

University of Tasmania School of Human Life Sciences



Locked Bag 1320 Launceston
Tasmania Australia 7250
Phone (03) 6324 5400; Fax (03) 6324 3658
<http://www.hls.utas.edu.au/>

20 September 2010

Gerald W Bowes, PhD
Manager, Cal/EPA Scientific Review Program
Office of research, Planning and Performance
State Water Resources Control Board
1001 I Street
Sacramento, CA 95814

Dear Dr Bowes,

Attached please find my review of the Draft Public Health Goal for Hexavalent Chromium in Drinking Water (August 2009)

I trust this provides the information required of me and fulfills my contractual obligation to the OEHHA.

Thank you for the opportunity to contribute to this process and I apologize again for my somewhat delayed response.

Sincerely yours,

A handwritten signature in black ink, appearing to read 'Elizabeth T. Snow', written in a cursive style.

Associate Professor Elizabeth T. Snow
School of Human Life Sciences

Scientific Review of Public Health Goal (PHG) for Hexavalent Chromium in Drinking Water

Having carefully read and evaluated the above mentioned document (PHG for Cr6) it is my considered opinion that the document is based on the best available scientific knowledge and that the conclusions reached are to the best of my knowledge and understanding both accurate and complete. The four points under consideration are noted below with my conclusions for each.

1. Accuracy of the information presented on metabolism, toxicity, mode(s) of action and exposure, including potential for carcinogenicity and reproductive toxicity following exposure to hexavalent chromium in drinking water.

OEHHA finds that the preponderance of evidence indicates that Cr6 is absorbed by the oral route, is genotoxic, is carcinogenic by multiple exposure routes, in multiple species, and requires the assumption that the environmental exposures to Cr6 pose a cancer risk to humans.

Decades of research on the metabolism and genotoxicity of Cr6 *in vitro*, in animal models and in human populations with industrial exposure to Cr6, primarily by inhalation, has shown conclusively that Cr6 is a mutagen and a genotoxic carcinogen. Cr6 is readily taken up by cells of all types and then undergoes reduction with the concomitant production of reactive species, including reactive Cr(V) and Cr(IV), with the end product being a variety of stable, membrane impermeable Cr(III) species. In addition to oxidative DNA damage, Cr(III)-DNA adducts and Cr(III)-mediated DNA-protein crosslinks are arguably among the most important types of mutagenic DNA damage produced by Cr6 uptake and metabolism. Most Cr3 species are not however taken up by cells and, in general, oral exposure to Cr6 leads to greater cellular uptake and a longer biological half-life than oral exposure to Cr3 species. Exposure to Cr6 in drinking water seems to produce quite variable uptake of the metal, which may depend heavily on individual differences in gastric pH and other metabolic variables. It has been argued that the rapid extracellular reduction of Cr6 to Cr3 species will prevent gastric uptake of Cr6, except at the highest levels of exposure. However, the data provided clearly show differential uptake and toxicity of Cr6 compared to Cr3 in both animal models and in humans, suggesting that some (variable) proportion of Cr6 is absorbed, even at low exposure levels. Clear evidence for carcinogenicity of Cr6 in drinking water relies on limited but sufficient data; the 2007 NTP rodent bioassays and one adequately documented human population exposure in China. This study, the only available study showing an excess of cancer in a human population exposed to Cr6 by an oral route (Zhang and Li 1997) was retracted by the editors of the journal because of lack of disclosure of financial conflicts of interest (Brandt-Rauf 2006), and also had a number of significant methodological issues, yet, having been re-evaluated by Beaumont et al. (Beaumont et al. 2008), still provides the best evidence to date of an increased incidence of stomach cancer in a human population with documented oral exposure to Cr6.

