Reviewer Comments for Derivation of Soil Remediation Ingestion Criteria for Cr^{+6} Based on the NTP Chronic Bioassay Data for Sodium Dichromate Dihydrate

The Risk Assessment Committee of the NJDEP Chromium Workgroup was charged with generating technical review comments for the document it had prepared for the cancer risk assessment of Cr^{+6} based on the NTP chronic bioassay for sodium dichromate dihydrate. The initial plan was to engage in a formal peer-review process. This process included the formulation of peer-review questions relating specifically to the document, identification of non-NJDEP peer-reviewers with specific qualifications in risk assessment and chromium and addressing potential conflicts of interest of those peer-reviewers. We identified three peer-reviewers with the appropriate expertise. Ultimately one of those reviewers was unable to engage in the review due to personal time constraints and asked to be excused. The responses of the other two reviewers, Gary Ginsburg, Ph.D. of the Connecticut Department of Health, and Mark Maddaloni, Dr.P.H., D.A.B.T. of U.S.EPA Region 2[1] to the conflict of interest questionnaire indicated no potential conflicts of interest. They both completed the review. Their reviews and or responses follow under "Summary of Peer-Review Comments."

During the course of the formal peer-review process, Dr. Lynn Flowers of the U.S.EPA's National Center for Environmental Assessment (NCEA) requested a copy of the draft review document and agreed to coordinate comments from NCEA staff on the document. In addition to general comments on technical toxicological and risk assessment issues, NCEA provided comments on the compatibility of the methodology in the document with NCEA cancer risk assessment policy. Those comments and our responses follow under "USEPA-NCEA Comments on NJDEP's "Derivation of Ingestion-Based Soil Remediation Criteria for Cr+6 Based on the NTP Chronic Bioassay Data for Sodium Dichromate Dihydrate"

In addition, informal comments were also received from NTP (Drs. Michelle Hooth and Mathew Stout). These comments and our responses follow under "NTP Comments."

Comments that have been incorporated into the final document are bolded.

[1] Although Dr. Maddaloni is a U.S.EPA employee, he undertook this review as an independent reviewer and not as an official representative of the U.S.EPA.

Summary of Peer-Review Comments for:
Derivation of Soil Remediation Ingestion Criteria for Cr^{+6} Based on the NTP Chronic Bioassay Data for Sodium Dichromate Dihydrate

1. *Is the review document clearly written and is it presented in a logical and useful manner? Please make specific suggestions for improving its clarity and presentation.*

General – Yes. Generally well written, transparent and logical

Specific –
1. Include description and discussion of status of NTP data review at EPA (also in Exec. Summary) and more specifics on differences. Consider moving from Appendix B.
2. Provide exposure context in the Introduction (status of oral Cr^{+6} exposure in NJ).
3. More background on previous oral studies. Is there a potency estimate from Chinese data?

Response –The apparent conflict between the EPA OPPTS assessment and the NCEA/IRIS guidance for carcinogen risk assessment as well as the fact that the OPPTS assessment appears to be (at the current time) strictly an internal document makes it difficult to know to what extent the OPPTS assessment should be compared in-depth to our assessment**. However, we will investigate the current status of the OPPTS assessment and as appropriate, provide additional discussion and comparison to our assessment.**
We will provide exposure context and some additional discussion on the Chinese data as appropriate.

2. *Does the review document provide a sufficient and useful summary of the NTP study and its results (http://ntp.niehs.nih.gov/files/546_web_FINAL.pdf)?*

General – Generally

Specific -
Reviewer 1
1.. Should provide discussion of evidence of cytotoxicity or necrosis to support the suggestion of toxicity-related hyperplasia
2. Provide a table of dose-response for water intake and body weight changes.
Reviewer 2
1. There appears to be a discrepancy between the statement in the NTP report that all sections in all animals were examined for tumors and the statement in the NJDEP document that not all small intestine tissues were examined in all mice.
Response –
- The conclusion in the NTP report that the diffuse hyperplasias were consistent with regenerative epithelial cell growth secondary to tissue injury is based on the microscopic pathology of the cellular organization that has previously been observed to occur with

tissue injury rather than on direct observation of tissue injury. There is not a great deal more that can be added to our reporting of this observation. **However, we will add a brief description of the cellular organization as the rationale for this conclusion**.

- As noted in our reply to reviewers' responses to question 4 below, we will add a table showing the relationship between body weight and Cr^{+6} drinking water concentration. However, the table suggested by the reviewer would show a general association between decreased water consumption or decreased body weight and increased tumor incidence. As discussed in our assessment document, the animals did not show signs of clinical dehydration. Furthermore, increased tumor incidence was seen in the absence of significantly decreased body weight. Therefore, associations of tumor incidence with body weight or water consumption appear to be secondary to potability issues and/or systemic Cr^{+6} toxicity. In the absence of evidence for a causal relationship we believe that such a table would not be informative and would be confusing.
- With regard to the apparent discrepancy in the number of animals examined for potential tumors in all sections of the small intestine, this arises because NTP provided two different, but complementary types of data tables for neoplasms. One type are Tables 6 and 13 in the NTP final report, that gave summary incidence data for oral neoplasms in the rat and intestinal neoplasms in the mouse, respectively. These tables give the combined total of neoplasms detected on gross and microscopic analysis. The other type of data table is the pathology tables giving the results of the microscopic analysis for each animal. These are found at http://ntp.niehs.nih.gov/go/29141. These tables agree in the number of neoplasms identified (i.e., in the numerator of the incidence ratio), but disagree slightly in the count of the number of animals examined in each of the different sections of the mouse intestine (i.e., duodenum, jejunum and ileum). That is, in the denominator of the incidence ratio. We had originally based our calculation of the incidence using the data from the pathology tables. Since not all animals were examined microscopically for neoplasms in all sections of the small intestine, this lead to the notion that some animals were partially at risk. To address this, we derived an upper and lower estimate of incidence based on the value of the denominator of incidence at each dose. However, subsequent discussion with NTP revealed information that was not obvious from these tables. Animals that were not examined microscopically in all sections of the small intestine fell into that category for two reasons. The first category is that they had undergone autolysis (i.e., decomposition) between the time of unplanned death and recovery for pathology. In some cases, this precluded microscopic examination, but not gross examination for neoplasms. The second category is that sections taken for preparation of slides did not include the relevant section of small intestine due to alignment issues. NTP provided information that all animals were examined grossly by multiple pathologists (beyond standard NTP procedure) and that is was unlikely that any intestinal neoplasms were missed due to the lack of microscopic examination. Therefore, in response to our explicit question, NTP has advised that correct denominator of incidence for the mouse intestinal tumors should be the number given in Table 13 of the NTP final report. In general, this number is 50 animals (i.e., the starting number of animals in each dose group). The only exceptions to this are animals that died prior to the appearance of the first intestinal tumor on day 451. Note that this discussion applies only to the denominator of the incidence ratio (i.e., the number of animals at risk). For the numerator of the incidence ratio (i.e., the number of tumors detected), the summary

number given by NTP in Table 13 (whether detected grossly or microscopically) is used as reported and was never in question. **Based on the foregoing information, we are changing the calculation of incidence as presented in Table 2 of our original document. It is no longer necessary to consider animals as partly at risk and therefore, we are eliminating the estimate of upper and lower incidence. This will also change the calculations of cancer potency (Table 3 of the original document) slightly.**

3. *Based on the summary information provided, does the NTP chronic bioassay of sodium dichromate dihydrate provide an appropriate and valid basis for derivation of a cancer potency estimate and associated soil remediation criterion for sodium dichromate and more generally, for Cr^{+6}?*

Reviewer 1 - Yes. The NTP study is a modern, state-of-the-art study which documents clear evidence of oncogenic effects without major caveats.
Reviewer 2 -

1. The question regarding the NJDEP soil remediation criterion should be separated from the question regarding the estimate of the cancer potency, since the former is dependent on NJDEP protocols and policies. With respect to the soil remediation criterion, a comparative assessment of natural/anthropogenic background would be informative
2. The NTP study provides an adequate basis for deriving cancer potency.
3. There is some concern regarding the dosing regimen with respect to Cr^{+6} reducing capacity and how it relates to human exposures.

