Update on Draft Chromium 6 Public Health Goal

The Office of Environmental Health Hazard Assessment (OEHHA) is required, under existing statute (SB 351, Ortiz, Chapter 602, Statutes of 2001), to develop a Public Health Goal (PHG) for chromium 6, also known as hexavalent chromium. The Department of Health Services (DHS) will use the PHG as the health basis to develop a regulatory Maximum Contaminant Level (MCL) for chromium 6.

The University of California has completed an external scientific peer review of a pre-release draft of OEHHA's chromium 6 PHG assessment. The peer reviewers were Dr. Leonard Bjeldanes, Nutritional Sciences and Toxicology, UC Berkeley; Dr. Roberto Gwiazda, Environmental Toxicology, UC Santa Cruz; and Dr. Michael Kelner, Department of Pathology, UC San Diego. OEHHA is revising its draft assessment based on the peer review comments. Upon completion of these revisions, OEHHA will release the draft for public review and comment, and will schedule a public workshop. OEHHA expects to release the draft document in fall 2005.

OEHHA considered both cancer and noncancer data in developing the pre-release draft PHG. There are insufficient data to reliably calculate a PHG based on a cancer study. Therefore, the draft PHG will be based on a noncancer endpoint.

The National Toxicology Program (NTP) currently is conducting a major toxicological study to help determine whether chromium 6 causes cancer when ingested. OEHHA and DHS were among the entities that petitioned NTP to undertake this study. OEHHA will review data from the NTP study when it becomes available and will make any necessary revisions to the PHG. In the meantime, OEHHA will finalize the public-review draft of the PHG based on noncancer health effects and proceed with the public review process.

Because OEHHA considers the pre-release draft a "pre-decisional" document, and it is undergoing revision, it is not being made available for distribution. However, OEHHA is releasing the external peer reviews of that document. Those reviews are attached.

Attachment

OEHHA
8/25/05

Review of the draft OEHHA document "Public Health Goal (PHG) for hexavalent chromium in drinking water."

Reviewer: Leonard Bjeldanes (prepared 5/12/05)

1. Accuracy of the information presented. The background information presented in the document appears to be comprehensive based on the extensive bibliography provided. The list does not include a recent comprehensive review of chromium environmental chemistry and biology (Zhitkovich, JCRT, 2005). However, in key areas of CrVI activities the document is selective in its use of certain published findings. For example, the Report reinterprets important published findings and comes to conclusions that are different from the conclusions drawn by the original authors. Furthermore, the Report has ignored major concerns stated in other published reviews about the value of certain studies in projecting cancer effects in humans.

2. Appropriateness of the approach and interpretations. One of the issues that is raised in this Report is whether CrVI is genotoxic at a site distant from the initial exposure site. On page 34 the case is made for the distant site of action of orally administered CrVI, in which the results of 9 primary studies are summarized in Table 2 on page 36. Unfortunately, only one of these studies (Kuykendall et al. 1996) was in humans and the result was negative. The Report dismisses this result because it was based on a short term exposure and then suggests that the leukocyte assay is a questionable marker. However, the authors of one of the papers sited to support the concerns over the leucocyte assay (Paustenbach et al. 1996), conclude that at concentrations of 10 mg CrVI/L or less in the drinking water of exposed humans, CrVI appears to be completely reduced to CrIII prior to systemic distribution.

A further issue relating to the applicability of the rodent studies to projection of human risk that is ignored in the Report relates to the well established pH dependance of the rate of conversion of CrVI to CrIII. Many studies have shown that whereas CrIII undergoes many biologically important reactions in vitro, it has very little effect on cells or in animals presumably because it is very poorly absorbed across cell membranes. In contrast, CrVI is said to be well absorbed into cells via the sulfate and phosphate anion channel. Thus, the degree of conversion of CrVI to CrIII is of key importance in the possible carcinogenic effects of ingested CrVI. According to the recent Zhitkovich review (JCRT, 2005), CrVI is readily reduced in highly acidic solutions containing any organic molecules with oxidizable groups, including the gastric juice. The rate of CrVI reduction increases as the pH is decreased. This pH effect on the conversion rate is used by Zhitkovich, and others, to explain the large differences in the biological activity of CrVI by different routes of exposure, i.e. high toxicity by inhalation and low toxicity by oral route. This pH dependence also suggests that oral CrVI is likely to be more highly absorbed in rodents than in humans because the pH of the rodent forestomach is about 4, whereas the pH of human gastric juice is only about 1. Thus, CrVI is likely to be much more rapidly reduced to CrIII in the human than in the rodent gut.

In addition, several investigators have questioned the relevance to humans of studies that show carcinogenic effects only in the rodent forestomach. The forestomach of the rodent is an absorptive, bacteria containing organ that is not present in the human. In any case,

the differences in gastric anatomy and physiology between man and rodents would be expected to lead to a much higher sensitivity of the rodent to the effects of oral CrVI. The fact that none of the extensive work on the lung carcinogenic effects of CrIII in occupationally exposed workers has shown an increase in stomach cancers adds further doubt on the oral toxicity of CrVI in humans.

3. Data evaluation and interpretation. The primary evidence provided in support of the claim of carcinogenic activity of oral CrVI in humans is based on two unpublished analyses of data published others.

The first analysis is of published data on the incidence of stomach cancer in workers occupationally exposed by inhalation (p. 46). Although none of the many individual studies reported a significant increase in stomach cancers, an unpublished meta-analysis done for the Report suggests a small increased relative risk of 1.22 in exposed workers. The individual studies showed broad variation in the results, including 2 studies that indicated decreased stomach cancer incidence in the exposed group. Whether the selection of the studies that were included in the OEHHA analysis and the method of statistical analysis were appropriate should be determined based on the standards of peer reviewed publication.