The carcinogenic potential of Cr6 in drinking water is best documented by the recent NTP study in which long term (2 year) exposure of both male and female mice to Cr6 in drinking water showed that Cr6 exposure by this route is capable of causing liver damage (chronic inflammation and fatty changes) at doses as low as 0.2 mg/kg-day and a dose-dependent increase in cancer of the small intestine (NTP 2007). Oral administration of Cr6 can also

produce genotoxicity in cells of the lung, kidney and liver, as documented in multiple additional rodent bioassays, including the preliminary 3-month NTP study (NTP 2007).

In contrast to the well-documented uptake and metabolism of Cr6 after oral as well as inhalation exposure; Cr3 is very poorly absorbed by cells, including cells lining the gastric mucosa. Nevertheless, Cr3 is an essential trace element and tannery workers exposed to Cr3 have been shown to exhibit an excess DNA damage in lymphocytes relative to controls (Medeiros et al. 2003; Zhang et al. 2008). The mechanism and efficiency of Cr3 uptake and the form of metabolism of nutritional Cr3 uptake merits further study, especially given the evidence that Cr3-DNA adducts are significantly mutagenic. Although the available evidence suggests that the Cr6 induced mutagenesis is likely to be the primary mechanism of carcinogenesis, there is as yet no definitive proof of this for oral exposure to Cr6. Recent data suggests that chromium may also produce epigenetic changes that may contribute to its carcinogenicity (Sun et al. 2009). The relative importance of an epigenetic mode of action is not known at this point in time.

In contrast to the documented evidence for carcinogenicity by multiple routes of exposure, the reproductive toxicity of oral exposure to Cr6 has not been demonstrated except at doses sufficient to cause significant maternal toxicity.

Based on these data it is clear that environmental exposure to Cr6 in drinking water can pose a potential risk for human carcinogenesis.

2. Selection of the NTP data set and supporting information for extrapolation to humans, particularly regarding interpretations of carcinogenicity data and mechanisms.

OEHHA concluded that the best available tumor data for the risk extrapolation was the significantly increased incidence of tumors of the small intestine in the male mice reported in the recent NTP lifetime drinking water studies of Cr6.

The genotoxicity and carcinogenicity of Cr6 has been studied for decades, however well documented studies showing an increase in cancer risk after oral exposure to Cr6 in drinking water have been lacking until recently. Prior to the recent NTP lifetime drinking water study the only available study of oral exposure to Cr6 was that of Borneff et al. (Borneff et al. 1968), which was seriously flawed and can not be used to evaluate dose response. The NTP study (NTP 2007) showed a significant, dose-dependent increase in tumors of the small intestine in male (and female) mice which can be used to extrapolate an estimated dose-response for Cr6-induced cancer in humans by the same route.

3. Appropriateness of risk assessment methodology use for extrapolation to human exposures to Cr6 in drinking water.

A cancer potency estimate of $0.6 \text{ (mg/kg-day)}^{-1}$ derived following standard guidance from the U.S. EPA and OEHHA (U.S. EPA 2005, OEHHA 2009), resulted in an extrapolated 1 in 10^6 lifetime cancer risk level for Cr6 in tap water of 0.06 ppb.

The standard guidance from the U.S. EPA and the OEHHA provides a well-defined protocol for extrapolating rodent toxicity and carcinogenesis data to humans. Given that the best available data shows evidence that Cr6 is a genotoxic carcinogen, the default protocol is to use a no threshold linear dose response based on the slope of the best available dose response for a cancer endpoint. The only available human exposure data showing an increase in cancer risk due to oral exposure to Cr6 in drinking water was a cohort in China first reported by Zhang and Li (Zhang and Li 1980) and reanalyzed by Beaumont et al. (Beaumont et al. 2008). This environmental exposure data is sufficient to show evidence of human cancer risk but cannot be used to reliably determine a quantitative risk estimate.