Response -

- We essentially agree with Reviewer 1.
- We agree with Reviewer 2 that if the basis for the cancer potency estimate is valid, the validity of the remediation criterion rests on NJDEP-specific policies and protocols.
- Reviewer 2 expands on this issue of the dosing regime in a subsequent response. We respond to this point below.

4. *Was the issue of possible dehydration and its potential impact on the assessment of carcinogenicity adequately addressed? In your opinion, could the decrease in water consumption at the highest doses be a factor in the occurrence of excess tumors?*

General - Both reviewers agree that the issue was generally adequately addressed. Reviewer 1 states that it is unlikely that the tumors are related to the palatability issue.

Specific –
Reviewer 1
1. The description on pg. 4 is confusing as it says both that there is a palatability effect and that there is evidence of an adverse (systemic) effect. The use of the term, “in part” in this context is inexact.
2. The question of whether decreased water consumption can cause the observed decrease in body weight should be specifically addressed.

3. The question of whether there is any evidence in general that decreased water consumption or tumors can promote carcinogenesis should be addressed.
4. The decrease in hematocrit at the highest dose in females (as opposed to the increase in hematocrit that would be consistent with dehydration) is not clear evidence of the lack of dehydration since the increase in hematocrit could be a treatment related effect that could mask concomitant dehydration.
5. The basis for the conclusion that smaller decreases in body weight at doses below the highest dose are due to palatability rather than a systemic effect is unclear.
6. The relevant NTP data should be presented to make this issue and its interpretation better understood.

Reviewer 2

1. While the reviewer is unaware of any direct effect of dehydration on tumor occurrence, the reviewer suggests the possibility that dehydration could lead to a decrease in gastric secretions resulting in a decrease in reduction capacity.

Response

- The wording, "in part" used to describe the contribution of decreased water consumption due to palatability issues appears in the NTP report. **We will change the text to attribute this statement to NTP.**
- The wording in the last paragraph on pg. 4 of our document is partly in error and also should be more informative. **We will insert a table showing all of the time-weighted average body weights and percent changes in body weights for rats and mice at all doses.**
- **We are unaware of any data that provide evidence that dehydration (or decreased water consumption) can potentiate tumors. We will state this explicitly.** With respect to the possibility raised by Reviewer 2 that reduced water consumption could have resulted in decreased production of gastric fluid that, in turn, resulted in a decreased reduction capacity, ultimately leading to an increased rate of tumors, we note the NTP's observation that water consumption (except in high-dose male mice) was constant on a body-weight basis through the first 20 weeks of dosing. This suggests that, like body weight, gastric fluid volume was proportional to drinking water intake. We also note that while female mice had 10 and 20% weight reduction at the second highest and highest doses, respectively, male mice had less than 2% weight reduction at the second highest dose. At that dose, however, tumor incidence was significantly increased. Furthermore, in the benchmark dose modeling that is the basis for the cancer potency slope estimate, the highest doses have less influence on the slope estimate than the second highest dose. This is particularly the case for the male mice that are the primary source for the slope estimate. This can be seen in the example in Fig. 5 of our document where the benchmark dose is almost identical to the second highest dose. Thus strongly suggests that whatever effect reduced fluid intake may have had on reduction capacity, it did not significantly influence the estimate of the cancer potency slope estimate.
- We agree in principle with Reviewer 1 that the significant decrease in hematocrit seen at the highest dose in male mice might be a systemic toxic effect of Cr^{+6} that could mask a dehydration-related tendency toward increased hematocrit. **We will note this. However, we will also note that no such change in henatocrit was noted at the second highest dose in male mice, nor in female mice at any dose. In any event the**

data from the highest dose in male mice, has a relatively small practical effect on our estimate of the cancer potency.

- Reviewer 1 raises a valid point with regard to the extent to which decreases in body weight can be attributed to palatability rather than a systemic effect on body weight per-se. NTP notes that when water consumption is adjusted on the basis of body weight, male and female rats and female mice drank approximately the same quantities of water per gram as the controls for the first 20 weeks of the study. High dose male mice, on the other hand, drank less water on a body weight basis than controls throughout the study. Thus, it seems likely that palatability was a major factor only for the high dose male mice. In the other groups where significant decreases in body weight did occur, body weight-adjusted water consumption appears to have remained relatively constant. This suggests that the decrease in body weight for those groups was due to a systemic toxic effect rather than decreased water consumption. **We will revise this section of the document to reflect this information.**
- **As stated previously, we will insert a table of body weight for each animal sex and water concentration.**

5. *Do you agree with the selection of the mouse as the key species for derivation of a human cancer potency estimate? If you disagree, please specify your reasons.*

General - Both reviewers agree with the selection of the mouse as the key species.

Specific –

Reviewer 1

There are more dose groups showing the carcinogenic response in the mice and the dose-response appears to be greater in mice than in rats. The difference in locations of tumors in mice and rats is interesting. It might be argued that the oral tumors in rats are more relevant to humans because the (relative) lack of reduction in the oral cavity could make the oral cavity more relevant for (environmentally relevant) low-dose exposures, whereas in the GI tract low dose exposure may be entirely reduced. However, the analysis in Appendix A demonstrates that Cr^{+6} can survive reduction in the GI tract. This helps mitigate the concern that the intestinal tumors may not be so relevant to humans.

Reviewer 2

It would be illuminating to see how the combined data from both sexes [in the mouse] influenced the slope factor derivation.

Response

- We agree with reviewer 1 that from a dose-response standpoint the mouse data show a stronger and more useful dose-response. We also agree that the evaluation of reduction in Appendix A indicates that significant amounts of Cr^{+6} escapes reduction in the GI tract regardless of the reduction capacity. As we discuss, this appears to be a function of kinetics (i.e., the kinetics of absorption versus reduction and the kinetics of gastric emptying versus reduction) rather than of chemical equilibrium.
- Reviewer 2 raises the issue of combining data across both mouse sexes in further comments. We address this issue below.

6. *Is the general approach in the review document for calculating the human cancer potency reasonable and (to the best of your knowledge) consistent with the 2005 USEPA Guidelines for Carcinogen Risk Assessment (http://oaspub.epa.gov/eims/eimscomm.getfile?p_download_id=439797)?*

General

Both reviewers agree that in general, the approach is consistent with the USEPA 2005 guidelines.

Specific –

Reviewer 2

1. The EPA Cancer Guidelines (2005) caution about using a maximum tolerated dose that causes significant (i.e., 10%) body weight loss or that results in pharmacokinetic changes that could influence cancer incidence.
2. Regarding the pharmacokinetic issue, the MTD for female mice was above the calculated Cr reducing capacity of the mouse GI tract [based on Table A-1 in our document] and the next highest for female mice and the MTD for the male mouse were uncomfortably close to the calculated reduction capacity.

Response

- The EPA 2005 Cancer Guidelines refers twice to body weight decreases (or decreases in weight gain) with respect to the maximum tolerated dose (MTD).

The first reference (pg 2-17) states that:

> "With regard to the appropriateness of the high dose, an adequate high dose would generally be one that produces some toxic effects without unduly affecting mortality from effects other than cancer or producing significant adverse effects on the nutrition and health of the test animals … If the test agent does not appear to cause any specific target organ toxicity or perturbation of physiological function, an adequate high dose can be specified in terms of a percentage reduction of body weight gain over the lifespan of the animals. The high dose would generally be considered inadequate if neither toxicity nor change in weight gain is observed. On the other hand, significant increases in mortality from effects other than cancer generally indicate that an adequate high dose has been exceeded. …Other signs of treatment-related toxicity associated with an excessive high dose may include (a) significant reduction of body weight gain (e.g., greater than 10%), (b) significant increases in abnormal behavioral and clinical signs, (c) significant changes in hematology or clinical chemistry, (d) saturation of absorption and detoxification mechanisms, or (e) marked changes in organ weight, morphology, and histopathology."

The second reference (pg. A-4) states that:

> "…the question often arises of whether a carcinogenic effect at the highest dose may be a consequence of cell killing with compensatory cell replication or of general physiological disruption rather than inherent carcinogenicity of the tested agent. …. If adequate data demonstrate that the effects are solely the result of excessive toxicity rather than carcinogenicity of the tested agent *per se*,

then the effects may be regarded as not appropriate to include in assessment of the potential for human carcinogenicity of the agent."

