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Derivation of an Ingestion-Based Soil Remediation Criterion for Cr\textsuperscript{6+} Based on the NTP Chronic Bioassay Data for Sodium Dichromate Dihydrate

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Abstract
Although the carcinogenicity of hexavalent chromium (Cr\textsuperscript{6+}, Cr(VI)) by inhalation has been known for a long time, there has been little evidence regarding the potential for the carcinogenicity and no ability to estimate cancer potency of Cr\textsuperscript{6+} by ingestion until recently. The release in 2008 of the National Toxicology Program's (NTP) chronic bioassay of rats and mice exposed to Cr\textsuperscript{6+} in drinking water provided clear evidence of cancer risk by ingestion and permits the estimation of the cancer potency and the associated soil remediation criterion. Dose-related increases in oral cavity tumors were observed in both sexes of rats and small intestine tumors were observed in both sexes of mice. Following USEPA guidance, NJDEP calculated a value for the human-equivalent cancer potency of 0.5 (mg Cr\textsuperscript{6+}/kg body weight/day)\textsuperscript{-1} based on the most sensitive species and sex (male mice). For a one-in-a-million (1x10\textsuperscript{-6}) lifetime cancer risk, this is equivalent to a daily dose of 1x10\textsuperscript{-6} mg Cr\textsuperscript{6+}/kg body weight/day. Based on NJDEP soil remediation standards guidance, this corresponds to a soil concentration of 1 ppm (part per million). The NTP study was scientifically sound in its design and execution. Taking into account the ability of the stomach to metabolize Cr\textsuperscript{6+} to the less toxic Cr\textsuperscript{3+} form, the NTP animal data are judged to be relevant to human exposure. As per the USEPA scheme for characterization of carcinogenic potential, it is concluded that Cr\textsuperscript{6+} is "likely to be carcinogenic to humans" by ingestion.

Introduction
The carcinogenicity of Cr\textsuperscript{6+} (hexavalent chromium, Cr(VI)) to the respiratory tract and particularly the lungs through the inhalation route of exposure has been known since the 1930's. The USEPA developed an inhalation carcinogenicity unit risk (potency) in the 1980's (USEPA, 1998). However, carcinogenicity by inhalation does not necessarily imply carcinogenicity by ingestion. Furthermore, different potencies for each route of exposure and different rates of exposure by each route can lead to different levels in soil (or other environmental media) that correspond to the same level of risk.

Historically, studies of workers employed in chromate production and related industries who were exposed to Cr\textsuperscript{6+} mostly through inhalation, have yielded equivocal evidence of ingestion-related cancers (NJDEP, 2006). A recent analysis of stomach cancer in a population in China exposed to high levels of Cr\textsuperscript{6+} in drinking water provides a stronger suggestion that Cr\textsuperscript{6+} can cause cancer by ingestion (Beaumont et al., 2000). However, that study does not lend itself to the development of an estimate of ingestion cancer potency or a soil remediation criterion. Prior to the National Toxicology Program (NTP) chronic bioassay, the only relevant animal study relating to the ingestion carcinogenicity of Cr\textsuperscript{6+} dealt with the co-carcinogenicity of Cr\textsuperscript{6+} and UV light in the production of skin tumors (Davidson et al., 2004; Uddin et al., 2007). It is difficult to apply the results of that study to environmental risk-based standard setting because of its unusual design and because Cr\textsuperscript{6+} is a co-carcinogen in that study rather than a direct carcinogen.

At the request of the State of California, the National Toxicology Program (NTP) a part of the National Institutes of Health, U.S. Department of Health and Human Services, undertook a two-year chronic bioassay of Cr\textsuperscript{6+} in mice and rats by ingestion in drinking water. The final peer-reviewed report of that study was released in July of 2008 (NTP, 2008). This is a state-of-the-art toxicology study that provides all of data and analysis necessary to derive a quantitative estimate of human cancer risk from ingestion. The data presented in the NTP study was used in the New Jersey Department of Environmental Protection's (NJDEP) risk assessment to derive a human cancer potency estimate for Cr\textsuperscript{6+} by ingestion and an associated soil remediation criterion.
Methods

The NTP study was conducted using sodium dichromate dihydrate (Na₂Cr₂O₇·2H₂O), a common, soluble compound containing Cr⁶⁺. Cr⁶⁺ occurs in two different ionic forms, the chromate ion (CrO₄²⁻) and the dichromate ion (Cr₂O₇³⁻). Previous studies on the health effects of the various forms of Cr⁶⁺ have indicated that both forms are essentially identical in their toxicology. The only significant difference between them is that the dichromate ion contains two moles of Cr⁺⁶ for each mole of dichromate, whereas the chromate ion has only a single mole of Cr⁺⁶ per mole of chromate. To avoid confusion on this count, the risk assessment is based on the dose of the underlying Cr atom rather than on either chromate or dichromate form. The resulting cancer potency and soil remediation criterion are applicable to all forms of Cr⁺⁶.

