Appendices to Technical Comments Regarding:

DRAFT Public Goal for Hexavalent Chromium in Drinking Water December 2010

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Appendices

Appendix A: Hexavalent Chromium [Cr(VI)] MOA Research Project To Inform EPA's IRIS Assessment.

Appendix B: ToxStrategies (2010) Technical Comments Regarding: EPA's Toxicological Review of Hexavalent Chromium In Support of Summary Information on the Integrated Risk Information System (IRIS).

Appendix C: Zacharewski, T. (2010) The U.S. EPA determined that the MOA for intestinal tumors involves a mutagenic MOA. Molecular data in this target tissue are lacking and are needed to discern whether the MOA is more likely related to mutagenicity or observed species differences in intestinal hyperplasia.

Appendix D: Summit Toxicology (2010) Comments on USEPA's Draft IRIS Report for Hexavalent Chromium. December 10, 2010.

Appendix E: De Flora, S. (2009) Comments on the draft document entitled "Public Health Goal for Hexavalent Chromium in Drinking Water", prepared by the California Environmental Protection Agency (Office of Environmental Health Assessment).

Appendix F. Texas Commission on Environmental Quality (TCEQ) (2010) Comments Regarding the U.S. Environmental Protection Agency Draft Toxicological Review of Hexavalent Chromium in Support of Summary Information on the Integrated Risk Information system (IRIS). Notice of Public Comment Period and Listening Session, 75 FR 60454, September 30, 2010, Docket ID No. EPA-HQ-ORD-2010-0540.

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Attachment A:

Hexavalent Chromium [Cr(VI)] MOA Research Project To Inform EPA's IRIS Assessment

December 22, 2010

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Background

The NTP drinking water study (NTP 2008) found that chronic consumption of drinking water containing 20 to 180 mg/L of hexavalent chromium [Cr(VI)] as sodium dichromate dihydrate (SDD) causes small intestinal tumors in mice, and that 60 to 180 mg/L of Cr(VI) in drinking water causes oral tumors in rats. The NTP study informs the hazard identification for cancer in the GI tract of rodents associated with high concentration exposures and provides a starting point for an oral cancer risk assessment. However, the NTP study does not provide critical MOA data or information on toxicodynamics and toxicokinetics in the low dose range; all of which are necessary to quantify risk to humans at environmentally-relevant exposures.

To fill these critical data gaps and expand upon the NTP study findings, the Cr(VI) Panel of the American Chemistry Council (ACC) undertook an effort to investigate the MOA underlying the development of mouse intestinal tumors and rat oral tumors reported in the NTP drinking water study. This effort is known as the Cr(VI) MOA Research Project.

The Cr(VI) MOA Research Project is based upon a hypothesized MOA that was developed in accordance with the EPA's MOA Framework outlined in the Agency's *Guidelines for Carcinogen Risk Assessment* (commonly referred to as the Cancer Risk Assessment Guidance) (U.S. EPA, 2005). Specifically, a MOA Framework was developed and used to identify data gaps in a hypothesized MOA, and based on this information, studies were designed to gather the data necessary to address these data gaps. Use of the MOA framework to identify data gaps and structure the research project is described in a recent publication by Thompson *et al*. (2011).

The NTP study raised numerous questions regarding how Cr(VI) caused tumors in animals (whether MOA is mutagenic or nonmutagenic), why disparate findings were observed in mice and rats, and how these findings in laboratory animals relate to humans, who are exposed at much lower levels and have notable differences in gastrointestinal anatomy and physiology. As acknowledged in the Draft Toxicological Review, there is considerable uncertainty in the Cr(VI) risk assessment (U.S. EPA, 2010). Furthermore, currently available data do not allow for definitive determination of MOA for the Cr(VI)-induced tumors in rodents exposed via the ingestion route.

Cr(VI) MOA Research Project

The MOA Research Project is designed to: 1) follow EPA's Cancer Risk Assessment Guidance (U.S. EPA, 2005) specifying the MOA framework; 2) extend the doses in the NTP drinking water study to lower doses anticipated in the environment; 3) investigate the MOA (whether mutagenic or nonmutagenic) associated with tumor formation in the target tissues identified by NTP; 4) generate pharmacokinetic data and models needed to

extrapolate between rodents and humans and from high to low doses. Thus, the overall goal for this research project is to provide critical information which would allow for refined human health risk assessment for ingested Cr(VI). The MOA Research Project is scheduled to be completed in the second quarter of 2011, which coincides with EPA's schedule for finalization of the Agency's Toxicological Review and IRIS update for Cr(VI).

The Cr(VI) MOA Research Project was developed under the guidance of an independent science advisory board (SAB) overseen by Toxicology Excellence for Risk Assessment (*TERA*).[1] Prior to beginning any experimental studies, an SAB panel provided review and guidance on the Cr(VI) MOA Research Project. The purpose of the panel review was to evaluate whether the proposed research strategy would provide the information that is necessary to adequately determine the MOA based on EPA's Cancer Risk Assessment Guidance (U.S. EPA, 2005). All of the results from this project will be peer-reviewed by the independent SAB and published in the scientific literature.

1. Cr(VI) MOA Research Project: Planned Studies and Studies in Progress

The Cr(VI) MOA Research Project is being conducted with mice and rats of the same strains that were tested in the NTP drinking water study. The in-life portion is complete. Animals were administered Cr(VI) at the concentrations tested by NTP, and at two lower concentrations thought to be more relevant for current human exposures. The Research Project comprises four multifaceted studies, including 1) comprehensive examination of the genomic responses to Cr(VI), and 2) biochemical investigations to evaluate mutations, genotoxicity and other potential key events in the MOA. The Project also includes 3) an *in vitro* high-content imaging study to characterize the potential species differences in tumor formation between rodents and humans, and 4) a pharmacokinetic study to develop the data supporting a physiologically-based pharmacokinetic models, an approach that EPA prefers for extrapolating cancer findings between species and from the high doses administered to rodents to environmentally-relevant human exposures which are several orders of magnitude lower (U.S. EPA, 2005). The pharmacokinetic study also includes determination of the gastrointestinal reductive capacity and rate using real gastric fluid and contents in mice, rats, and humans.

This work is being conducted by a highly qualified team of experts, including the study director and research laboratory that conducted the NTP drinking water study at Southern Research Institute, experts in risk assessment and pharmacokinetic modeling, ToxStrategies, Inc. and Summit Toxicology, Inc., and academic experts from several universities including Michigan State University, George Washington University Medical Center, and University of Cincinnati Medical Center.

[1] http://www.tera.org/Peer/Chromium/Chromium.htm

The MOA Research Project is currently on-going. Preliminary results of the histopathology, and some of the biochemical analyses are provided here. Additional details on the project and findings form the toxicogenomics are provided in Attachments C and D of the ACC comments. These attachments were prepared by Dr. Tim Zacharweski of Michigan State University and Summit Toxicology. Additional work on DNA modifications, including Cr-DNA binding and chromosomal aberration, and mutation analyses are on-going at George Washington University Medical Center but are not available to date (see Attachments E of the ACC comments).

2. 90-day Subchronic Rodent Toxicity Studies of Cr(VI) in Drinking Water

In early 2010, the 90-day subchronic toxicity studies of Cr(VI) administered in drinking water as SDD to $B6C3F_1$ mice and F344/N rats were started. These studies were conducted using the experimental conditions of the original NTP drinking water study to the extent possible.

Given the absence of a notable sex difference in the tumorigenic response of the NTP drinking water study, only female mice and rats were used. A total of 6 dose groups plus the control group were evaluated. In addition to the doses tested in the NTP drinking water study, two Cr(VI) concentrations lower than those tested in NTP study were included to better inform the MOA and risk assessment at lower doses. The specific concentrations administered *ad libitum* in the 90-day drinking water studies were: 0.1, 1.4, 5, 20, 60, or 180 mg/L Cr(VI) in mice (which are equivalent to 0.3, 4, 14, 60, 170, or 520 mg/L SDD), and 0.1, 1.4, 20, 60, or 180 mg/L Cr(VI) in rats (which are equivalent to 0.3, 4, 60, 170, or 520 mg/L SDD). Preliminary findings from these studies are described below.

Preliminary histopathology and biochemical findings

For mice, test article-related microscopic lesions were observed in the small intestine of animals in the 60, 170, and 520 mg/L dose groups, and included crypt epithelial hyperplasia, histiocytic cellular infiltration of the villous lamina propria, cytoplasmic vacuolization of the villous epithelium, multinucleated syncytia of the villous lamina propria, villous atrophy, and apoptosis. In general, the incidence and/or severity of lesions, particularly crypt epithelial hyperplasia and villous atrophy, were greater in the duodenum than the jejunum on Days 8 and 91, and effects were similar but more severe and/or occurring at lower dose on Day 91 than on Day 8. No test article-related microscopic lesions were observed in the oral mucosa of any of the dose groups on Days 8 and 91.

Preliminary biochemical analysis of the mouse data indicate that compared to the control group, the higher dose groups exhibited decreased GSH/GSSG ratio (**Figures 1 and 2**) in the mouse duodenum, jejunum, and plasma and increased 8-isoprostanes in the mouse duodenum. The ratio of GSH/GSSG decreases as glutathione is oxidized, and a decreased GSH/GSSG ratio is suggestive of an oxidative stress response. No statistically significant

changes were observed for GSH/GSSG ratio in the ileum. Similarly, 8-isoprostanes levels, a measure of lipid peroxidation, in mouse duodenal tissue were elevated as a function of dose. Isoprostanes are compounds that are formed via free radical-induced lipid peroxidation, and are considered to be reliable markers of increased oxidative stress in both humans and animals. The increases in 8-isoprostane observed in the duodenum of mice in the current study suggest that oxidative stress may have played a role in the microscopic changes observed in that tissue.