We agree that the highest dose in female mice significantly exceeded the guideline of 10% body weight loss (reduction in body weight gain) and that the second highest dose in female mice marginally exceeded this value (10.1%). However, NTP noted that none of the animals (including the high dose female mice) showed signs of clinical toxicity and all appeared in good health (including those enumerated in the Cancer Guidelines). Thus, it could be argued that the decrease in body weight at the highest dose in female mice was an isolated effect that did not have obvious implications directly or indirectly on the carcinogenic potential of Cr^{+6}. Nonetheless, the significant exceedance of the 10% guidance value for decrease in body weight and the possibility that significantly decreased body-weight reflects a systemic effect of Cr^{+6} argues for focusing on the male mice as the primary data set for derivation of the cancer potency. **We will note the issues related to the exceedance of the MTD as well as the implications of this for focusing primarily on the male mice. However, we will also investigate the relationship of the dose response with combined male-female data sets (and male-female with high-dose females excluded) to the male-only data set.**

7. *Do you agree with the selection of the control mouse time-weighted average body weight as the representative body weight value for calculation of the cancer potency estimate? If not, please suggest an alternate approach.*

General - In general, both reviewers agree that this approach is valid as applied to the body-weight scaling calculation in Table 3 of the peer-review draft (See addendum response of Reviewer 1).

Specific –

Reviewer 2

The approach of using a time-weighted average body weight in the scaling calculation is appropriate, but it creates a minor inconsistency given that the human body weight portion of that calculation does not represent a time-weighted average since an adult body weight of 70 kg is assumed.

Response

Based on discussions with USEPA, we confirmed that USEPA's informal policy is to us the default human body weight of 70 kg for the dose scaling from animals to humans. However, they recognize that this approach is somewhat inconsistent with the use of a time-weighted average animal body weight in the same calculation. Given the allometric scaling of body weight, the practical difference between the use of a value of 70 kg and 59 kg is only about 4%. **Thus, for consistency with USEPA methodology, we will use the USEPA default value of 70 kg. However, note that in column 11 of Table 4, we continue to use the value of 59 kg for the calculation of the daily mass of Cr^{+6} ingestion corresponding to a 1 x 10^{-6} risk. This is an NJDEP requirement.**

8. *Do you agree with the approach taken in the review document for estimating an upper and lower tumor incidence?*

General – As per the response to question 2, both reviewers point out the apparent discrepancy between the NTP statement that all animals were examined for tumors in all sections of the small intestine and the results of the microscopic examination of the individual animals as presented in the NTP Pathology Tables (http://ntp.niehs.nih.gov/index.cfm?objectid=5FE88732-F1F6-975E-70FA764DD21980C2).

Specific – Reviewer 1 suggests that given the small difference in outcome that results from using either the upper or lower estimate of incidence and suggests that the document would be more clear and concise if the more inclusive estimate of incidence were used and the alternative were simply referenced in a footnote.

Response

Given the clarification from NTP (discussed above), we have identified a single denominator for incidence ratio at each dose in each sex. This change is now reflected in Tables 3 and 4.

9. *Are the steps taken in calculating the cancer potency (summarized in Table 3) appropriate and correct?*

General – Both reviewers agree that the steps, themselves, are correctly laid out and organized.

Reviewer 1

Reviewer 1 raises that question of whether a local physico-chemical interaction, rather than a systemic rate-based process is controlling the risk in the case of the mouse small intestinal tumors. In the former case, allometric scaling (i.e., $(\text{body-weight})^{3/4}$ may not be appropriate, whereas, it would be appropriate in the latter case. The reviewer suggests that alternatively, allometric scaling could be justified on the basis that the processes of intestinal absorption and "lower transport" may be quicker in mice on the basis of body weight. The reviewer asks if there is any literature on this. Additionally, the reviewer asks if there is a pharmacokinetic model for rodents and humans and if so, what the implications of such a model are for scaling body weight.

Reviewer 2

With above caveat regarding upper and lower estimates of incidence, the steps appear appropriate and correct.

Response

We agree that a strictly local (i.e., point-of-contact) process would not be appropriately modeled cross-species based on allometric scaling. However, in agreement with the reviewer's "alternate" approach, we believe that there are several key processes governing the carcinogenicity of ingested Cr^{+6} in the gastrointestinal tract of rodents and humans that are best described as physiological processes that are appropriately modeled across species using allometric scaling. These include the reduction of Cr^{+6} in the

stomach and small intestine, gastric emptying, and intestinal emptying. In addition, depending on the specific mode(s) of action of Cr^{+6} ingestion carcinogenicity, this list could also include absorption from the lumen into cells of the small intestine and or stomach. **We will add a brief discussion about the physiological processes that are consistent with allometric scaling including gastric and intestinal emptying. We will also discuss these processes in the context of the kinetic considerations involved with the reduction capacity of the gastrointestinal tract.**
O'Flaherty (1996) and O'Flaherty et al. (2001) have published PBPK models of Cr^{+6} for rats and humans respectively. These models address absorption of Cr^{+6} from the gastrointestinal tract as a first order rate constant. However, these models are not specifically concerned with the local processes in the gastrointestinal tract and so, do not appear to model the individual processes listed above.

10. Do you agree with the use of benchmark dose modeling to determine the point-of-departure (POD)?

Reviewer 1
Yes. The data are sufficient to support the use of the benchmark dose approach and the BMR of 0.1 is appropriate.
Reviewer 2
The point-of-departure (POD) could have been extracted directly from the male mouse incidence data with the lowest concentration (85.7 mg/L) corresponding to a significant increase in tumors identified as the POD.

Response
Reviewer 2 is suggesting that the POD could have been identified as the LOAEL. If one were to follow such an approach, it would be more appropriate to choose the NOAEL (28.6 mg/L). The Cr^{+6} dose at this concentration is 0.91 mg/kg/day. This dose is comparable to the range of approximately 1-2 mg/kg/day for the BMDLs for the male mice. Thus, the reviewer is correct that, from the standpoint of quantitative risk assessment, the use of the NOAEL could have been justified. However, the validity of such an approach relative to the benchmark dose approach is only known after the NOAEL is compared to the BMDLs. Therefore, we believe that use of the benchmark dose approach is informative and is bolstered by reference to the NOAEL.

11. Please comment on the appropriateness of choosing a BMDL value from a single model as the basis for a POD. Is there more or less rationale for choosing a value based on a summary statistical approach (as described in the review document) for choosing the POD compared to choosing a BMDL value from a single model? If you favor choosing a BMDL value from a single model, should that value be based on the single best-fitting model, or should it be the based on the model that yields the lowest BMDL (given an acceptable fit)? The USEPA has an informal policy of preferentially using the BMDL from the multistage cancer model (one of the BMDS model choices) for deriving the POD in cancer potency calculations (providing that that model gives an adequate fit). In your opinion, should that model be given precedence in this analysis?

Reviewer 1
The calculation using several models was helpfully transparent in showing that the choice of model makes little difference and there is no mechanistic basis for choosing one over another. The presentation of the array and the rounding of the soil remediation value to 1-2 ppm is technically the most accurate approach.

Reviewer 2
Does not feel strongly, and ultimately the choice makes a very modest impact on the slope factor. That being said, the model that proves the best fit for the data should be employed.

Response
With revisions of the denominator of the incidence (i.e., number of animals at-risk) based on additional information from NTP, nearly all of the models for the male mice converge on a single value. Therefore, the choice of a model or models is no longer a salient issue.

12. Do you agree with the choice of the male mice for determination of the cancer potency and soil remediation criterion? If not, please provide a justification for choosing the female mice.

Reviewer 1
More discussion would be useful as to why NJDEP differs with (OPPTS) EPA given that EPA chose the female mouse. Also, consideration should be given combining the male and female mouse data to see if the combined data set yields a more robust and better fitting model. However, it would probably not make a difference and may not be worth the effort.

Reviewer 2
Generally agrees that the male mouse is more suitable given a smoother dose-response curve, lack of physiologic alteration at the MTD and lack of exceedance of the calculated reduction capacity at the MTD (as opposed to females). Still it would be interesting to look at the combined male and female data.

Response
We have examined approaches to combining the male and female data sets and comparing the results to those obtained the male and female data analyzed separately. The results of these additional analyses are presented in Tables 4c and 4d and discussed in the related text.

