groundbreaking risk assessment methods. Failure to use the soon-to-be available MOA data will be seen by future risk assessors as a great opportunity missed.

10 Conclusions

The Draft December 2010 Public Health Goal document is deficient in a number of aspects. The document fails to address a number of comments submitted in early 2009 by peer reviewers on a 2008 draft version of the PHG and comments submitted in September 2010 by five peer reviewers on the Draft August 2009 PHG document. A number of the same flaws in the PHG document are also evident in EPA's recent Toxicological Review of Cr(VI) (EPA, 2010). The Texas Commission on Environmental Quality provided comments on the EPA document and most, if not all, of these comments are applicable to the Draft Dec. 2010 PHG document. The TCEQ comments are included as Appendix F.

The document lacks consideration of the ubiquitous and widespread presence of Cr(VI) in both groundwater and drinking water supplies. To date, no human cancers or other adverse health effects have been attributed to natural background levels of Cr(VI) in drinking water.

There is a lack of any formal consideration of the MOA of Cr(VI). This is a critical flaw in the PHG document, especially when one considers that the MOA forms the overarching conceptual framework for cancer risk assessment (EPA, 2005a). Interspecies differences in toxicokinetics of Cr(VI) were not considered appropriately, and there was a failure to recognize that effects seen in rodents are portal-of-entry effects. Nonlinear toxicodynamic effects of Cr(VI) that likely underlie the cancer response were not examined. These effects include oxidative stress, inflammation, and disruption of gene networks that regulate the cell cycle. Instead, the Draft Dec. 2010 PHG document <u>implicitly assumes</u> that Cr(VI) produces tumors by a mutagenic MOA, stating "[o]nce inside cells, Cr VI has been shown to damage DNA. The finding of **genotoxicity** in the liver following oral administration of Cr VI is consistent with both the toxicokinetic findings and the **proposed DNA-damaging mechanism** of action (cf. p. 95)." Nowhere do we find the words *mutagenic mode of action*.

Genotoxicity, or more correctly, DNA-reactivity and DNA damage are not equivalent to mutagenicity, as pointed out by Prof. Rossman. OEHHA must make a finding of

mutagenicity to justify linear extrapolation of the benchmark dose to calculate the cancer potency slope under U.S. EPA guidelines. If OEHHA cannot make this finding, then it must state that it could not make a determination of the MOA and then OEHHA has the option to default to the linear extrapolation method of analysis.

OEHHA fails to consider nonlinearity and the possible presence of a threshold. Although Appendix A, titled "Carcinogenic Threshold?," in the Draft Dec. 2010 PHG document addresses the idea of a threshold, this appendix considers only reductive capacity and absorption, and fails to take into account epigenetic changes that underlie the tumor response and that likely do have thresholds. The lack of consideration of MOA also prevents exploration of the use of precursor effects as recommended in EPA's *Guidelines for Carcinogen Risk Assessment* (EPA, 2005a).

The document uses two highly flawed studies in mice and humans respectively (Borneff *et al*., 1968; Zhang and Li, 1987) in an attempt to establish a link between Cr(VI) exposure and gastrointestinal cancer in humans. The use of these studies is in direct contradiction to the advice of an expert panel convened by the University of California in 2001 (OEHHA, 2001b).

The use of the age-sensitivity adjustment detailed in OEHHA (2009) is inappropriate because of lack of consideration of MOA. In addition, the calculations that employed this adjustment are difficult to replicate because the necessary information is scattered throughout the document and the explanation of some of the assumptions is insufficient.

The uncertainty associated with dose-response modeling is not explored. The narrative and tables describing the modeling is very brief and difficult to follow. The number of animals at risk for the various dose groups in NTP (2008) was changed from those in the Draft August 2009 PHG document without explanation.

An attempt is made to impeach the results of the Gatto *et al*. (2010) meta-analysis that found no association between occupational exposure to Cr(VI) and gastrointestinal cancer in humans. Although the Draft Dec. 2010 PHG document made several suggestions to "improve" the meta-analysis, it is unlikely that any of these suggestions would alter the results.

OEHHA should conduct a quantitative analysis of the uncertainty in the assumptions and values used in the calculation of the PHG, including a full discussion of these in the section on Risk Characterization. This point is especially true for a highly influential document such as this one (*e.g.* OMB, 2003).