A similar concern is raised in the analysis of the published findings concerning the incidence of stomach cancers in a Chinese population that was exposed to CrVI presumably from contaminated water. The OEHHA report disregards one study (Fryzek et al.) that found no increased cancer rates in a human population that appears to have been exposed at least to low level CrVI. In addition, in the analysis of the single human study considered (Zhang and Li, 1997), the OEHHA report rejects the reconsidered conclusion of the authors that there is no significant association of the stomach cancer rates with CrVI exposures in the populations in the vicinity of an alloy plant. Although the data show a high level of CrVI contamination of well water in the area, along with an increase in total cancer and stomach cancer, information on the level of CrVI exposures is lacking. Nor are data provided on levels of other possible carcinogens in the water, including other metals and nitrite. Furthermore, in the effort to explain the comparative decrease in cancers in areas in the closest proximity to the plant, the report proposes that alternate drinking water sources were used in these areas because the water was unpalatable. Whether this actually occurred and to what extent is the subject of speculation in the Report. Since the re-analysis of the Zhang and Li study is a cornerstone of the OEHHA case for the carcinogenic activity of oral CrVI in humans, this analysis, too, must be subjected to full peer review by specialists in the field.

A further area of concern in the OEHHA report is the manner in which the PHG was derived. Since useable information on the level of CrVI exposure is insufficient for purposes of developing a PHG based on human studies, the proposal was based on the single chronic oral treatment rodent study that has been published (Borneff et al., 1968). This reviewer does not have ready access to this article, which is in German. Nevertheless, according to the analysis provided in the OEHHA report and in other publications, there are several reasons to suggest that the value of the paper for the purpose of developing this PHG is seriously compromised. Among the weaknesses of the paper are the following: 1) The use of a detergent in this study could modify the stability of CrVI and increase its absorption in the stomach. 2) The smallpox outbreak might have

increased the sensitivity of the rodents to the CrVI as evidenced by the fact that the small increase in stomach tumors was seen only in the parent generation, which was the generation that was most strongly affected by the outbreak. 3) Only a single very high dose of CrVI was used which might have overwhelmed the capacity of the stomach juice to reduce CrVI to CrIII and provides no information from which to experimentally derive a dose/response relationship. 4) The very high concentration of CrVI in the water apparently resulted in avoidance of the water and dehydration of the rodents that was serious enough to result in cannibalism, apparently to obtain fluids from cagemates. This dehydration might also have increased the sensitivity of the animals to CrVI.
It is important to note, as well, that Borneff et al. apparently did not interpret the results as showing an increase in stomach cancer from CrVI or from an augmentation of the carcinogenic activity of the established forestomach carcinogen, benzo[a]pyrene.

4. Appropriateness of the risk assessment methodology used. In spite of the fact that only a single highly flawed rodent cancer study is available and was considered for the analysis, the OEHHA report goes on to derive a PHG using the most highly conservative linear computational model. Whether the results of the Borneff et al. study should be considered at all for this risk analysis, and certainly whether it is appropriate to use the linear projection model based on results for a single high dose protocol in rodents, are highly doubtful. The evidence for stomach carcinogenicity of ingested CrVI in humans is very weak and there is compelling evidence for a threshold effect on CrVI because of the preabsorptive conversion to CrIII in gastric acid.

5. Other major and critical information that should be considered. In addition to the additional information cited in the foregoing discussion, this OEHHA analysis should address the issues that have been raised in previous reviews concerning the safety of CrVI and which have not found credible bases to lower the current interim acceptable levels of CrVI exposure from drinking water. For example, one review points out that the EPA's oral reference dose for CrVI is derived from an absence of effect in rats receiving 25 mg/L in their drinking water for one year. Although this reviewer was not able to find the original report, clearly this study would be highly relevant to the present purpose.

6. The appropriateness of uncertainties in the PHG calculation. As indicated in the forgoing discussion, I suggest that the PHG calculation is based on specious experimental and epidemiological findings and, in any case, is unjustifiably conservative.
In summary, the data show that CrVI is a lung carcinogen in industrially exposed humans. Whether these workers also experience an increased incidence of stomach cancer has not been established in the peer-reviewed literature although the incidence data have been available for over two decades. Also, no peer-reviewed, published study has asserted that oral CrVI produces increased levels of cancer in any organ including forestomach cancers in rodents or stomach cancers humans. It is only by the reinterpretation of the published findings that the OEHHA report concludes that CrVI is a stomach carcinogen by the oral route. The OEHHA report goes on to use the highly conservative, linear projections of CrVI safe doses based on its own specious re-interpretations of the published data. This is a highly inappropriate and inadequate consideration of the uncertainties in the available biological and exposure information.

Review of the document:

"Public Health Goal for hexavalent chromium in drinking water"
Prepared by the Office of Environmental Health Hazard (OEHHA) of the California Environmental Protection Agency in January 2005

Reviewer: Dr. Roberto Gwiazda, Environmental Toxicology, University of California, Santa Cruz

Summary:
This document recommends the establishment of a public health goal (PHG) of 0.2 ppb for hexavalent chromium in drinking water to insure a lifetime risk level of at most 10^{-6} to stomach cancer due to water ingestion. This estimate is based on results of the Borneff et al 1968 study that showed a higher incidence of stomach cancer in mice exposed to 500ppm of potassium chromate in drinking water for a maximum of 880 days. While there are some lingering doubts about the validity of the findings due to the occurrence of a mice pox infection with high mortality in the course of the study, the precautionary principle would dictate that this finding of a relationship between oral uptake of Cr(VI) and stomach cancer incidence be accepted, especially since this is the only animal study where Cr(VI) was administered for such extended period of time. In addition, the review conducted by OEHHA of the humans studies of Zhang and Li is supportive of a link between oral Cr(VI) and stomach cancer. Results of the meta-analysis of occupational studies conducted by OEHHA are weakly supportive of such a link. The calculation of the PHG fully ignored the presence of a reducing capacity of Cr(VI) to Cr(III) by saliva, stomach, gastric juices, food stuff and intestinal bacteria. This reducing capacity becomes even more relevant at low concentrations of Cr(VI) in water. This factor must be included in the process of estimating the PHG.

Review:
This basis for the establishment of the PHG consists of three main lines of evidence:

- The study by Borneff et al. 1968, which investigated the carcinogenicity of chromate in three successive generations of mice.
- A meta-analysis of occupational studies where the incidence of stomach cancer is related to ingestion of inhaled Cr(VI)-bearing particles.
- The human study of Zhang and Li, 1987, where the incidence of stomach cancer in a group of villages in China in the vicinity of an alloy plant is related to contamination of groundwater with Cr(VI).