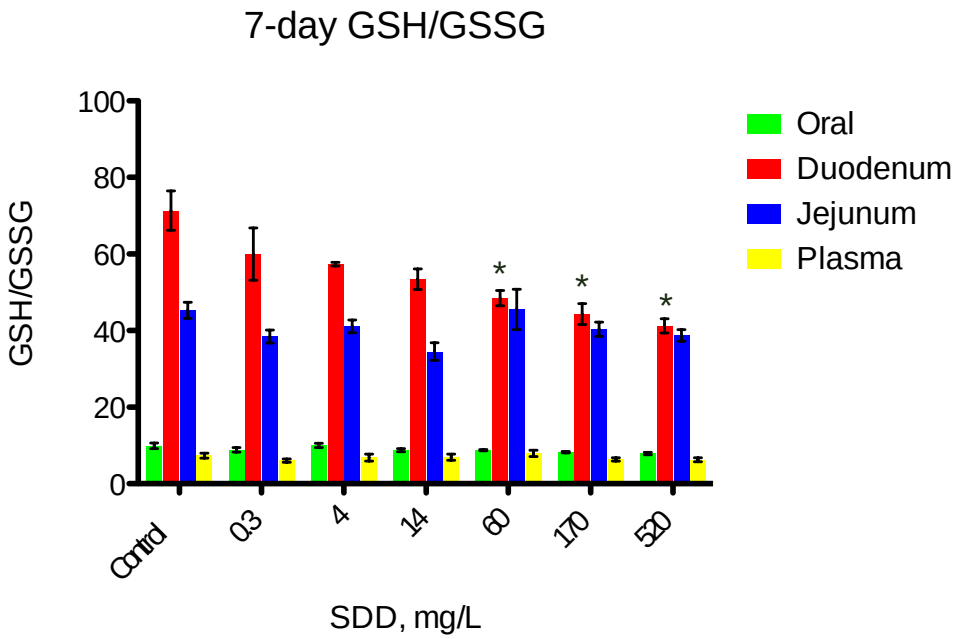

Figure 1. 7-day GSH/GSSG preliminary results for B6C3F$_1$ mice administered Cr(VI) as SDD in drinking water

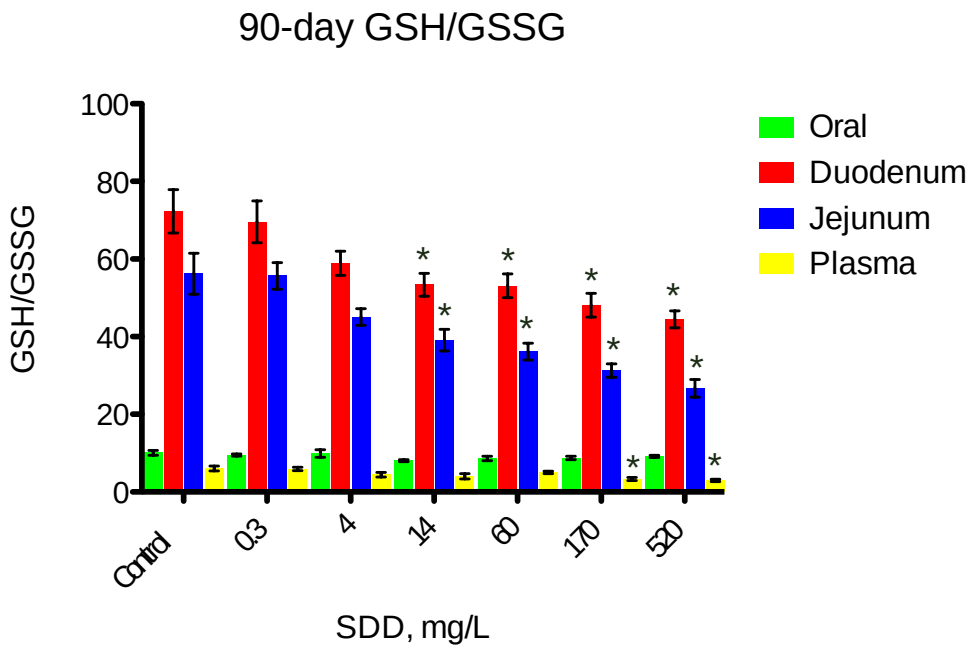

Figure 2. 90-day GSH/GSSG preliminary results for B6C3F$_1$ mice administered Cr(VI) as SDD in drinking water.

Preliminary toxicogenomics findings

Along with histopathology and biochemical evaluation, genomics analyses were also conducted to provide critical insights into the key events associated with Cr(VI)-induced tumor formation observed in the NTP drinking water study. Experience with other compounds has demonstrated that the collection of dose-response data for genomic alterations can provide the necessary evidence for identifying the key components of the MOA and for characterizing dose-dependent transitions. Gene expression microarray measurements were performed in the buccal cavity and small intestine of female F344/N rats and female B6C3F$_1$ mice following 7 and 90-day exposure at the doses identified above. Microarray technology was used to allow simultaneous evaluation of thousands of genes, thus providing a snapshot of toxicity. Changes in gene expression were assessed by pathway or function in an effort to determine potential MOAs associated with toxicity following exposure to Cr(VI) in drinking water.

As described in more detail in comments prepared by Dr. Tim Zacharewski's (Attachment C of ACC comments), Cr(VI) elicited more gene expression changes at Day 8 compared to Day 91 in the mouse small intestine, and genes differentially expressed at Day 91 were a subset of genes differentially expressed at Day 8. Notably, Cr(VI) elicited more gene expression changes in the mouse small intestine compared to the rat at Day 8, and some genes that were induced in rat were repressed in mice, and vice versa. This differential regulation may provide key insights as to the MOA. Moreover, Myc-mediated cell cycle progression and

hyperplasia, Nrf2-mediated oxidative stress, and apoptosis with the induction of Cdc2 and Bad and the suppression of Apaf1 were observed. Myc is a gene that codes for a multifunctional, nuclear phosphoprotein involved in cell cycle progression, apoptosis, and cellular transformation; DNA modifications at myc-gene loci are involved in a wide array of mammalian neoplasms (Steiner *et al*., 1996). Nrf2 is a transcription factor that regulates the expression of many antioxidant responses (Li and Kong, 2009). Cell cycle-regulated protein kinase Cdc2 mediates apoptosis with phosphorylation of the protein Bad (Donovan *et al*., 2002), and Apaf1 is a key regulator of the mitochondrial intrinsic pathway of apoptosis (De Zio *et al.,* 2010). These preliminary results are consistent with reported histopathology (i.e., hyperplasia, apoptosis) and biochemical assay results (i.e., reduced GSH/GSSG ratio).

MOA Research Project Informs the EPA IRIS Toxicological Assessment of Cr(VI)

Data from both the 90-day and 2-year NTP drinking water studies (2008) indicate that diffuse intestinal hyperplasia occurred in the duodenum of mice at all doses examined and as early as 90 days of exposure (NTP, 2007, 2008). The presence of hyperplasia with and without tumor formation suggests that Cr(VI) induced cell proliferation independent of DNA damage. Thus, cell proliferation observed in mice is thought to be a necessary precursor for the development of intestinal neoplasms. However, the current scientific knowledge is insufficient to definitively characterize the MOA and toxicokinetics of ingested Cr(VI).

The MOA data gaps that were identified in Thompson *et al.,* (2011), before the research project was initiated, included:

- reduction kinetics for Cr(VI) in the rodent stomach, particularly at lower exposures;
- measures of Cr(VI) absorption into the target tissue (i.e., small intestine);
- species differences in toxicokinetics and toxicodynamics;
- understanding the significance (if any) of histiocytic infiltration in mice and rats observed at all doses of the NTP (2008) drinking water study;
- the role of oxidative stress and inflammatory response in the small intestine;
- a no observable adverse effect level (NOAEL) for hyperplasia in the mouse target tissue;
- a mechanistic basis for interspecies differences in proliferative response;
- measures of DNA damage in the target tissue;
- the timing of Cr(VI)-induced mutations, and whether mutations occur early or late in the carcinogenesis process.

Key events in the MOA as proposed by Thompson *et al.,* (2011) are presented in **Figure 3.** The data gaps are represented as dashes in the Figure 3, with specific details listed above.

	New Doses		NTP Doses			
SDD, mg/L **(Cr(VI), mg/L)**	**0.3** **(0.1)**	**4** **(1)**	**14** **(5)**	**60** **(20)**	**170** **(60)**	**520** **(180)**
Saturate RC	--	--	**(X)**	**(X)**	**(X)**	**(X)**
Uptake in intestines	--	--	**(X)**	**(X)**	**(X)**	**(X)**
Oxidative stress Inflammation	--	--	--	--	--	--
Cell proliferation 90 Days 2 Years	--	--	**--** **X**	**X** **X**	**(X)** **X**	**X** **X**
Intestinal DNA damage 3 months 9 months 24 months	-- -- --	-- -- --	-- (-) DeFlora --	-- (-) DeFlora --	-- -- **(X)**	-- -- **(X)**
Mutagenesis	--	--	--	--	**X**	**X**
Tumors 9 months 2 Years	-	-	(-) DeFlora -	(-) DeFlora -	**X**	**X**

Figure 3. Current data gaps in MOA and toxicokinetics of Cr(VI).

-- = no data available; X = known effects; (X) = assumed effects
Reductive capacity (RC)

The Cr(VI) MOA Research Project was designed to fill these data gaps and provide critical data necessary to develop a scientifically defensible risk assessment for oral exposures to Cr(VI). Preliminary findings provide some information to address data gaps as indicated in **Figure 4**, where new data from the MOA research are in red. Although the project is not sufficiently complete to address all of the data gaps, and critical data for toxicokinetics, and some aspects (e.g., DNA modifications and mutagenicity) are still under development, Figure 4 provides an example of how the MOA Research Project study findings will be used to address data gaps in the MOA and provide the tools needed to refine human health risk assessment.

	New Doses		NTP Doses			
SDD, mg/L	**0.3**	**4**	**14**	**60**	**170**	**520**
Saturate RC	--	--	**(X)**	**(X)**	**(X)**	**(X)**
Uptake in intestines	--	--	**(X)**	**(X)**	**(X)**	**(X)**
Oxidative stress[1] Inflammation	(-)	(-)	*X*	*X*	**X**	**X**
Villus Cytotoxicity & Cell proliferation[2] 8 Days 90 Days 2 Years	 (-) (-)	 (-) (-)	 (-) (-) **X**	 (-) **X** **X**	 (-) **X** **X**	 **X** **X** **X**
Intestinal DNA damage 3 months (8-oxo-dG) Multiple other measures 9 months 24 months	 (-) ? -- --	 (-) ? -- --	 (-) ? (-) DeFlora --	 (-) ? (-) DeFlora --	 (-) ? -- **(X)**	 (-) ? -- **(X)**
Mutagenesis	--	--	--	--	**X**	**X**
Tumors 9 months 2 Years	 -	 -	 (-) DeFlora -	 (-) DeFlora -	 **X**	 **X**

Figure 4. Filling data gaps in MOA with preliminary data.

-- = no data available; X = known effects; (X) = assumed effects
X based on new data from MOA study, (-) negative
1 Decreased GSH/GSSG at 14 mg/L and higher, 8-iso significantly increased at 520 and possibly 170 mg/L, no inflammatory response with cytokines or chemokines, but genomics suggests inflammatory response.
2 Based on histopathology, further analysis of genomics may result in additional findings.

