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 2008

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

Summary:

This document recommends the adoption of a concentration of 0.06 μg/L hexavalent chromium in drinking water as a public health goal that would ensure at most a lifetime risk of one in a million for cancer in the gastrointestinal tract due to ingestion and inhalation of drinking water. The estimate is based on the results of the National Toxicology Program study of 2007 (NTP, 2007) that evaluated the cancer rates of rodents exposed to chromate in drinking water during their lifetimes. This study positively identified a strong link between exposure to hexavalent chromium in drinking water and various cancers in the gastrointestinal tract. Results of this study were used to calculate human exposure protective levels using a cancer slope factor derived from the linear extrapolation to zero of the lower boundary of the dose associated with a ten percent increase in tumors (LED_{10}) that was estimated from dose-response curves fitted to the rodent data. In addition to the NTP 2007 study, the document presents other evidence to demonstrate that CrVI is a carcinogen via the oral route, such as the Borneff et al., 1968 rodent study, and the Beaumont et al., 2008 human epidemiological study that linked exposure to high levels of CrVI in drinking water with increased risk of stomach cancer in rural villages in China. Moreover, the premise that oral exposure to CrVI can cause cancer is further supported by the presentation in this document of evidence from different studies that show that: a) CrVI is not fully reduced to CrIII in the stomach and is at least partly absorbed as CrVI into the bloodstream, b) CrVI is incorporated into cells and c) CrVI in cells is genotoxic. Other evidence includes rodent oral CrVI exposure studies that showed DNA damage distal to the port of entry

Overall, the document convincingly demonstrates that indeed there is a relationship between exposure to CrVI via the oral route and the development of cancer in the gastrointestinal tract. However, the weakest aspect of the estimate of the human protective level is the very crude approach followed to calculate it. The slope factor calculated via a linear extrapolation to zero of the lower boundary level of the ED_{10} ignores two issues that are not incorporated under this approach but that may yield a different protective level (lower or higher) if included: namely, the existence of sensitive populations and the extent to which the reducing capacity of the gastrointestinal tract may have different efficiencies in the conversion of CrVI to CrIII depending on the amount of CrVI in the stomach. Because of these unknowns it is uncertain whether the PHG provides adequate public health protection.

Detailed review:

Main Comments

A) Reduction capacity of the saliva and gastric fluids:

I had reviewed the 2005 PHG document for CrVI and, in that instance, I had remarked the absence of a consideration of the reduction capacity of the saliva and gastric fluids in the estimation of the protective level of CrVI in drinking water. It was my opinion that in the process of calculating the oral cancer slope factor by extrapolating to zero a CrVI dose that is associated with a certain incidence of cancer in an animal study, there is an unwarranted assumption that the efficiency of saliva and gastric fluids to reduce CrVI to CrIII is the same in the presence of nanogram amounts of CrVI in the human stomach resulting from exposure to drinking water as it is in the presence of milligram amounts of CrVI in the rodent stomach resulting from high CrVI doses in the rodent studies. There is no information to support this assumption of linearity. The scaling of mouse to human by the 0.75 power to yield a human equivalent to the mouse LED_{10} only lowers the point of departure (POD) that marks the beginning of extrapolation to lower doses but it does not address the behavior of the reduction capacity of saliva and gastric fluids as the amount of CrVI in the stomach is reduced. It is assumed with the approach followed in 2005 and here in this PHG estimate that the fraction of CrVI that is reduced to CrIII is the same at high exposures, at the point of departure, at lower exposures and at the protective level

The document addresses this issue at the beginning and presents evidence demonstrating that despite the high reducing capacity of the stomach and gastric fluids there are indications that this mechanism fails to neutralize all CrVI. For example: Initially, the documents presents evidence that demonstrates that hypothetically even with oral exposures to high levels of Cr in fluids, the combined reducing capacity of saliva and gastric juices should exceed the maximum amount to be expected to be ingested with any water or beverages because levels higher than 5 mg/L would not be drinkable due to bad taste. Yet, as the document shows, several lines of evidence indicate that despite this excess reducing capacity of saliva and gastric fluids, not all CrVI is converted to the more innocuous CrIII. The data to support this argument are: a) Increased absorption of chromium (measured as increased urinary excretion of total chromium) when orally given as chromium VI (6.9% absorption) vs CrIII (0.13%absorption) or even CrVI reduced in orange juice (0.6% absorption) (Kerger et al., 1996). b) Different tissue distribution after oral CrVI administration than after oral CrIII administration, which indicates that Cr is in different form in the bloodstream depending on the speciation of the administered chromium. After oral CrVI administration, chromium accumulates in liver, spleen and kidney but only in the kidney after CrIII oral exposure. C) Different half-lives of chromium in RBC's following CrVI vs CrIII administration, with longer half-life after CrVI exposure (though the evidence in this regard is not so definite).