The report relies on these three pieces of evidence to first demonstrate that indeed ingestion of Cr(VI) is carcinogenic, i.e can lead to stomach cancers. Based on this finding, it then utilizes the data from these studies and a series of assumptions to calculate the cancer potency of orally ingested Cr(VI).

A) Evidence of the carcinogenicity of Cr(VI) by ingestion:

Borneff et al, 1968: Overall, the evidence in this study that Cr(VI) produced stomach cancers is mixed. F0 animals indeed show a higher incidence of tumors than controls (22% Vs 3.6%), and this result is highly statistically significant. Despite the fact that F1 and F2 animals do not show a higher rate of stomach cancer than controls, inclusion of F1 and F2 animals in the overall statistical analysis does not change the significant outcome.

However, there are several issues with this study which diminish the credibility on the finding that oral Cr(VI) induces stomach cancer:

1) The outbreak of mouse pox that lead to high mortality. In ideal conditions, one would abandon this study and would begin anew with a new set of animals. If other animal studies that evaluated oral carcinogenicity of Cr(VI) were available this would be the proper course of action. However, in the absence of a study similar to Borneff et al. 1968, where animals were dosed over most of their lifetime, any information that can be gleaned from it, flawed as it is, should be considered, though in a skeptical manner.

The issue to consider is if the outbreak of mouse pox is directly related to the higher incidence of stomach cancer in F0 animals. According to the report "…there is no evidence that the increase in tumors observed in the female mice were due to the virus…(pg. 41)". However, this is a poor justification of a disconnection between the ectromelia outbreak and the incidence of tumors. The fact that evidence of a causal relationship has not been provided should not be taken as an indication that a causal relationship does not exist. For example, it is noted (pg. 41) "…that there is no evidence that the forestomach of the mouse is a site where mousepox lesions occur…" yet, there is no evidence that mouse pox lesions in the forestomach of this particular mouse strain do not occur. The report also notes that the identified papillomas had a branched morphology distinct from those that would result from the mouse pox infection and that if these lesions were a result of infection a similar number would have been observed in the control animals. While this argument may be true, it is also possible that there was a larger and faster uptake of Cr(VI) at the location of the small pox lesions in the forestomach (if they existed). This higher Cr(VI) tissue load would have triggered tumor growth later in life after the small pox infection was over and the small pox lesions had healed. The possibility of increased susceptibility to the carcinogenicity of Cr(VI) as a result of exposure to small pox virus should not be discarded either. In summary, the possibility of a higher incidence of stomach tumors in F0 animals because of the ectromelia infection should not be discarded.

2) The incidence of tumors in F1 and F2 is not different than in controls, in contrast to the observation in F0 animals. Some explanations are offered in the report to account for this difference: F1 and F2 animals received a smaller cumulative dose of chromate, tumor growth in F1 animals was delayed due to the infection, and in F2 animals vaccination could have delayed tumor development. The latter two reasons are plausible but they highlight the limitations of using a study with an infection of the magnitude observed in Borneff et al., 1968. In addition, the lack of report of preneoplastic lesions in F1 and F2 animals is

troublesome. It would be expected than in a study that investigates tumor lesions this type of outcome would be looked at. The report says (pg 43) "…preneoplastic lesions were not reported in mice administered benzo(a)pyrene. The reason for this is unknown, but it likely due to the same factor in mice exposed to chromium and those exposed to benzo(a)pyrene …" This is a very speculative statement and no further justification is provided. For example, the contrary argument is equally reasonable: They were not looked at in the benzo(a)pyrene treatment since this was a positive control and there was certainty about the carcinogenicity of benzo(a)pyrene treatment, whereas in the chromate treatment there were looked at as an indication of the potential of Cr(VI) to induce tumors, but they were not found.

One explanation offered for the lack of an effect in F1 and F2 animals is that their cumulative dose was not large enough to induce tumor growth. However, in page 40 it is stated, that tumor growth had already begun in F0 mice at the time of the infection. The cumulative dose at that time was 240 mg chromate, whereas F2 generation had received at the end of the study 510 mg, enough to initiate the growth of stomach carcinoma, but this development was not seen. On the other hand, the report mentions that Borneff and coworkers estimated 700 mg as the minimum chromate cumulative dose for the expression of chromate's carcinogenic effect. Is there a minimum cumulative dosage needed to induce stomach tumor growth (240 mg, 700 mg, lifetime?). If so, how is a minimum cumulative dosage incorporated in the oral potency calculation (Table 13)? Since it is not possible to state with certitude the reasons for the lack of a chromate treatment effect on F1 and F2 animals, which contradict results from F0 animals, the apparent carcinogenic effect on F0 should be accepted with a large dose of skepticism.

3) If it is accepted that the higher incidence of stomach cancer in F0 animals is due to ingestion of Cr(VI), this finding should not be necessarily interpreted as proof that Cr(VI) is an oral carcinogen in humans since the location of the mice tumors is precisely in an anatomical element that is absent in humans. Discussion of the correct pH of the forestomach is only relevant to oral carcinogenesis of Cr(VI) in mice, not in humans. But it should be noted that regardless of the pH of the forestomach, the Cr(VI) load was so large at 500 ppm that even in acidic conditions the reducing capacity of the forestomach might have been overwhelmed. Thus extrapolation of the results of this study, done at close to the maximum tolerable dose, to evaluate the oral potency of Cr(VI) in humans, without taking into consideration the ratio of Cr(VI) dose to the reducing capacity of saliva, gastric juices, intestinal bacteria and food, is highly questionable.

In summary: The preponderance of the evidence would support that F0 animals contracted tumor cancers due to Cr(VI) exposure. However, it is possible that these might have happened because of either higher Cr(VI) uptake through mouse pox lesions or higher sensitivity to Cr(VI) toxicity due to the infection. In addition, the very high Cr(VI) dose may have overwhelmed the reducing capacity of the stomach, facilitating Cr(VI) uptake.

Meta-analysis of occupational studies:
Pooling individual Cr(VI) occupational inhalation studies, none of which proofs conclusively a link between inhalation Cr(VI) exposure and stomach cancer, to increase the power to detect a link between Cr(VI) inhalation and stomach cancer is a legitimate approach, provided these studies are controlled for potentially confounding variables.
There are two reservations to this approach as applied here.

