SOUTHERN CALIFORNIA WATER COMMITTEE

October 29, 2009

Mr. Michael Baes
Pesticide and Environmental Toxicology Branch
Office of Environmental Health Hazard Assessment
1515 Clay Street, 16th floor
Oakland, CA 94612

Attn: PHG Project

Re: Comments on OEHHA's "Draft Public Health Goal for Hexavalent Chromium in Drinking Water"

Dear Mr. Baes:

I write on behalf of the Southern California Water Committee. We are a non-profit organization with a broad membership, including water agencies and professionals in the field of water resources management. We are writing to provide comments on the proposed "Draft Health Goal for Hexavalent Chromium in Drinking Water" ("Draft PHG"), which the Office of Environmental Health Hazard Assessment ("OEHHA") released for public comment on August 20, 2009.

OEHHA proposes a Draft PHG of 60 parts per trillion, a drastically low value, which, for the reasons summarized in this letter, we believe to be inappropriate and ill advised given the state of the science and the potential economic impacts. In addition, the goal is so low that it is immeasurable with existing standard technology used by commercial laboratories, which could result in much of California's water resources being considered impaired and could affect the availability of 30-65 percent of California's water supplies (according to analysis of data from the Department of Public Health and estimates by OEHHA at the October 14, 2009 public workshop on the Draft PHG). To adopt an immeasurably low value as a PHG that may make it impossible for water purveyors to meet a subsequently promulgated enforceable drinking water standard would be bad public policy, would needlessly compromise public confidence in the water resources, and would set a dangerous precedent.

OEHHA's risk assessment process is unreliable for multiple reasons, which we summarize below. Also attached and incorporated with my letter are additional technical objections as well as copies of the documents cited in the attached comments.

California law requires the agency to prepare the risk assessment "using the most current principles, practices, and methods used by public health professionals who are experienced practitioners in the fields of epidemiology, risk assessment, and toxicology." Cal. Health & Safety Code § 116365(c)(1). Unfortunately, OEHHA has failed to do so in preparing this Draft PHG, as also recognized by the Department of Toxic Substances Control ("DTSC") in a memorandum dated October 23, 2008, analyzing the pre-release version of the Draft PHG. Furthermore, OEHHA has not rectified past mistakes made in previous draft PHGs for chromium, which were pointed out by past reviewers of these documents. For example, three reviewing bodies - the 1996 Risk Assessment Advisory Committee (made up of 34 nationally renowned scientists), the 2001 external peer review panel (comprising University of California experts), and the 2005 external scientific peer reviews (also consisting of University of California experts) - were critical of previous draft PHGs because they contained hypotheses that were too speculative and did not constitute good science.

These mistakes pointed out by the external peer reviewers, including the reviewing University of California bodies mentioned above, have been repeated in the current Draft PHG. Although its own procedures and California statutes require it to respond to the issues raised by external peer reviewers, OEHHA has not adequately done so in the Draft PHG. Importantly, by continuing to ignore EPA's 2005 Guidelines for Cancer Risk Assessment by failing to provide a range of risks associated with the PHG, OEHHA ignores the recommendation of University of California reviewer Dr. Michael Kelner that OEHHA comply with these guidelines by providing a range of risks rather than just one value for risk, which provides a sense of certainty that does not actually exist.

DTSC, in its memorandum, also remarks on OEHHA's non-compliance with EPA guidance in preparing the Draft PHG. EPA guidance requires that where appropriate scientific data is available, an agency use other methodologies to assess carcinogenesis. Both DTSC and University of California peer reviewers recommended that OEHHA include in the Draft PHG an analysis of alternative approaches to calculate cancer risk, as set forth in EPA guidance. OEHHA did not use any other methods to do so. OEHHA instead improperly used a default linear extrapolation procedure, in which the results of a study in which rodents are exposed to high doses are linearly extrapolated across five orders of magnitude of dose to estimate the risk to humans from much lower environmental exposures. Such a linear extrapolation method is extremely conservative, which leads to inappropriate overestimation of the cancer risk of ingested hexavalent chromium, as DTSC points out. OEHHA should have analyzed all available data to determine whether alternatives such as a non-linear analytical approach would have been appropriate.

OEHHA's error in defaulting to the linear extrapolation procedure instead of determining whether other alternatives could have been more appropriate is compounded by its failure to push back release of the Draft PHG until the release of currently ongoing studies that will provide additional information. For example, EPA is using its Integrated Risk Information System ("IRIS") program to evaluate human health risk from chromium on an expedited basis, and the Hamner Institute is evaluating a non-linear "mode of action" ("MOA") approach for the same purposes. These studies, when available, will provide additional scientific data to OEHHA to help it determine whether the best and most scientifically valid method to analyze risk from chromium is linear extrapolation, as OEHHA prematurely decided, or a non-linear MOA approach. DTSC appreciated the significance of the Hamner Institute's MOA studies, stating in its memorandum That they "are prerequisites to any revisions to the OEHHA public health goal for [hexavalent chromium."

By refusing to wait for release of EPA's and the Hamner Institute's information, OEHHA further violates the California Health & Safety Code requirement to "us[e]the most current principles, practices, and methods" in its risk assessment. This information soon will be readily available, and the short time delay in obtaining it is well outweighed by its value. Furthermore, it is possible, if not probable, that OEHHA may have made decisions that could have led to calculation of a more reasonable standard than 60 parts per trillion, undetectable through standard commercial laboratory procedures, had it used the most current information. A more appropriate PHG that still protects public health is possible, as evidenced by the Agency for Toxic Substances and Disease Registry's recent calculation of a daily dose that is five hundred times the amount calculated in the Draft PHG.

In addition to these flaws, OEHHA overlooks the advice of the Risk Assessment Advisory Committee and the two university of California external peer review panels not to rely on hypotheses that are excessively speculative. One such hypothesis that completely lacks scientific basis regards speculation that the hypothesized presence of bacteria in the digestive tracts of some humans, but not others, may aggravate health effects from chromium. In its memorandum, DTSC stated that this bacterial infection hypothesis "is speculative, lacks relevance to developing the PHG and it should be eliminated from the document as it is speculation." Reliance on this speculative hypothesis causes OEHHA to make improper findings regarding ingestion-caused cancer in humans based on tumor findings in animal studies, as well as to avoid reconciliation of incongruent studies that do not support OEHHA's decision to default to the linear extrapolation method.

OEHHA's evaluation of the value of the scientific data upon which it relies is faulty. Not only does OEHHA rely on scientific studies that have been superseded by more recent studies, it also reinterprets or ignores other analyses that do not support its own conclusions. As just one example, in the Draft PHG, OEHHA relies heavily upon the 1987 Zhang and Li analysis of the human health effects of chromium in water supplies in China. The

lead co-author of this study further assessed the data in a 1997 study, finding no statistically relevant relationship between stomach cancer in humans and consumption of hexavalent chromium-containing water. In addition, in a peer-reviewed study recently published in 2009, Kerger et al. further evaluates the original 1987 data and failed to identify a dose-response relationship or even a rational pattern of association of cancer-related mortality with exposure to chromium in the water. Despite this, OEHHA continues to reject this 1997 study, for which it was criticized by the University of California external peer review panel in 2005, and ignores the Kerger study, as neither support its conclusion. OEHHA continues to rely - inappropriately - on the 1987 study to support its position. Multiple other similar examples exist.

In summary, these flaws and the use of improper scientific procedures result in the preparation of a scientifically unreliable, overly conservative Draft PHG. With the Draft PHG set so far below current detection limits, and given that a large portion of California's water supply contains hexavalent chromium in amounts higher than this proposed level, OEHHA unnecessarily and inaccurately raises doubts about the safety of California's water resources.

OEHHA must conform to the requirements of California law and the standards of the scientific community. It is therefore essential that OEHHA reevaluate its methods and prepare a revised draft PHG "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."

I appreciate the opportunity to provide these comments on the Draft PHG, and I would look forward to discussing the points I have raised in more detail, if you wish.

Sincerely yours,

Ronald Gastelum
Interim Executive Director

Enclosures

Cc: Joan Denton, Director, OEHHA

Comments on Draft Public Health Goal for Hexavalent Chromium Prepared By Office of Environmental Health Hazard Assessment

I. Executive Summary

On August 20, 2009, the Office of Environmental Health Hazard Assessment (OEHHA) of the California Environmental Protection Agency (CalEPA) released for public comment the draft Public Health Goal (draft PHG) for Hexavalent Chromium (Cr(VI) or Cr^{+6}) of 60 parts per trillion (ppt). A careful review of the draft PHG shows fundamental flaws in the risk assessment process and science used by OEHHA to propose the draft PHG. If promulgated as a drinking water standard, it may not be achievable or even measurable with current technology. Further, by not following the most current principles of risk assessment in developing this draft PHG, OEHHA will create confusion between its practices and the guidance and health assessments provided by other federal and state agencies charged with protecting human health and the environment.