All this evidence is supportive of the contention that some CrVI escapes reduction and is absorbed into the bloodstream. However, the document does not discuss to what extent the same phenomenon of some CrVI escaping reduction and being absorbed into the bloodstream takes place at very low doses of hexavalent chromium. In all studies presented throughout the document, either human or animal studies, the doses of administered cromate are several orders of magnitude higher than the doses that would be taken if drinking water were to meet the PHG guideline. For example, in Kerger et al 1996, the exposure was an acute bolus dose of 5 mg at a concentration of 10 ppm cromate, which is 42 10^6 times larger than the amount a person would be exposed in a day, or 16000 times larger than the amount a person would be exposed over a lifetime, with drinking water meeting the PHG guideline. Kerger et al 1996, found increased Cr VI absorption, which the PHG takes as indication that indeed the reducing capacity is not 100% efficient in neutralizing hexavalent chromium to trivalent chromium. But, it is not clear that the relative higher absorption of CrVI vs CrIII (6.9% vs 0.13%) would be as high if the dose of hexavalent chromium were much smaller. I am not arguing that *all* CrVI is reduced and that there is a threshold dose above which CrVI starts to be absorbed. The criticism is directed at the assumption that the proportions of CrVI reduced and absorbed are the same regardless of dose. Competing kinetics between absorption and reduction may facilitate an increased *fractional* absorption of CrVI as the amount of CrVI increases, such that CrVI dose and CrVI absorption are not linearly related. This is directly addressed in the document in page 12 where it is argued that the linear increase of Cr concentration in tissue with CrVI dose (Anderson et al., 2002) is evidence that there is no threshold for absorption of CrVI and the linearity of Cr concentration in tissue would support the premise that there is a constant fraction of CrVI absorbed regardless of dose. However, the same results would be observed from absorption of CrIII after full reduction of CrVI in the stomach and this study can not distinguish between the two possibilities

Consideration of non-linearity in the reduction efficiency of stomach and gastric fluids with dose may yield a higher PHG.

B) Sensitive Populations:

There are two sensitive populations that are not included in the estimate of the one in a million lifetime cancer risk: carriers of Helicobacter pylori and people with anomalous stomach pH regulation. It is noted that animals in the NTP 2007 study were free of H. Pylori. As noted at the end of the document, a more realistic scenario, at least to evaluate the oral carcinogenicity of CrVI in carriers of H. pylori would utilize infected animals. This study would most likely yield a lower point of departure for linear extrapolation to zero and result in a lower PHG estimate.

The document recognizes the existence of other groups of sensitive individuals: those with a variety of conditions that result in reduced gastric capacity production. The equation of page 97 does not consider these sensitive subpopulations either. At this point there is no sufficient information to quantify

the higher risks that these populations may be exposed to due to CrVI in drinking water. The only certainty is that their inclusion in the cancer risk estimate would yield a lower protective level of CrVI in drinking water than the current one that does not incorporate them specifically.

C) Uncertainity

The document extensively discusses the unknowns involved in many of the parameters that are to be considered and included in the PHG estimate. However this discussion does not translate into a quantifiable measure of uncertainity. In other words: what is the degree of confidence in the PHG value? Can OEHAA quantify the uncertainity and say "There is X probability that a value as low as this PHG would protect 1 in a million"?

Other comments:

Absorption:

The absorption section is muddled and could be improved. The paragraphs are not thematically separated nor are the arguments built consistently on the basis of the previous paragraphs. These could be rewritten by leading each paragraph with the main point that is being made and each conclusion built on the foundation set by the previous paragraph

1- Urinary excretion is a good marker of absorption
2- More Cr is absorbed after CrVI administration than after CrIII administration based on urinary profiles
3- RBC and plasma profiles after CrVI administration are different than after CrIII administration and support the notion than CrVI is not fully reduced before absorption
4- There is a higher absorption of CrVI than CrIII in the duodenum CrVI
5- There is evidence of dermal absorption of CrVI

There is a great deal of enumeration of studies but the order they are presented is not conducive to support an argument because the argument is spread over several paragraphs that address this and other arguments as well. For example: The very important observation of a difference in Cr tissue distribution after CrIII and CrVI administration via **oral** route should be presented in a single paragraph or more, rather than intermingled with the argument about the different patterns of distribution of Cr after CrVI administration via different routes

Kerger at el 1996, it is an important study which conclusively demonstrates different gastrointestinal absorption for CrVI and CrIII oral exposures. The authors interpretation is however at odds with the PHG document (and mine) interpretation, claiming instead that with CrVI administration there is a higher absorption of CrIII-ligand bound complexes, not of CrVI. They quote some of the

same references that are cited in the PHG document to argue the opposite, namely that metal bound organic complexes absorption is not increased. The PHG document should clarify this further.