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Conclusions

In keeping with EPA's Cancer Risk Assessment Guidance (U.S. EPA 2005), it is critical that the Agency's cancer assessments be based on the best available science. The findings from the Cr(VI) MOA Research Project studies will provide a scientifically supportable basis for determining the MOA for rodent tumors, a quantitative evaluation of interspecies (rodent as compared to human) toxicokinetics, quantitative measures of gastric reductive rate and capacity for rodents and humans, and the information that EPA prefers to use as the basis for cancer risk assessment (U.S. EPA 2005). In absence of these data, the Draft Toxicological Review has relied upon uncertain and default approaches for quantitative risk assessment. To address public health concerns regarding wide-spread, low-level Cr(VI) contamination, EPA should seek to provide the most scientifically robust and refined risk assessment possible for ingestion of Cr(VI), and as such, we urge EPA to consider and include the findings of the Cr(VI) MOA Research Project in the final Toxicological Review for Cr(VI).

References

De Zio, D., Bordi, M., Tino, E., Lanzuolo, C., Ferraro, E., Mora, E., Ciccosanti, F., Fimia, M., Orlando, V., Cecconi, F. (2010). The DNA repair complex Ku 70/86 modulates Apaf1 expression upon DNA damage. Cell Death Prolif. Doi:10.1038./cdd.2010.125.

Donovan, N., Becker, E.B.E., Konishi, Y., and Bonni, A. JNK phosphorylation and activation of BAD couples the stress-activated signaling pathway to the cell death machinery. *J. Biol. Chem*. **25**, 40944-49.

Li, W., Kong, A-N. (2009). Molecular mechanisms of Nrf2-mediated antioxidant response. *Mol. Carcinog.* **48**, 91-104.

National Toxicology Program (NTP). (2007). NTP technical report on the toxicity studies of sodium dichromate dihydrate administered in drinking water to male and female F344/N rats and B6C3F1 mice and male BALB/c and am3-C57BL/6 mice. January 2007 National Institutes of Health Public Health Service U.S. Department of Health and Human Services. Accessed at: /http:// ntp.niehs.nih.gov/ntp/htdocs/ST_rpts/TOX72.pdfbm94934.

NTP. (2008). Final technical report on the toxicology and carcinogenesis studies of sodium dichromate dihydrate in F344/N rats and B6C3F1 mice. Accessed at: /http://ntp.niehs.nih.gov/files/546_web_FINAL.pdfS.

Steiner, P., Rudolph, B., Muller, D., Eilers M. (1996). The functions of Myc in cell cycle progression and apoptosis. *Prog. Cell Cycle Res.* **2**, 73-82.

Thompson, C., Haws, L, Harris, M. Gatto, M., Proctor, D. (2011). Application of the U.S. EPA mode of action framework for purposes of guiding future research: A case study involving the oral carcinogenicity of hexavalent chromium. *Toxicol. Sci.* DOI:10.1093/toxsci/kfq320.

United States Environmental Protection Agency (U.S. EPA). (2005). Guidelines for carcinogen risk assessment, EPA/630/P-03/001F. Risk Assessment Forum: U.S. Environmental Protection Agency, Washington, D.C.

U.S. EPA. (2010). Toxicological Review of Hexavalent Chromium. (CAS No.18540-29-9). In Support of Summary Information on the Integrated Risk Information System (IRIS) (External Review Draft). *U.S. Environmental Protection Agency, Washinton, D.C.*

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Attachment B:

Technical Comments Regarding:

Toxicological Review of Hexavalent Chromium In Support of Summary Information on the Integrated Risk Information System (IRIS) (September 2010)

December 22, 2010.

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EXECUTIVE SUMMARY

ToxStrategies, Inc. has prepared these comments regarding the U.S. Environmental Protection Agency's (EPA's) *Draft Toxicological Review of Hexavalent Chromium (CAS No. 18540-29-9) In Support of Summary Information on the Integrated Risk Information System (IRIS)* (Draft Toxicological Review) on behalf of the Hexavalent Chromium [Cr(VI)]] Panel of the American Chemistry Council (ACC).

The Draft Toxicological Review presents an updated risk assessment including a mode of action (MOA) evaluation for Cr(VI)-induced tumors by the ingestion route, an oral cancer slope factor and an oral reference dose (RfD) based on the findings of a 2-year National Toxicology Program (NTP, 2008a) drinking water study. In conducting the assessment, EPA concluded that rodent tumors were induced by a mutagenic MOA. Although the NTP study provides clear evidence that Cr(VI) induced tumors in rodents, the concentrations administered in the study are far in excess of typical environmental exposures (approximately ~1,000-fold greater) and may have overwhelmed the normal protective mechanisms that are thought to limit the carcinogenic potential of Cr(VI) following ingestion (Proctor et al., 2002b; De Flora, 2000). Thus, the MOA of ingested Cr(VI) at high doses may not be operative at environmentally relevant doses, and the slope of the dose-response curve at high doses may not be consistent with that at low-doses.

Dose-dependent transitions in the MOA are important considerations in risk assessment, as called for by the National Research Council in *Science and Decisions: Advancing Risk Assessment* (NRC, 2009), and should be considered for the risk assessment of ingested Cr(VI). Further, the disparate findings observed in rats and mice in the NTP study raise important questions about differences in toxicokinetics and toxicodynamics between species and the most appropriate manner of extrapolating the NTP study findings to humans, who are exposed at much lower levels, and have notable differences in gastrointestinal (GI) anatomy and physiology as compared to rodents.

EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) (Cancer Risk Assessment Guidance) calls for evaluation of the MOA and human relevance. However, as acknowledged in the Draft Toxicological Review, considerable uncertainty exists in the data that are currently available to discern the MOA. Recognizing that there are limitations in the data available to definitively determine the MOA, an extensive MOA research project was launched by the Cr(VI) Panel of ACC. As described in Thompson *et al.* (2010), this research project was developed based on a comprehensive assessment of the pertinent literature concerning the oral carcinogenicity of Cr(VI) in context of EPA's Cancer Risk Assessment Guidance (U.S. EPA, 2005a). This research, scheduled to be completed by mid-2011 with findings published and presented as developed, will provide a substantially more robust data set for assessing the human cancer risk posed by ingestion of Cr(VI). Specifically, the MOA Research Project will provide:

1. Critical toxicokinetic information, especially at environmentally-relevant exposures, such as reduction kinetics for Cr(VI) in the rodent stomach, and target tissue dose in the small intestine and oral mucosa;

2. Species-specific physiologically-based pharmacokinetic (PBPK) models which are EPA's preferred method for interspecies extrapolation (U.S. EPA, 2005a) to inform extrapolations between species and from high to low doses;

3. Data necessary to better determine the MOA underlying the specific tumors observed in mice and rats in the NTP drinking water study, whether mutation occurred early or late in the carcinogenic process, and relevance of the MOA to humans.

We have the following specific comments on the Draft Toxicological Review. These comments are outlined in the main body and key comments are summarized below:

- **Environmental characterization of Cr(VI) is inadequate.**

Cr(VI) exists at low levels throughout the environment. For example, in California, approximately one-third of the drinking water supply contains Cr(VI) at low levels, and most of the impacted drinking water are thought to be due to natural sources. In addition, widespread, but low level, occurrence of Cr(VI) has recently been found in urban house dust. The characterization of Cr(VI) occurrence and current human exposures is inadequate in the Draft Toxicological Review. In addition, the discussion of analytical detection limits is not current. As an important practical issue, the Agency should consider that health-based screening levels for Cr(VI), which are based on the proposed oral cancer slope factor such as the EPA Regional Screening Levels (RSLs), are lower than the current limits of detection, and naturally occurring levels of Cr(VI) in the environment.

- **There are significant data gaps in current scientific knowledge of Cr(VI) toxicokinetics that need to be addressed.**

Results of the NTP (2008a) study provide evidence of portal-of-entry effects, which should not be scaled across species using body weight scaling ($BW^{3/4}$). Also, differences in neoplastic and non-neoplastic effects observed in the GI tract of rats and mice of the NTP (2008a) study raise numerous questions regarding the MOA and toxicokinetics of ingested Cr(VI). Data gaps in the toxicokinetics that have been identified include:

- reduction kinetics for Cr(VI) in the rodent stomach, particularly at lower exposures;
- measures of Cr(VI) absorption into the target tissue (i.e., small intestine); and
- species differences in toxicokinetics.

Correct and quantitative characterization of bioaccessibility and bioavailability are critical for human risk assessment at environmentally relevant exposures.

- **Refined physiologically-based pharmacokinetic (PBPK) models capable of reasonably estimating internal dosimetry in rodents and predicting internal dosimetry in humans are critical for the risk assessment of Cr(VI)**.

In the Draft Toxicological Review, EPA used $BW^{3/4}$ scaling to extrapolate between species. However, this approach is not well supported for portal-of-entry effects (U.S. EPA, 2006b) because the dose at the target tissue will be greatly influenced by kinetic factors such as absorption and reduction in the stomach. The disposition of ingested Cr(VI) is dependent on competing rates of reduction, absorption and gastric motility, and interspecies differences in these toxicokinetic parameters might significantly impact the target tissue dose (Thompson *et al*., 2010).

Tissue chromium data and non-neoplastic findings from the NTP (2008a) study suggest that all of the doses of Cr(VI) administered in the NTP (2008a) study exceeded the reduction capacity of the mouse stomach (Thompson *et al.,* 2010). Importantly, studies involving lower doses of Cr(VI) compared to those used in the NTP (2008a) study have demonstrated a threshold for chromium tissue accumulation between 3 and 10 mg/L (Sutherland *et al.,* 2000; NTP 2007). Collectively, these findings raise questions about the appropriateness of using the default $BW^{3/4}$ scaling and linear extrapolation of risk at high concentrations to the very low concentrations that are environmentally-relevant.

EPA's preferred method for interspecies extrapolation is PBPK modeling (U.S. EPA, 2005a). However, currently available models for Cr(VI) lack target tissue compartments, and important pharmacokinetic parameters such as gastric reduction capacity. In light of this serious data gap, efforts are underway as a part of the Cr(VI) MOA Research Project to collect data necessary to refine published PBPK models for use in risk assessment. These models will provide a more scientifically robust basis for development of an oral RfD and cancer slope factor for Cr(VI).

- **Current data do not support a definitive determination that Cr(VI) is acting through a mutagenic MOA. The current scientific data are equally supportive of a non-mutagenic MOA. As such, Age-Dependent Adjustment Factors (ADAFs) should not be applied.**

Investigators have reported that many mutagenic chemicals induce tumors at multiple sites and with relatively short time to tumor development (McCarroll *et al*., 2010; U.S. EPA, 2007). In contrast, drinking water administration of Cr(VI) in the NTP (2008a) study resulted in hyperplasia—secondary to epithelial injury, and long time to tumor formation with most tumors only identified at the final sacrifice. Further, chromium tissue accumulation occurred at high concentrations in organs such as the liver, without the induction of a carcinogenic response. These factors are clearly suggestive of a non-

mutagenic MOA, where mutation is unlikely to be the *initiating* key event but rather occurs subsequent to other key events.