NTP exposed mice and rats to constant concentrations of Cr⁺⁶ in their drinking water for two years. There were 50 animals of each sex exposed to four different concentrations of Cr⁺⁶ plus an unexposed control group for each species and sex. NTP calculated the dose (mg Cr⁺⁶/kg body weight/day) from the water consumption and the measured body weight of the animals. At termination of the study, or when an animal died, all animals were examined for gross and microscopic pathology of all major organ systems and for blood pathology.

Results

Compared to control animals, decreased body weight occurred at the highest dose in each species and sex. In female mice, the decrease was 20%. This is considered to be an indication of toxicity in the female mice. In all other animals, the decrease in body weight was less than 10% and is not considered toxicologically significant. There was little difference in survival between high dose and control animals and clinical signs were normal at all doses. The only significant toxicity in either species was a statistically significant increase in benign and malignant tumors of the oral mucosa and tongue in male and female rats and of the small intestine in male and female mice. Figure 1 (a-d) shows the incidence of these tumors. The tumors were statistically significantly elevated compared to the controls at the highest dose in rats and at the two highest doses in mice. In the mice, hyperplasia (irregular growth of tissue) of the small intestine was noted at all doses. NTP judged this to be a response to tissue injury from Cr⁺⁶ exposure. Overall, NTP judged that the results showed clear evidence of carcinogenicity in both species and both sexes.

NJDEP Risk Assessment of the NTP Study Results

General approach

The approach used to derive the cancer potency from the NTP data follows USEPA guidance (USEPA, 2005a). As per that guidance, the tumor incidence was based on the sum of benign and malignant tumors under the assumption that the benign tumors have the capacity to become malignant over time. The tumor incidence at each dose took into account the number of tumors observed in the unexposed control animals. The cancer potency is calculated as the slope of the line that begins at zero dose and extends to a point on the graph of dose versus tumor incidence below which there are no longer useful data. This is referred to as the point of departure (POD). Consistent with the USEPA guidance, the POD was calculated using benchmark dose modeling (USEPA, 2000). Through fitting mathematical functions to the data of dose and tumor incidence, benchmark dose modeling permits the estimation of the dose corresponding to a given target value for tumor incidence. In this assessment, the benchmark dose modeling was used to estimate the lower 95% confidence limit on the dose corresponding to a 10% increase in tumors. That point was taken as the POD.

Figure 1. Incidence of oral tumors in rats and small intestine tumors in mice

![Graph showing incidence of oral tumors in male and female rats.](image)
Selection of key species
In both rats and mice, tumors increased in response to increased Cr\(^{6+}\) dose. In mice, however, this increase was greater and occurred at lower doses of Cr\(^{6+}\). Because the mouse is the more sensitive species, the mouse data were selected for the derivation of the cancer potency.

Results of the POD calculation
The USEPA benchmark dose software used to calculate the POD allows the calculation of the POD with several different mathematical functions. Benchmark dose modeling was carried out for male and female mice separately using the full data for each and for the male and female mouse data combined. In addition, because of the non-cancer toxicity in the high-dose female mice that resulted in the significant loss of body weight, benchmark dose modeling was also carried out for the female mice and for male and female mice combined, excluding the high dose females. Nearly all the models gave close fits to the male mouse data and the PODs calculated from these models were nearly identical. For the female mouse data as well as for the combined data sets, however, none of the models fit the data comparably to the male mouse data. Figure 2 is an example of the fit of the models to the male mouse data. The male mice were, therefore, used to estimate the cancer potency.

Calculation of the human cancer potency
The slope of the straight line from the POD to the point of zero-dose (the purple line in Figure 2) gives the cancer potency for the male mouse ((mg Cr\(^{6+}\)/kg body weight/
day)^1). This was converted mathematically to the dose at one-in-a million (1x10^-6) cancer risk (mg Cr^6/kg body weight/day) and converted to the human dose taking into account differences in body weight and metabolic rate. The corresponding human cancer potency is 0.5 (mg Cr^6/kg body weight/day)^1 and the corresponding dose at 1x10^-4 lifetime cancer risk is 1.9x10^-6 mg Cr^6/kg body weight/day. To put the cancer potency of Cr^6 in perspective, this is one-third as potent as arsenic by ingestion.