California Health and Safety Code Section 116365(c)(1) specifically requires that OEHHA employ the most current practices and methods used by health science experts when proposing a new PHG. In the past, OEHHA has been criticized for not using sound science in the development of PHGs. Three recent examples are the 1996 Risk Assessment Advisory Committee (comprised of 34 nationally known scientists), the 2001 Scientific Review and 2005 peer review provided by scientists at California universities on earlier draft chromium PHGs. Each of these bodies of scientists and their reviewers criticized OEHHA for using overly speculative hypotheses or for not using sound science as the basis for public health decisions.

Unfortunately, OEHHA has repeated these same mistakes in its draft PHG for Cr(VI).

First, OEHHA did not comply with its own and the U.S. Environmental Protection Agency's (EPA) procedures for calculating the draft PHG. As pointed out by the Department of Toxic Substances Control (DTSC) in an October 23, 2008 memorandum on the PHG (Berry, 2008) (the DTSC memorandum), the method employed by OEHHA to calculate the PHG ignored recent advances in assessing carcinogenesis. EPA guidance specifically requires alternate means of assessing the results of cancer bioassays where appropriate scientific data is available. In contrast, OEHHA ignored all other options for calculation of cancer potency and simply adopted the EPA's default "linear extrapolation" procedure for this draft PHG. This default procedure linearly extrapolates the results of a high-dose exposure rodent study across five orders of magnitude of dose to estimate the human cancer risk from far lower environmental exposures. According to DTSC, the default methods employed by OEHHA are highly conservative and improperly overestimate the carcinogenic potency of ingested hexavalent chromium. If OEHHA followed the appropriate procedures, it would analyze all the available data to determine whether the weight of evidence favored alternative conclusions such as a non-linear analytical approach. In fact, DTSC and scientific peer reviewers from the University of California (UC) suggested that an analysis of alternative approaches should have been included in the draft PHG documents. OEHHA improperly refused to do so.

Second, OEHHA did not adequately respond to several important issues raised by the University of California peer reviewers of the draft PHG. One key example regards the comments of Dr. Michael Kelner of UC San Diego's Medical Center. Dr. Kelner strongly recommended that "all the NTP [National Toxicology Program] 2007 studies need to be analyzed and slope factors derived for each study by an accepted methodology. Then the mean median (preferably) slope factor is to be utilized for subsequent calculations. NOT the 95% confidence interval." Essentially, Dr. Kelner was urging OEHHA to follow EPA's 2005 Guidelines for Carcinogen Risk Assessment and to provide a range of risks to inform decision-makers. By not doing so, OEHHA's work projects a false sense of certainty. The issues raised by the peer reviewers point to fundamental flaws in OEHHA's approach. OEHHA needs to address these important issues. In fact, it is required to so by its own procedures.

Third, OEHHA has relied on exactly the kind of overly speculative theories that it was warned not to use by both the Risk Assessment Advisory Committee and the prior peer reviewers in the 2005 peer review. With absolutely no scientific basis, OEHHA speculates that adverse effects of chromium may be exacerbated by the hypothesized presence of bacteria in the digestive tracts of some human populations, but not others. DTSC has said this so-called Helicobacter Hypothesis "is speculative, lacks relevance to developing the PHG and it should be eliminated from the document as it is speculation." For OEHHA, this pure speculation is the primary basis for linking tumor findings in animal studies to the possible occurrence of stomach cancer in humans ingesting chromium in water. Further, OEHHA uses this speculation to avoid acknowledging the disparate results of the various studies that would otherwise call into question OEHHA's decision to default to a linear dose-response extrapolation. Without this guess work about bacteria, OEHHA would not have an adequate basis for choosing a 60 ppt PHG. Instead it would have come to the same conclusion as DTSC, *i.e.*, "that ingested doses of Cr^{+6} that are insufficient to produce local irritation, tissue damage, inflammation and regenerative hyperplasia are also without additional carcinogenic risk."

Fourth, for this draft PHG, OEHHA erred in its scientific evaluation of the data in published studies in several ways. OEHHA relied on studies that have been superseded by more recent findings. It also chose to reinterpret other studies that do not fit its own conclusions. And OEHHA ignored data that did not support its conclusion. A good example of all three of these problems is OEHHA's evaluation of the 1987 Zhang and Li assessment of chromium pollution of water supplies in China. This was one of the major studies relied upon by OEHHA in developing the draft PHG. In 1997, the lead co-author of the 1987 study expanded the assessment of the data and found no statistically relevant link between stomach cancer in humans and consumption of water containing Cr(VI) (Zhang and Li, 1997).

OEHHA did an internal reevaluation of the 1987 study data (which was not peer-reviewed). The 2005 PHG scientific peer reviewers criticized OEHHA's rejection of the 1997 study, noting OEHHA's effort to explain the comparative decrease in cancers in areas in the closest proximity to the plant as "the subject of speculation." Since the re-analysis of the 1987 Zhang and Li study was a cornerstone of the OEHHA case for the carcinogenic activity of oral Cr(VI) in humans, their "analysis too, must be subjected to full peer review by specialists in the

field." OEHHA subsequently published a peer-reviewed internal OEHHA reevaluation (Beaumont *et al.*, 2008), and while this evaluation has been cited for its "serious limitations in the data and the methods of analysis" (Smith, 2009), OEHHA cites its own study and continues to rely on the original 1987 brief report. A recent peer-reviewed and published study further evaluating the original 1987 data for the exposed villages and comparing the cancer rates to nearby areas with no Cr(VI) in groundwater did not find a dose-response relationship or a coherent pattern of association of lung-, stomach-, or all-cancer mortality with exposure to Cr(VI)-contaminated groundwater (Kerger *et al.*, 2009). Thus, OEHHA apparently disregards the more recent studies – both of which did not support OEHHA's hypothesis on an association of stomach cancer in humans drinking Cr(VI)-impacted water. There are other examples where OEHHA similarly reevaluated published data and studies to support OEHHA's hypothesis that the 2008 Peer Reviewers noted as "overreaching" and that the DTSC memorandum concluded inadequately addressed the weight of evidence.

Fifth, OEHHA has ignored the fact that analyses and studies are underway that could call into question their adoption of the default linear extrapolation procedure. EPA is evaluating chromium risk on an expedited basis through its Integrated Risk Information System (IRIS) program. In addition, OEHHA is monitoring studies by the Hamner Institutes that will help determine whether the linear extrapolation method it chose or a more scientifically valid non-linear "mode of action" (MOA) approach is the more appropriate risk analysis method for chromium. DTSC recognized the importance of the Hamner Institutes program in addressing the mode of action of chromium and said that the studies should be "prerequisites to any revisions to the OEHHA public health goal for Cr^{+6}." By issuing a draft PHG without waiting for this information, OEHHA is not taking account of the most up-to-date science.

If OEHHA had used the most current cancer risk assessment tools and processes and fairly evaluated the most current chromium literature, it may well have made different decisions at critical decision points in the development of this draft PHG. Such choices and actions would likely have resulted in an analysis similar to that conducted by the federal Agency for Toxic Substances and Disease Registry (ATSDR), which identified a daily dose that is 500-fold higher than the draft PHG, yet still protective of human health (ATSDR, 2008). Ignoring good scientific procedures, OEHHA has now proposed a numerical goal that is well below levels that can be detected using standard commercial laboratory techniques.

OEHHA has drafted a PHG that is not scientifically reliable and therefore is not helpful to public health and water agencies trying to protect the public. These flaws are not without cost. As DTSC stated, "there are serious consequences associated with overly conservative analysis that fail to account for a carcinogenic MOA." As discussed by EPA's Dellarco and Baetcke (2005), application of an MOA framework to data generated from appropriate studies can also be very informative to risk assessors and policy makers. OEHHA's failure to use the latest risk assessment methods accepted by health science experts can dangerously skew future decisions regarding water supply, water quality treatment technology, and testing and monitoring methodology. By proposing a draft PHG that is so far below currently detectable levels, OEHHA has unnecessarily called into question the safety of California's water supply. Given the potentially enormous consequences to the State of California, it is essential that OEHHA be

required to rigorously follow the most current procedures and apply the most up-to-date science before adopting a PHG for chromium. Accordingly, OEHHA should re-evaluate its draft PHG, consistent with its processes, "using the most current principles, practices, and methods used by public health professionals" and the absolute best science. Once that is done, a new and scientifically valid draft PHG should be reissued and peer reviewed.

II. Background

The National Toxicology Program (NTP) conducted a carcinogenic and toxicological study of Cr(VI) in response to requests from members of the California Congressional delegation. California health and regulatory agencies also supported NTP conducting this study. California officials were concerned that they lacked information on the oral route of exposure for Cr(VI), as what information was available was insufficient to set a safe drinking water standard. The NTP study was aimed at determining carcinogenic impacts from high-dose chronic exposures to rats and mice. The NTP study, completed in July 2008, was not intended to, and did not, recommend a particular dose or regulatory exposure level. Going beyond risk assessment of oral chromium exposures to management of the risk of chromium in drinking water, OEHHA, in the draft PHG, applied certain key assumptions about dose-response relationships and other factors and then extrapolated the NTP results to calculate a draft PHG of 60 ppt for Cr(VI) that would give a theoretical risk level of 1×10^{-6} (one in a million).