Although the Draft Toxicological Review has documented genotoxicity and mutagenicity in many systems, the most relevant studies (i.e., *in vivo* drinking-water exposure studies) were negative in both humans and animals (Shindo *et al*., 1989; De Flora *et al.,* 2006, 2008; Mirsalis *et al*., 1996; Kuykendall *et al*., 1996; NTP, 2007). This suggest that genotoxicity and mutagenicity observed *in vitro,* or at very high doses administered by irrelevant routes of exposure (i.e., gavage and intraperitoneal injection), are not relevant to the MOA that is likely operational for environmentally-relevant drinking water exposures. Further, the only available study to examine genotoxicity in the target tissue of the small intestine (De Flora *et al*., 2008) demonstrated that, at exposures far higher than current drinking-water standards and expected human exposures, Cr(VI) did not cause target tissue genotoxicity. These findings clearly raise questions as to whether Cr(VI) could be acting through a mutagenic MOA at relevant exposures. Thus, in accordance with EPA's *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b), which specifies that ADAFs are to be applied only when the MOA is known to be mutagenic, it is inappropriate to apply ADAFs in the risk assessment of Cr(VI).

- **EPA's consideration of alternative MOAs for ingested Cr(VI) is inadequate.**

In contrast to recommendations in EPA's Cancer Risk Assessment Guidance), the Draft Toxicological Review fails to adequately address alternative MOAs. More specifically, EPA summarily dismisses a non-mutagenic MOA based on proliferation, citing a lack of necrosis in the NTP (2008a) study, and fails to adequately consider the evidence supporting an equally plausible alternative MOA based on epigenetic modifications. Recent reviews by Chiu *et al.* (2010) and Nickens *et al*. (2010) indicate great variations in the MOA for Cr(VI) between tissues and species, yet the findings of these reviews have not been adequately considered in the Draft Toxicological Review.

As discussed extensively in Thompson *et al.* (2010), the weight of evidence suggests that the MOA for Cr(VI)-induced tumors in the mouse small intestine likely involves proliferative pressure, overloading epithelial cells with Cr(III) as a result of exposures to very high doses of Cr(VI), leading to genomic instability and late-stage mutations and tumors. This MOA is expected to be operational only at extremely high doses, which cause hyperplasia, and not at environmentally relevant doses. Considering that hyperplasia was observed in mice, but not rats, and that it preceded tumor development in both dose and time, evidence supports that proliferation is a necessary key event in the MOA, which will only occur at very high doses.

Given the weight of the evidence supporting plausible alternative MOAs, EPA should expand upon the current assessment presented in the Draft Toxicological Review and offer a balanced consideration of non-mutagenic MOAs.

- **The Draft Toxicological Review concludes that, for *all* routes of exposure, a mutagenic MOA is operative; however, this determination is not scientifically supported or clearly described.**

The Draft Toxicological Review presents background information and justification for hazard identification and dose-response assessments of *ingested* Cr(VI) only, and not of dermal and inhalation exposures (U.S. EPA, 2010d; p.1). An assessment of the MOA for inhalation or dermal routes is not provided in the Draft Toxicological Review. Thus, without evaluation of the cancer health affects or mechanistic data associated with inhalation and dermal routes of exposure, the carcinogenic MOA for these routes of exposure cannot be offered. The Agency should retract the position that Cr(VI) acts by a mutagenic MOA or offer a comprehensive review.

- **The significant uncertainties associated with the epidemiologic data available for Chinese villagers exposed to Cr(VI) by ingestion limits any reasonable conclusion of an increased cancer risk among humans from oral exposure.**

In EPA's summary of the Overall Weight of Evidence the Agency concluded that "*Cr(VI) is 'likely to be carcinogenic to humans' via the oral route of exposure based on…, and evidence of an association between oral exposure to Cr(VI) and stomach cancer in humans*" (U.S. EPA, 2010d; pp. 199-200). The only study reviewed by EPA that reports an association between stomach cancer in humans and oral exposure to Cr(VI) is the original Zhang and Li (1987) study of Chinese villagers and a subsequent study relying on the same data by Beaumont *et al.* (2008). Based on the significant limitations and uncertainties associated with the original study data, as discussed in detail herein, it is a clear overstatement to consider these data as "evidence" of an association. The quality of data from these studies is especially poor and the interpretation of results is questionable. Additionally, the reanalysis by Kerger *et al.,* of these same data found 1) no dose-response using three dose metrics, and 2) that the increased risk among villagers with Cr(VI) exposure was not significant when compared to villagers without Cr(VI) exposure. Furthermore, all other environmental epidemiology studies have not found an increased risk of cancer in humans with environmental exposure, including a very recent Health Consultation for New Jersey residents potentially exposed to Cr(VI) in soil as Chromite Ore Processing Residue (COPR) (NJDHSS/NJDEP, 2010). Finally, it is contradictory to conclude, as EPA has, that data from the Chinese studies are not sufficient to support a conclusion regarding the presence or absence of a dose-response relationship, but to use these same data to conclude that Cr(VI) is likely to be carcinogenic in humans via the oral route of exposure (U.S. EPA, 2010d; p. 215).

- **A recently published literature review and meta-analysis of GI-tract cancers in Cr(VI)-exposed workers found no increased risk of oral, esophageal, stomach, small intestine, colon and rectal cancers, drawing into question the relevance of tumors observed in the mouse small intestine to humans (Gatto *et al.,* 2010).**

Well over 100 epidemiological studies of Cr(VI)-exposed workers have been published; although most have focused on lung cancer, dozens have also reported on cancers outside the respiratory system. A systematic literature review of GI-tract cancers among workers with known occupational exposure to Cr(VI), and meta-analysis of 32 studies published since 1950, did not find an association between Cr(VI) exposure and GI tract cancer (Gatto *et al*., 2010). Gatto *et al.* (2010) identified only three studies reporting small-intestine cancer among Cr(VI)-exposed workers, and all three reported a very small number of cases (n<3) and none reported a significant increase. The findings of Gatto et al. (2010) are important for assessing human relevance, but the study was not cited by EPA in the Draft Toxicological Review.

- **Although EPA's effort to summarize all relevant literature is applauded, a more transparent review process is needed**.

The Draft Toxicological Review states that the literature search is current through September 2010. However as discussed in more detail in our comments that follow, many important publications were not included. Given that the inclusion and exclusion of certain papers could significantly impact the conclusions reached by EPA, it is critical that EPA specify and adhere to specific inclusion criteria for papers evaluated in the Agency's Hazard Identification process. In January 2010, the Cr(VI) Panel of ACC submitted additional references of peer-reviewed literature for EPA to review and consider in the development of Agency's IRIS review of Cr(VI) (Attachment F of ACC's comments). However, most of these papers, which are critical to a comprehensive evaluation of Cr(VI), are not cited in the Draft Toxicological Profile, indicating that the current assessment is incomplete.

INTRODUCTION

On behalf of the Hexavalent Chromium [Cr(VI)]] Panel of the American Chemistry Council (ACC), ToxStrategies, Inc. has prepared comments regarding the U.S. Environmental Protection Agency's (EPA's) *Draft Toxicological Review of Hexavalent Chromium (CAS No. 18540-29-9) In Support of Summary Information on the Integrated Risk Information System (IRIS)* (Draft Toxicological Review).

The Draft Toxicological Review presents an updated risk assessment including a mode of action (MOA) evaluation for Cr(VI)-induced tumors by the ingestion route, an oral reference dose (RfD) and an oral cancer slope factor based on observations of tumors in the small intestine of mice in the 2-year National Toxicology Program (NTP, 2008a) drinking water study. Comments offered herein address all aspects of the Draft Toxicological Review. However, our greatest concern with the risk assessment, as noted throughout these comments is associated with the inadequate data available to determine the MOA at environmentally-relevant doses, extrapolate from rodents to humans, and thus quantify dose-response in the low dose range.

EPA's Cancer Risk Assessment Guidance (U.S. EPA, 2005a) calls for evaluation of the MOA for the purposes of risk assessment, both qualitatively and quantitatively, the risk to humans. As acknowledged in the Draft Toxicological Review, considerable uncertainty exists in the data currently available to conduct cancer risk assessment for ingested Cr(VI). Sufficient evidence exists to suggest that Cr(VI) may have caused tumors in the mouse small intestine by a non-mutagenic MOA, and that the MOA which caused tumors in the NTP study mice is likely to be operational only at the high doses administered in that study.

Discussed in detail herein, the NTP studies (NTP 2008a; 2007) provide evidence to support a proliferative MOA; however such an MOA was dismissed by EPA because necrosis was not reported in the NTP studies. It should be noted that proliferation occurs by many mechanism that do not involve necrosis, and while the NTP studies did not characterize the intestinal tissue as necrotic, NTP clearly states in both the 90-day and 2-year bioassays that the diffuse hyperplasia appeared to be secondary to tissue injury. Regenerative hyperplasia preceded tumor formation in both dose and time in the NTP (2008a) mice, but did not occur at any dose in rats, suggesting that proliferation, which is clearly a high dose effect, is a key event necessary for tumor formation (Thompson *et al.,* 2010).

EPA's *Guidelines for Carcinogen Risk Assessment* states:

> A nonlinear approach should be selected when there are sufficient data to ascertain the mode of action and conclude that it is not linear at low doses and the agent does not demonstrate mutagenic activity or other activity consistent with linearity at low doses. Special attention is important when the data support a nonlinear mode of action but there is also a suggestion of mutagenicity. Depending on the strength of the suggestion of mutagenicity, the assessment may justify a conclusion that mutagenicity is not operative a low doses and focus on a nonlinear approach or alternatively, the assessment may use both linear and nonlinear approaches (U.S. EPA, 2005a, p. 3-22).

Although we acknowledge that more data are needed to quantify toxicokinetics and dose-response in the low dose range, the current weight of scientific evidence does not support several key assumptions in the Draft Toxicological Review. Specifically, the available data do not definitively support a mutagenic MOA, that the tumor rates observed at the high doses, which overwhelmed the reductive capacity of the rodent stomach, can be linearly extrapolated orders of magnitude to the lower doses to which humans are exposed, or that the extrapolation of these portal-of-entry effects between species can be conducted using $BW^{3/4}$ scaling. Because widespread, low level human exposure to Cr(VI) exists, it is imperative that the Agency avoid default approaches and use the best science available for the risk assessment of ingested Cr(VI).