13. Please comment on the Weight of Evidence for Characterization of Ingestion Carcinogenicity to Humans. Taking into account the discussion in Appendix A of the document, do you agree with the statement in the document that "The mechanism(s) of Cr^{+6} carcinogenicity responsible for the observed tumors in the mouse small intestine are likely to be relevant to the potential for carcinogenicity in the human gastrointestinal system."? Do you agree with characterization (as per the USEPA

2005 Guidelines for Carcinogen Risk Assessment) of oral exposure to Cr^{+6} as "likely to be carcinogenic to humans?"

Reviewer 1

The multiple lines of evidence detailed in Appendix A are sufficient to conclude that that it is unlikely that some gastrointestinal threshold phenomenon controls the availability of $Cr^{+6}$to gastrointestinal tract tissues. It now appears that Cr^{+6} is stable enough to penetrate beyond the stomach to lower gastrointestinal targets as well as systemic sites. The rapid uptake of Cr^{+6} would likely also occur at low environmental doses in humans. I agree with the 2005 cancer classification as "likely."

Reviewer 2

The weight of evidence of ingestion carcinogenicity to humans from the NTP study is indeed strong. However, the shape of the dose-response curve as it relates to environmental exposures likely to be encountered by humans is arguably the weakest link in this document – as evidenced by the lack of positive human epidemiological data except at extremely high (>20 mg/L) drinking water concentrations (Zhang and Li, 1996). The NTP study suggests that as the Cr^{+6} reducing capacity of the mouse GI traact is approached or exceeded, carcinogenic response increases. This is to be expected as the equilibrium between Cr^{+6} and Cr^{+3} will favor Cr^{+6} as the amount of Cr^{+6} in the GI tract increases. So I agree with the statement "The mechanism(s) of Cr^{+6} carcinogenicity responsible for the observed tumors in the mouse small intestine are likely to be relevant to the potential for carcinogenicity in the human gastrointestinal system." However, I question the magnitude to which this mechanism will be operable in a human exposure environment that strongly favors the reduced form of chromium in the GI tract. It is interesting that the rat did not experience intestinal tumors. Appendix A might have benefited from an analysis of the rat GI reducing capacity relative to the dose the animals received.

Response

- We agree with the response of Reviewer 1
- We agree with Reviewer 2's endorsement of the relevance of the mechanism of Cr^{+6} to humans.
- We disagree with Reviewer 2 that the lack of observed of epidemiological evidence of Cr^{+6} ingestion carcinogenicity is evidence of the potential lack relevance of the shape of the dose response curve to human exposure. We are aware of only the single investigation of the Chinese population by Zhang and Li (and its several iterations and contentious versions) that relates to a population with a specific known ingestion exposure. All other epidemiological studies of non-respiratory cancer from Cr^{+6} relate to occupational cohorts where the primary route of exposure was inhalation. In such studies the extent of ingestion exposure was unknown and unquantifiable. Thus the lack of evidence of low dose human ingestion carcinogenicity is due to lack of study rather than rather than lack of effect. "Absence of evidence is not evidence of absence."
- We agree with Reviewer 2 that as the fraction of estimated reducing capacity that is consumed by ingested Cr^{+6} in the NTP study increases, the carcinogenic response increases. However, we do not agree that this necessarily indicates that the increase in

the carcinogenic response is caused by the decrease in residual reduction capacity. The extent to which the carcinogenic response increases is primarily a function of the dose. If the amount of Cr^{+6} that reached the target tissues increased as a function of dose despite the fact that sufficient residual reduction capacity remained, then the consumption of a portion of the reduction capacity would not be the factor determining the carcinogenic response. Based on our assessment of the data from several independent perspectives, we believe that, in fact, adequate reduction capacity remains in the mouse stomach, except possibly at highest dose in female mice. It is important to understand that the determination of whether the reduction capacity of the stomach is exceeded is not, *per se*, the critical factor in determining the relevance of the mouse data to human exposure. Rather, the critical factor is the balance between the rate of reduction in the stomach and the rate of transport of the Cr^{+6} from the stomach into the small intestine. The calculation we presented in Appendix A is a logically reductive scenario. That is, for the sake of simplicity, we compared the hourly rate of Cr^{+6} in the stomach to the hourly input of Cr^{+6} assuming that the hourly dose of Cr^{+6} remains in the stomach for the entire hour. Such a static situation is not realistic because during that period, the stomach is continually discharging its contents to the small intestine. As quoted in our document, O'Flaherty et al. (2001) make a similar point with respect to absorption of Cr^{+6} from the stomach: "The greater absorption of Cr^{+6} than Cr^{+3}does not imply that the reduction capacity of gastric juice was exceeded, but rather that absorption from the gastrointestinal tract is so rapid that it is able to compete effectively with reduction in the stomach." **Thus, a more realistic calculation would have be to compare the hourly reduction capacity to the mass of available Cr^{+6} integrated over that time period given the half-life of the mouse gastric emptying time. Unfortunately, it does not appear that sufficient data are available to carry out such a calculation. We will more thoroughly explain that the issue of reduction capacity is a kinetic rather than an equilibrium consideration and we will expand the calculations in Table A-1 to include such kinetic considerations.**

- We dealt with the question of whether the lack of gastrointestinal tumors in the rat implies a greater reduction capacity in the rat compared with the mouse in Appendix A. We noted that, based on data presented by NTP (urine Cr concentration vs. drinking water Cr^{+6} concentration), the rate of Cr^{+6} uptake from the GI tract in rats was more than three times that in mice. Thus, while it is not clear why the mice developed GI tract tumors and the rats did not, reduction capacity does not appear to be the critical factor.

14. Please comment on the statement in the document that there is insufficient evidence to support the conclusion of a mutagenic mode of action as described in the USEPA Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens. EPA/630/R-03/003F (http://oaspub.epa.gov/eims/eimscomm.getfile?p_download_id=459042).

Reviewer 1

The document does not describe the strengths and weaknesses of the gene-tox data for Cr^{+6} (e.g., is it a mutagen, clastogen, does it form DNA adducts, in what tissues?). The document refers to hyperplastic changes in GI tissue as indicative of tissue injury but the document does not describe any evidence showing necrotic and cytotoxic changes in

these tissues. Why is so much emphasis put on this possibility to the exclusion of other mechanisms that can induce hyperplasia (e.g., mitogenesis, mutation leading to proliferative clones). Has the inhalation cancer evidence pointed to an MOA that might be relevant for GI tissue? It appears that the possibility of a mutagenic MOA has been dismissed too readily. A careful weight of evidence determination is called for because it will affect how children's cancer risk is addressed.

Reviewer 2

This is a debatable point. The EPA's genetic activity profile (GAP) analysis makes an arguably compelling case for a mutagenic mode of action.

Response

- With respect to the specific evidence for necrotic changes in the mouse intestinal tissue, NTP makes specific note of the presence of diffuse hyperplasia in the mouse duodenum that was present at all doses. This indicates that the hyperplasia was not a secondary, high dose effect. NTP states that, "Compared to controls, the duodenal villi of exposed mice were short, broad blunt and lined by densely packed, tall columnar epithelial cells that were more basophilic than the shorter epithelial cells lining the duodenum villi of the controls. The epithelial cells and cell nuclei were often piled up in multiple layers along the long axis of the villi. Intestinal crypts were often elongated and generally appeared to contain increased number of epithelial cells with increased numbers of mitotic figures. Collectively, these lesions are considered consistent with regenerative hyperplasia secondary to previous epithelial cell injury." **We have now provided this information in the document**.
- We agree that there is clear evidence that Cr^{+6} has a mutagenic potential, and that Cr^{+6} can act as a mutagen, both *in vitro* and *in vivo*. Among the factors to consider in determining the mode of action, the Supplemental Guidance to the 2005 USEPA's carcinogens guidance document specifically lists "presence of proliferative lesions, for example, hepatic foci, or hyperplasia." The relevant question here is not specifically about mutagenic potential, but rather about the specific criteria for assuming a mutaginc mode of action (MOA). That is, to some extent, a policy decision as well as a scientific decision. As the context for assuming a mutagenic MOA stems from the USEPA's 2005 guidance documents, the specific criteria that should be applied in making such a determination should also be made within the context of the USEPA's guidance. To date, those criteria have not been formalized.