III. OEHHA Did Not Apply State-of-the-Art Principles and Practices for Assessing Potential Carcinogenic Risk To Humans, Nor Did It Follow Current National and International Regulatory Program Guidelines.

As pointed out in the DTSC memorandum on the draft PHG, methods used by OEHHA in developing the draft PHG "are default protocols that were outlined in the 1985 California Department of Health Services *Guidelines for Carcinogen Risk Assessments and Their Scientific Rationale*." Fundamental to the evolution of cancer risk assessment over the last three decades has been the increased understanding of the biology of cancer and the identification of key events in carcinogenesis. Through the mid-1980s, national and international assessments of human cancer hazard and risk depended primarily on lifetime assays in rodents of potentially carcinogenic agents. Inherent in rodent-based assessments was the assumption that the observation of tumors in laboratory animals could be meaningfully extrapolated to identify potential human carcinogens and, by the use of mathematical models, to provide upper-bound estimates of risk at human doses of regulatory significance. During the same period, the potential significance of mutagenesis in carcinogenesis was becoming accepted by the scientific community.

Subsequently, it has become increasingly apparent that an appreciable number of chemicals cause cancer in laboratory animals by processes that do not involve direct interaction with DNA. These developments in understanding of the biological basis of carcinogenesis in both laboratory animals and humans have benefited risk assessment processes by providing more data on the toxicokinetics and toxicodynamics of suspect carcinogenic agents. Consideration of the biological processes involved in the carcinogenesis of specific compounds has led to the concept of mode of action.

A postulated MOA for carcinogenesis is a biologically plausible sequence of key events leading to an observed effect supported by robust experimental observations and mechanistic data. It describes key and obligatory cytological and biochemical events – that is, those that are both measurable and necessary to the observed carcinogenicity – in a logical framework. Mode of action contrasts with mechanism of action, which generally involves a sufficient understanding of the molecular basis for an effect and its detailed description so that causation can be established in molecular terms.

As described by EPA (Dellarco and Baetcke, 2005), early experience with the original mode of action framework (Sonich-Mullin *et al.*, 2001; EPA, 1999) proved it to be valuable for evaluating how an agent leads to a tumor response in laboratory animals. In 2002, EPA co-sponsored a project with the International Life Sciences Institute Risk Science Institute (ILSI-RSI) to develop a framework to evaluate the human relevance of laboratory animal tumors. An expert group convened by ILSI-RSI developed the decision logic for understanding the biological events leading to an animal tumor response and how these events relate to humans (Cohen *et al.*, 2004). This human relevance framework was illustrated with several examples. EPA called upon risk assessors to include mode of action information in a framework structure so that "facts could be laid out in a logical manner and serve as the basis on which conclusions regarding postulated mode of action and human relevance can rest."

In understanding the MOA for tumorigenesis by Cr(VI) as the basis for a sound risk assessment, it is worth noting that the 2005 EPA guidelines recommend evaluation of noncancer events that appear to be key events in the carcinogenic process. The guidelines state:

> *Dose-response assessment procedures based on tumor incidence have seldom taken into account the effects of key precursor events within the whole biological process due to lack of empirical data and understanding about these events. In this discussion, response data include measures of key precursor events considered integral to the carcinogenic process in addition to tumor incidence. These responses may include changes in DNA, chromosomes, or other key macromolecules; effects on growth signal transduction, including induction of hormonal changes; or physiological or toxic effects that include proliferative events diagnosed as precancerous but not pathology that is judged to be cancer. Analysis of such responses may be done along with that of tumor incidence to enhance the tumor dose-response analysis. If dose response analysis of nontumor key events is more informative about the carcinogenic process for an agent, it can be used in lieu of, or in conjunction with, tumor incidence analysis for the overall dose-response assessment.*

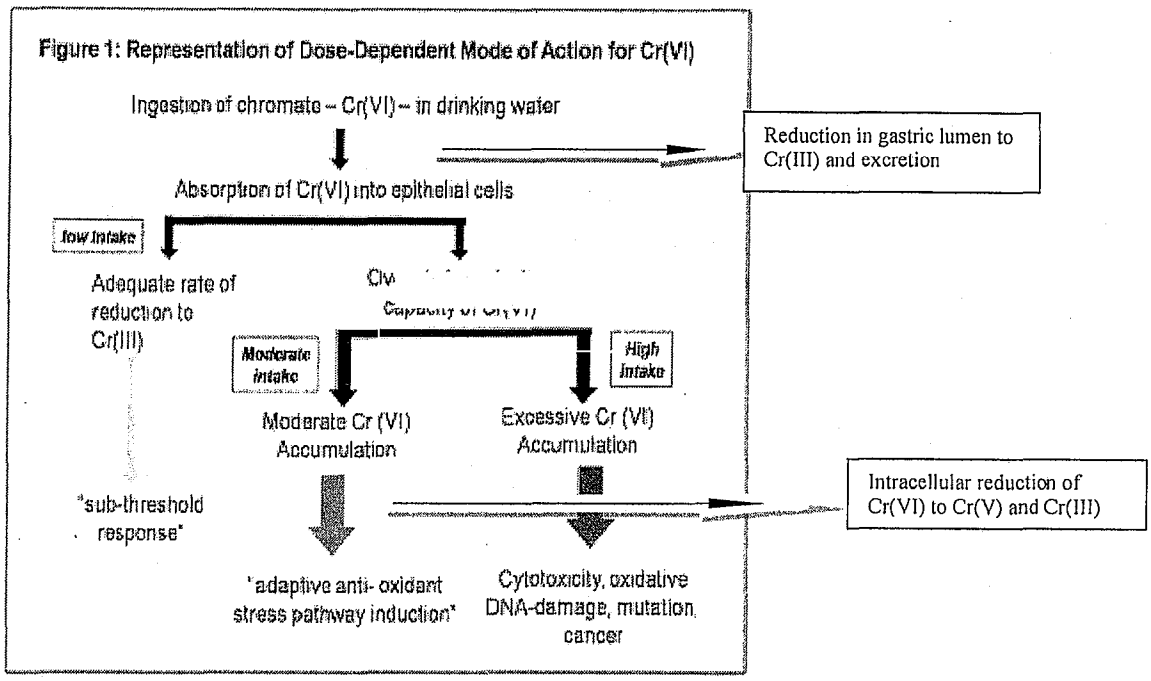
The Human Relevance Framework (HRF) for cancer provides an analytical tool to enable the transparent evaluation of the data, identification of key data gaps, and structured presentation of information that would be of value in the further risk assessment of the compound, even if human relevancy cannot be excluded. Using this framework and existing literature, it is the purpose of these comments to point out where scientifically credible information could lead to a different action than that taken by OEHHA at critical decision points in the development of the

PHG – since, as DTSC pointed out its memorandum, it "is important to remember the differences between the basic principles versus the default assumptions made in the 1985 guidance and to realize that the guidance should be modified in order to be consistent with current scientific principles (and not vice-versa)." Using this framework guidance would be consistent with California Health and Safety Code Section 116365(c)(1), which requires OEHHA to prepare the risk assessment "using the most current principles, practices, and methods used by public health professionals who are experienced practitioners in the fields of epidemiology, risk assessment, and toxicology." The elements are recognized as those required to distinguish a mere association from a causal relationship.

The elements of the framework analysis include:

- Postulated Mode of Action
- Key Events
- Concordance of Dose-Response Relationships
- Temporal Association
- Strength, Consistency and Specificity of Association of Tumor Response with Key Events
- Biological Plausibility and Coherence
- Other Postulated Modes of Action

A biologically plausible MOA can be postulated from existing data when the framework is applied. In doing so, it is recognized that there are uncertainties in the sequencing and dose-response for key events but that these uncertainties will be addressed in the Hamner Institutes' study. In summary, prolonged exposure to Cr(VI) above 14.3 mg/L sodium dichromate induces sustained cytotoxicity and cell proliferation that, as described by NTP, is regenerative hyperplasia secondary to epithelial injury. In the 2008 peer review comments of Dr. Bjeldanes, he noted his concerns about the high level of Cr(VI) in the drinking water in the NTP study and recognized that the lesions identified in the small intestine of the mouse are often considered to be pre-cancerous. This MOA has been established for other chemicals, e.g., chloroform and cacodylic acid. As a result of genetic changes within this proliferating cell population, neoplasia emerges. The genetic changes are postulated to be secondary to the cytotoxicity, metaplasia, and hyperplasia that are clearly induced by Cr(VI). Cr(VI) has been found to be genotoxic in some *in vitro* and *in vivo* test systems but was not acting as a direct mutagen.