A. Incomplete Characterization of Cr(VI) in the Environment

The Draft Toxicological Review does not include a discussion of the low-level, widespread naturally-occurring Cr(VI) that is present in the environment and should be revised accordingly. This information is readily available from the published literature and on the websites of the states of California and New Jersey.

In section 2.2, the Draft Toxicological Review states, "*Natural occurrence of hexavalent chromium is rare as it is readily reduced by organic matter in the environment*" (U.S. EPA, 2010d; p.14). Although this is true for some environmental conditions, Cr(VI) has been found to occur naturally and prevalently in groundwater throughout California, and likely also exists in other areas around the country due to natural sources, but monitoring for low levels of Cr(VI) (less than the current total Cr Maximum Contaminant Level [MCL] of 0.1 µg/L) in drinking water is not commonly performed.

1. Low level of Cr(VI) in the environment

In recent years, there has been increased public concern about the presence of Cr(VI) in drinking water. Such concerns prompted the California Department of Public Health (CDPH) [formerly Health Services (DHS)] to adopt a requirement for monitoring of Cr(VI) in all large public water supplies. This monitoring was required after the Office of Environmental Health Hazard Assessment (OEHHA) identified Cr(VI) as posing a cancer risk when ingested and was mandated as part of California EPA's program to establish a Public Health Goal (PHG) for Cr(VI). Specifically, in 2001, the CDPH adopted a requirement for monitoring Cr(VI) in all large public water supplies. Since then, the CDPH has collected monitoring data from over 7,000 drinking water sources in California and has documented Cr(VI) detections at or above the 1-µg/L detection limit in about one-third of monitored sources [in excess of 12,000 drinking water samples with detectable Cr(VI)]. The available data, which are current through February 2009, reports Cr(VI) levels of 1–5 ppb in 65% of those sources, levels of 6–10 ppb in 20.7%, and levels of 11-20 ppb in 10.5% (CDPH, 2009).

Using water monitoring data obtained from the CDPH website (http://www.cdph.ca.gov/certlic/drinkingwater/Pages/Chromium6.aspx) and geographical information system (GIS) software, CDPH estimated average Cr(VI) levels detected in drinking water by County for the State of California from 2001 to 2003. The results of this analysis are presented in Figure 1.

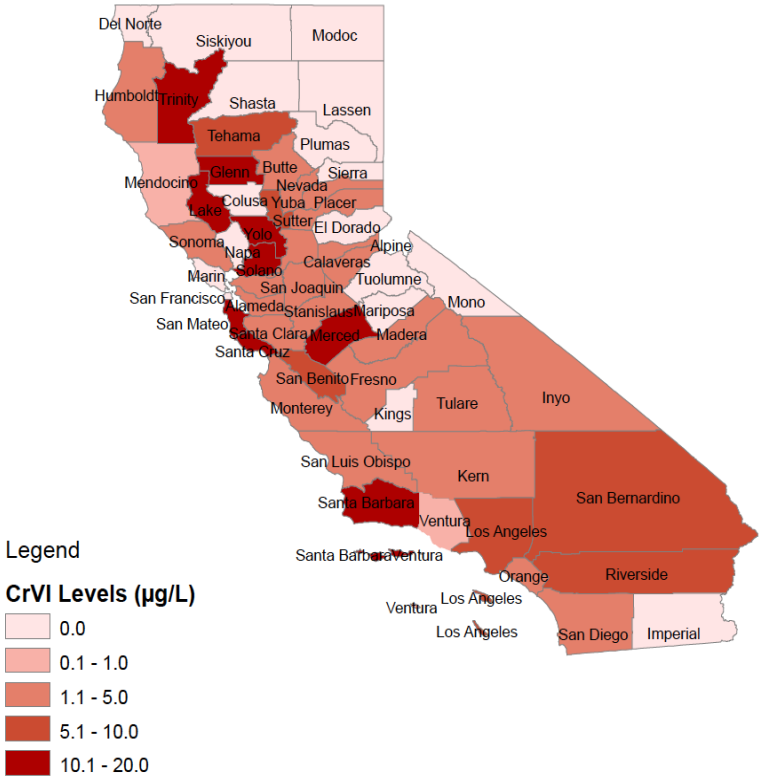

Figure 1. Mean levels of measured Cr(VI) in drinking water by county in California, 2001-2003. Mean of all detected measurements

Source: California Department of Public Health. Chromium-6 in Drinking Water Sources: Sampling results. http://www.cdph.ca.gov/certlic/drinkingwater/Pages/Chromium6sampling.aspx.

In addition, recent sampling of drinking water suggests that Cr(VI) occurs naturally in groundwater at many locations around the world (Gonzales *et al*., 2005; Ball and Izbicki, 2004; Kulongoski and Belitz, 2005; Oze *et al*., 2007; Boufounos *et al*., 2009). A recent study reported unusually high concentrations of Cr(VI) in groundwater collected from the Adamantina Aquifer which supplies at least 54 municipalities located in the northwestern region of the State of São Paulo, southeast Brazil (Boufounos *et al.,* 2009). An investigation conducted in the municipality of Urânia, where the highest concentrations of chromium were detected regionally, demonstrated that the origin of the Cr(VI) contamination is natural. This conclusion was based on the observation that the highest concentrations of chromium were detected in aquifer sandstones (average of 221 mg/kg) and also in pyroxenes (6000 mg/kg), one of the main heavy minerals found in aquifer sediments. Further supporting the researchers conclusion that the Cr(VI) was naturally occurring is the fact that no other possible diffuse or point sources of contamination were observed in the study area. Stratification of groundwater quality was observed, and the highest concentrations of Cr(VI) were detected at the base of the aquifer (0.12 mg/L)—inconsistent with there being a source at the surface—and where groundwater shows favorable redox and pH conditions.

Recent data also indicates that low level Cr(VI) exposure in urban house dust is widespread. Stern *et al.* (2010) compared Cr(VI) in house dust in Jersey City, New Jersey (an area with historical chromate production) to selected background communities with no known sources of Cr(VI). Samples were collected from living areas, basements and window wells. Cr(VI) was detected in dust from all Jersey City and background houses. In the Jersey City homes, the mean (±SD) Cr(VI) concentration for all samples was 3.9±7.0 μg/g (range: non-detect–90.4 μg/g), and the mean Cr(VI) loading was 5.8±15.7 μg/m^2 (range: non-detect–196.4 μg/m^2). In background homes, the mean Cr(VI) concentration of all samples was 4.6±7.8 μg/g, (range, 0.05–56.6 μg/g). The mean loading was 10.0±27.9 μg/m^2 (range, 0.22–169.3 μg/m^2) (Table 1). There was no significant difference between Cr(VI) dust concentrations in Jersey City and background locations. Stratification by sample location within houses and sampling method gave similar results. Lower concentrations in window well samples suggest transport from outside is not the major source of indoor Cr(VI). The authors conclude that there appears to be a widespread low level background of Cr(VI) in household dust that is not elevated in Jersey City homes despite its historic chromite ore processing residue (COPR) contamination. The researchers conclude that it is possible that house dust, in general, is a source of Cr(VI) exposure.

Table 1. Concentration and loading of Cr(VI) in house dust in Jersey City and background locations (from Stern et al. 2010)

Location	Number of samples	Concentration (μg/g)			Loading (μg/m^3)		
		Mean	Std. Dev.	95th percentile	Mean	Std. Dev.	95th percentile
Jersey City							
Droyers point	78	2.1	3.2	6.9	1.5	2.6	4.9
Freedom	24	6.6	9.2	32.1	8.1	7.1	20.6
Garfield	48	3.4	4.4	14.4	6.1	15.2	15.4
Lafayette	38	3.6	4.1	9.7	8.3	18.0	69.4
Society Hill	29	3.5	2.8	9.4	4.0	6.0	13.8
Other	75	5.6	11.1	17.3	8.8	24.3	23.5
All Jersey City samples	292	3.9	7.0	11.7	5.8	15.7	18.2
Background locations	60	4.6	7.8	13.4	10.0	27.9	33.1

These data indicate that the natural occurrence of Cr(VI) in the environment is not rare and that ingestions exposures to Cr(VI) at low levels are likely widespread and common.

2. Bioaccessibility of Cr(VI) from environmental exposures

EPA has not adequately addressed the bioaccessibility of Cr(VI) in the Draft Toxicological Review. Broadway *et al* (2010) recently published a study that examined the bioaccessibility of Cr(VI) in 27 Glasgow soil samples, including samples contaminated with chromite ore processing residue (COPR). Note, in this case, bioaccessibility refers to the extraction of Cr(VI) in simulated GI and lung conditions, not extracellular reduction prior to

absorption as discussed in Section 3 of the Draft Toxicological Review. Clarifications for these terms are needed. Presumably, EPA intended to discuss extraction of Cr(VI) from soil in GI conditions in section 2, and the inclusion of data from Broadway *et al.* would have been insightful.

In the study, the oral bioaccessibility of chromium was typically greater by a factor of 1.5 in simulated stomach conditions as compared with simulated conditions of the stomach and intestine together, and on average, the oral bioaccessibility of chromium in the stomach was 5% of total chromium in soil. Importantly, Broadway *et al*. also noted that the use of total chromium concentrations in human health risk assessment models with the assumption that all chromium is present as Cr(VI) was likely to produce a very conservative assessment.

3. Limitations of analytical methods are not sufficiently discussed

The speciation of chromium is important to accurately characterize exposure and toxicity. The Draft Toxicological Review notes, "*The level of detection and ability to detect speciation versus total chrome in various environmental and biological compartment is a critical consideration when evaluating the relevance and reliability of any given environmental or toxicological data*" (U.S. EPA, 2010d; p. 15). However, the Draft Toxicological Review does not include a comprehensive evaluation of current, state-of-the-science information related to analytical methods and analytical challenges. The information on analytical methods is dated and incomplete. For example, Table 2-4 lists methods for analysis. Analysis by IC is listed as having a detection limit of 93 µg/L, but EPA Method 218.6, the Agency IC method, achieves a Method Detection Limit of 0.3 µg/L in drinking water.[1] Also, it should be noted that the reference for the limit of detection in this table is labeled as "MISSING". The contract laboratory required detection limit (CRDL) for Cr(VI) by the colorimetric method (EPA 7196A) is 20 µg/L, but reported as 50 µg/L in Table 2-4.[2] This information is readily available on EPA's own website, and it is recommended that internal Agency resources be used to update this section. In addition, numerous publications have reported limitations of the current analytical methods for Cr(VI), especially at low concentrations (U.S. EPA, 1991; Vitale *et al*., 1997; ATSDR 2008; Sores *et al.,* 2010)

A discussion concerning potential analytical problems, detection limits, and uncertainties would greatly improve the chemical and physical information in the Draft Toxicological Review and would provide clarification as to why, in many cases, studies cited in the Draft Toxicological Review were limited to reporting only total chromium. Characterization of potential health risks from ingestion of Cr(VI) based on the proposed EPA risk assessment will be problematic, as health-based screening levels for drinking water will be lower than the detection limit for any analytical method. For instance, applying EPA's risk assessment

[1] http://water.epa.gov/scitech/swguidance/ethods/bioindicators/upload/2007_07_10_methods_method_218_6.pdf

[2] http://www.epa.gov/region9/qa/pdfs/dqi/hexavalent.pdf

presented in the Draft Toxicological Review results in a drinking water Regional Screening Level of 0.043 μg/L for water, and 0.3 mg/kg, for soil (U.S. EPA, 2010a), both of which are far below the detection limit of EPA methods.