The overall recommendations of these comments are that OEHHA begin with a good faith commitment to examine the MOA with sound scientific reasoning and develop the risk assessment upon which the PHG is based with careful consideration of the human relevance of the effects seen in rodents.

11 References

Arakawa, H., Wu, F., Costa, M., Rom, W. and Tang, M. S. (2006). Sequence specificity of Cr(III)-DNA adduct formation in the p53 gene: NGG sequences are preferential adduct-forming sites. *Carcinogenesis* **27**, 639-45, bgi249 [p ii] 10.1093/carcin/bgi249.

Arivarasu, N. A., Fatima, S. and Mahmood, R. (2008). Oral administration of potassium dichromate inhibits brush border membrane enzymes and alters anti-oxidant status of rat intestine. *Arch Toxicol* **82**, 951-8, 10.1007/s00204-008-0311-0.

Armienta-Hernandez, M. A. and Rodriguez-Castillo, R. (1995). Environmental exposure to chromium compounds in the valley of Leon, Mexico. *Environ Health Perspect* **103 Suppl 1**, 47-51.

Barton HA, Cogliano VJ, Flowers L, Valcovic L, Setzer RW and Woodruff TJ. 2005. Assessing susceptibility from early-life exposure to carcinogens. *Environ Health Perspect* **113**, 1125-1133.

Beaumont, J. J., Sedman, R. M., Reynolds, S. D., Sherman, C. D., Li, L. H., Howd, R. A., Sandy, M. S., Zeise, L. and Alexeeff, G. V. (2008). Cancer mortality in a Chinese population exposed to hexavalent chromium in drinking water. *Epidemiology* **19**, 12-23, 10.1097/EDE.0b013e31815cea4c
00001648-200801000-00004 [p ii].

Bednar, C. M. K., C. (1991). Inorganic Contaminants in Drinking Water Correlated with Disease Occurrence in Nebraska. *J Am Water Resour Assoc* **27**, 631-635.

Berry, D.L. (2008) Memorandum to Jeff Wong. Hexavalent Chromium Public Health Goal. California Department of Toxic Substances Control , October 23, 2008.

Boobis, A. R., Cohen, S. M., Dellarco, V., McGregor, D., Meek, M. E., Vickers, C., Willcocks, D. and Farland, W. (2006). IPCS framework for analyzing the relevance of a cancer mode of action for humans. *Crit Rev Toxicol* **36**, 781-92, X822044Q4N364552 [p ii] 10.1080/10408440600977677.

Boobis, A. R., Daston, G. P., Preston, R. J. and Olin, S. S. (2009). Application of key events analysis to chemical carcinogens and noncarcinogens. *Crit Rev Food Sci Nutr* **49**, 690-707, 914016321 [pii] 10.1080/10408390903098673.

Borneff, J., Engelhardt, K., Griem, W., Kunte, H., Reichert, J. (1968). [Carcinogens in water and soil. XXII. Experiment with 3,4-benzopyrene and potassium chromate in mice drink]. *Arch Hyg Bakteriol* **152,** 45-53.

Bucala, R. and Donnelly, S. C. (2007). Macrophage migration inhibitory factor: a probable link between inflammation and cancer. *Immunity* **26**, 281-5, S1074-7613(07)00184-7 [pii] 10.1016/j.immuni.2007.03.005.

Chiu, A., Shi, X. L., Lee, W. K., Hill, R., Wakeman, T. P., Katz, A., Xu, B., Dalal, N. S., Robertson, J. D., Chen, C., Chiu, N. and Donehower, L. (2010). Review of chromium (VI) apoptosis, cell-

cycle-arrest, and carcinogenesis. *J Environ Sci Health C Environ Carcinog Ecotoxicol Rev* **28**, 188-230, 927101365 [p ii] 10.1080/10590501.2010.504980.

Chung, W. C., Jung, S. H., Lee, K. M., Paik, C. N., Kwak, J. W., Jung, J. H., Yoo, J. Y., Lee, M. K. and Chung, I. S. (2010). Genetic instability in gastric epithelial neoplasias categorized by the revised Vienna classification. *Gut Liver* **4**, 179-85, 10.5009/gnl.2010.4.2.179.

Danielsen, T. E., Langard, S. and Andersen, A. (1996). Incidence of cancer among Norwegian boiler welders. *Occup Environ Med* **53**, 231-4.