This postulated MOA for Cr(VI) is mainly based on observations of consistent, non-linear dose-response relationships for all three key events (sustained cell injury, cell proliferation, and tumors) and concordance of incidence of diffuse hyperplasia in other regions of the intestinal tract (NTP, 2007). Studies on Cr(VI)-induced neoplasia from inhalation exposure provide consistency with recent observations with oral exposures, *e.g.*, chronic tissue injury (Nettesheim and Szakal, 1972; Derelanko *et al.*, 1999). *In vivo* and *in vitro* studies as to the mechanism of toxic and carcinogenic actions provide support that the reduction reactions involving intracellular Cr(VI) are not tissue dependent. Oral exposure of animals to Cr(VI) but not Cr(III) results in irritation and histopathological changes to tissues including cell injury, death, and regeneration (NTP, 2007; NTP, 2008). Following three-month exposures to Cr(VI), dose-responses in duodenal histiocytic infiltration of the duodenum in rats and epithelial hyperplasia and histiocytic cellular infiltration of the duodenum in mice were observed (NTP, 2007). After two years of exposure, dose-responses in duodenal histiocytic infiltration in rats and duodenal epithelial hyperplasia and histiocytic cellular infiltration in mice were observed. Diffuse epithelial hyperplasia, considered by NTP to be consistent with regenerative cell growth secondary to tissue injury, was present in all treated mice (NTP, 2008). Evidence for liver tissue injury in rats and mice exposed to oral Cr(VI) has been reported to result from acute, intermediate, and chronic oral exposures to Cr(VI) (NTP, 2007; Acharya *et al.*, 2001; Rafael *et al.*, 2007).

While citing use of EPA's 2005 risk assessment guidelines, it appears that OEHHA has missed the "changing paradigm" as described by EPA (Schoeny, 2007) that was ushered in with these guidelines and their supplemental guidance. This point was confirmed in the DTSC memorandum on the draft PHG, which stated that "the 1985 default methods ignored recent advances in interspecies scaling and evaluations of the mode of action (MOA or

toxicodynamics) ... that are used routinely by other regulatory agencies in derivation of toxicity factors for a wide range of materials." In sharp contrast to the cancer risk assessment framework used to demonstrate causation discussed above, OEHHA's apparent relevant risk assessment steps for the draft PHG were:

- Metabolism and Pharmacokinetics
- Toxicology
- Dose-Response Assessment
- Calculation of the PHG
- Risk Characterization
- Appendix A – Carcinogenic Threshold? [Note: there is no discussion of threshold in Appendix A. This is a discussion of the reducing capacity of the stomach documenting Cr(VI) absorption into the body. As questioned by the DTSC memorandum, it "is unclear how this discussion contributes to the understanding of a threshold-based dose-response relationship for ingested chromate." It is DTSC's position that "the most likely threshold effect is the ability of the hexavalent chromium to elicit dose-dependent overt tissue damage, chronic inflammation and local regenerative hyperplasia."]
- Appendix B
    - Mouse Cancer Study of Borneff *et al.*, 1968
    - The Helicobacter Hypothesis

Contrary to current cancer risk assessment guidance, no specific mode of action was identified or discussed to support the dose-response model used by OEHHA for the draft PHG. OEHHA states that taken together, "the toxicity and cancer studies in humans and animals, plus the mechanistic, toxicokinetic and genotoxicity studies, provide sufficient reason for concern regarding the carcinogenic potential of this toxicant in humans" (p. 97). Based on this, OEHHA assumed the default model to be a linear dose-response. In OEHHA's response to Dr. Kelner's 2008 peer review comments that were critical of the overestimate of risk, OEHHA states that the mode of action is unknown, and thus the default linear extrapolation applies. In its memorandum, DTSC criticized the draft PHG because "there is no *a priori* reason to accept the OEHHA assumption the Cr^{+6}-induced tumors of the gastrointestinal tract in rodents can be most accurately with statistical model that is linear at low-dose."

Contrary to current cancer risk assessment guidance, no specific mode of action was identified or discussed to support the dose-response model used by OEHHA for the draft PHG. OEHHA states that taken together, "the toxicity and cancer studies in humans and animals, plus the mechanistic, toxicokinetic and genotoxicity studies, provide sufficient reason for concern regarding the carcinogenic potential of this toxicant in humans" (p. 97). Based on this, OEHHA

assumed the default model to be a linear dose-response. In OEHHA's response to Dr. Kelner's 2008 peer review comments that were critical of the overestimate of risk, OEHHA states that the mode of action is unknown, and thus the default linear extrapolation applies. In its memorandum, DTSC criticized the draft PHG because "there is no *a priori* reason to accept the OEHHA assumption the Cr^{+6}-induced tumors of the gastrointestinal tract in rodents can be most accurately with statistical model that is linear at low-dose." In support of DTSC's position is EPA's 2005 risk assessment guidelines, which state:

> *When adequate data on mode of action provide sufficient evidence to support a nonlinear mode of action for the general population and/or any subpopulations of concern, a different approach – a reference dose/reference concentration that assumes that nonlinearity – is used. The POD [Point of Departure] is again generally a BMDL [Benchmark Dose Level] when incidence data are modeled. A sufficient basis to support this nonlinear procedure is likely to include data on responses that are key events integral to the carcinogenic process. This means that the POD may be based on these precursor response data, for example, hormone levels or mitogenic effects rather than tumor incidence data.*

The mechanistic and genotoxicity studies that OEHHA discusses fit the MOA identified above (Figure 1). OEEHA discusses the "postulated mechanism(s) of Cr(VI)-induced DNA damage that include: (1) indirect free radial DNA damage; (2) direct metal-mediated oxidative DNA damage; and (3) direct metal-DNA binding" (p. 42). OEHHA indicates that "hexavalent chromium carcinogenesis is thought to be mediated through this DNA damage" (p. 42), but recognizes that Cr(VI) may not be the species that directly causes DNA damage. Despite identifying the role of genotoxicity in the observed Cr(VI) oral carcinogenesis and recognizing that DNA damage may not occur at environmental exposure levels (p. 74), OEHHA failed to use EPA's mutagenic framework guidance document and defaulted to the linear dose-response model. This EPA guidance is important to the development of a PHG since it expands and clarifies discussions on characteristics to be evaluated to determine a chemical's potential for a "mutagenic mode of action for carcinogenicity" and whether or not a linear model applies. It should be noted that in OEHHA's draft response to peer review comments (OEHHA, 2009), OEHHA recognizes that the "possibility of a threshold for carcinogenic effects of Cr VI is an important consideration" and appears to believe it followed the most recent carcinogen guidelines and its own principals, concluding that "if there is evidence that an agent acts through a genotoxic mechanism (as there is for Cr VI), no threshold for effect is assumed." Clearly, OEHHA is out of touch with the current cancer guidelines and supplemental guidance.

It should be noted that OEHHA did not differentiate between genotoxicity and mutagenicity – which is a very important distinction. If OEHHA had utilized the concepts in EPA's Framework for Determining a Mutagenic Mode of Action for Carcinogenicity, it would have helped them determine whether or not the data support a finding of a mutagenic mode of action for carcinogenicity. The Framework also addresses the adverse endpoints of mutagenicity. OEHHA does not make one reference to Cr(VI)'s mutagenicity in the entire document – with the exception of Appendix A, where it was noted that mutagenicity tests

"have revealed that hexavalent chromium is cytotoxic to *E.coli* at concentrations of 10 to 15 ppm (Lantzsch and Gebel, 1997) or 100 to 150 ppm (Olivier and Marzin, 1987)." While not discussed by OEHHA, the finding of cytotoxicity is also important when considering different modes of action that may be operating over different dose ranges, as stated in EPA's 2005 risk assessment guidelines and referenced in the draft PHG. Such cytotoxicity supports application of a nonlinear dose-response model per EPA's 2005 risk assessment guidelines. Specifically, the guidelines state that "**depending on the strength of the suggestion of mutagenicity, the assessment may justify a conclusion that mutagenicity is not operative at low doses and focus on a nonlinear approach, or alternatively, the assessment may use both linear and nonlinear approaches.**"

In vivo genotoxicity studies indicate that there are exposures below which DNA damage would not be produced locally or systemically following ingestion of Cr(VI). Daily administration of Cr(VI) as chromate for up to 20 mg/L for nine months did not increase the frequency of DNA-protein crosslinks or produce oxidative DNA damage in mouse forestomach, glandular stomach, or duodenum (De Flora *et al.*, 2008). Micronuleus formation in bone marrow or peripheral blood in mice administered up to 500 mg/L (chromate) in drinking water for up to 210 days was not increased. No genotoxic effects in fetal liver or peripheral blood were observed in treated pregnant mice receiving up to 10 mg/L (chromate) in drinking water (De Flora *et al.*, 2006). The results of incidences of four micronucleus tests conducted in the three strains of mice from 2007 NTP were predominately negative. In Study 1 (up to 1000 mg/L dichromate in drinking water for three months), no significant increases were seen in micronucleated normochromatic erythrocytes in peripheral blood samples from male or female B6C3F1 mice. In Study 2 (up to 250 mg/L chromate in drinking water for three months), a significant exposure concentration-related increase ($P < .001$) in micronucleated normochromatic erythrocytes was seen in am3-C57BL/6 male mice (transgenic for PhiX17am3). An equivocal increase in micronucleated erythrocytes was noted in male B6C3F1 based on a small increase in micronucleated normochromatic erythrocytes that did not reach statistical significance. No increase in micronucleated changes normochromatic erythrocytes was observed in male BALB/c mice. No significant effect of sodium dichromate dihydrate exposure on the percentage of and polychromatic erythrocytes was observed in any of the three micronucleus tests conducted in Study 2 (Bucher, 2007). None of this information is discussed in the draft PHG.