The last paragraph of page 11 is very confusing: "…The amount of hexavalent chromium recovered… " it is in fact total chromium recovered. Further down: "This is probably due…." It is not clear what 'This' is referring too. "… Administration of less than 10 mg/day…did not result in an increase…" with dose? compared to what?

The last paragraph of this section in page 13 is aimless. What it the message of this paragraph?

Distribution:

The observation that there is absorption of CrVI when administered in the 6+ species is supported by a different tissue distribution and urinary half-lives after CrVI and CrIII administration. However, there is an apparent inconsistency in the fact that the half life of Cr in RBC's after intraperitoneal or intravenous CrVI dosing does not match the half life of Cr in RBC's after oral CrVI administration. It is argued that blood carries Cr immediately from the point of oral absorption to the liver preventing a blood buildup of CrVI. Critics would argue that the Cr RBC time profile is not consistent with CrVI in blood and the increase in liver CrVI is in fact evidence for absorption of complexes of CrIII-organic ligands. The PHG document should address this interpretation

Genetic Toxicity

(Page 40) The case is made that despite the fact that the reducing capacity of the stomach should completely reduce the dose a human receive from drinking California waters, genotoxic effects were observed in distant tissues in rodents chronically administered by gavage doses "…not likely to overwhelm the reductive capacity of the stomach, intestines, and blood…", such as 1 mg/kg-d or 2.5 mg/kg-d. Further, at the end of the page this information is quoted again indicating that in these oral studies CrVI was not fully reduced, and DNA damage was observed. First, it is not know what the reducing capacity of the rodent stomach is. Second, this argument fails to account for the peculiarities of a gavage study. Assuming that a 0.25 Kg rat has a stomach with a 3mL volume and the dose is 2.5 mg/kg-day at one treatment a day, the concentration of the solution injected in the stomach is 250000ppb. Can the reducing capacity of the rat stomach (which is produced throughout the day) instantaneously reduced an acute bolus of this magnitude before some of it is absorbed? This concentration is 50,000 the concentration of California drinking water of 5 ppb. Or conversely, the typical per day dose of a 70 Kg California resident assuming 5 ppb CrVI in water, and 2 Liter consumption per day, is 0.00014 mg/Kg-d or 18,000 times less than that given in that rodent study. The comparisons made in the aforementioned quotations are not appropriate. At the very least the mention of California drinking water in this context should be eliminated because it is misleading.

Borneff study

The document discusses extensively the Borneff et al., 1968, study. The amount of space devoted to this study is not justified and it appears that this extensive presentation and discussion are a leftover from previous PHG's documents were Borneff et al. 1968, was the only animal study that could be used to demonstrate that oral CrVI is carcinogenic and to calculate an oral cancer slope factor. This is not the case anymore and it is puzzling that given the amount of uncertainty surrounding the results of this study so much space and speculation is devoted to it, in contrast to the study of Beaumont et al 2008, which is the only human study that shows a relationship between CrVI environmental exposure and oral cancer, but receives a mere two paragraphs of attention.

The document addresses the difference in cancer incidence between F0 and F1 animals in Borneff et al,1968, and hypothesizes that infection with H pylori in F0 resulted in higher cancer incidence in these animals compared to F1 animals. This hypothesis is plausible but it is not provable. There is much space devoted to speculation about the possibility that this infection would explain the cancer rates, but this discussion does not add to the PHG estimate. Since it remains in the realm of possibility, it can not be used as evidence for the carcinogenicity of oral CrVI. I would recommend that the document sticks to the known facts. It is not possible 40 years later to know if Borneff 's animals were infected or not, and by dwelling on a possibility we'll never know for sure, nothing is added to prove that CrVI is an oral carcinogen or to calculate a protective level

Cancer of Ingestion- and digestion related organs reported in occupational studies

The analysis of the occupational studies is fairly inconclusive and at most suggestive of a link between CrVI exposure and stomach cancer. Given the very little weight that this analysis carries OEHHA should consider not including this analysis in the PHG document. The PHG document reports that the occupational studies reveal an increase in the incidence of stomach cancers with hexavalent exposure. However, the evidence presented is not compelling enough to make such a statement.