1. It would seem that a meta-analysis would be the most powerful tool to pool independent studies when all of them show the same trend in outcome but statistical significance is not reached because of the small number of subjects in each study. This is not the case here: Of the 10 studies, 2 show an average lower risk for stomach cancer for workers exposed to inhaled Cr(VI), 2 show almost no effect (Roseman 1996, and Satoh, 1981), 5 show a positive relationship, and 1 has such large confidence intervals that the average rate ratio is meaningless (Deschapms, 1995). In this situation, the meta-analysis, rather than pooling the data from all studies together to raise the power of detection, pits data of negative studies against data from positive studies, canceling them out. A test for heterogeneity of findings was done and it did not reveal significant differences in the outcomes (p=0.22, page 51), however, the rate ratios in the great majority of studies are so close to 1 (i.e. the link between inhalation of Cr(VI) and stomach cancer is very weak), that the impact of the large within-study variances of a few of the studies (Deschaps, 1995 Franchini 1983, and Okubo 1977) overwhelms the variability in rate ratios between studies such that the heterogeneity test is negative. In this situation, it would be advisable to: (i) evaluate the assumption of heterogeneity in a stepwise fashion (would not including Deschapms, 1995, yield significant heterogeneity across the remaining studies?, for example). Results of sensitivity tests, which can be run with the Compare2 computer program (page 49), are not presented. It would also be informative to include the weight of each study in the overall rate ratio in table 3. (ii) Assume heterogeneity and ran a random effect model. This needs to be more thoroughly investigated in the report.
   The overall net effect is so small that it is plausible the average rate ratio would have been less than one, had one more study been included or excluded.

2. Among the criteria for selection of appropriate studies the report cites, age, calendar time, race and gender. However, equally or more important, none of these studies controlled for important risk factors for stomach cancer: These included: gastritis, the presence of the ulcer producing bacteria Helicobacter Pylori, smoking or tobacco use. It could be argued that the higher impact of one of these factors in a particular study would be weighed by another study with a lesser impact of the same factor, such as overall the impact of these risk factors averages out in the final analysis. This argument is not convincing, not only because controlling confounding variables was one of the

criteria for the selection of the individual studies but also because the overall standardized mortality ratio of 1.23, (95%CI: 1.02-1.47) is so close to 1 that minor adjustments in the rate ratios of the individual studies to account for risk factors of stomach cancer could easily bring below 1 the 95% confidence interval lower limit (1.02).

Zhang and Li, 1987 Human study:

The report presents a very comprehensive survey of the available knowledge on the release of hexavalent chromium from the JinZhou plant in theLianing province of China, the concomitant elevated Cr(VI) levels in groundwater downstream from the plant, and its evolution with time. Very credible arguments are presented to explain the geographic distribution of the Cr(VI) groundwater plume, and the reasons for the presence of more wells with high Cr(VI) levels farther from the plant than in the nearer villages. The report states that in areas with wells with very elevated Cr(VI) the population may have refrained from groundwater consumption for drinking because of the obvious color discoloration or because of intervention by public health authorities. But in areas with lower groundwater concentration of Cr(VI) due to plume dispersion, more people may have been exposed because of their lack of awareness about the risk. This argument convincingly rebuts the observation that the villages with the highest levels of hexavalent chromium had the lower cancer rates (assuming there is a link between ingested Cr(VI) and cancer). Furthermore, as stated in the page 77, comparison or cancer rates with distance from the factory are very speculative given the lack of clear information to characterize the pattern and magnitude of exposure.

The comparison of stomach cancer rates in the exposed villages with the whole province as a whole, while informative, appears to be misguided. The assumption on such analysis is that the only difference between the two groups is Cr(VI) exposure through groundwater. In other words, had the villagers not been exposed to Cr(VI) their stomach cancer rates would have been indistinguishable from the rates in the whole province. Clearly, this can not be assumed because there are variables that may have an impact in stomach cancer rates that are ignored in this comparison but could be substantially different between the villages and the whole province: exposure to pesticides, urban vs rural living, nutritional status, smoking, exposure to other contaminants in air, food or water, access to health care, etc. The comparison with the group of villages considered not exposed is a much more robust approach, under the assumption that the above-mentioned variables would not differ significantly between exposed and non-exposed villages. According to this analysis, the report convincingly makes the case that indeed Cr(VI) exposure in groundwater at the levels reached in these villages appeared to increase the incidence of stomach cancer to a statistically significant level (RR = 1.81, 95% CI= 1.08-2.98). This finding is important in providing substantial supporting evidence for the tenet that ingestion of high levels of Cr(VI) can indeed lead to stomach cancer. In this

particular case, however, the significance of this finding is tempered by the lack of data on the extent of individual exposures and incidence of stomach cancer as opposed to mortality. Total absence of information of the prevalence of other risk factors for stomach cancer in these villages, introduces a high level of uncertainty in linking the Cr(VI) exposure and Cr(VI) intake via groundwater.
Of the three lines of evidence presented in the report linking ingestion of Cr(VI) and stomach cancer incidence, the reevaluation by OEHHA of the studies of Zhang and Li, is the strongest of all.

B) Cancer potency of orally ingested Cr(VI)
Borneff et al, 1968. The report utilizes the nominal Cr(VI) dose (500ppm) given to the animals that showed a higher incidence of stomach cancer than controls (F0 animals) and assumes in the calculation in table 13 that this is the dose that over the lifetime of the mouse lead to that outcome. However, F0 animals were not exposed over their lifetime, nor did F1 or F2 animals. One less conventional alternative is to use the minimum cumulative dose necessary to induce stomach cancer and calculate the daily dose that over a mouse lifetime would induce stomach cancer.
For example: According to page 40, 700 mg cromate (1 mg /day for 700 days) were necessary for the induction of tumors
Dose = 1 mg/day * 700 days / 1460 days * 0.27 /0.031 kg = 4.17 mg/kg/day (assuming lifetime of the mouse = 4 years =1460 days)
The utilization of the daily dose to calculate the oral potency of oral Cr(VI) is not clear. This study utilized only one dose. How can then a dose-response be constructed to estimate the ED_{10} and its confidence limits from only one point unless the dose-response is linear? If that is the case, this information should be included and more details should be provided (how is the confidence limit estimated?)