OEHHA correctly points out that Cr(VI)-mediated DNA damage can be eliminated by preventing oxidative stress and free radical formation (p. 42). This is a key step or critical event in the MOA framework (Figure 1). If OEHHA had followed EPA's current cancer risk assessment guidance and framework, it would have determined that if the critical step (oxidative stress/free radical formation) is prevented, then the more deleterious effects such as mutation and tumor formation would not occur and the nonlinear model applies. Toxicologists who prepared the DTSC memorandum on the draft PHG agree and find that "it is clear that tumor development is related to local inflammation and hyperplasia in the target tissue." Further, DTSC states that 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."

Clearly, OEHHA recognized the important role of irritation/inflammation, cytotoxicity, hyperplasia in tumor formation (pp. 42, 134), yet it failed to develop the logical and well-established hypothesis for Cr(VI) mode of carcinogenic action, *i.e.*, sustained cell injury, death, and repair (Figure 1). OEHHA mentioned the NTP's findings of a significant and dose-related increase in diffuse hyperplasia in mice duodenum. OEHHA cited the NTP's findings "that collectively, these lesions are considered consistent with regenerative hyperplasia secondary to previous epithelial cell injury." But no discussion was presented by OEHHA as to the role of this finding in the observed tumorigenesis. As stated in the DTSC memorandum on the draft PHG, that is "highly indicative of a promotional mechanism that begs the discussion of a threshold dose-response." The genetic changes are postulated to be secondary to the cytotoxicity, metaplasia, and hyperplasia that are clearly induced by Cr(VI). The only discussion of the relationship between persistent cell injuries, hyperplasia, and tumor formation in the draft PHG is when OEHHA speculates on the role of stomach bacterial infection (Helicobacter) and incidence of stomach cancer, *i.e.*, OEHHA's Bacterial Infection Hypothesis.

While not stated, if OEHHA's evaluation of the weight of evidence of "all available data were insufficient to establish the mode of action" (EPA, 2005), then OEHHA should have presented alternative analyses. Specifically, OEHHA should have presented results based on both a linear <u>and</u> nonlinear approach as part of its risk characterization process. Such an analysis would help provide risk managers and decision-makers with a perspective on the uncertainty inherent in the numerical value of OEHHA's draft PHG. A calculation based solely on the linear dose-response model presents the draft PHG as if it were "the number" that would be protective of human health. Specifically, OEHHA should have followed the 2005 EPA guidance, *i.e.*, **"where alternative approaches with significant biological support are available for the same tumor response and no scientific consensus favors a single approach, an assessment may present results based on more than one approach."** Such an analysis would provide a range of values that better reflect the uncertainty in the single value calculated by OEHHA. In addition, it would have addressed one of Dr. Kelner's major criticisms of the draft PHG.

Instead of providing both the linear and nonlinear response assessment that could have been conducted in light of the uncertainties in the MOA and according to EPA's 2005 guidelines, OEHHA appears to have ignored other possible mechanisms and studies that did not support its predetermined linear model approach. This default approach results in a five orders of magnitude extrapolation from the minimal dose producing tumors (non-cancer) in mice to a one-in-a-million risk to humans – a point that each of the 2008 peer reviewers were concerned about. For example, Dr. Kelner quoted from EPA's guidelines (2005) that traits that overestimate risk include when "linear extrapolation is used as a default and extends over several orders of magnitude." Dr. Bjeldanes expressed concern as to the method used to derive the draft PHG and the lack of consideration for "the likelihood of a threshold for Cr(VI) biological activity." The weakest aspect of the estimate of the draft PHG is OEHHA's "very crude approach followed to calculate it," as stated in the 2008 peer review comments of Dr. Roberto Gwiazda (UC Santa Cruz).

Applying the benchmark dose (BMD) approach (a nonlinear dose extrapolation) to the NTP mouse duodenal hyperplasia data, as was done by ATSDR (2008) and as provided for in

EPA's 2005 guidelines, results in BMDL10 values of 0.09 to 0.13 mg Cr(VI)/kg/day. Consistent with EPA practices, an uncertainty factor of 100-fold could be applied to account for extrapolation from animals to humans (10x) and for intra-human sensitivity (10x). The resulting reference dose would be approximately 0.001 mg/kg/day. The results of applying the linear and nonlinear dose-response models yield values with more than a 500-fold difference in daily doses, with proportional differences in the corresponding drinking water criteria, *e.g.*, OEHHA's draft PHG of 60 ppt (using the linear model) and ATSDR's Minimum Risk Level of 35,000 ppt (using the BMD approach).

OEHHA did not use the most current principles and practices in determining the non-cancer health-protective dose (HPD). Rather than using the benchmark dose/nonlinear approach on the mouse data for the noncancer risk assessment, OEHHA identified the lowest adverse effect level (LOAEL) from the rat study (female liver – mild chronic inflammation, fatty changes) and applied a 1,000-fold uncertainty factor that included 10x to account for the lack of a no observed adverse effect level (NOAEL). OEHHA guidance calls for the use of BMD over NOAEL/LOAEL. The DTSC memorandum on the draft PHG found that the NTP subchronic data incorrectly identified the NOAEL as an LOAEL, and DTSC criticized OEHHA for applying 1,000-fold uncertainty factors in developing the HPD.

IV. OEHHA Should Respond to All Comments Made By All of the Peer Reviewers.

While OEHHA has labeled the response to peer reviewers comments as "draft" and states that these comments and responses are provided in the "spirit of the open dialogue among scientists that is part of the process under Health and Safety Code Section 57003," the draft PHG reflects severely limited responses to critical peer reviewers comments, which is not in the spirit of the Health and Safety Code. In essence, the University of California peer reviewers found that OEHHA "failed to demonstrate that the scientific portion of the proposed rule [draft PHG] is based upon sound scientific knowledge, methods, and practices." In this case, California Health and Safety Code Section 57004(d)(2) directs that the formal response to comments shall include OEHHA's basis for disagreement with the reviewers, as well as why it determined that its own interpretation is based on sound scientific knowledge, methods, and practices.

In particular, Dr. Kelner strongly recommended that "all the NTP 2007 studies need to be analyzed and slope factors derived for each study by an accepted methodology. Then the mean median (preferably) slope factor is to be utilized for subsequent calculations. NOT the 95% confidence interval." Dr. Kelner was urging OEHHA to use the mean or median ED10 as described in EPA's 2005 guidelines, *e.g.*, "risk assessors should calculate, to the extent practicable, and present the central estimate and the corresponding upper and lower statistical bounds (such as confidence limits) to inform decision makers." OEHHA viewed this as part of a formal uncertainty analysis for which guidelines for cancer risk extrapolation from animal data have never been provided. OEHHA states that it acknowledges the "various uncertainties inherent in cancer risk assessment" in the Risk Characterization section of the draft PHG document (pp. 96-98), but there is no quantitative assessment of uncertainty in the value of the draft PHG, nor is there any discussion or quantitative estimate of the large "uncertainty factor" in the equation used to calculate the PHG (pp. 95-96). However, there are EPA policies and guidelines for risk characterization that include uncertainty analysis. If Dr. Kelner's

recommendations and EPA risk characterization guidelines were followed, risk managers and decision-makers would be better informed of the uncertainty in the value of the draft PHG. The issues raised by the peer reviewers point to fundamental flaws in OEHHA's approach.

OEHHA concludes the four-part risk assessment process with risk characterization (pp. 96-98). Risk characterization "is considered to be a conscious and deliberate process to bring all important considerations about risk, both the **likelihood of the risk** but also the **strengths and limitations of the assessment** and a **description of how others have assessed the risk into an integrated picture** (p. 9; EPA, 2000)." Also, "the goal of risk characterization is to clearly communicate the key findings and their strengths and limitations so its **use in decision making can be put into context with the other information critical to evaluating options for rules,** regulations and negotiated agreements (*e.g.*, economics, social values, public perception, policies, etc.)" (p. 9; EPA, 2000).

V. OEHHA Should Not Base Public Health Decisions on Overly Speculative Hypotheses.

As pointed out by Seiber *et al.* (1996), what is important is that OEHHA "adopts a rational approach that includes evaluations based on all available and valid scientific data, and that the agency does not base public health decisions on overly speculative hypotheses." Contrary to the recommendations in this report and peer reviewers' comments on the 2005 draft PHG, OEHHA has developed a widely speculative hypotheses that inflammation caused by bacteria may be additive to, or synergistic with, adverse effects of hexavalent chromium-produced irritation on the stomach such that inflammation may "help push an individual along the path to stomach tumors" (OEHHA, Comments for the NTP Cr(VI) Public Meeting, July 24, 2002). Furthermore, OEHHA's approach is far from valid or conventional and dismisses valid scientific data (see Section IV).