De Flora, S. (2000). Threshold mechanisms and site specificity in chromium(VI) carcinogenesis. *Carcinogenesis* **21**, 533-41.

Enterline, P. E. (1974). Respiratory cancer among chromate workers. *J Occup Med* **16**, 523- 6.

Farinati, F., Cardin, R., Cassaro, M., Bortolami, M., Nitti, D., Tieppo, C., Zaninotto, G. and Rugge, M. (2008). Helicobacter pylori, inflammation, oxidative damage and gastric cancer: a morphological, biological and molecular pathway. *Eur J Cancer Prev* **17**, 195-200, 10.1097/CEJ.0b013e3282f0bff5 00008469-200806000-00003 [pii].

Fingleton, B., Carter, K. J. and Matrisian, L. M. (2007). Loss of functional Fas ligand enhances intestinal tumorigenesis in the Min mouse model. *Cancer Res* **67**, 4800-6, 67/10/4800 [pii] 10.1158/0008-5472.CAN-06-4473.

Fryzek, J. P., Mumma, M. T., McLaughlin, J. K., Henderson, B. E. and Blot, W. J. (2001). Cancer mortality in relation to environmental chromium exposure. *J Occup Environ Med* **43**, 635-40.

Gatto, N. M., Kelsh, M. A., Mai, D. H., Suh, M. and Proctor, D. M. (2010). Occupational exposure to hexavalent chromium and cancers of the gastrointestinal tract: a meta-analysis. *Cancer Epidemiol* **34**, 388-99, S1877-7821(10)00053-6 [pii] 10.1016/j.canep.2010.03.013.

Gonda, T. A., Tu, S. and Wang, T. C. (2009). Chronic inflammation, the tumor microenvironment and carcinogenesis. *Cell Cycle* **8**, 2005-13, 8985 [pii].

Hill, R., Leidal, A. M., Madureira, P. A., Gillis, L. D., Cochrane, H. K., Waisman, D. M., Chiu, A. and Lee, P. W. (2008). Hypersensitivity to chromium-induced DNA damage correlates with constitutive deregulation of upstream p53 kinases in p21-/- HCT116 colon cancer cells. *DNA Repair (Amst)* **7**, 239-52, S1568-7864(07)00351-5 [pii] 10.1016/j.dnarep.2007.10.001.

Hirose, T., Kondo, K., Takahashi, Y., Ishikura, H., Fujino, H., Tsuyuguchi, M., Hashimoto, M., Yokose, T., Mukai, K., Kodama, T. and Monden, Y. (2002). Frequent microsatellite instability in lung cancer from chromate-exposed workers. *Mol Carcinog* **33**, 172-80, 10.1002/mc.10035 [pii].

Holmes, A. L., Wise, S. S. and Wise, J. P., Sr. (2008). Carcinogenicity of hexavalent chromium. *Indian J Med Res* **128**, 353-72.

Hou, L., Savage, S. A., Blaser, M. J., Perez-Perez, G., Hoxha, M., Dioni, L., Pegoraro, V., Dong, L.

M., Zatonski, W., Lissowska, J., Chow, W. H. and Baccarelli, A. (2009). Telomere length in peripheral leukocyte DNA and gastric cancer risk. *Cancer Epidemiol Biomarkers Prev* **18**, 3103-9, 1055-9965.EPI-09-0347 [pii] 10.1158/1055-9965.EPI-09-0347.

Iaia, T. E., Bartoli, D., Calzoni, P., Comba, P., De Santis, M., Dini, F., Farina, G. A., Valiani, M. and Pirastu, R. (2006). A cohort mortality study of leather tanners in Tuscany, Italy. *Am J Ind Med* **49**, 452-9, 10.1002/ajim.20309.

Julien, E., Boobis, A. R. and Olin, S. S. (2009). The Key Events Dose-Response Framework: a cross-disciplinary mode-of-action based approach to examining dose-response and thresholds. *Crit Rev Food Sci Nutr* **49**, 682-9, 914014390 [pii] 10.1080/10408390903110692.

Kahn, H. D. and Stralka, K. (2009) Estimated daily average per capita water ingestion by child and adult age categories based on USDA's 1994-1996 and 1998 continuing survey of food intakes by individuals. *J Expo Sci Environ Epidemiol* **19**, 396-404. 10.1038/jes.2008.29

Kerger, B. D., Richter, R. O., Chute, S. M., Dodge, D. G., Overman, S. K., Liang, J., Finley, B. L. and Paustenbach, D. J. (1996). Refined exposure assessment for ingestion of tapwater contaminated with hexavalent chromium: consideration of exogenous and endogenous reducing agents. *J Expo Anal Environ Epidemiol* **6**, 163-79.