First: the number of studies that report a rate ratio significantly above 1 for stomach cancer after chromium occupational exposure is 3 out of 25, close to the frequency expected from a significance level of 0.95 (1 study out of 20 wrongly rejecting the null hypothesis when in fact the null hypothesis is true).

Second: In reaching the conclusion that most studies show an increased risk of stomach cancer, the confidence intervals of the rate ratios are ignored. If the report chooses to follow this approach and consider the rate ratios independently of their confidence intervals, it should be consistent and address the contradictory observation that on the basis of the rate ratios alone 25% of the studies would support a protective role of CrVI exposure against stomach cancer! But this logical conclusion is ignored. It seems that a more rigorous statistical approach to pool the studies, than just counting studies with rate ratios larger and smaller than 1, should be pursued. This analysis would take into consideration

the confidence intervals of the rate ratios. The conclusion that the occupational studies 'reveal' (page 75) an overall increase of stomach cancer is overreaching.

Third: The report chose to highlight stomach cancers as the most notable outcome: "…The most notable results were for stomach cancer, for which most studies found excess risk…(page 66)". However, following the PHG approach of adding up the number of studies with rate ratios larger than 1 (and disregarding confidence intervals) there are outcomes that are even more notable than stomach cancer: For stomach cancer, 70% (18 out of 26 studies, Table 7) have RR >1

For 'All digestive', 70% (18 out of 26 studies, Table 7) have RR >1

For Esophagus, 73%, (8 out of 11 studies, Table 7) have RR>1

For Rectum, 75% (12 out of 16 studies, Table 7) have RR>1

Unless a statistical analysis is followed that pools the results from all studies and confirms the relationship between stomach cancer and hexavalent chromium exposure, the report should downgrade (page 75, 5th paragraph) the conclusion of the existence of a link between occupational CrVI exposure and stomach cancer to a level of "suggestive" of a link between the two, or fully eliminate this analysis.

Ingestion studies

The Beaumont et al 2008 study deserves much more attention than two paragraphs and meaningless map!

Dose Response modeling

The modeling of the female data of the NTP 2007 study is not used for the calculation of cancer potency because "the male data used in the modeling was more robust". OEHAA should reconsider this. Examination of the cancer incidence response with dose from the NTP study suggests a different response according to gender, with males appearing to have a more linear response through the dose range and with female data showing an apparent higher sensitivity at lower doses and saturation in cancer incidence at a lower dose than the males. Does this indicate a gender specific difference in the response shape and sensitivity? Female data should be considered, the LED_{10}'s are lower than those derived from the male data, and the most conservative approach would suggest taking that data into account.

Figures should be provided with all model fits and with the data for female and male mice cancer incidence with dose (in mg/kg-d). What is the reason to consider the multistage model over others for the estimate of the cancer slope factor? Perhaps, a more appropriate choice would be a weighed average of all models, each model LED_{10} weighed by a factor that incorporates the chi square value

Other comments:

Page 7: Temperature of the water would probably have been closer to soil temperature, since most likely it is coming from the pipes buried in the ground

End of page 17: Trivalent Chromium is largely excluded from cells. Isn't CrIII a micronutrient? Please clarify.

Page 36: "…Bigaliev et al (1977)…observed chromosomal aberrations…" in what tissues?

End of same paragraph: Cheng et al 2000, correlations were only observed in the lung!

Page 40: "…Cancers arising from exposures of rodents….via inhalation, ip, intramuscular … etc…" The outcomes in these studies were not cancer, and no intramuscular studies reported genotoxicity (see page 37 first paragraph)

Page 60: "… The reduced water consumption appears to be consistent with the reduced weight gain in these animals…" This is not the case: Female mice drank as much as controls from week 15 and never gained enough weight. Male mice drank less than controls from week 15 but gained as much weight.

Page 65: End of 3rd paragraph. Add how many studies were evaluated and how many met the criteria

Page 83: Last sentence should say Table 13, not Table 17.

Page 84: is $PY_{a,d}$ "number person-years at risk' (measure of exposure) or expected number of cancers (measure of effect, according to the last sentence on page 83?. Last paragraph says Table 13 and it should be Table 12.

Page 88: First paragraph: it is not clear where the numbers are coming from:

"slope based upon excluding the highest two exposures is 38% above that in Analysis 1…": These are $2.66X10^{-1}$ vs $2.58X10^{-1}$ or 3% apart. When all doses are included, there is 38% difference: $2.45X10^{-3}$ vs $3.77X10^{-3}$. Later on, not clear where 88% and 33% come from.