Although OEHHA's Bacterial Infection Hypothesis is overly speculative, it serves as the basis for developing the draft PHG for Cr(VI) in drinking water of 60 ppt. Nevertheless, in the draft PHG documentation, OEHHA included the 2007 NTP study results and revived their Bacterial Infection Hypothesis to ultimately tie three key studies together to support OEHHA's preconception that Cr(VI) is carcinogenic to humans at environmentally relevant doses. OEHHA attempts to present a cohesive story for Cr(VI) oral carcinogenicity by picking what fits their hypothesis but ignoring critical information that does not. The entire basis for the derivation of the cancer potency slope factor and thus the draft PHG lies with three studies, as discussed below.

A. Borneff, J., Engelhardt, K., Griem, W., Kunte, H. and Reichert, J. "Carcinogens in water and soil. XXII. Mouse drinking water experiments with 3,4-benzopyrene and potassium chromate." *Arch Hyg Bakteriol* (1968) [translated from German].

OEHHA's 2005 PHG peer reviewers discounted the use of the Borneff *et al.* (1968) study for cancer risk assessment purposes for multiple reasons, including the high mortality associated with mouse pox outbreak in the study animals. For example, Dr. Bjeldanes called the study

"highly flawed" in his peer review comments of 2005. Nonetheless, OEHHA attempts to redeem the study and put certain findings "in a positive light" to fit their needs (p. 122). In the DTSC memorandum on the draft PHG, DTSC criticizes OEHHA for placing significant weight on the Borneff *et al.* study, indicating that although it "may be historically interesting, the study is qualitative at best" and that "the results should not have been reproduced and should be viewed as anecdotal."

Apparently, OEHHA did not respond to the original 2005 peer reviewers' comments, as the 2008 peer review draft PHG contained an unjustified and extensive presentation and discussion, according to Dr. Gwiazda's 2008 peer review comments. Dr. Gwiazda indicated his puzzlement on the amount of space devoted to the study "given the amount of uncertainty surrounding the results." In response, OEHHA moved the "extensive discussion" of the Borneff *et al.* study to an Appendix. However, OEHHA felt consideration of the Borneff *et al.* study should be used in a weight-of-evidence approach to "better understand why Cr VI is an oral carcinogen." OEHHA cites the study in the Risk Characterization section of the draft PHG document as the "study in mice provided limited data regarding increases in tumors ..." in support of a cancer endpoint in animals (p. 97). In contrast, as stated by DTSC, the "fact that only a single dose level was examined precludes any identification of a dose-response relationship, a key piece of evidence required in any assessment of causality."

In the Borneff *et al.* study, there was an increase in stomach cancers (n=2) and nine (9) benign stomach tumors in the parent generation. OEHHA's analysis of the study's data included combining the malignant and benign stomach tumors in all three generations mice in order to find statistical significance (p. 121). Increases in stomach cancer in the first (F_1) and second (F_2) generations were not observed. The authors point to a threshold for the stomach cancer that was not observed in the first generation offspring (F_1), *i.e.*, the offspring did not receive high enough doses of Cr(VI) to produce tumors. However, OEHHA discounts the authors' explanation of the study findings and promotes their own speculative hypothesis that an unidentified bacterial infection in the parental generation (F_0) was in part responsible for stomach tumor formation. Stomach cancer was not produced in the first generation (F_1) because the bacterial infection was not passed to offspring due to Cr(VI) bactericide activity (p. 126).

B. Zhang, J.D., Li, X.L. "Chromium pollution of soil and water in Jinzhou." *Zhonghua Yu Fang Yi Xue Za Zhi (Chinese Journal of Preventive Medicine* 21(5):262-4 (1987) [translated from Chinese].

OEHHA relies on the original publication regarding the Chinese population associated with drinking water contaminated with Cr(VI) (Zhang and Li, 1987) and OEHHA's reassessment of the Chinese data (Beaumont *et al.*, 2008) to draw the connection between exposure to Cr(VI) and stomach cancer in humans and various cancer sites in experimental laboratory animals. In doing so, OEHHA ignores other publications that do not support OEHHA's contention of the causal link between oral exposure to Cr(VI) and cancer in humans. A recent publication characterized data from an ecological cancer mortality study of a population where the average lung-, stomach-, and all-cancer mortality rates for the three agricultural villages without Cr(VI) in groundwater were not statistically different from those found by Zhang and Li for the five

agricultural villages with Cr(VI) in groundwater. Also, three surrogate measures of dose of Cr(VI) in village drinking water did not significantly correlate with cancer mortality rates in the five exposed villages. Further, the industrial town in which the Cr(VI) source was located had different demographics and a different pattern of stomach and lung cancers compared to the adjacent agricultural villages, regardless of Cr(VI) groundwater exposure.

The results of other local investigations on cancer mortality and genotoxicity in the exposed populations have been reviewed. The overall findings in the studied population do not indicate a dose-response relationship or a coherent pattern of association of lung-, stomach-, or all-cancer mortality with exposure to Cr(VI)-contaminated groundwater (Kerger *et al.*, 2009). Kerger *et al.* is not even referenced or discussed by OEHHA.

OEHHA's position on the Zhang and Li (1987) study is consistent with the previous draft PHG, where the peer reviewers were critical of the selectivity of OEHHA. Dr. Bjeldanes commented on OEHHA's speculation and rejection of conclusions by study authors in his 2005 comments. In Dr. Bjeldanes' 2008 comments, he voiced remaining concerns about the "interpretation of these results for the present purpose." Furthermore, Dr. Bjeldanes submitted for OEHHA's consideration a study (Bednar and Kies, 1991) where "no association was found between low levels of Cr(VI) in drinking water (up to 10 ppb) with total cancer mortality." OEHHA dismissed this study and Dr. Bjeldane's criticism of OEHHA's selectivity stating "the analysis was not specific to Cr(VI)" and that this makes it "difficult to compare the findings to those of Beaumont *et al.* (2008) of a relationship between hexavalent chromium in water and increased risk of stomach cancer." [Note: The study of the Chinese population has severe limitations on exposure and analysis.] Regarding the previous 2005 draft PHG, peer reviewer, Dr. Gwiazda commented on OEHHA's speculations when it compared cancer rates with distance from the factory "given [the] lack of clear information to characterize the pattern and magnitude of exposure."

OEHHA does recognize that the data underlying Zhang and Li (1987) have several important limitations that included lack of exposure data and a short observation time (14 years) after residents first noticed the yellow color of the water. However, OEHHA draws on the Bacterial Infection Hypothesis to overcome some of these limitations in order to continue relying upon the positive association of increased stomach and over all cancer rates with drinking Cr(VI)-tainted water. Specifically, OEHHA speculates that the uncharacteristic short duration of exposure and latency period for development of cancer is because the villagers were likely infected with the bacteria (*Helicobacter pylori*), *i.e.*, due to the Bacterial Infection Hypothesis. To OEHHA, the brief exposure duration and short latency period before the stomach cancer that was detected in the Chinese villagers is "reminiscent of the short exposure and latency period for stomach tumors in Mongolian gerbils following administration of MMG and MNU" (p. 135). In the Risk Characterization section of the draft PHG document, OEHHA states "the available human studies provided limited information on the dose-response relationship for hexavalent chromium by the oral route" (p. 97). OEHHA then acknowledges that "cancer potency values based on a dose-response relationship could not be reliably calculated from the findings of Zhang and Li (1987)" (p. 97).

C. National Toxicology Program. "National Toxicology Program Technical Report on the Toxicity Studies of Sodium Dichromate Dihydrate (CAS No. 7789-12-0) Administered in Drinking Water to Male and Female F344/N Rats and B6C3F1 Mice and Male BALB/c and *am3*-C57BL/6 Mice" (2007).

While using the data for small intestinal tumors in the NTP study to develop the cancer potency slope factor for Cr(VI), OEHHA speculates that the findings of tumors in the small intestine, in contrast to the stomach (where OEHHA reports tumors were found by Borneff *et al.* (1968) and Zhang and Li (1987)), is due to the fact that the NTP mice were free of bacterial infection (p. 136). It follows, that if these bacteria colonies were present, OEHHA would predict that there would have been tumor formation in the forestomach similar to that identified in Borneff *et al.*. (1968). OEHHA's Bacterial Infection Hypothesis requires the co-existence of Cr(VI) and acid-resistant bacteria in the stomach of the Chinese study population and the mice in the Borneff *et al.* (1968) study in order for tumors to be formed – conditions that are speculative and impossible to prove or disprove, as acknowledged by OEHHA.