The best available data for determining a risk estimate for oral exposure to Cr6 is the NTP rodent cancer data (NTP 2007) which can be used to estimate a linear dose response (the oral cancer potency) which can then be extrapolated to provide the best estimate of human risk. It should be noted that a linear fit to the NTP data is the default protocol as defined by the U.S. EPA and OEHHA and that the data could equally well be fitted to a nonlinear, supralinear (concave) or 'hockey stick' response model. Additional inhalation exposure to Cr6 in water droplets during showering was also considered, but was found to contribute only a small fraction to the total risk from oral exposure to Cr6 in the drinking water. Using this prescribed methodology, a proposed public health guidance (PHG) value of 0.06 ppb Cr6 was calculated to give a maximum lifetime cancer risk of 1 in a million.

4. Identification of the uncertainties in the risk assessment and proposed PHG calculation.

Considering the uncertainties, OEHHA believes the incorporation of health protective assumptions provides an acceptable level for public health protection.

There are a number of important uncertainties inherent in this estimation of the risk for human disease due to environmental exposure to Cr6 in drinking water. There are very little data showing human health effects due to exposure to any but the very highest doses of hexavalent (or trivalent) chromium in drinking water. Acute toxicity has only been reported at levels of Cr6 sufficient to turn the water yellow and make it distinctly unpalatable to both humans and rodents. Only one cohort with a measurable increase in the prevalence of cancer in humans due to exposure to Cr6 in drinking water has been reported; and this study is sufficiently flawed as to make its reliability suspect. Other studies of environmental and occupational exposure to Cr6 or Cr3 with an oral (or dermal) route of exposure give very limited evidence of an adverse cancer risk. This could be because of small cohort sizes, as only those studies with a relatively large exposed population (e.g. Knutsson et al. 2000) gave evidence for a significantly increased risk for stomach cancer due to Cr6 exposure. There is also only one reliable study of rodent carcinogenesis due to exposure to Cr6 in drinking water, the 2007 NTP study. In this study increased cancer risk was only seen at very high levels of Cr6, at doses of greater than 14 ppm Cr6 in a lifetime exposure study. Based on this study, along with very limited evidence of tumor response at lower levels of Cr6, there is very limited evidence for a linear dose response. It is more likely, due to the high probability of extracellular conversion of the Cr6 to the much less toxic Cr3, that uptake and bioavailability of the Cr6, in itself, will exhibit a non-linear (threshold) dose response. A low dose, linear response also assumes a lack of DNA repair and other protective mechanisms with an expected maximum protective effect at low dose. An epigenetic mechanism for cancer is also

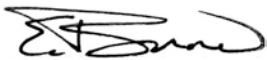
expected to give rise to a non-linear dose response and evidence for this type of mechanism for chromium is increasing as more laboratories explore this option.

The essential nature of Cr³ as a required trace element should also be considered as oral Cr⁶ is expected to be reduced to Cr³ for which there must be some sort of uptake mechanism in order to supply this nutrient to the body. There must also be some sort of mechanism for the intracellular utilization of some forms of Cr³, which, given that Cr³ is the intracellular end product of Cr⁶ metabolism, implies a possible threshold limit for Cr³ (and thus Cr⁶) toxicity within the cell. The questions of Cr³ uptake, metabolism and toxicity all require further study and have important implications with regard to chromium toxicity at low doses.

Inherent variability in metabolism and uptake of chromium species is another significant unknown. The possibility has been raised that infection with *Helicobacter pylori* can raise stomach pH and thus lead to the slower or incomplete extracellular conversion of Cr⁶ to Cr³, leading to an increased uptake of Cr⁶. This would be expected to make *H. pylori* sufferers more susceptible to Cr⁶ induced stomach cancer. Although there is almost no evidence at this time that *H. pylori* does in fact play a role in Cr⁶ uptake by humans or rodents, this possibility needs to be tested and other susceptible populations will need to be identified.

However, given these uncertainties, and with the understanding that it is incumbent on the OEHHA to deliver a PHG value that is maximally health protective, it is my belief that the estimated PHG value of 0.06 ppb provides an acceptable minimum value for public health protection.

Sincerely,



Elizabeth T Snow, PhD

Associate Professor of Molecular and Environmental Health
School of Human Life Sciences
University of Tasmania
Launceston, Tasmania 7250
Australia

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