Zhang and Li, 1987 Human study: This estimate suffers from the limitation that there is no information on individual exposures, and therefore the entire range of Cr(VI) concentrations in groundwater exceeding background is used to calculate potency. As a result upper and lower estimates differ by a factor of 50, with the highest potency calculated on the basis of a Cr(VI) groundwater content (50 ppb) below current US federal guidelines (100 ppb) and equal to the current maximum contaminant level for California. In the absence of precise data on exposure, estimation of a range is the only option available to estimate the oral potency from these human studies. However, this range of estimates should not be over-interpreted, and unfortunately that is the case in the report: For example: page 85: "…it should be noted that carcinogenic potency estimate for Cr(VI) from the Borneff et al., 1968 study fell within the range of possible potencies from the Zhang and Li study…"; page 100: "…The potency from the animal study was within the range of potencies based on the results of Zhang and Li…" These assertions carry very little weight because the range of potencies estimated from the Zhang and Li study were based on groundwater Cr(VI) levels that were essentially background to a level where the water is undrinkable. No surprise

then that the oral potency from the Borneff study fell within the range of potencies calculated from the largest possible range of Cr(VI) that can be observed in groundwater.

Meta-analysis of occupational studies: The estimate of oral potency of Cr(VI) from the meta-analysis relies on the study of Gibbs 2000 to postulate what the report calls: (page 84)"…a reasonable estimate of the mean level of hexavalent chromium in air in an occupational setting…" To utilize a single Cr(VI) airborne concentration value, derived from a single study not even included in the meta-analysis, and associate it with an increased risk ratio of stomach cancer is highly questionable. In occupational settings airborne concentrations are highly variable depending on what step of the manufacturing process workers are working at, and what safety measures are in place to minimize exposure (ventilation, air masks, etc). These conditions were probably different across all studies. The concept of a "mean level of hexavalent chromium in air in an occupation setting" is not justified. (Also, the value used in the report from the Gibbs study is for all workers and not for those that developed cancer, who were exposed to average 0.29 mg/m^3-yr instead of 0.134). To assert that humans are equal or more sensitive to Cr(VI) carcinogenicity than mice (page 85), on the basis of the comparison of the oral potency derived from Borneff et al 1968, with the oral potency derived from this meta-analyses, is simply not credible. The complete absence of any information about airborne levels of Cr(VI) in any of the studies included in the meta-analysis makes such comparison a futile exercise.

Calculation of the PHG: Overall utilization of the potency derived from the Borneff et al study appears justified, not because it is a very credible estimate but because the estimates based on the human studies are too uncertain. This study has several problems which were reviewed above but the precautionary principle would dictate that in the absence of a better animal study and until the NTP study is completed oral potencies derived from Borneff et al 1968, should be used for the purpose of establishing a PHG for Cr(VI).
However, the main problem with the approach utilized here to derive a health protective level is its total lack of acknowledgement of the Cr(VI) to Cr(III) reducing capacities of the saliva, stomach, gastric juices, food stuff and intestinal bacteria. In all studies quoted in the report that evaluated oral Cr(VI) carcinogenicity, the Cr(VI) concentrations in water were very high and likely to overwhelm this reducing capacity. The report rightly states (page 13):"…the widespread distribution of chromium into tissues following hexavalent chromium administration indicates that although reduction is likely to be occurring in the blood, it is not occurring at fast enough rate to prevent hexavalent chromium from reaching and being taken up by tissues…." This probably also applies to Cr(VI) in the stomach. The key word in this statement is "at fast enough rate" . This summarizes the dilemma of extrapolating the results of studies with very high doses to chronic exposure to Cr(VI) in groundwater. The reducing mechanisms that are overwhelmed under high doses of Cr(VI) are still operative at much lower doses. In calculating the average lifetime daily dose that would be protective, the

presence of the reducing mechanism is fully ignored, even though its existence is acknowledged throughout the report, (page 15 for example: "…Due to its slow rate of absorption, an oral dose of hexavalent chromium would be expected to be largely converted to trivalent chromium in the stomach and plasma…"). In other words: if there were absolutely no mechanisms for reducing Cr(VI) to Cr(III) the calculation of the PHG would have taken the same form and arrive to the same result. This glaring omission could be based on the assumption of the absence of a threshold for stomach cancer due to ingestion of Cr(VI), but studies quoted in the report point to the existence of a fixed stomach capacity of reduction, and substantiate that the stomach does indeed reduce Cr(VI). Where is this fact included in the calculation of risk?

Several facts are brought up in the report to argue that even in the presence of a reducing mechanism in the stomach, Cr(VI) is not fully reduced. One evidence for this is different Cr tissue distributions after Cr(VI) and Cr(III) oral administration in animals. However, this is observed with very high doses of oral Cr, which likely overwhelm the reducing capacity of the stomach.

It is also noted a longer urinary half life elimination after Cr(VI) administration than after Cr(III) administration in humans, 39 hours in Cr(VI) Vs 10 hours in Cr(III), which the report attributes to higher liver Cr levels. Accumulation in the liver is typical for Cr(VI) and not Cr(III) and this longer half life would indicate that Cr(VI) resided in blood and was taken by the liver before being converted to Cr(III) in the gut or plasma or incorporated into RBC's. Furthermore, in addressing the evolution of RBC Cr content after oral administration in humans studies the report notes that in some individuals Cr RBC levels remained elevated, indicating the probable RBC uptake of Cr(VI). However, the issue that is not fully addressed in the report in the discussion of the reducing capacity of the stomach (pages 13, 14 and 15) is the ratio of the dose administered in these studies vs the reducing capacity of the stomach. If this capacity is overwhelmed, clearly Cr(VI) will not be reduced and instead will be absorbed. But water Cr(VI) concentrations in animals studies which show differential tissue accumulation with Cr(VI) were much higher than the doses humans would ever be exposed under current guidelines (Sutherland et al., 2000, 3000-5000 ppb, Thomann et al., 100000ppb). Similarly, the doses of oral Cr(VI) in humans studies were very large: 5000 and 10000 ppb (Finley et al., 1997, Kerger et al., 1997). This can be contrasted with a reducing capacity of saliva of 0.7-2.1 mg/day and gastric juice of 84-88 mg/day (DeFlora et al., 1997). While some Cr(VI) is absorbed in the humans studies because of competing kinetics between reduction and absorption, at the current guideline of protection for California (50 ppb) and a daily water intake of 2L, the ratio of reducing capacity Vs Cr(VI) intake is 88000000ng /100000 ng = 880, extremely in favor of reduction. Admittedly, some absorption will occur because of competing kinetics and because some Cr(VI) must remain in the redox equilibration. The important point is that the estimate of the PHG completely ignores the reducing capacity of saliva, stomach, gastric juices, food stuff and intestinal bacteria and assumes that the fraction of ingested Cr(VI) in drinking water at 0.2 ppb that remains at 6+ oxidation state in the gut, is