When confronted with lack of concordance in tumor locations between NTP (2007), Borneff *et al.* (1968), and Zhang and Li (1987) (mouse intestine, mouse forestomach and human stomach, respectively), OEHHA evokes the Bacterial Infection Hypothesis. While the difference in tumor locations between Borneff *et al.* (1968) and Zhang and Li (1987) suggests that, at the least, mice may not be an appropriate model for humans, OEHHA postulates that Helicobacter infections are producing a "de facto" aglandular epithelium "(reminiscent of the rodent forestomach) prior to the occurrence of gastric cancer in humans." Therefore OEHHA speculates that "the rodent forestomach may be an appropriate model for tumors of the human stomach" (p. 137) – albeit as long as the bacterial infection is present. Given OEHHA's speculative hypothesis on the role of bacteria in cancer, OEHHA encourages NTP to use rodents that are infected with Helicobacter in future cancer bioassays (p. 138). In addition to noting that the NTP mice that developed intestinal cancer were bacteria-free, OEHHA ties the intestinal tumor location to the Helicobacter infection since it is characterized by the occurrence of metaplasia in the stomach – "a transformation of the stomach into a tissue that resembles intestine" (p. 136). It should be noted that all OEHHA's speculation on tumor formation is focused on the occurrence of tumors in mice and humans but does not mention or attempt to explain the tumors produced in the oral cavity of the rat in the NTP study. This is another example of OEHHA selecting data that fits its hypothesis and ignoring data that does not fit.

As another example of OEHHA's bias, OEHHA overstates the weight of evidence of Cr(VI) human carcinogenicity by the inhalation route and for the ingestion route and draws conclusions that are overreaching for the occupational exposures. OEHHA states that the International Agency for Research on Cancer (IARC) (1990) concluded that Cr(VI) is a "strong" carcinogen for the respiratory tract, while the document concluded that "there is sufficient evidence in humans for the carcinogenicity of Cr(VI) compounds as encountered in the chromate production, chromate pigment industry and chromium plating industries." It should be noted that in these occupational settings, the high Cr(VI) exposures often resulted in ulcers and perforations of the nasal septum. Based on Dr. Gwiazda's 2008 peer review comments on the peer review version of the draft PHG document, OEHHA "ignored" the confidence intervals of the

epidemiological rate ratios in reaching its (OEHHA's) conclusion that most occupational studies showed an increase risk of stomach cancer. Dr. Gwiazda commented that if OEHHA chose to include the analysis, it should be "consistent and address the contradictory observation that on the basis of the rate ratios alone, *e.g.*, 25% of the studies would support a protective role of Cr(VI) exposure against stomach cancer!"

But this logical conclusion was ignored. In response to Dr. Gwiazda's comment, the analysis was retained but revised to indicate the rate ratios above and below 1 for stomach cancer and then included the rate ratios for other sites. As pointed out in the DTSC memorandum, analyses of these same data by Cole and Rodu (2005) indicated there were no significant increases in stomach or gastrointestinal tumors associated with Cr(VI) exposure. Similar conclusions have been reached by others including IARC (1990), the World Health Organization (1988), Cohen *et al.* (1993), and De Flora (2000). However, these conclusions were ignored by OEHHA, and in doing so, OEHHA neglected a key criterion for determining the causative link between Cr(VI) exposure and cancer, *i.e.*, consistency of study results.

VI. OEHHA Should Provide a Complete Review of the Literature and Select Only the Literature That Supports Its Hypothesis.

In addition to placing an emphasis on understanding the underlying mode of action, providing a weight-of-evidence narrative, considering both linear and nonlinear extrapolations, another key feature of EPA's 2005 guidelines is "an increased emphasis on analyzing data before invoking default options." The EPA guidelines also call for an informative discussion of the scientific evidence, including a summary of the quality of the data and the degree of confidence that is placed in the risk estimates (*i.e.*, risk characterization).

As discussed above, OEHHA relies on the original publication on the Chinese population associated with drinking water contaminated with Cr(VI) (Zhang and Li, 1987) and OEHHA's reassessment of the Chinese data (Beaumont *et al.*, 2008) to draw the connection between exposure to Cr(VI) and stomach cancer in humans and various cancer sites in experimental laboratory animals. In doing so, OEHHA ignores other publications that do not support OEHHA's contention of the causal link between oral exposure to Cr(VI) and cancer in humans, including the recent publication on the study population by Kerger *et al.* (2009). Furthermore, in his 2008 peer review of the draft PHG, Dr. Bjeldanes brought the Bednar and Kies (1991) drinking water study to OEHHA's attention. In this study of 453 communities in Nebraska, no association was found between low levels of chromium in drinking water and total cancer mortality. OEHHA affirmed the finding and agreed that the data could be examined but cast doubts on the results. OEHHA believes the analytical method likely did not measure Cr(VI) but rather total chromium (Cr(VI) and Cr(III)). OEHHA ignores similar problems with the exposure assessment of the Chinese study population.

Another example of OEHHA's selective interpretation of the literature can be found in its description of the role of Cr(VI) reduction to Cr(III) in the stomach and in cells – the subject of Appendix A,, "Carcinogenic Threshold?" to OEHHA's draft PHG document. Appendix A is intended to provide support for OEHHA's default to a linear extrapolation model because a

fraction of ingested Cr(VI) is absorbed into the body – escaping the body's first line of defense, *i.e.*, gastrointestinal reduction of Cr(VI) to Cr(III). OEHHA points to the 2007 NTP study showing the dose-related systemic absorption of orally administered Cr(VI) in mice being inconsistent with the research of De Flora and others that OEHHA characterizes as the "assertion that hexavalent chromium absorption occurs only when the reducing capacity of the GI tract is exhausted." Contrary to OEHHA's interpretation of the literature, the studies published by researchers such as De Flora and others do not suggest that the detoxification pathways are 100% efficient or unsaturable. These researchers' contributions to the literature indicate that the reduction of Cr(VI) to Cr(III) in the gastrointestinal tract limits the bioavailability and attenuates the potential for adverse effects of Cr(VI) compounds *in vivo*. Apparently, OEHHA takes the position that since Cr(VI) can be absorbed into the body, inferring that there is no threshold for Cr(VI) carcinogenicity via ingestion. This is a critical OEHHA determination that ignores other mechanisms that attenuate the bioavailability and potential adverse effects of Cr(VI), including DNA damage (Sedman *et al.*, 2006). The high rate of reduction of very low concentrations of Cr(VI) to Cr(III) effectively detoxifies Cr(VI) since Cr(III) is not readily taken up by cells, *i.e.*, it is not bioavailable. This markedly changes the shape of the dose-response curve at low doses because the reduced Cr(VI) is no longer bioavailable.

The literature supports the attenuation in the bioavailability and potential adverse effects of Cr(VI). The first defense against Cr(VI) after oral exposure is reduction to Cr(III) in the gastric environmental where gastric juice and ascorbate play important roles (De Flora *et al.*, 1987; Samitz, 1970). The absorption fraction of Cr(VI) was higher when administered directly into the duodenum (approximately 10%) compared to when it is ingested (approximately 1.2%), indicating an important role of the stomach in reducing Cr(VI) to Cr(III) (Anderson *et al.*, 1983).

The absorbed fraction of Cr(VI) undergoes intracellular reduction through a number of steps, ultimately yielding Cr(III). The diffusion of Cr(VI) into the cell, the reduction to Cr(III) and the complexing to nucleic acids and proteins causes the concentration equilibrium to change so that more Cr(VI) diffuses through the membrane (Aaseth *et al.*, 1982). Reactive intermediates formed during the intracellular reduction of Cr(VI) to Cr(III) and oxidative reactions as well as complexes formed with critical target macromolecules by Cr(III), mediate Cr(VI) toxicity and carcinogenicity (Chen and Shi, 2002; Costa, 2003; Costa and Klein, 2006; Ding and Shi, 2002; Jeejeebhoy, 1999; Levin and Lay, 2005; Liu and Shi, 2001; O'Brien *et al.*, 2003; Shrivastava *et al.*, 2002; Zhitkovich, 2005; Bridgewater *et al.*, 1998; Dai *et al.*, 2009; Tully *et al.*, 2000). The rapid reduction of Cr(VI) to Cr(III) with Cr(V) and Cr(IV) as intermediates involves intracellular reductants such as ascorbate, glutathione, or amino acids (Zhitkovich *et al.*, 1996; Liu *et al.*, 1997; Blankenship *et al.*, 1997).

At the cellular level, during Cr(VI) reduction, a diverse range of genetic lesions are generated including some that likely promote a terminal cell fate, such as apoptosis or terminal growth arrest and other lesions that are potentially pre-mutagenic. Cytotoxicity, reactive oxidative stress, and DNA damage have been linked in dose- and time-dependent fashion to Cr(VI) exposure (Dana *et al.*, 2001; Wang *et al.*, 2006; Patlolla *et al.*, 2009). Cell toxicity from Cr(VI) exposure can be blocked by free radical scavengers indicating that oxygen radicals play a key role in chromium toxicity (Hojo *et al.*, 2000; Luo *et al.*, 1996; Tsou *et al.*, 1996; Ueno *et al.*,

1995). These effects support Cr(VI)-induced cytotoxicity and the possibility of epithelial cell death and regenerative proliferation in response.