15. Please comment on the Characterization of Uncertainty. Does this discussion adequately address the significant uncertainties in this assessment?

Reviewer 1

The discussion is coherent and generally adequate. Points raised in previous comments (appropriateness of allometric scaling, mutagenic MOA) should be added. Also, despite the discussion in Appendix A, an ongoing uncertainty will be the relative ability of the human stomach to reduce Cr^{+6} prior to transit to the intestine and uptake into tissues. The text could be expanded to indicate why this is less of an uncertainty given the discussion in Appendix A. The human Cr^{+3}/Cr^{+6} absorption study of Kerger et al. (1996) should be more fully described. If the evidence [presumably from the discussion in Appendix A] is

used to support a presumption of systemic absorption, it should be noted that NTP found no evidence of systemic tumors although the Cr^{+6}/UV-light study did suggest a systemic tumor promoting effect. The extent to which systemic targets may be at risk may still be an uncertainty.

Reviewer 2
The analysis of uncertainty thoroughly addresses the potentially significant source of uncertainty regarding the reducing capacity in the NTP study and its relevance to human environmental exposure. The direction and magnitude of the uncertainty associated with the exposure assumptions used to derive the soil remediation criterion relative to those inherent in the toxicity assessment would be informative and within the purview of a thorough uncertainty analysis. Bioavailability issues and non-ingestion routes of exposure and their contribution to overall cancer risk could be included in this discussion.

Response
- The question of the appropriateness of allometric scaling relates specifically to the calculations in Appendix A. Since those calculations figure only indirectly in the main text of the assessment, we believe that, for the sake of clarity, the discussion of allometric scaling is best conducted in Appendix A In addition, we have added an additional discussion in Appendix A regarding the kinetics of gastric emptying compared to the rate of gastric reduction. We believe that consideration of the kinetics of gastric emptying make the choice of allometric scaling less of a critical uncertianty.
- **We will include the residual uncertainty regarding the reduction ability of the human stomach relative to the mouse stomach in the context of the discussion in Appnedix A.**
- **We will provide more detail about the relative absorption of Cr^{+3}/Cr^{+6} from Kerger et al. (1996) .**
- We do not intend to make a specific case for systemic (i.e., non-GI) cancer risk from Cr^{+6} ingestion. **However, we will include this as an uncertainty.**
- We agree with Reviewer 2 that, in general, the uncertainties associated with exposure issues are as much a part of the overall uncertainty as are those associated with toxicity. However, from the standpoint of site remediation, exposure assumptions are dictated by NJDEP policy. Thus, the purpose of including the exposure aspects in the document is largely to provide an indication of the practical significance of the toxicity assessment. The uncertainties associated with the exposure assumptions (including Cr^{+6} bioavailability) are, indeed, generic and, as such, outside the realm of this document.

16. Appendix A - Does the discussion in Appendix A adequately address concerns that the doses in the NTP study may have exceeded the Cr^{+6} reduction capacity of the mouse gastrointestinal tract in a manner that is not relevant to human environmental exposure?

Reviewer 1
Yes.

Reviewer 2

Appendix A provides a detailed analysis of chromium reducing capacity in the NTP study and its relevance to human environmental exposure, however reducing capacity may not be the only kinetic factor that ultimately impacts toxicodynamics. The tissue concentration data indicate that Cr^{+6} is being absorbed across the range of doses employed in the study. These data support the position that tumors are not solely associated with exceeding Cr^{+6} reducing capacity. It is apparent from Figures A-1e and A-1f of Appendix A that the plasma and erythrocytes chromium concentration is sublinear indicating saturation of the Cr^{+6} systemic uptake mechanisms. This phenomenon is consistent with the postulated uptake mechanism of facilitated diffusion via anion channels. Conversely, Figures A-1c and A-1d demonstrate that at higher Cr^{+6} dosing concentrations the concentration of chromium in non glandular and glandular stomach tissues follows a supralinear relationship. These apparent dichotomous kinetic trends between chromium concentration in blood (plasma and erythrocytes) and stomach tissues could be explained by the addition of direct uptake of Cr^{+6} into the tissue lining the gastric lumen. It would be instructive to see if the same trend (i.e., supralinear) held for tissues (e.g., duodenum) lining other sections of the mouse gastrointestinal tract. The relevance to human environmental exposures is such that in a scenario where both reducing capacity and uptake are far from saturated, the Cr^{+6} ion concentration and kinetic profile in the human intestinal lumen might add further uncertainty to the low-dose extrapolation of cancer potency.

Response

While we appreciate the incisiveness of Reviewer 2's analysis, we believe that it is important to point out the large uncertainty (error bars), particularly at the highest doses in each of the plots in Figure 1. This makes conclusions about the shape of the dos-tissue concentration curve at high doses, and therefore, conclusions about saturation kinetics of transport, likewise, highly uncertain. Reviewer 2 may well be correct that, at least in the GI tract, both the reducing capacity and the absorptive capacity are unsaturated adding additional uncertainty to low-dose extrapolation. However, we do not believe that the available data permit a more detailed assessment of this point.

17. Please provide any additional comments that you believe will improve the review document.

Reviewer 1

None

Reviewer 2

Overall, a well-reasoned approach with a level of uncertainty that is within the realm typically encountered when developing toxicity factors for environmental contaminants. Additional comments are provided below:

P.2 The use of the term "control mice" in the Davidson wt al. (2004) study seems misplaced as these mice were treated with either potassium chromate or UV light.

P.5 (Paragraph 1, line 12) Omit 'in"

P.15 (Line 5) Replace "model" with "models"

P.17 (Paragraph 3, line 4) The sentence beginning "It might,.." is repeated.

P.22 (Paragraph 1, line 16) There is some tortured wording in the sentence that begins "The absence…." Additionally, it would seem that the absence of a statistically significant increase in tissue accumulation would support (rather than not support as the sentence indicates) the hypothesis that the threshold for reductive capacity was not exceeded at the doses used in the NTP study.

P.23 The Cr+6 reduction rate for humans (10mg/hr) is a maximum value based on mealtimes. A weighted value that also accounts for fasted states should be considered for use in scaling to mice.

Response

Given both the rate of gastric emptying and the rate of the Cr^{+6} reduction reaction in the stomach, it does not appear that the fasting period in either humans or mice are relevant to the potential for Cr^{+6} reduction.

USEPA-NCEA Comments on NJDEP's *"Derivation of Ingestion-Based Soil Remediation Criteria for Cr+6 Based on the NTP Chronic Bioassay Data for Sodium Dichromate Dihydrate"*

February, 2009

Thank you for the opportunity to review the assessment. We are providing general comments, and comments specific to the mode of action analysis and cancer quantification. Finally, a few editorial comments are included for your consideration. Please call either Lynn Flowers (703-347-8537) or Ted Berner (703-347-8583) if you have any questions.

General Comments

In general, the document was clearly written, understandable, and well organized, and it was, for the most part, consistent with EPA's risk assessment methodologies.

1. *The body weight decrement observed in the female mice at the end of the NTP study is quite large (i.e., 20%) relative to the body weight decreases seen in the male mice (8.2%) and the male (7.5%) and female (8.0%) rats. Although some discussion of this issue is currently included in the document, further investigation of this issue may be warranted.*

Response
As noted in our responses to the peer-review comments, we recognize that it is likely that the 20% decrease in body weight in the high-dose female mice resulted from a systemic toxic effect rather than from palatability issues. We also recognize that this is an indication that this group exceeded the MTD. There is no direct indication that this effect contributed to the tumor incidence, and no such issues arise with respect to the male mice. There does not appear to be any basis to further directly investigate the implications of this effect on the estimation of the cancer potency. **However, as noted in our responses to the peer-review comments, we have investigated computational approaches that combine the male and female incidence data with and without the dose corresponding to the 20% decrease in body weight.**

2. *In the NTP study, the neoplastic response in rats occurred in the oral cavity, while in mice, the neoplastic response occurred in the GI tract. It would seem that further discussion of this lack of site concordance might be useful given that the route of exposure in these two species was identical.*