the same fraction that remains in this oxidation state after ingestion of water with 135000ppb Cr(VI)in the Borneff et al study.

Minor comments:

Page 3: A few sentences about the role of Cr(III) as a nutrient could be added.
Page 5: It would be advisable to keep consistent units: there is ppb, μg/L, and μg/Kg (page 6)
Page 7: The paragraph starting with Finley et al, 1996a (also← in addition to what?) should be at the end of the OEHAA shower assessment.
Page 9: Cr(VI)absorption from the gut 1%, however last paragraph of page 10 quotes four sources all of them with absorption above 1%.
Page 11: Anderson et al 2002 Toxicological Sciences does not exist. (wrong ref?). Regardless, "…These results also do not indicate that oral absorption of hexavalent chromium only occurs (begins) when the reducing capacity of the stomach is exceeded, given the lack of an apparent threshold when absorption occur…" what is the basis to state that the absorption was of hexavalent chromium and not of trivalent chromium after reduction in the gut?
Page 12: "…which is function of its solubility…" Solubility of what? Perhaps only relevant to inhalation?
Page 13: Top. There is a contradictory statement: on one hand, as Cr(III) administration increases, transferrin binding sites and then albumin binding sites get saturated, at that point or earlier low affinity binding to RBC kicks in. On the other hand: "… Increased blood levels of chromium following oral administration of Cr(III) in the blood were associated wit the plasma fraction..." In the latter RBC binding is ignored. A mixed message is conveyed in this paragraph. Especially considering page 15:"… At the high doses administered Cr(III) may have sorbed on the surface proteins of the RBC…"
Bottom: "… While chromium was detected in high levels in … RBC's…when hexavalent chromium was administered…" In contrast:
Very bottom: "…Oral administration of hexavalent chromium revealed …much lower levels in RBC…" Is this difference due to different routes of administration? Please clarify.
Page 14: How consistent is high content of Cr in the liver (presumably Cr(VI)) and at the same time low Cr content in RBC's? Why isn't Cr(VI) sequestered in RBC's in its passage to the liver? Wouldn't this indicate that in fact there was reduction to Cr(III) in blood and liver accumulated the Cr(III), even though the kidney appears to be the organ of excretion of Cr(III)? This would need a bit of a more expanded discussion. Accumulation of Cr(VI) in liver without an increase in RBC Cr is not consistent with RBC's uptake of Cr(VI).
Eliminate the sentence alluding to nuclear medicine, it's confusing and diverts attention from the fact that the decrease in the blood Cr content is not that expected from RBC turnover.
Page 15: " …By contrast, following administration of Cr(VI)…" By what route?
Page 16: "…The increase in absorption as reflected in an increase in plasma and erythrocyte levels… appears to indicate that the hexavalent form of the metal is

orally absorbed…" Why an increase in plasma indicate absorption of Cr(VI)? It would indicate absorption of Cr(III) or conversion of Cr(VI) to Cr(III) in blood.
In the same paragraph: "… The behavior of administered trivalent chromium –low plasma…" Why lower plasma Cr content is expected with Cr(III) than with Cr(VI) administration? This does not appear consistent, as written, with the 1st paragraph of page 13.
A statement is made that toxicokinetic studies in humans do not support complete conversion of Cr(VI) to Cr(III) in the stomach. On the same page it is noted that " The administered Cr(VI) at first glance appears to somewhat behave as if trivalent chromium has been administered…" further, it states: "… it appears that the bulk of the chromium in the blood was probably trivalent chromium …" it is not only until the end of the page that one makes sense of all the paragraphs on this page: the differences in urinary half life are because of liver retention of only Cr(VI),which would indicate a differential absorption between Cr(VI) and Cr(III). This meandering argument needs to be fully rewritten. However, note that these results do not negate the capacity of the stomach to reduce Cr(VI). The emphasis as written is on whether ALL Cr(VI) is converted. This is an issue of secondary importance because the fraction of Cr(VI) converted is a function of the magnitude of the dose. The most important outcome of the human studies is the evidence pointing to reduction in the gut and this finding is minimized in the text, as written.
Page 27: Dose in the different studies is expressed in different units: ppm, mg/Kg-day. If information were available, it would be helpful if the doses were also expressed in the same units across studies.
Page 40: a breakdown of tumor numbers per generation should be provided
"…were exposed to more than 700 chromate…" Is this chromate or K_2chromate?
Page 41: " the high early mortality in the F1 generation .. and the shorter life spans of F1 and F2 generations are a concern…" However, is the total number of animals in F1 and F2 that were evaluated for stomach cancer larger than the number of F0 animals? Perhaps the shorter lifetime would be the only valid argument. High mortality affected also F0 yet cancers were detected.
Page 42: "… The level of hexavalent Cr did not appear to have achieved the MTD…" yet page 25 states "The NTP designated 400 ppm as the MTD for male and female mice". Borneff gave 500 ppm.
"… the MTD is 'the highest dose of the test agent during the chronic study that can be predicted not to alter animal's longevity from effects other than cancer'…" There is no information in the Borneff study on whether longevity was affected or not since the study was not conducted over the lifetime of the animals, in addition to the confounding effect of the lethal infection.
Page 56: all the figures taken from Zhang and Li are simply awful. No scale appears on the map and Shi Li Tai and Yang Xing villages appear reversed in the legend. See following comments
Page 57: "…proportion of wells in 1965 with non-detectable hexavalent chromium…" what level is defined as detectable Vs undetectable?