B. Gaps in EPA's Toxicokinetic Consideration of Cr(VI) for the MOA Analysis

In the NTP (2008a) study, most of the tumors in mice occurred in the duodenum, the portion of the small intestine in closest proximity to the stomach. In the small intestine, with greater distance from the point where the stomach empties, fewer tumors were observed. This is an important observation for evaluation of the MOA as it demonstrates a portal-of-entry effect. Tumors in the small intestine appear to be related to direct contact of the small-intestine epithelial tissues with high doses of Cr(VI) and illustrate that species-specific variability in GI anatomy and physiology are critical to understanding the relationship between observations in mice and relevance to low-concentration exposure in humans. For the purposes of risk assessment and extrapolation between rodents and humans, Cr(VI) dose in the target tissue (i.e., duodenal epithelium) needs to be quantified.

The Draft Toxicological Review states, "*Consideration of the bioaccessibility, bioavailability, and biochemistry of ingested Cr(VI) are* ***critically important*** *key toxicokinetic determinants of the MOA*" (emphasis added) (U.S. EPA, 2010d; p.49). We concur, but are concerned that although EPA recognizes the importance, these parameters are not taken into account in the risk assessment. As evidenced by the toxicokinetic data, the reduction of Cr(VI) in the stomach before interaction with target tissues in the small intestine is an important key event. The study of Donaldson and Barreras (1966), which clearly demonstrates the importance of reduction in the stomach prior to entering the small intestine, is discussed in detail in section 3.1.1 and 3.1.2 of the Draft Toxicological Review, yet the findings are not incorporated in any way in the risk assessment. Extracellular reduction of Cr(VI) to Cr(III) in the lumen should be taken into account using PBPK models for accurate risk assessment. While PBPK models are currently available, we concur with EPA that that these models are currently of limited utility for risk assessment (U.S. EPA, 2010d; p.63).

The findings of the NTP (2008a) study highlight the need for additional pharmacokinetic (PK) data to determine target tissue dose and to allow for extrapolation from the high doses that overwhelmed the reductive capacity of the rodent stomach (>5,000 μg/L) and likely resulted in sustained high-dose exposure to the small intestine, to the very low levels of exposure to Cr(VI) that occur in the environment (<5 μg/L). Furthermore, the fact that the concentrations of Cr(VI) administered in the NTP study (2008a) greatly exceed expected human exposures raises questions regarding dose-response and risk to humans at environmentally-relevant exposures. Currently available data are insufficient for one to determine the dose at which Cr(VI) is sufficiently reduced to limit key events in the small intestine that subsequently lead to tumor formation.

Moreover, questions remain as to whether Cr(VI) is taken up more rapidly or sequestered in tissues of the small intestine of the mouse as compared to the rat, and whether interspecies differences in kinetics produced the observed differences in tumor formation. As stated in the Draft Toxicological Review:

> *The accuracy of any dose-response analysis would be improved by greater rigor afforded the characterization of the reduction capabilities, and this may be especially important for interspecies extrapolation. This will likely need to involve a more physiologically-based description of GI uptake as reduction is a function of physiological factors affecting lag time (peristalsis) and spatial distribution in the GI tract* (U.S. EPA, 2010d; p. 49).

We concur with this statement and are developing data to fill this critical data gap as part of the Cr(VI) MOA Research Project (See Attachment D of the ACC comments). We believe that it is premature to finalize development of an RfD or oral cancer slope factor without this information.

Active transport of inorganics and minerals and absorption occur primarily in the duodenum. Hence another important question to consider is whether the epithelium of the mouse small intestine absorbs Cr(VI) more efficiently than the mouse epithelium of the stomach or forestomach, which might explain why tumors were not observed in the stomach or forestomach of the mouse, even though these tissues are expected to have experienced higher exposures than the small intestine. If Cr(VI) was acting by a mutagenic MOA, one would assume that tumors would form in these tissues for which the exposure was greatest and significant accumulation of chromium (Cr) occurred.

1. Additional data needed on the bioaccessibility of ingested Cr(VI)

A 2002 Society of Toxicology (SOT) presentation provides rates of Cr(VI) reduction using real and simulated gastric fluid for varying human exposure conditions (Proctor *et al*., 2002a). This study is discussed in comments prepared by Dr. Sean Hayes of Summit Toxicology (Attachment D of ACC comments). Proctor *et al.* (2002a) was not cited in the Draft Toxicological Review but provides data that are important for evaluating bioaccessibility of ingested Cr(VI). Importantly, it provides data on the rate of reduction, as well as the capacity for reduction under varying GI conditions. While data for reduction capacity is well described in the Draft Toxicological Review; reduction rate should be discussed in greater detail because the rate of reduction must be considered relative to the rate of absorption and GI transit rate to assess internal dosimetry. Total reduction capacity has limited application in quantitative risk assessment for Cr(VI) but reduction rate may be used to parameterize PBPK models and assess target tissue dose under varying conditions.

In simulated gastric conditions, Proctor *et al*. (2002a) measured a half-life of 0.7 to 10 minutes for the reduction of Cr(VI) to Cr(III) depending on gastric conditions. Proctor *et al.* (2002a) found that low concentrations of Cr(VI) would be reduced in real human stomach fluid within minutes under fasting conditions, that reduction capacity was greatly increased with the addition of food and that reduction capacity was increased at lower pH. Importantly, an expanded and advanced *ex vivo* reduction rate study is underway as part of

the Cr(VI) MOA Research Project. This study involves use of real human, rat and mouse gastric fluid in fasted and fed conditions and advanced analytical techniques to refine the measures of reduction. These studies will be published in the peer-reviewed literature, presented at the 2011 SOT conference and will be made available to EPA as developed.

EPA includes a claim by Zhitkovitch (2005) that that reductive capacity measures of De Flora *et al.* may have been overestimated due to the addition of acid for the colorimetric analysis (U.S. EPA, 2010d; p.24). Zhitkovitch provides no data to back-up this comment, thus EPA should consider this statement as speculative. Even if capacity were to be overestimated, it does not discount the overwhelming evidence that Cr(VI) is readily reduced to Cr(III), significantly limiting the bioaccessibility and toxicity of Cr(VI).

Finally, we note that on page 211, when considering the evidence for a threshold for carcinogenicity, EPA cites the discussion of Stout *et al.,* (2009) wherein the authors conclude that tumors occurred at doses that are less than an estimated mouse stomach reductive threshold proposed by Stout *et al.,* (2009). However, this values is based on the DeFlora *et al.,* (1997) reduction capacity data. This is the same data Zhitkovitch (2005) argues is overestimated. It is contraditory for EPA to challenge the validity of these reduction capacity data based on the discussion of Zhitkovitch (2005), and then rely on the same data analyzed, as analyzed in the discussion of Stout *et al.,* (2009), to argue that no threshold exists due to reductive capacity.

We do not concur with the mouse reduction rate estimates proposed by Stout *et al.,* (2009), and believe that there is clear evidence that all doses of the NTP study saturated the reductive capacity of the stomach. As discussed in Thompson *et al.,* (2010), the reductive capacity as estimated by tissue accumulation data, appears to occur around 3 mg/L in rodents. Hence, the evaluations of Stout *et al.,* (2009) of the NTP data with a lowest dose of 5 mg/L, will not be able to observe the reductive threshold which appears to fall at lower doses. This confusion regarding low dose toxicokinetics empasizes the need for additional toxicokinetics data in the environmentally-relevent dose range.

[Note the Draft Toxicological Review states that the reaction is calorimetric, rather than colorimetric on page 24, which is a typographical error.]

2. Bioavailability of Cr(VI) in the low-dose range is critical for human risk assessment

The key events of chromium uptake and intracellular biochemistry are critical to its subsequent disposition and elimination from the body. EPA states that "*The key mechanisms of bioavailability that determine internal tissue dose include the following: uptake through cell membranes, intracellular distribution and binding to cellular macromolecules*" (U.S. EPA, 2010d; p.25). However, because Cr(VI) is much more readily absorbed into cells than Cr(III), the extracellular reduction of Cr(VI) to Cr(III) in the gut lumen before cellular absorption (bioaccessibility) is a critical kinetic process limiting

toxicity. Thus the bioavailability at high doses will not be the same as that at the lower doses.

The most critical measures of Cr(VI) bioavailability are those observed at environmentally-relevant (i.e., low) exposures in drinking water, not at high doses or by other routes of exposure (i.e., dermal, inhalation). The toxicokinetics section in the Draft Toxicological Review should be revised to primarily consider studies of lower levels of exposure that are more relevant to human exposures and focus on observations in the low dose range. The lowest drinking water concentration in the 2-year NTP bioassay of Cr(VI) was 5 mg/L Cr(VI) (NTP, 2008a). Notably the 5 mg/L Cr(VI) tested in the NTP (2008a) study exceeds the 95th percentile for Cr(VI) concentrations in U.S. drinking water by approximately 300-fold (**Figure 2**). As discussed in more detail in our comments that follow, as well as in Thompson *et al.* (2010), the NTP study data are not informative regarding bioaccessibility or bioavailability in the low dose range because even the lowest dose in the NTP drinking water study likely exceeded the reductive capacity of the rodent stomach.