Kim, H. K., Choi, I. J., Kim, C. G., Kim, H. S., Oshima, A., Yamada, Y., Arao, T., Nishio, K., Michalowski, A. and Green, J. E. (2010). Three-gene predictor of clinical outcome for gastric cancer patients treated with chemotherapy. *Pharmacogenomics J*, tpj201087 [pii] 10.1038/tpj.2010.87.

Komarova, E. A., Krivokrysenko, V., Wang, K., Neznanov, N., Chernov, M. V., Komarov, P. G., Brennan, M. L., Golovkina, T. V., Rokhlin, O. W., Kuprash, D. V., Nedospasov, S. A., Hazen, S. L., Feinstein, E. and Gudkov, A. V. (2005). p53 is a suppressor of inflammatory response in mice. *FASEB J* **19**, 1030-2, 04-3213fje [pii] 10.1096/fj.04-3213fje.

Langard, S. and Norseth, T. (1979) Cancer in the Gastrointestinal Tract in Chromate Pigment Workers. *Arh Hig Rada Toksikol* **30**, 301-4.

Lengauer, C., Kinzler, K. W. and Vogelstein, B. (1998). Genetic instabilities in human cancers. *Nature* **396**, 643-9, 10.1038/25292.

Maltoni, C., Lefemine, G., Ciliberti, A., Cotti, G. and Carretti, D. (1981). Carcinogenicity bioassays of vinyl chloride monomer: a model of risk assessment on an experimental basis. *Environ Health Perspect* **41**, 3-29.

Maltoni, C; Lefemine, G; Ciliberti, A; et al. (1984) Experimental research on vinyl chloride carcinogenesis. In: *Archives of research on industrial carcinogenesis. Vol. 2*. Maltoni, C; Mehlman, MA, eds. Princeton, NJ: Princeton Scientific Publishers, Inc.

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.

Meek, M. E., Bucher, J. R., Cohen, S. M., Dellarco, V., Hill, R. N., Lehman-McKeeman, L. D., Longfellow, D. G., Pastoor, T., Seed, J. and Patton, D. E. (2003). A framework for human relevance analysis of information on carcinogenic modes of action. *Crit Rev Toxicol* **33**, 591-653.

Meek, M. E. (2008). Recent developments in frameworks to consider human relevance of hypothesized modes of action for tumours in animals. *Environ Mol Mutagen* **49**, 110-6, 10.1002/em.20369.

Montanaro, F., Ceppi, M., Demers, P. A., Puntoni, R. and Bonassi, S. (1997). Mortality in a cohort of tannery workers. *Occup Environ Med* **54**, 588-91.

Morgan, J. W. and Prendergast, T. (2000) Community Cancer Assessment in Hinkley California, 1988-1993 at http://www.dscsp.com/pdfs/HinkleyCancerAssessment.pdf

National Research Council (NRC) (2007) *Toxicity Testing in the 21st Century: A Vision and A Strategy.* Available at http://www.nap.edu/catalog/11970.html. Accessed June, 2009.

National Research Council (NRC). (2009). *Science and Decisions: Advancing Risk Assessment Approaches Used by the U.S. EPA*. Available at: http://www.nap.edu/catalog.php?record_id=12209. Accessed January 20, 2009.

National Toxicology Program (NTP). (2007). NTP technical report on the toxicity studies of sodium dichromate dihydrate administered in drinking water to male and female F344/N rats and B6C3F1 mice and male BALB/c and am3-C57BL/6 mice. January 2007 National Institutes of Health Public Health Service U.S. Department of Health and Human Services. Accessed at: http:// ntp.niehs.nih.gov/ntp/htdocs/ST_rpts/TOX72.pdfbm94934.

National Toxicology Program (NTP). (2008a). Final technical report on the toxicology and carcinogenesis studies of sodium dichromate dihydrate in F344/N rats and B6C3F1 mice. Accessed at: http://ntp.niehs.nih.gov/files/546_web_FINAL.pdfS.

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.