Page 58: Table 5 should come after figure 11. In this figure the value for Nuer River village should be the real value from figure 8b and not the one from figure 8a.

Page 61: The report makes mention that in the original paper results from two villages were mistakenly reversed. The original error is reproduced in figure 6, in the headers of figure 9 and 10, and in Table 7. The report carries this error forward in Table 5 by preserving the wrong geographical order of the villages (and including erroneously the information from figure 8b and not from figure 8a in the fraction of non impacted wells for Nuer River Village). However in the first paragraph of page 61 the village is mentioned by its right name "…results reported for ShiLiTai…" Leaving the wrong information in the report but correcting it afterwards (page 69) is very confusing. It should be stated from the beginning that there is an error in the original papers and present the correct information throughout.

Page 62: A scale and better graphics are needed in this map

Page 63: 2nd paragraph: the quotation from Zhang and Li had already been cited in page 54. What about use of groundwater during the dry season? It seems that the dry period is when most water would be drawn

Page 64: Statements in this page are inconsistent. The map shows large areas (where are the villages here?, scale? Names?) with high Cr content, yet: page 63"…. Contaminant concentration…decreased quickly… stable for many years"…(page 64)…" the groundwater contamination level became close to or below drinking water standards…" Immediately follows a map with areas with [Cr] between 1000 to 5000 ppb!, and a full paragraph outlining the very high levels found in different places over time. The apparent contradiction between the levels reported and the conclusions of Zhang and Li should be explicitly stated, and the reader should not be left trying to make sense of the inconsistent reports

Page 84: bottom denominator should be 70 years not 70 days

Page 87: Third line should be "… in cumulative exposure units of (mg/m3-yr)$^{-1}$…" Last paragraph should be before explanations of the different fittings

Page 89: Table 17 CrO3 exposure units should be $\mu g/m^3$-yr

Page 93: First paragraph; quoted figures do not agree with results from tables 18 and 19

"…slope based upon excluding the highest two exposure categories is 32% above that of Analysis 1…" But 2.66/ 2.58 is 3% higher

"…slope based upon excluding the highest dose category is 88% above…" but 3.1/3.17 ~1, is the same.

"…and the slope based upon all categories is 39% above that in analysis 1…", but 2.45/ 3.77 = 35% lower

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Attn: Hexavalent chromium review

Ms. Caraway:

I have reviewed the June 2004 draft entitled "Public Health Goal for Hexavalent Chromium in Drinking Water".

Unfortunately, I have some serious concerns as to how the final threshold of 0.2 ppb was derived.

The initial portion of the document (the review and historical section) is very good and very comprehensive. Overall, the data provided on toxicity, kinetics, metabolism, and routes of potential exposure is quite accurate with one exception. This sole exception regards the carcinogenic potential of hexavalent chromium, both the selection of key studies (or data sets) and subsequent interpretation of this data.

I have major concerns as to the derivation of the proposed threshold for hexavalent chromium content in drinking water. Specifically, my primary concern is how the 0.2 ppb value (see page 100) is obtained due to numerous flaws in the two studies that provide the supporting data for this value. My concerns are not with the equations utilized, or with the subsequent calculations (the overall approach utilized), but the data extracted from non-representative two studies used as the primary input for the calculations. The overall approach used in the document is acceptable but it is the key studies that are used to derive the 0.2 ppb threshold that are severely flawed and therefore not acceptable.

I am going to limit my comments to the two studies that support this 0.2 ppb threshold value, as this is the primary thrust of the document. Note that these are the <u>only</u> two supporting studies for this threshold value of 0.2 ppb.

The two studies used to support the 0. 2 ppb values are a chronic animal study (referred to as Borneff et al., 1986 in the document) and a human oral exposure study (referred to as Zhang and Li, 1987). Both of these studies have major faults as described next.

Borneff study

The Bornheff study was a three-generation study designed to determine the carcinogenicity of hexavalent chromium by oral route in mice.

The most major problem with this study is that a mousepox virus (ectromelia) outbreak occurred and the majority (> 500 or ~ 80%) of animals died. Inoculation of the appropriate vaccine did not occur for almost 2 months after the outbreak started.

In the few surviving Fo mice there were 2 malignant stomach tumors (2 of 66).

However, there were also 9 benign tumors (papillomas) in the Fo mice. So the authors of this PHG document merge these two tumor classes (malignant and non-malignant) to get a combined rate of 11/66. The rational for combining malignant and non-malignant tumors in mice is not provided. It is not uncommon for rodents to develop non-malignant tumors during a life-span study. I would also like to point out that this combined rate equals 16.6% (11 of 66) and not the 22%, which is cited in some portions of document (line 8, paragraph 3, page 40). In the control mice the incidence is 2 of 79 animals or ~3%.

There was no increased tumor incidence in the F1 and F2 generation mice as compared to control animals. In the PHG document this lack of tumor growth is attributed to a delay of tumor growth formation due to the development of the mousepox epidemic. However, the F2 generation mice were never exposed to the virus, so this explanation is not valid for the F2 generation.

One other major concern is the derivation of the oral potency or slope factor based on the Borneff study (see page 82). I could verify other calculations in the document, but not this set of calculations (which is critical to the 0.2 ppb threshold). The document states that the mouse ED10 of 3.42 mg/kg-day was converted to the human dose LED10 of 0.51 mg/kg-day using the scaling relationship of human to rodent body weight. I presume the superscript ¾ refers to the exponential factor derived by Kleiber in 1932. However, the mouse to human dose decreases by only a factor of 6.7 (3.42 to 0.51). The ratio for human to rodent body weight, based on a 70 kg standard for humans and a 0.02 kg standard for mice, results in a conversion factor > 100. This implies that a correction factor of some unspecified value was utilized in deriving the human dose from the mouse dose, but this factor is not mentioned in the document, or that an error was made in the calculations. I would need access to the actual calculations to determine how the 0.51 human dose was derived. Verifying this LED10 of 0.51 for humans is critical if one intends to use the 0.2 ppb threshold.