**Calculation of the corresponding NJDEP soil remediation criterion**

The calculation of the NJDEP soil remediation criterion concentration follows directly from the human equivalent dose corresponding to 1x10^-6 lifetime cancer risk by applying the exposure assumptions in the NJDEP Soil Remediation Standards Basis and Background document (NJDEP, 2008) (i.e., exposure duration = 30 years (from 1 year-old to 31 years old); body weight (integrated over the 30 years) = 59 kg; integrated soil rate (integrated over the 30 years) = 114 mg/day). The resulting soil concentration of Cr^6 is 1 part per million (ppm).

**Weight of evidence for characterization of carcinogenicity to humans**

The results of the NTP study clearly show that ingestion of Cr^6 in drinking water resulted in tumors in both sexes of rats and mice. This strongly suggests that a similar potential exists for humans ingesting drinking water or soil. The NTP study was well designed and well executed with no significant problems that raise questions about the validity of the results. The animals remained in good health and did not appear to develop cancer because of other toxicities related to the exposure. The tumors in both rats and mice occurred in the alimentary system. In both the male and female mice there was a clear relationship between Cr^6 dose and tumor incidence. As outlined below, the evidence supports a hypothesis that the observed tumor incidence is relevant to human exposure at reasonably anticipated environmental levels. Although there are differences in the acid level of mouse and human stomachs, it does appears that stomach acidity is the predominant factor in the ability of Cr^1 to act as carcinogen. Thus, the mouse appears to be a reasonable model for the carcinogenic potential of ingested Cr^6 in humans. The ability of Cr^6 to cause tumors in the mouse small intestine is likely to be similar in the human gastrointestinal system. In addition, the ability of Cr^6 to act as a carcinogen in the gastrointestinal tract is not surprising given its known ability to cause cancer in the human respiratory tract.

Under the USEPA Guidelines for Carcinogen Risk Assessment (USEPA, 2005a), these observations are consistent with the characterization of oral exposure to Cr^6 as “likely to be carcinogenic to humans.”

**Weight of evidence for the carcinogenic mode of action (MOA) of Cr^6**

Under current USEPA guidance (USEPA, 2005a; 2005b), if it is determined that a chemical is a carcinogen through a mutagenic mode of action (MOA) the cancer potency is divided by a factor of 10 to account for the observed increase in potency of such chemicals during early life. This is referred to as an age-dependent adjustment factor, ADAF. The criteria for concluding that a mutagenic MOA is operative have not been formalized. Among the necessary criteria is evidence that a chemical interacts with DNA to produce tumors. There are considerable data indicating the ability of Cr^6 to react directly with DNA. However, the hyperplasia observed in the mouse small intestine suggests that tissue damage and regeneration could have played a role in the formation of tumors in the in the NTP study. Given the absence of clear criteria for determination of a mutagenic MOA and given the evidence for at least one other possible MOA, the ADAF is not applied in this assessment.

**Characterization of uncertainty**

Although it is not clear why the rats and mice developed tumors in different organs, in general and with respect to USEPA guidance, the occurrence of tumors in different organs in different species is not considered to weaken the assumption of cancer risk to humans.

It is known that the human stomach has a large capacity to reduce Cr^6 to the much less toxic Cr^3 form (De Flora et al., 1987; 1997). This raises the possibility tumors occurred in the mouse small intestine because the doses in the NTP study were large enough to overwhelm the reduction capacity of the stomach for Cr^6. According to this hypothesis, smaller doses, such as those likely to be received from contaminated soil or drinking water would not overwhelm the capacity of the stomach and would therefore, not lead to tumors. In other words, there would be a threshold for tumors from ingested Cr^6 and there would be no cancer risk as long as the threshold was not exceeded. Several independent lines of evidence, however, indicate that the reduction capacity of the mouse stomach in the NTP study was not exceeded and that the small intestine tumors developed despite the intact reduction capacity. This evidence is developed fully in Appendix A of the full report. In brief, the following observations support this conclusion:

1. Applying the data on the reduction capacity of the human stomach to mice and comparing that capacity to the doses in the NTP study suggests that at most, the reduction capacity was exceeded only at the highest dose in female mice. This is a worst-case scenario since it assumes that the dose of Cr^6 remains in the stomach until the reduction is complete.
2. In fact, the Cr^6 does not remain in the stomach until the reduction is complete. There is an emptying of the stomach into the small intestine that is rapid compared to the rate of Cr^6 reduction. Therefore, even low doses of Cr^6 can escape reduction because they are passed into the small intestine before they have a chance to be chemically changed.
3. We examined data from NTP on the accumulation of Cr in various organs of the mouse at the doses of Cr^6 used in the NTP cancer study. Those data show no evidence that the rate of Cr accumulation in tissues increased as would be expected if there was a
threshold for the production of tumors within the range of the doses in the NTP study.