We acknowledge this lack of concordance. There does not appear to be an obvious explanation for this, but as discussed in the document, it does not appear that this results from a greater reduction capacity in the rat GI tract compared to the mouse. We note the lack of concordance in our section on the Characterization of Uncertainty. We note that this may arise from differences in the actual MOA in each species given the observed

hyperplasia in the mouse GI tract and the lack of hyperplasia in the rat oral cavity. It is not clear what else can be said to clarify the lack of concordance. We do note, however, that the EPA's 2005 Guidance for Carcinogen Risk Assessment explicitly states that: "Target organ concordance is not a prerequisite for evaluating the implications of animal study results for humans. Target organs of carcinogenesis for agents that cause cancer in both animals and humans are most often concordant at one or more sites (Tomatis et al., 1989; Huff, 1994). However, concordance by site is not uniform. The mechanisms of control of cell growth and differentiation are concordant among species, but there are marked differences among species in the way control is managed in various tissues… Thus, an animal response may be due to changes in a control that are relevant to humans but appear in animals in a different way." **We will, however, briefly note the lack of concordance along with a reference to the foregoing information from the 2005 Guidelines.**

3. As noted in Appendix B, EPA's Office of Prevention, Pesticides and Toxic Substances (OPPTS), in conjunction with a re-registration eligibility decision (RED) on copper-chromate-arsenical (CCA) pesticides, concluded that Cr+6 is mutagenic, while NJDEP stated that, despite evidence for the mutagenic potential of Cr+6, they could not conclusively determine that Cr+6 operates via a mutagenic mode of action (MOA). Without addressing which conclusion has greater support, it really makes no difference for dose-response modeling purposes, as linear extrapolation is deemed appropriate in both cases.

Response
We agree that for the purposes of determining the method of extrapolation, the determination of whether a mutagenic MOA is operative is a moot point since the 2005 Guidance defaults to linear-from-POD approach in either case. However, the determination of the MOA has practical import in determining whether an age-dependent adjustment factor (ADAF) should be applied.

4. NJDEP determined a range of cancer slope factors (0.41-0.51 per mg/kg/day) by drawing a line from a POD that was estimated from modeling the intestinal tumors in mice treated with sodium dichromate. There is a slight discrepancy in the reporting of these CSFs between the Executive Summary and the rest of the document. In the Executive Summary, a range of CSFs from 0.41-0.51 per mg/kg/day is reported. In Table 3, a range of CSFs for males (0.3-0.7 per mg/kg/day) and females (0.26-1.16 per mg/kg/day) are reported. There is also mention of the mean weighted CSF of 0.41 per mg/kg/day on page 15 (paragraph 2), but the origin of the CSF of 0.51 per mg/kg/day in the Executive Summary could not be found in any other place in the document.

Response
Table 3 (now tables 4a-d) is definitive. The Executvie Summary is clearly in error. **We will correct the range reported in the Executive Summary.**

Comments on cancer quantification:

5. In converting the sodium dichromate doses reported by the NTP to Cr^{+6} doses, NJDEP employed a conversion factor of 0.35 as the fraction of the sodium dichromate molecular weight contributed by chromium. In actuality, this fraction is 0.40. Using the correct fraction yields Cr+6 doses that are about 13 percent higher than the doses employed by NJDEP and presented in Table 1. Employing the corrected Cr+6 doses in the fitting of the multistage model, with the two-stage model exhibiting the best fit, resulted in CSFs that were about 10 percent lower than those presented in Table 3 of the document. It is suggested that the Cr+6 doses presented in Table 1 be corrected, and the modeling redone using these corrected doses.

Response

Our calculation of the Cr^{+6} water concentration and doses was based on the mass of Cr as a percentage of sodium dichromate *dihydrate*. The dihydrate form of the chemical was the specific form used by NTP. The molecular weight of the dihydrate form of the chemical is 290 AMU. The molecular weight of the anhydrous form of the chemical is 262 AMU. Cr^{+6} is 40% of the mass of the anhydrous form, but 35% of the dihydrate form. We believe that the conversion we employed, based on the dihydrate form is correct. **We will, however, change Table 1 to specifically indicate that the chemical in question is sodium dichromate dihydrate.**

6. NJDEP fit all of the dichotomous dose-response models available in the EPA's Benchmark Dose Software (BMDS) to the mouse tumor incidence data. For cancer endpoints, IRIS typically uses only the multistage model to estimate CSFs. However, the impact of fitting all of the dichotomous models versus only the multistage model on the estimated CSF is negligible. The NJDEP CSF estimate was characterized to be in the range of 0.41 to 0.51 $(mg/kg\text{-}day)^{-1}$ based on the tumor incidence in male mice, while the estimated CSF using the same data, but based on the multistage model alone, is in the range of 0.45 to 0.46 $(mg/kg\text{-}day)^{-1}$, which falls right in the middle of the range identified by NJDEP. If the goal of NJDEP's analysis is to be consistent with IRIS practice, then the multistage model should be used.

Response

We appreciate the fact that the use of EPA's preferred benchmark dose model to the exclusion of the other possible models will make little difference in either the CSF or the soil remediation value. However, we believe that, given the lack of an objective biological basis for model selection, it is good risk assessment practice to examine and present the outcome with all of the available models. Furthermore we committed (elsewhere in response to reviewers' comments) to analyze the dose-response using the combined male and female mouse data. The outcome of that analysis may mot be the same with respect to the representativeness of the multistage model.

7. On page 7 of the document, NJDEP is commended for evaluating the impact of the choice of animal body weight on the estimate of the CSF. On page 8, NJDEP

is also commended for calculating CSFs under two different incidence scenarios (i.e., upper and lower incidence estimates); although these two incidence scenarios tuned out to be not that different.

Response
Thank you.

8. On page 10, in the section of the document entitled, "Results of POD Calculations," the NJDEP notes that for female mice, none of the fitted models yield a "strong fit" to the data. In fact, all of the fitted models using the female mouse data, including the multistage model, exhibited statistically significant ($p < 0.1$) lack of fit. Because of this fact, it might not be prudent to use, or even report, any of the modeling results for female mice. One option NJDEP may want to consider is to drop the highest dose in the females and refit the models.

Response
We agree that the female mouse data, by themselves, are not sufficiently robust to support the derivation of a cancer potency. We presented them in order to demonstrate this. We have committed, elsewhere in response to reviewers' comments, however, to examine the benchmark dose modeling for the combined male and female data sets (with and without the highest dose for female mice**). We have compared the robustness of the model fits for the combined data sets against those for the male-only models. See Tables 4c and 4d and related text.**

9. At the top of page 17, it is not relevant to compare the BMDL estimates from the male versus female mouse data, and then use this comparability to conclude that the resulting CSF estimates are robust and reliable, especially given that all of the dose-response models fit to the female mouse data exhibited statistically significant ($p < 0.1$) lack of fit.

Response
We agree. **We have removed the statement about the robustness of the POD based on similarities between the values for the male and female mice while continuing to note the similarities. We have, however, also included a discussion of the results of the benchmark dose modeling of the combined male-female data sets here.**

10. In the section of the document entitled, "Characterization of uncertainty," which begins on page 17, NJDEP may want to consider including a summary of the uncertainties associated with the choice of animal body weight and the use of high versus low incidence estimates previously discussed on pages 7 and 8.

Response
As detailed in the sensitivity analysis in the original document, the maximum difference in the human cancer potency estimate that would derive from use of the high dose animals (lowest body weight) as opposed to the control mice (the value used in the

document) would be 6% for female mice and less than 5% for male mice. Given the results of this sensitivity analysis, we do not consider the choice of the summary body weight for the allometric scaling of potency from animals to humans to be a sifficiently significant uncertainty to warrant discussion in the Uncertainty section of the document.

11. In Appendix B on page 29, it is probably worth pointing out that another difference in the approach that OPPTS used versus the one NJDEP employed was that OPPTS chose a mouse body weight of 30 grams, while NJDEP used a mouse body weight of 50 grams. Also in Appendix B, NJDEP states that "… this document used the current approach recommended in the USEPA Cancer Guidelines (USEPA, 2005a) that calls for the slope to be calculated from a straight line extending from the point-of-departure (POD) to the point corresponding to zero incremental dose-zero incremental response." However, in making this statement, NJDEP failed to make clear that the Cancer Guidelines also state that in drawing a straight line from the POD to the origin, the line needs to be corrected for any background response.