OEHHA overlooked relevant *in vivo* genotoxicity data published in peer-reviewed journals during the development of the draft PHG. For example, OEHHA indicated that data on DNA damage in the oral cavity or gastrointestinal tract was needed to reduce the uncertainty in the draft PHG. Negative data does exist demonstrating that daily administration of Cr(VI) to mice at doses up to 20 mg/L (chromate) for nine months did not increase the frequency of DNA-protein crosslinks or cause oxidative DNA damage in the gastrointestinal tract (De Flora *et al.*, 2008). Data demonstrating the lack of systemic genotoxicity in erythrocytes in bone marrow and peripheral blood of mice administered up to 500 mg/L of chromate in drinking water for 210 days (De Flora *et al.*, 2006) was ignored. Furthermore, there was no recognition that Cr(VI) at concentrations up to 10 mg/L (chromate) administrated in the drinking water of pregnant mice did not produce genotoxic effects in fetal liver or peripheral blood. Curiously, the results of the four genotoxicity tests (micronucleus) conducted by NTP on three strains of mice receiving Cr(VI) in the drinking water at chromate concentrations up to 1,000 mg/L for three months were not considered by OEHHA (Bucher, 2007).

VII. Rather than Applying "Uncertainty" Factors Ranging Over Five Orders of Magnitude in Developing the Draft PHG, OEHHA Should Identify the Key Data Gaps and Acknowledge Ongoing Studies That Would Reduce the Uncertainty in OEHHA's Risk Assessment.

OEHHA's draft PHG is, at its core, an attempt to define the lowest safe level or no significant risk level from exposure of contaminants to humans based on extrapolations from a study of the effects of high dose exposure of Cr(VI) to mice. These kinds of extrapolations, by dose and species, while necessary, have been surrounded by intense discussion and debate. Such extrapolations must be conducted cautiously, with knowledge of the complexities involved, particularly when the margin of exposure from the doses experienced by humans is much lower than that tested by NTP. Nonetheless, OEHHA has calculated a new draft PHG based on the NTP study while overlooking important technical considerations.

Neither the draft PHG nor any high-dose animal study provides direct evidence for safe dose levels for humans. Rather, setting a standard requires determining the mode of carcinogenic action and using a dose-response model to extrapolate from the effects of high dose exposures. The selection of the appropriate approach for deriving the safe low dose concentration is among the most critical decisions in conducting a risk assessment. According to EPA's cancer risk assessment guidance, the first step in selecting the appropriate dose-response model is determining the MOA, the biological processes that cause tumor formation. Different understandings of the MOA drive how the data are extrapolated to the human dose. A "weight of evidence" evaluation of all relevant data should be conducted to identify the MOA for a tumor site. A linear extrapolation is the approach used when the weight of evidence of all available data are insufficient. The linear approach is to draw a straight line between a point of departure from observed data (*e.g.*, a tumor) and the origin (zero dose) using a number of mathematical models. This is the most conservative approach, yielding the most restrictive possible standard other than

zero and, as stated in the DTSC memorandum on the draft PHG, the linear model is "highly conservative and may greatly over-estimate the potency of Cr^{+6} via the oral route."

OEHHA's decision regarding the uncertainty in these matters resulted in conservative assumptions where others may have assumed differently, resulting in less conservative assumptions that would not include the use of a linear model. The linear model makes the controversial assumption that there is basically no safe level of exposure. OEHHA's use of the linear model departs from the scientific and regulatory communities that recognize there are levels of exposure to substances, which are both genotoxic and carcinogenic, below which cancer incidence is not increased.

OEHHA believed that "many sources of uncertainty are reflected by the large combined uncertainty factor used in the calculation of the proposed PHG" (p. 98) – five orders of magnitude to be precise. Some additional or better studies are needed, according to OEHHA, although the nature of those studies is not specified. Such studies are being conducted at the Hamner Institutes. The Hamner Institutes research (2009a) focuses on a series of studies associated with five key areas that affect evaluation of a MOA for Cr(VI): (1) a 90-day-in-life study to assess histological responses of the buccal and intestinal epithelium over six drinking water concentrations; (2) genomic studies on tissues from the 90-day study to assess dose response for alterations in gene families over the broad dose range; (3) pharmacokinetic modeling to evaluate expected non-linearities in epithelial tissue dose of Cr(VI); (4) *in vivo* mutation analysis from the exposed animals; and (5) high data content in vitro studies to differentially assess the relative dose response for oxidative stress and DNA damage in relevant epithelial cells from rodents and humans. A sixth part of the proposal integrates these *in vivo* and *in vitro* studies into an MOA-based risk assessment for Cr(VI). This integrated body of information will provide quantitative relationships between dose and response for multiple endpoints, and the differential dose-response relationships for these endpoints will support development of a more robust cancer risk assessments for Cr(VI) based on a better understanding of dose-dependent transitions in the MOA over broad ranges of dose. The pharmacokinetic (PK) models will evaluate expected tissue concentrations at the site of contact (*i.e.*, at the epithelial cells within the alimentary tract for Cr(VI)). Cumulatively, these data are expected to lead to and add support to a non-linear cancer risk assessment based on a point-of-departure calculation from the two-year or 90-day exposure and estimation of the margin of exposure between the point-of-departure Cr(VI) tissue levels and current Cr(VI) tissue levels associated with ambient environmental exposures in the general population.

OEHHA indicated that "if better studies of hexavalent chromium toxicity, dose-response, and exposure become available, the uncertainties associated with the risk assessment can be reduced" (p. 98). OEHHA was an observer at the Scientific Advisory Board (SAB) review of the Hamner Institutes' Cr(VI) MOA Study Protocols and had an opportunity to provide input into this important research filling data gaps in the existing oral Cr(VI) database. DTSC recognizes the importance of these ongoing studies in OEHHA's PHG efforts. Specifically, DTSC notes the importance of PK modeling in the risk assessment process, as well as the need to assess MOA using PK and genomics that are part of the Hamner Institutes program (2009a). As stated by DTSC in its memorandum, the Hamner Institutes' program is "collecting the genomic and

pharmacokinetic parameters necessary to determine the MOA and to scale properly the delivered Cr^{+6} dose to target tissues properly from rodents to humans."

Therefore, it would seem that OEHHA is aware that the Hamner Institutes' studies that would augment the presently available information, which OEHHA finds limiting, would allow OEHHA to reduce the uncertainties in the draft PHG.

VIII. OEHHA Should Take No Action to Finalize the PHG Until It Can Review the Results of the Ongoing Research, Including the Hamner Institutes' Research Into the MOA.

While other scientists may disagree, it is OEHHA's opinion that the weight of evidence supports a linear response for extrapolating tumor site (small intestine) data in mice produced by Cr(VI) in the drinking water. This determination, in conjunction with OEHHA's faulty or selective interpretation of the 2005 EPA guidelines, leads to the application of a procedure for calculating cancer potency that linearly extrapolates across five orders of magnitude of cancer incidence from the NTP data to the estimated human dose for one-in-a-million cancer incidence. While never defined as "uncertainty" in the text of the PHG document, OEHHA recognized the existence of many sources of uncertainty as reflected by the large combined "uncertainty factor" used (but not shown) to calculate the proposed PHG. OEHHA acknowledges that when and if better studies of Cr(VI) toxicity, dose-response, and exposure become available, the uncertainties associated with the OEHHA risk assessment can be reduced.

The Hamner Institutes' research (2009a) promises to advance significantly the understanding of the specific mechanisms that account for tumor formation from exposure to Cr(VI), an issue that is critical to determining risk and ultimately to setting a safe exposure level. The research proposal should lay out the key events for the hypothesized and alternative MOAs, identify measurable endpoints for each key event, and describe how the various endpoints being measured in the various assays help to evaluate those key events in the context of the modified Hill criteria/MOA framework.

The Hamner Institutes' SAB panel noted that the following are likely key events:

- Cr(VI) is taken up by the cell and causes oxidative stress as it is reduced to Cr(III)
- In the cell, Cr(III) forms DNA adducts, complexes, and cross-links
- Cytotoxicity and inflammation, and direct DNA reactivity can all result from oxidative stress
- Gene mutation
- Tumor formation

Critical to OEHHA's draft PHG determination, the Hamner Institutes' SAB unanimously agreed that oxidative stress is a key event in the carcinogenicity of chromium (Hamner Institutes, 2009b).

New studies that shed light on MOA are of great importance to regulators in setting remediation standards. Point in fact, in its Interim Genomics Policy, EPA encourages the use of toxicogenomics, a key element of the Hamner Institutes' Cr(VI) program (Hamner Institutes, 2009a) in a weight of evidence approach for risk assessment (EPA, 2002). Recently, in its document "An Approach to Using Toxicogenomic Data in U.S. EPA Human Health Risk Assessments: A Dibutyl Phthalate (DBP) Case Study," EPA has outlined the approach for utilizing toxicogenomic data in a chemical risk assessment (EPA, 2009). This document advances the effort to devise strategies for using genomic data in risk assessment by defining an approach, performing a case study, and defining critical issues that need to be addressed to better utilize these data in risk assessment. There is no reason that OEHHA should not have the benefit of these new MOA studies before adopting the final value of the PHG for Cr(VI). More specifically, if the Hamner Institutes-hypothesized MOA is further validated, it substantially changes key assumptions of the draft PHG that would result in recalculation of the draft PHG. OEHHA should postpone finalizing the draft PHG or any action to re-set its own standards until it has the full benefit of the Hamner Institutes studies (2009a).

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