- The observation of hyperplasia in the mouse small intestines even at the lowest dose of Cr\(^{6+}\) also shows that within the range of doses in the NTP study, there was no threshold below which, no Cr\(^{6+}\) escaped reduction in the stomach.

- The observation that even a dose that was 3% of the lowest dose in the NTP study, Cr\(^{6+}\) was transported to the skin and was able to act as a co-carcinogen (Davidson et al., 2004) also suggests that low doses of Cr\(^{6+}\) can escape reduction in the stomach.

In the NTP study, tumors were only observed in the oral cavity (rats) and small intestine (mice), data on accumulation of Cr in other tissues in the NTP study as well as other studies raises the possibility that Cr\(^{6+}\) has the potential to cause tumors in other locations in the body. At the present time, this is merely a hypothesis.

As with all other animal studies used to derive estimates of cancer risk to humans at low levels of exposure, it was necessary to extrapolate the observed NTP data across five orders of magnitude to estimate the one-in-a-million lifetime cancer risk to humans. This is a significant uncertainty, but one that is inherent in all such assessments.

**Putting the Findings of this Risk Assessment into Context**

Prior to the NTP study, the NJDEP soil remediation criterion for Cr\(^{6+}\) for the ingestion route of exposure was based on non-cancer effects (USEPA, 1998); a value of 240 ppm. In February 2007, the NJDEP chose to apply an interim soil cleanup criterion of 20 ppm to all sites contaminated with Cr\(^{6+}\) from chromate production waste (http://www.state.nj.us/dep/dsr/chromium/crmorlift200702.pdf). Although this value initially applied only to inhalation exposure on industrial sites, it was chosen as a general criterion because it was the lowest NJDEP remediation criterion then in use. Based on the ingestion cancer potency estimated from the NTP study, ingestion exposure to soil containing 20 ppm Cr\(^{6+}\) would correspond to a lifetime cancer risk of two-in-a-hundred thousand (2x10\(^{-5}\)) compared to a risk of one-in-a-million (1x10\(^{-6}\)) for soil containing 1 ppm Cr\(^{6+}\). While a 1 ppm soil remediation criterion is one-twentieth of the 20 ppm interim remediation criterion, it should be noted that both values fall within the risk range of one-in-a-million to one-in-ten-thousand (1x10\(^{-4}\)) often applied to the setting of standards and guidelines for exposure to carcinogens for the protection of public health.

Risk-based criteria and standards for the protection of public health, and particularly those that derive human cancer risk from animal studies should not be viewed as precise predictions of health outcomes. This is the case for several reasons. The first is that the levels of risk (such as one-in-a-million excess cancer risk) that are deemed to be appropriate levels at which to protect public health, are sufficiently low that one would never be able to detect such a small increase in risk under real-world conditions. The second reason is that the process of deriving these criteria and standards encompasses many uncertainties and gaps in knowledge. To address these uncertainties, it is necessary for risk assessments to make certain assumptions. The assumptions that are selected are chosen because they are both scientifically plausible and protective of public health. When there are choices to be made among several scientifically plausible options, the ones that are selected are generally those that are more likely to protect public health. Thus, the treatment of uncertainties in these risk assessment tends to make the resulting criterion or standard more “conservative” rather than less “conservative.” In the context of hazardous site remediation, “conservative” means that the uncertainty about the true value of the number that is derived in the risk assessment results in a value that is more weighted toward the protection of public health.

The extent to which it would be practical to apply a soil remediation criterion of 1 ppm depends on two factors, the ability to reliably and reasonably measure Cr\(^{6+}\) in soil at that level using reasonably available analytical techniques, and the background level of Cr\(^{6+}\) in soil in the absence of a specific source of contamination. At present, it is not known whether there is a background level of Cr\(^{6+}\) in NJ soils. Relatively high levels of Cr\(^{6+}\) are known to occur naturally in soil under very specific soil conditions. In order to consider the practical implications of a soil standard based on a criterion of 1 ppm, it is necessary to investigate whether soil concentrations around 1 ppm could be widespread, particularly in urban soils that are subject to diffuse sources of contamination. NJDEP is currently undertaking a study to better define urban background levels for Cr\(^{6+}\) in soil.

**References**


USEPA (2005a). Guidelines for Carcinogen Risk Assessment. EPA/630/P-03/001F.