OEHHA (1999) Public Health Goal for Chromium In Drinking Water, California Environmental Protection Agency. At www.oehha.ca.gov/water/phg/pdf/chrom_f.pdf

OEHHA (2000) *Air Toxics "Hot Spots" Program Risk Assessment Guidelines Part IV Exposure Assessment And Stochastic Analysis Technical Support Document*, Chapter 8 at http://www.oehha.ca.gov/air/hot_spots/finalStoc.html#download

OEHHA (2001a) Announcement of Public Meeting Requesting Public Input on the Review of Scientific Questions Regarding the Potential of Chromium 6+ to Cause Cancer When Ingested. At http://www.oehha.ca.gov/public_info/public/chr6meets.html

OEHHA (2001b) Scientific Review of Toxicological and Human Health Issues Related to the Development of a Public Health Goal for Chromium (VI). A Report Prepared by the Chromate Toxicity Review Committee. Panelists: Flegal, R., Last, J., McConnell, E.E., Schenker, M., and Witschi, H. at http://oehha.ca.gov/public_info/facts/pdf/crpanelrptfinal901.pdf

OEHHA (2009) *Technical Support Document for Cancer Potency Factors: Methodologies for derivation, listing of available values, and adjustments to allow for early life stage exposures* (May 2009) at http://www.oehha.ca.gov/air/hot_spots/tsd052909.html

Office of Management and Budget (OMB). (2003) Circular A-4, Regulatory Analysis at http://www.whitehouse.gov/omb/circulars_a004_a-4/

O'Flaherty, E. J., Kerger, B. D., Hays, S. M. and Paustenbach, D. J. (2001). A physiologically based model for the ingestion of chromium(III) and chromium(VI) by humans. *Toxicol Sci* **60**, 196-213.

Portier, C. J. and Bailer, A. J. (1989). Testing for increased carcinogenicity using a survival-adjusted quantal response test. *Fundam.Appl.Toxicol.* **12**, 731-737.

Rugge, M., Bersani, G., Bertorelle, R., Pennelli, G., Russo, V. M., Farinati, F., Bartolini, D., Cassaro, M. and Alvisi, V. (2005). Microsatellite instability and gastric non-invasive neoplasia in a high risk population in Cesena, Italy. *J Clin Pathol* **58**, 805-10, 58/8/805 [pii] 10.1136/jcp.2004.025676.

Sablina, A. A., Budanov, A. V., Ilyinskaya, G. V., Agapova, L. S., Kravchenko, J. E. and Chumakov, P. M. (2005). The antioxidant function of the p53 tumor suppressor. *Nat Med* **11**, 1306-13, nm1320 [pii] 10.1038/nm1320.

Seed, J., Carney, E. W., Corley, R. A., Crofton, K. M., DeSesso, J. M., Foster, P. M., Kavlock, R., Kimmel, G., Klaunig, J., Meek, M. E., Preston, R. J., Slikker, W., Jr., Tabacova, S., Williams, G. M., Wiltse, J., Zoeller, R. T., Fenner-Crisp, P. and Patton, D. E. (2005). Overview: Using mode of action and life stage information to evaluate the human relevance of animal toxicity data. *Crit Rev Toxicol* **35**, 664-672.

Summit Toxicology (2010) Comments on USEPA's Draft IRIS Report for Hexavalent Chromium. December 10, 2010.

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.

Tomko, R. J., Jr., Bansal, P. and Lazo, J. S. (2006). Airing out an antioxidant role for the tumor suppressor p53. *Mol Interv* **6**, 23-5, 2, 6/1/23 [pii] 10.1124/mi.6.1.5.

ToxStrategies (2010) Technical Comments Regarding: EPA's Toxicological Review of Hexavalent Chromium In Support of Summary Information on the Integrated Risk Information System (IRIS) Appendix B.

U.S. EPA (1997) *Exposure Factors Handbook (Final Report*) EPA/600/P-95/002F a-c at http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=12464

U.S. EPA (2000) *Benchmark Dose Technical Guidance Document.* EPA/630/R-00/001. External Review Draft. At http://www.epa.gov/ncea/pdfs/bmds/BMD-External_10_13_2000.pdf

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

U.S. EPA (2005b). *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens,* EPA/630/R-03/003F. Risk Assessment Forum: U.S. Environmental Protection Agency, Washington, D.C.