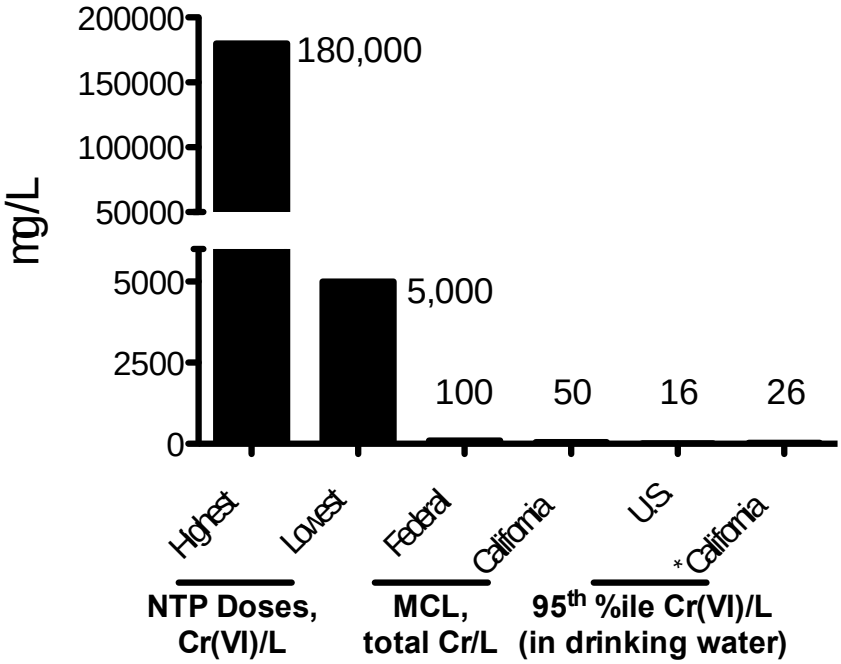

Figure 2. Drinking water concentrations of rodents in the NTP 2-yr bioassay and in U.S. drinking water. The Maximum Contaminant Level (MCL) is for total chromium, not speciated for Cr(VI). The fraction of total chromium that is Cr(VI) in drinking water is expected to be highly variable. However, if 100% of the total chromium in drinking water at the MCL were Cr(VI), the NTP doses would be higher than the standards by >50-fold (Federal) and >100-fold (California). 95th percentile for Cr(VI) in U.S. drinking water was computed from over 4,500 measurements taken nationwide between 1978 and 1996. Of these values, nearly 50% reported values of zero (assumed to be below the detection limit), which were replaced with 1 μg/L.

* Screening of over 7,000 drinking water sources in CA indicated Cr(VI) ≥ the detection limit of 1 μg/L in about 1/3 of the sources. The 95th percentile shown for Cr(VI) in CA drinking water was computed only from those sources with Cr(VI) ≥ 1 μg/L. This value was computed from over 12,000 measurements (excluding non-detects) taken between 1997 and 2008a (updated 02/17/10); the data are available at: (http://www.cdph.ca.gov/certlic/drinkingwater/Pages/Chromium6sampling.aspx).

The NTP 21-day toxicokinetic study evaluated kinetics at lower Cr(VI) concentrations (NTP, 2007). Shown in **Figure 3**, NTP (2007) data indicate no elevation of blood Cr concentrations in three rodent species exposed to ≤ 3 mg/L Cr(VI) for 21 days. Sutherland *et al.* (2000) also suggest that dose-dependent transitions in Cr disposition occur in rats at concentrations exceeding 3 mg/L Cr(VI) in drinking water for 9 months. As cited in the Draft Toxicological Review, Sutherland *et al.* (2000) found significant tissue accumulation at the 3 and 10 mg/L Cr(VI) exposure levels, with the effect most pronounced at 10 mg/L Cr(VI) (U.S. EPA, 2010d, p. 36), but no increases of Cr in any tissue at 0.5 mg/L.

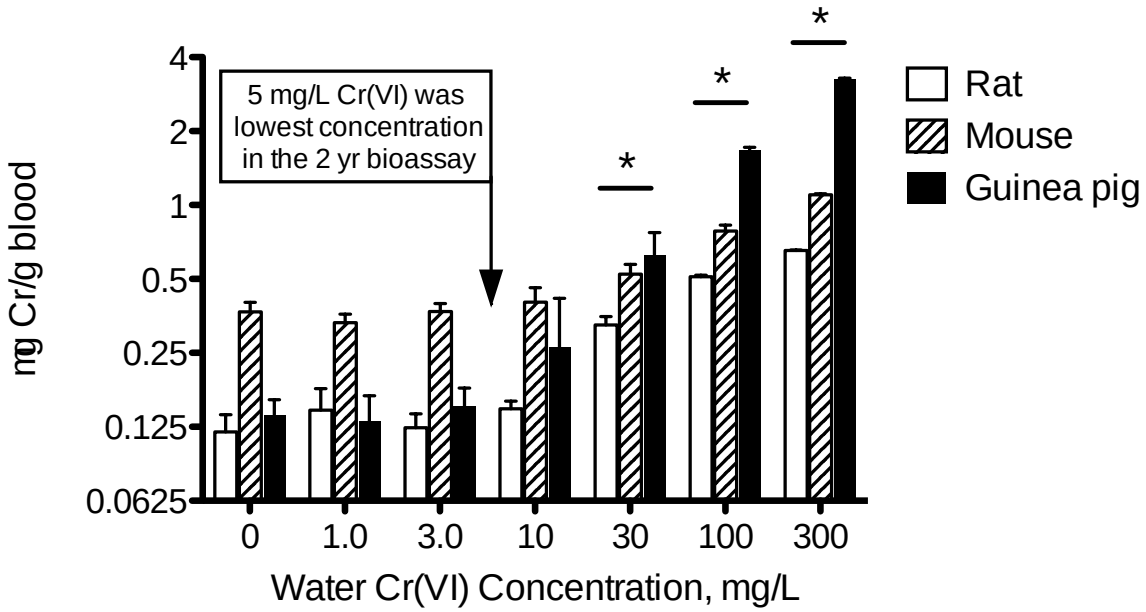

Figure 3. Data represent whole blood total Cr concentrations in rodents after 21 days of exposure (NTP, 2007). Concentrations ≥ 30 mg Cr(VI)/L (*) resulted in significant (p<=0.05) increases in blood Cr relative to control group by Dunnett's test. Tests for linear trend were positive for all species as argued in Stern (2010); however trend tests were negative for all three species when the highest three concentrations were omitted.

Thus, these data suggest that at lower doses, Cr(VI) is sufficiently reduced to the less-absorptive Cr(III) in the stomach such that no increase in Cr in tissues occurs. Additionally, these data suggest that there is a threshold for reduction at exposures below those tested in the NTP (2008a) study.

Collins *et al.* (2010) and Stout *et al.* (2009a) argue that the reductive capacity of the stomach is not exceeded at any dose of the NTP study due to the lack of a sublinear accumulation of Cr in tissues at lower doses. This argument is presented on page 211 of the Draft Toxicological Review. However, a linear trend in tissue accumulation would also be expected if all of the doses of Cr(VI) tested exceeded the reductive capacity of the GI tract as may likely be the case in the NTP drinking water study (2008a) (Thompson *et al.,* 2010). Had the NTP drinking water study included lower doses of Cr(VI), non-linear accumulation of Cr in tissues would likely have been observed was in these other studies, which by the argument posed by Stout *et al.* and Collins *et al.*, would support the existence

of a threshold based on the reductive capacity of the upper GI tract. With regard to Sutherland *et al*. (2000), the Draft Toxicological Review notes that "*a proportion of the Cr(VI) escaped extracellular reduction in the GI tract and became bioavailable for systemic distribution*" and proposes an alternative mechanism that "*Cr(III) formed in the GI tissues and absorbed was not cleared in the kidneys and was taken up by the cells*" (U.S. EPA, 2010d, p. 36). However, these observations are speculative because Cr was reported as total Cr and not speciated, further emphasizing the need for additional data to characterize the toxicokinetics of ingested Cr(VI). Finally, because no increase in Cr tissue concentrations occurred in any tissue at the lowest dose tested (0.5 mg/L) by Southerland *et al*., this clearly supports the existence of non-linearity in the toxicokinetics in the low dose range, and that extrapolation from the high doses tested in the NTP drinking water study to extremely low doses should be based on a nonlinear approach.

3. Consideration of interspecies differences in Cr tissue data

As discussed in the Draft Toxicological Review, species differences in tissue Cr accumulation has been observed in several studies. Tissue data from both NTP (2007, 2008a) studies indicate that far more Cr(VI) was absorbed in the mouse than the rat, suggesting that the rat had increased capacity to reduce Cr(VI) to Cr(III) in the stomach. The mice had significantly higher concentrations of Cr in the stomach, liver and blood than rats (**Figures 3 and 4**). Although intestinal Cr tissue concentrations were not measured in the NTP (2008a) study, female mice exhibited 5.6-fold and 3.6-fold higher concentrations in liver and stomach, respectively, than male rats (Collins *et al.,* 2010). The notable differences in liver and stomach concentrations between species suggest that the target tissue dose in the small intestine of mice was likely greater than that in rats. This may explain, at least in part, why hyperplasia was observed in the mouse duodenum at all doses in NTP (2008a) study but not in the rat. However, as demonstrated by Thompson *et al.* (2010) and reproduced here in Figure 4, the difference in tissue concentration is not merely a function of differences in administered dose.

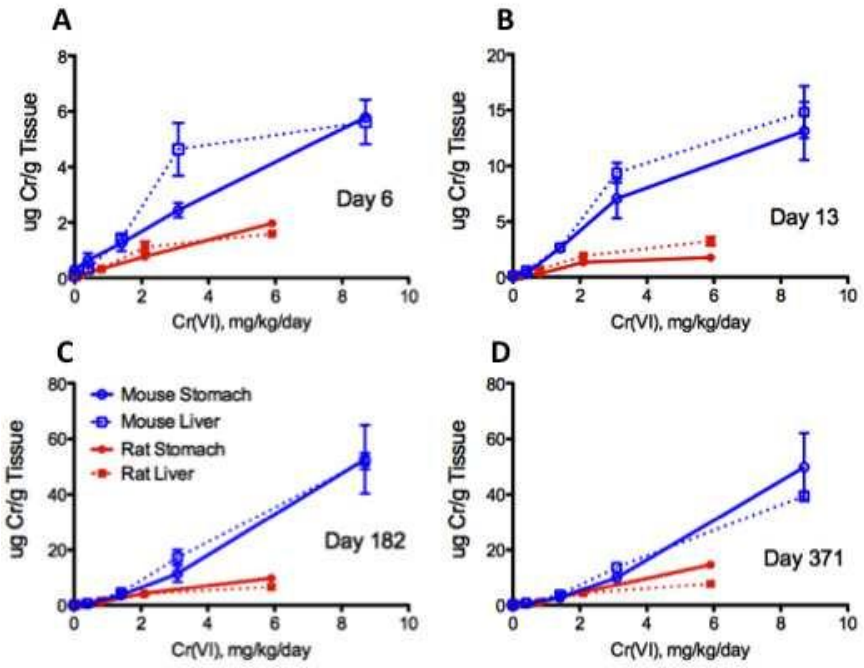

Figure 4. Total Cr in the glandular stomach and liver in male rats and female mice after 6 (A), 13 (B), 182 (C) and 371 (D) days of exposure. Tissue concentration data are mean mg total Cr/g tissue ± standard error reported in tabular form in NTP (2008aa). The mg/kg/day dose is the average daily ingested dose of Cr(VI) over the study duration as reported in Stout *et al.* (2009).