Response

We will note the differences in body weight assumptions between our assessment and that of EPA-OPPTS. We will also add the language concerning the correction of the linear slope from POD for background response.

12. As indicated in the document, there may be some uncertainty in using the CSF derived from mice intestinal tumors for humans based on possible differences in Cr+6 toxicokinetics between two species. There is evidence that Cr+6 is reduced to Cr+3 largely under acidic conditions in the human stomach compared to rodent stomach conditions. Also, there is evidence that Cr+6 is well-absorbed compared to Cr+3 and it is unstable. After absorption, Cr+6 is converted to Cr+3 relatively fast in liver, thereby decreasing the availability of Cr+6 in blood and systemic tissues. Better understanding of the toxicokinetic differences between the species (reduction constants, absorption differences) is important. There is a lengthy discussion on these aspects in Appendix A. Perhaps, the data needed to understand the toxicokinetic differences from PBPK modeling between species are not adequate and, as such, this is a research issue that could be pointed out in the document. There are no methods established that would allow for reporting the chromium in tissues according to the oxidation state. The available data in the literature report the chromium in tissues as total chromium with no differentiation between Cr+3 and Cr+6.

Response

We agree that a better understanding of inter-species toxicokinetic differences with respect to Cr uptake, transport and metabolism would be useful. We have addressed these concerns, albeit indirectly, in Appendix A. We believe that the lack of more direct information on these differences, however, is not an impediments to the derivation of a reasonable and applicable estimate the cancer potency from the NTP data within the context of current practice in derivation of cancer potency estimates.

Comments on mode of action discussion:

1. The mode of action conclusions seem to rely on the interpretation of the Cancer GLs as requiring "unambiguous" evidence of a mode of action (see pg 6, 1st para under "General Approach...."). Instead, as is the case for Cr+6, it would be reasonable to assume that a mutagenic MOA is operative, based on a fairly extensive database of in vitro, in vivo and human evidence of genotoxicity as well as carcinogenicity (including in occupationally exposed humans). There is significant evidence for mutagenicity in a variety of assay systems including gene mutations (reverse mutations, base pair substitutions and frameshift mutations) in various strains of *S. typhimurium* and *E. coli*, and DNA-protein cross links, DNA adducts, chromosomal aberrations, DNA strand breaks and sister chromatid exchanges in mammalian cell lines (animal and human). In vivo studies have reported micronucleus formation, DNA protein cross links, DNA alterations in rats and mice and in various organs including lung, liver, kidney etc. In addition, some but not all occupational studies have reported higher levels of chromosomal aberrations or sister chromatid exchanges in workers exposed to Cr+6. All the above evidence indicates a mutagenic MOA for Cr+6.

Response

As we have stated in response to other comments, we agree that Cr^{+6} can act as a mutgen. At the same time we also note the presence of diffuse hyperplasia indicative of necrosis and regeneration. This is suggestive of the possibility of a non-mutagenic MOA. It is currently unclear what the criterion are and should be for assuming that a mutagenic MOA is operative. Therefore, we have not drawn any firm conclusions as to the MOA.

2. The document's section on weight of evidence (p 16) does note the existence of "considerable data indicating the ability of Cr+6 to react directly and indirectly with DNA". A few studies as such are cited; however, the database actually includes dozens of papers (a PubMed search of "Cr+6 genotoxicity" retrieved >50 articles) that could inform the issue. There are two options for addressing this deficit. The first is to review the available studies, at least in summary fashion, and provide a tabular presentation of the primary data on genotoxicity. Alternatively, rather than reviewing the primary literature in detail, the NJDEP document could reference a few of the recent reviews where such data are reviewed and summarized: Salnikow and Zhitkovich, Chemical Res and Toxicol 21(1) 28-44, 2008; Sedman et al, J Environ Sci and Health Part C 24: 1, 155-182, 2006; O'Brien et al, Mut Res 533: 3-36, 2003; and IARC 1997. In brief, these articles describe effects such as DNA adducts, cross links, strand breaks, mutation, DNA base damage, genomic instability, etc, all of which are supportive of a determination that a mutagenic MOA is operative. Finally, there is a GAP profile (attached) and an ATSDR Toxicological Profile (also attached) that includes a summary of the available mutagenicity and genotoxicity studies on Cr+6 that was recently released for public comment.

Response

As per our previous response, the issue regarding the MOA has less to do with documenting the studies that establish that Cr^{+6} can be a mutagen and more to do with the criteria for assuming a mutagenic MOA for the purposes of risk assessment. We have cited several fairly complete review articles that clearly make the case that Cr^{+6} *can* act as a mutagen. In the absence of making a case that a mutagenic MOA should be assumed, we do not believe that it is necessary to fully document the supporting evidence.

3. Page 16, the weight of evidence for a carcinogenic mode of action section *states "….no evidence to suggest that the mouse intestinal tumor resulted from MOA that is specific only to tissue disrupting effects at high doses with a clear threshold for tumor induction…." "…..also, because of considerable uncertainty regarding the specific MOA by which Cr caused either the mouse intestinal tumors or the rat oral mucosal tumors, there is insufficient evidence to support the conclusion of a mutagenic mode of action…."* Cr+6 has been found to be mutagenic in vivo in the lung, liver, brain and bone marrow of rats and/or mice and enhances DNA damage and micronucleus induction in humans. Although there are no studies specifically in the target tissue (mouse intestine or rat oral mucosa), there is significant evidence supporting the mutagenic MOA in other organs. Furthermore, since Cr+6 produces tumors in different species, genders and is a multisite carcinogen, a mutagenic MOA for the tumors in rats and mice is plausible because there is convincing evidence that Cr+6 exposure, via ingestion or inhalation, can have systemic effects that are distant from the site of exposure. Furthermore, there is no convincing data for an alternative MOA. Considering all the above information and available genotoxicity data, a more thorough MOA analysis should be conducted before concluding lack of mutagenicity for Cr+6.

   It would be appropriate to point out in the text that Cr+6 does not act like a "classic" mutagenic carcinogen, such as benzo[a]pyrene. For example, the time to tumor is very long (450 days). Generally, there is agreement that the reductive intracellular metabolism of Cr+6 to Cr+3 is what contributes to DNA damage and mutagenicity, and that the mutagenic and tumorigenic responses only occur when the reductive capacity of cells is exhausted. Along these lines, De Flora and his coworkers have argued the point that this evidence leads one to envision a threshold response even though they have conceded that Cr+6 is mutagenic in vivo if it can reach remote sites and be reduced to Cr+6.

Response

With respect to the first part of this comment, we have explained our position regarding the possibility of a mutagenic MOA in response to other reviewer comments. With respect to the second part of this comment, we do not agree with this interpretation. While it may well be correct that the mutagenic activity of Cr^{+6} is secondary to the reduction of Cr^{+6} to Cr^{+3}, multiple lines of evidence presented in Appendix A of our document indicates that the tumors in the mouse small intestine were produced without exhausting the reduction capacity of the gastrointestinal tract. Rather, the ability of Cr^{+6}

to reach the intestinal cells is dependent on the kinetics of gastric emptying rather than the exceedance of the gastric reduction capacity and this reduction likely occurs intracellularly in the intestinal cells rather than in the gut lumen.

Specific Editorial Suggestions

1. **Throughout the document, NJDEP refers to "… the ingestion carcinogenicity of hexavalent chromium …" where the more correct terminology would be "… the carcinogenicity of hexavalent chromium via ingestion …"**

2. **In several places in the document, NJDEP refers to a "cancer potency factor" where the currently accepted EPA terminology is "cancer slope factor."**

3. **In the discussion of the Borneff (1968) study on page 2, a suggestion is made to include the chemical formula for potassium chromate – K_2CrO_4.**

4. **In discussing the NTP study design on page 3, it would be useful to discuss why the female mice were exposed to sodium dichromate dihydrate concentrations that were, for the most part, two-fold higher than the concentrations to which the male mice were exposed.**

Response
We had already stated that the selection was based on a an earlier NTP sucbronic study. **We have now added that the selection was based on an estimate of the MTD from that study**

5. **In Table 1 on page 4, for clarity and consistency, a suggestion is made to change the heading of the second and sixth columns of the table to read "Cr^{+6} water conc. (mg/L)," rather than "chromium water conc. (mg/L)".**

6. **Also in Table 1, a footnote should be added to the third and seventh columns of the table to indicate that the sodium dichromate dose values (in mg/kg-day) were reported by the NTP.**

7. **In the graphs presented in Figures 1 through 4 (page 33), NJDEP noted that the tumor incidences of the rats and mice were not plotted on the same scale. In addition, the doses were not plotted on the same scale. For ease of comparison, a suggestion is made to redo these plots, so that both incidences and doses are plotted on the same scale across all four graphs.**

8. **It is odd that the tables are embedded in the text of the main document, while Figures 1 through 4 follow Appendix B. A suggestion is made to move Figures 1 through 4 into the body of the document.**

Response
This is a minor point, but we believe that given the number and size of the figures, this would produce too much of a disruption to the flow of the text.