U.S. EPA (2006) Approaches for the Application of Physiologically Based Pharmacokinetic (PBPK) Models and Supporting Data in Risk Assessment. EPA/600/R-05/043F. August, 2006. At http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=157668

U.S. EPA (2008) Child-Specific Exposure Factors Handbook. EPA/600/R-06/096F at http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm.deid=199243.

U.S. EPA. (2009) *Exposure Factors Handbook (External Review Draft) 2009 Update*. EPA/600/R-09/052A at http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=209866#Download

U.S. EPA (2010a). Toxicological Review of Hexavalent Chromium. (CAS No.18540-29-9). In Support of Summary Information on the Integrated Risk Information System (IRIS) (External Review Draft) at http://cfpub.epa.gov/ncea/iris_drafts/recordisplay.cfm?deid=221433

U.S. EPA (2010b) News Release: EPA Places Four IRIS Assessments on Hold Pending Review at http://yosemite.epa.gov/opa/admpress.nsf/0/b64d44f06a56d5b285257742007c5002?OpenDocument

Valko, M., Rhodes, C. J., Moncol, J., Izakovic, M. and Mazur, M. (2006). Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chem Biol Interact* **160**, 1-40, S0009-2797(05)00433-3 [pii] 10.1016/j.cbi.2005.12.009.

Wang, X. F., Xing, M. L., Shen, Y., Zhu, X. and Xu, L. H. (2006). Oral administration of Cr(VI) induced oxidative stress, DNA damage and apoptotic cell death in mice. *Toxicology* **228**, 16-23, S0300-483X(06)00498-7 [pii] 10.1016/j.tox.2006.08.005.

Yamashita, K., Sakuramoto, S. and Watanabe, M. (2011). Genomic and epigenetic profiles of gastric cancer: Potential diagnostic and therapeutic applications. *Surg Today* **41**, 24-38, 10.1007/s00595-010-4370-5.

Yao, H., Guo, L., Jiang, B. H., Luo, J. and Shi, X. (2008). Oxidative stress and chromium(VI) carcinogenesis. *J Environ Pathol Toxicol Oncol* **27**, 77-88, 779c060611675f49,5dca8c313f0c7d56 [pii].

Zhang, J. D. and Li, X. L. (1987). [Chromium pollution of soil and water in Jinzhou]. *Zhonghua Yu Fang Yi Xue Za Zhi* [Chinese Journal of Preventive Medicine] **21**, 262-4.

Zhang, J. D. and Li, S. (1997). Cancer mortality in a Chinese population exposed to hexavalent chromium in water. *J Occup Environ Med* **39**, 315-9.

Zhang, L., Hou, Y., Ashktorab, H., Gao, L., Xu, Y., Wu, K. and Zhai, J. (2010). The impact of C-MYC gene expression on gastric cancer cell. *Mol Cell Biochem* **344**, 125-35, 10.1007/s11010-010-0536-0.

Zhitkovich, A. (2005). Importance of chromium-DNA adducts in mutagenicity and toxicity of chromium(VI). *Chem Res Toxicol* **18**, 3-11, 10.1021/tx049774+.

Appendices

Appendix A: Hexavalent Chromium [Cr(VI)] MOA Research Project To Inform EPA's IRIS Assessment.

Appendix B: ToxStrategies (2010) Technical Comments Regarding: EPA's Toxicological Review of Hexavalent Chromium In Support of Summary Information on the Integrated Risk Information System (IRIS).

Appendix C: Zacharewski, T. (2010) The U.S. EPA determined that the MOA for intestinal tumors involves a mutagenic MOA. Molecular data in this target tissue are lacking and are needed to discern whether the MOA is more likely related to mutagenicity or observed species differences in intestinal hyperplasia.

Appendix D: Summit Toxicology (2010) Comments on USEPA's Draft IRIS Report for Hexavalent Chromium. December 10, 2010.

Appendix E: De Flora, S. (2009) Comments on the draft document entitled "Public Health Goal for Hexavalent Chromium in Drinking Water", prepared by the California Environmental Protection Agency (Office of Environmental Health Assessment).

Appendix F. Texas Commission on Environmental Quality (TCEQ) (2010) Comments Regarding the U.S. Environmental Protection Agency Draft Toxicological Review of Hexavalent Chromium in Support of Summary Information on the Integrated Risk Information system (IRIS). Notice of Public Comment Period and Listening Session, 75 FR 60454, September 30, 2010, Docket ID No. EPA-HQ-ORD-2010-0540.