There are also some minor issues with the Borneff study that I could not resolve during my review.

One example is that tumor development is not assigned to an individual animal. It is not clear whether the benign tumors described arose in separate animals or if they occurred in animals also having malignant tumors. Basically does one animal has two tumors? Both a malignant tumor (carcinoma), and also a non-malignant papilloma? If the latter is occurring (two tumors in a single animal) , this markedly alters the data (tumor rate) and subsequent calculations. The oral potency factor, currently assigned as 0.19 $(m/kg\text{-}day)^{-1}$ would change dramatically, and there would be a corresponding rise in the threshold value.

A second example is that a detergent was used as a vehicle for chromium. This raises the issue as to whether there was a synergistic interaction between the two agents.

A third example is that the fertility of surviving mice was adversely affected, as the number of offspring from F1 generation mice was severely depressed.

A fourth concern is the lack of detection of pre-neoplastic forestomach lesions in this study. With other agents that induce forestomach tumors in mice, there is also an incidence of pre-neoplastic stomach lesions. Why is there no reported incidence of pre-neoplastic forestomach lesions in the Borneff study?

The document goes to considerable lengths to dismiss all these concerns, but the reality of the situation is that no one knows the truth about the Borneff study (and probably never will). The development of mousepox in this carcinogenicity study is a fatal flaw in the validity of this study.

The document also states because "the study began with rather large numbers of animals, enough animals survived allowing a sufficient sensitivity to detect a carcinogenic response" (page 41).

I disagree with that statement. I believe that 66 animals is <u>not enough</u> for a carcinogenicity study. One needs a sufficient number of animals not only for sensitivity but to ensure that the tumor incidence noted is reliable and not a statistical aberration.

Zhang and Li study.

To support using the high tumor incidence value noted in the Bornheff animal study, the documents cites one exposure assessment study of human oral intake of hexavalent chromium that was done many years ago in China (Zhang and Li study). Note that there is only one human oral intake study for chromium, although there are many inhalation studies, which complicates the problem.

This human study in China has many flaws, including the fact that the <u>one of original</u> authors published a follow up study that indicated their original analysis/interpretation was flawed and that there was NOT an increased cancer rate. OEHHA believed that this second report included questionable interpretations (exactly which interpretations are suspect is not explicitly stated).

To overcome this problem, the authors of this document do an independent review and analysis using four unpublished reports. I do not now where the original content of the reports nor would anyone else - one has only the OEHHA authors interpretation and summary available. After extensive discussion they conclude the work is valid and supports the mouse study described above. One would have to spend hours sifting through these unpublished reports and raw data OEHHA is using to determine the validity of their reasoning and subsequent conclusions.

Even if one agrees with the unpublished data and subsequent interpretations, in addition to the numerous problems associated with this study (exact content of chromium, water consumption per person, use of unsubstantiated and unpublished documents, etc), it should be noted that one major flaw exists with the Zhang and Li study.

There exists the question as to what else was in this water?

Hexavalent chromium is not the only toxic metal that contaminates sites associated with a chrome plating facility. Both lead and arsenic contamination is commonly associated with chrome plating facilities in the United States. Both of these metals can also cause the symptoms described in the residents of the Nuer River Village. Indeed, when I read the symptoms described in the residents, my first thought was arsenic poisoning. The development of the mouth ulcerations in arsenic poisoning is very common. In contrast, in the few reported cases of acute chromium poisoning the development of oral ulcerations is not described. Arsenic is also associated with the development of lung and stomach cancer (perhaps even more so than hexavalent chromium).

There is absolutely no description of this problem in the document. All associated adverse medical findings are associated to chromium. One cannot, however, dismiss the relevance of concomitant presence of other heavy carcinogenic metals because their presence at toxic concentrations has been documented at contaminated sites associated with a chrome plating facility in the Unites States.

SUMMARY

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

1) A very flawed mouse study in which the majority of mice died from a virus and for which the incidence of malignant and non-malignant tumors was combined to achieve a high rate for the Fo generation mice (remember the F1 and F2 mice did not have an increased incidence of tumors). The influence of the mousepox virus on the results will never be known and cannot be dismissed. In addition, regardless of the influence of the viral epidemic on tumor development and growth, the number of surviving F1 mice the virus is too small to be reliable.

Also, the derivation of the human oral potency dose needs to be examined.

2) A single report of a flawed human assessment study, which even the one of original authors subsequently concluded may not be correct. The relevance of concomitant presence of other heavy carcinogenic metals is not addressed (and can probably never be addressed at this late date).

My main concern is that this draft represents a Public Health Document describing goal(s) for limiting hexavalent chromium content in drinking water, and as such is a regulatory document. Regulatory documents should be based on solid peer-reviewed scientific evidence in which the raw data and initial conclusions (number of tumors per individual, death rate, etc.) are solid and non-controversial. What can happen next is that the derivation of the calculations becomes controversial. As an example, what biosafety or overall uncertainty factor should be incorporated into the calculations? Should one use the traditional value of 3,000 or should a more conservative value of 10,000 be employed (as in this document)? What value should be assigned to the relative source contribution? Should one use a 100% value, or a less stringent value?

In this case the initial raw data and conclusions are not solid and quite controversial.

Please note that a large section of the document does use the standard approach of solid peer-reviewed scientific data and derives a different or higher threshold than 0.2 ppb. This is stated as 3 ppb (see page 101) and this threshold is primarily derived from NTP subchronic studies of mice in the mid 1990's. Yes, these NTP studies have limitations, but the results are consistent with reports by others.

I would like to point out that the 3 ppb value (presented on page 101), however, can still be considered controversial because in deriving this value the authors of this document employ an overall uncertainty factor that is 3.3+ fold higher than what is traditionally used. The result is a 3.3+ fold lower threshold value than what other scientists may calculate. Using a traditional safety factor of 3,000 (and not 10,000) would result in a threshold of 10 ppb (and not 3 ppb)

I personally, however, would have no problem defending the 3 ppb but would have serious reservations about defending the 0.2 ppb based on the numerous flaws in the studies supporting this value. In contrast, other scientists may feel that the 10 ppb threshold is more appropriate based on published results of hexavalent chromium.

Sincerely,

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