9. **On page 8, in reference to Table 2, NJDEP notes that "… the estimated number of animals at risk between the two approaches is relatively small resulting in a maximum difference of 8% in the incidence ratio." However, the maximum difference in the incidences in Table 2 is about 2%. This discrepancy should be addressed.**

Response
Given the additional information obtained from NTP (see above) the upper and lower approach to the incidence estimation has been eliminated. DONE

10. **Table 3 (page 12) is quite dense and difficult to read. A suggestion is made to reformat this table, and possibly consider breaking it up into multiple tables. Of course, as mentioned above under "General Comments," EPA would typically only fit the multistage model to tumor incidence data, and thus presenting the output from all of the other dichotomous models in the table would not normally be an issue. Also, for the multistage model, the table should indicate which stage of the model was ultimately selected.**

Response
We have broken Table 3 (now Table 4) into 4 sub-tables. We continue to believe that none of these models has an *a priori* claim to being more appropriate for modeling Cr^{+6} cancer dose-response. However, given that following incorporation additional information on incidence ratios from NTP the models clearly converge on the same BMDL value, the choice of the model is moot. With respect to stages of the cancer multistage model, the BMDS software integrates the several stages of the model into a single response function. This is different from the historical application of this model where only the coefficient (slope) of the linear portion of the model is applied (after adjustment by the other stages).

NTP Comments on NJDEP's *"Derivation of Ingestion-Based Soil Remediation Criteria for Cr+6 Based on the NTP Chronic Bioassay Data for Sodium Dichromate Dihydrate"*

Comments from Dr. Michelle Hooth

1. Page 4, Table 1- You indicated in your email that you miscalculated the concentration of Cr (as Cr) in the water as a mass percent of sodium dichromate, however these values look correct and are the same ones we reported.

Response

In its comments, EPA suggested that the concentration of Cr in drinking water as a function of the concentration of sodium dichromate in drinking water was miscalculated since we assumed that Cr^{+6} constituted 35% of the molecular weight of the parent chemical, but EPA assumed that it constituted 40%. This also affected the dose estimate expressed as mg Cr^{+6}/kg/day. The confusion arises because we calculated the weight of the parent compound based on the molecular weight of the dihydrate form that NTP used, while EPA calculated the molecular weight based on the anhydrous form. From NTP final report, it is clear to us that NTP based it's concentrations on the molecular weight of the dihdrate form. We, therefore, believe that our calculations (based on the same basis) were and remain correct.

2. Page 5- 2nd paragraph- The tumors of the oral mucosa or tongue seen in exposed rats have a very low historic incidence..... The historical control incidences are for the combination not the tongue alone.

3. Calculation of tumor incidence- I already sent you the correct numbers for the female mice in Table 2.

4. Page 14- Under "Calculation of the soil concentration..."- 1st paragraph last sentence should say column 12 of table 3 (instead of column 11).

5. **Page 18- 1st line- delete "in the"**

6. **Page 21- 2nd paragraph- The chromium picolinate tissue distribution data for mice are presented in the respective technical report. Also see Matt's comments below.**

Response

We have added the Cr-picolinate data for the female mice and recalculated both the male rat and female mouse data from tables M1 and M2 of the Peer-Review Report for Cr-Picolinate.

7. **Page 22- 2nd paragraph- "....that a threshold for reductive capacity of the mouse gastrointestinal tract was exceeded- remove "not"**

8. **Page 23- 1st paragraph- delete "by multiplying" DONE**
9. **Page 23- Last paragraph- Table 4 should be Table A-1**,
next line- delete "shows that"
11. Appendix B- Is the OPPTS risk assessment publically available? I looked on the website and could not find the document.

Response
Not as of this writing, but it is expected to be finalized and posted.

Comments from Dr. Mathew Stout

1. Page 5-first paragraph-the decreases in hematocrit and other hematologic lesions were characterized by an erythrocyte microcytosis (in rats this was an anemia)-this provides evidence of systemic exposure.

Response
At this point in the text, the focus is on the lack of dehydration rather than on the occurrence of systemic effects.

2. Page 2-second paragraph-consider replacing "in study control" with concurrent control.

3. Page 6-second paragraph and several locations-The available evidence suggests that Cr3 reacts with DNA following intracellular reduction, and that oxidative damage can also occur during reduction-I am not aware of evidence that Cr6 reacts with DNA (a good review is O'Brien, et al. 2003).

Response
We have received detailed comments from EPA regarding mutagenesis and the possibility of a mutagenic mode of action (MOA). Those are dealt with in response to EPA's comments.

4. Page 17-fourth paragraph-the sentence "It might, therefore, be hypothesized that the tumors observed in the NTP study reflect a threshold mechanism that functions only after this reduction capacity is exceeded, and that such a mechanism is not relevant to human environmental exposure." is repeated-remove one of them.

5. Page 21-second paragraph-Consider expressing the difference in administered dose between Cr3 and Cr6 as 1.7 times larger instead of 70% larger. The mouse doses to use in comparing chromium uptake between Cr6 and Cr3 are 36.73 mg/kg for Cr3 and 13.2 mg/kg for Cr6, so the disparity in tissue uptake between Cr6 and Cr3 is even larger for mice than rats.

6. **Page 22- With a supralinear (not sublinear) dose response, changes in the slope reflect a decrease in the rate of Cr accumulation with increasing dose.**

Response
The comment is correct. **We will change sublinear to supralinear.**

7. **Page 24-For the section entitled "Effect of pH on reduction capacity for Cr6" the first part of the paragraph (to the sentence starting with "Thus, it appears that reduction capacity is affected to some extent by pH..." is confusing.**

Response
We have rewritten this paragraph to make it less confusing.

8. Page 25-third paragraph-too much emphasis may have been placed on mouse urine data-for example, even when normalizing to internal dose, mouse liver Cr is much higher than rats but is close in kidney with rats:

* Rat liver: 516 mg/L=8.95 mg/kg; Day 182 Cr=6.650 microg/g tissue--(0.74 microg/g)/(mg/kg)
* Mouse liver: 516 mg/L=13.2 mg/kg; Day 182 Cr=52.047 microg/g tissue--(3.94 microg/g)/(mg/kg)
* Rat kidney: 516 mg/L=8.95 mg/kg; Day 182 Cr=15.263 microg/g tissue--(1.70 microg/g)/(mg/kg)
- Mouse kidney: 516 mg/L=13.2 mg/kg; Day 182 Cr=17.487--(1.32 microg/g)/(mg/kg)

Response
It is true that, contrary to urine and to a lesser extent kidney, mouse liver accumulated more Cr than rat liver. Since our point was that even though the rats did not get gastrointestinal tumors from Cr^{+6}, they absorbed more Cr than did the mice, the greater retention of Cr in the mouse liver might be seen as evidence to the contrary. However, as the reviewer notes, the comparison of Cr retention in rat and mouse kidney presents a different picture than liver. There are many possible reasons why a tissue in a given species may absorb more or less Cr than the same tissue in a different species. As we have stated, however, urine integrates whole body absorption, and should not, therefore, be subject to tissue-specific factors.

8. Page 36 - Female mouse kidney graph does not match ours (in our graph, for the 172 mg/L dose, the concentration in higher at 182 days relative to 371 days, whereas at 516 mg/kg, Cr is higher at 371 days relative to 182 days). Also, for all the graphs, the x axis title is Cr6 in mg/L but the numbers correspond to sodium dichromate dihydrate concentrations.

Response

We have checked the data underlying the graph against the raw animal-specific data received from NTP. We do not find a discrepancy. **We have changed the x-axis labels.**