Source: Thompson *et al.* (2010)

Differences in the Cr tissue concentrations may reflect differences in extracellular reductive capacities in the GI tract of the two species and/or differences in surface area or membrane transport. Gastrointestinal reductive capacity is determined, in part, by gastric fluid composition, pH and gastric acid production rate (Thompson *et al*., 2010). Quantification of reductive capacity is important for understanding the differential responses observed in rats and mice, as well as for evaluating the potential human relevance of intestinal tumors observed in mice. Humans, with greater gastric acid production capacity than rodents are expected to be able to reduce more Cr(VI) to Cr(III) in the stomach, and thus to have an increased ability to detoxify Cr(VI). Species differences in GI tract anatomy and physiology are described in detailed in Thompson et al (Supplemental Table 2 of Thompson *et al.,* 2010) and are reproduced here as Table 2.

Table 2

Inter-species Comparison of Gastric Anatomical and Physiological Parameters						
GI Parameter	**Rat**	**Mouse**	**Swine**	**Dog**	**Humans**	**Considerations for Interspecies Extrapolation**
Diet: Carnivore or Herbivore	Herbivore[1]	Herbivore[1]	Omnivore with herbivorous tendencies[1]	Carnivore[1]	Omnivore with carnivorous tendencies[1]	As herbivores, both rats and mice consume a plant-based diet characterized by bulk and high fiber content. Gastric acid production increases and and pH decreases by species in a association with a more carnivorous diet.
Gastric Emptying: Incomplete or Complete	Incomplete[1,2]	Incomplete[1,3]	Incomplete[1]	Complete[1]	Complete[1]	In contrast to humans, the rodent stomach empties its contents onto the intestine in an incomplete manner. Species-specific data for reduction rate and capacity are critical for internal dosimetry.
Gastric Acid Production:						
Mean Output Rate (uEq/h)	10-92[4,5,6,7]	0.24-8[7,8,9,10]	2,800-10,000[11,12,13]	100[7,11,14,15]	2,000-5,000[1,11,14,16]	Based on the basal and peak rates of gastric acid output, the mouse stomach is expected to have a lower reductive capacity than that of the rat and other species examined here. As a result, a greater fraction of Cr(VI) would be transported to lower GI compartments in the mouse than the rat, consistent with the observations of the NTP study. In comparison, the basal and peak rates of gastric acid output are fairly high in humans. Even with surface area scaling (body weight)$^{3/4}$, mean and peak acid outputs for the mouse are substantially lower compared to the human gastric outputs. Peak gastric output for the carnivorous dog, relative to other species, suggests that diet is an important factor affecting species difference in gastric acid production and also likely capacity and rate of Cr(VI) reduction.
Peak Output Rate (uEq/h)	110-312[5,17,18]	20.8-28[7,8,9,10]	30,100[12,13]	39,000[7,15]	18,000-34,500[1,11,16,19]	
Scaled Mean Output Rate[a,b] (uEq/h/kg$^{3/4}$)	16.8-154.7	2.3-75.7	138.6-495.1	21.0	82.6-206.6	
Scaled Peak Output Rate[a,b] (uEq/h/kg$^{3/4}$)	185.0-524.7	196.7-264.8	1,490.4	8,199	743.8-1425.6	
Relative Size of Gastric Stomach	~10%[1]	~10%[1]	~33%[1]	~70%[1]	~33%[1]	The rodent gastric stomach represents a smaller fraction of their GI tract relative to omnivores and carnivors.
Gastric pH:						
Fasted	3.9 [20]	4.0 [20]	1.6-1.8 [10,14]	1.5 [10,14]	1.7 [1,11,14]	The capacity for reduction is increased at lower pH for solutions with consistent concentrations of reducing
Fed (during meal)	3.2 [20]	3.0 [20]	<2 [10,14]	2.1 [10,14]	5 [1,11,14]	Humans have a higher pH in the fed state compared to the fasted state, but the opposite is true for rats and mice. The higher protein human diet has a buffering effect on gastric pH.

a Mean body weights used to calculate adjusted mean and peak gastric output rates are 0.50 kg (F344 strain rat), 0.05 kg (B63CF1 strain mouse), 55 kg (sw ine), 8 kg (beagle), and 70 kg (adult male).
b Mean and peak gastric output rates adjusted by body weight were calculated by dividing the upper and the lower bound values of gastric output rates by (mean body weight kg)3/4

Source: Thompson *et al.* (2010)

1 de Zwart, L. L., Rompelberg, C. J. M., Sips, A. J. A.M., et al. 1999. Anatomical and physiological differences between various species used in studies on the pharmacokinetics and toxicology of xenobiotics. The National Institute of Public Health and the Environment, Bilthoven, the Netherlands: RIVM Report 623860 010.

2 Kennedy, C. and Palmer, L.S. 1927. The fundamental food requirements for the growth of the rat: coprophagy as a factor in the nutrition of the rat. J Biol Chem 76, 607-622.

3 Sakguchi, E. 2003. Digestive strategies of small hindgut fermenters. Anim Sci J 74, 327-337.

4 Kitamura, M., Sugamoto, S., Kawauchi, S., et al. 1999. Modulation by endogenous nitric oxide of acid secretion induced by gastric distention in rats: enhancement by nitric oxide synthase inhibitor. J Pharmacol Exper Therap 291, 181-187.

5 Kuwahara, Y., Sunagawa, Y., Imoto, Y., and Okabe, S. 1990. Effects of orally administered human epidermal growth factor on natural and delayed healing of acetic acid-induced by gastric ulcers in rats. Japan J Pharmacol 52, 164-166.

6 Takeuchi, K., Ukawa, H., Konaka, A., et al. 1998. Effect of nitric oxide-releasing aspirin derivative on gastric functional and ulcerogenic responses in rats: Comparison with plain aspirin. J Pharm Exp Therap 286, 115-121.

7 Tibbitts, J. 2003. Issues related to the use of canines in toxicologic pathology --issues with pharmacokinetics and metabolism. Soc Toxicol Pathol 31 Supp, 17- 24.

8 Friis-Hansen, L., Sundler, F., Li, Y., et al. 1998. Impaired gastric acid secretion in gastrin-deficient mice. Am J Physiol 274, G561-568.

9 Ito, S. and Schofield, G.C. 1974. Studies on the depletion and accumulation of microvilli and changes in the tubulovesicular compartment of mouse parietal cells in relation to gastric acid secretion. J Cell Biol 63, 364-382.

10 Wang, K.S., Komar, A.R., Ma, T., et al. 2000. Gastric acid secretion in aquaporin-4 knockout mice. Am J Physiol Gastrointest Liver Physiol 279, G448- G453.

11 Dressman, J.B. and Yamada, K. 1991. Animal models for oral drug absorption. In: Pharmaceutical Bioequivalence (Eds. Welling, P.G., Tse, F.L.S., and Dighe, S.V.). Marcel Dekker, Inc., New York, NY.

12 Merritt, A.M. and Kolts, B.E. 1983. Simultaneous pancreatic, gastric and hormonal responses to 2-deoxyglucose in the conscious pig. Q J Exp Physiol 68, 69- 75.

13 Moeller, H. and Sewing, K.-Fr. 1974. Gastric acid secretion in conscious miniature pigs. Arch Pharmacol 283, 83-92.

14 Karali, T.T. 1995. Comparison of the gastrointestinal anatomy, physiology, and biochemistry of humans and commonly used laboratory animals. Biopharm Drug Dispos 16, 351-380.

15 Soldani, G., Del Tacca, M., Ernardini, C., et al. 1984. Evidence for two opposite effects of clonidine on gastric acid secretion in the dog. Arch Pharmacol 327, 139-142.

16 Walsh, J.H., Richardson, C.T., and Fordtran, J.S. 1975. pH dependence of acid secretion and gastrin release in normal and ulcer subjects. J Clin Invest 55, 462-468.

17 Runfola, M., Rossi, S., Panunzi, S., et al. 2003. Botulinum toxin in gastric submucosa reduces stimulated HCL production in rats. BMC Gastroenterol 3, 26.

18 Wallmark, B., Larsson, H., and Humble, L. 1985. The relationship between gastric acid secretion and gastric H+, K+-ATPase activity. J Biol Chem 260, 13681-13684.

19 Fordtran J.S. and Walsh, J.H. 1973. Gastric acid secretion rate and buffer content of the stomach after eating--results in normal subjects and in patients with duodenal ulcer. J Clin Invest 52, 645-657.

20 McConnell, E.L., Basit, A.W., Murdan S. 2008. Measurements of rat and mouse gastrointestinal pH, fluid and lymphoid tissue, and implications for in-vivo experiments. J Pharm Pharmcol 60, 63-70.

4. Uncertainties in the current understanding of Cr kinetics

As acknowledged in the Draft Toxicological Review, there are notable data gaps that limit current understanding of Cr kinetics. Studies with speciated Cr measurements are needed because much of the general knowledge on Cr kinetics have been inferred from studies comparing Cr(III) to Cr(VI) compounds. The need for additional pharmacokinetic research to determine internal dosimetry in the target tissues is recognized in the Draft Toxicological Review:

> *"Quantitative descriptions of the pathways and mechanisms for this distribution, however, has been constrained by detection limits and costs of the analytical methods and would be especially informed by time course studies of speciated chromium content*" (U.S. EPA, 2010d; p.26).

There are only three studies (Kargacin *et al*., 1993; NTP, 2007; Stout *et al*., 2009) that have compared Cr kinetics across species. None of these studies evaluated target tissue or exposures in the environmentally-relevant range. The data to characterize the kinetics and MOA of Cr(VI) are limited and indicate the need for species-specific PBPK models for the oral disposition of Cr(VI).

Because not all tissues were routinely evaluated in Cr toxicokinetic studies, blood, urine, and fecal excretion data have been used as the basis for understanding the kinetics of Cr(VI), but there is considerable uncertainty in concluding whether Cr(VI) or trivalent chromium [Cr(III)] is systemically absorbed from these data. At higher concentrations, several studies have found non-specific binding of Cr(III) to proteins on the outside of red blood cells (RBCs) to be significant. Edel and Sabbioni (1985) observed that 15% of Cr(III) in blood was associated with RBCs at 24 hours post-administration. In a study by Gao *et al.* (1993), up to 35% of Cr(III) in blood was associated with RBCs.

Human studies demonstrate variability and uncertainty in characterizing the absorption of Cr(VI) and underscore the need for refined PBPK models. A better understanding of Cr(VI) disposition in rodents through the development of PBPK models would better inform the disposition of Cr(VI) in humans, as well as provide insight as to whether the MOA for mouse tumors is relevant to humans, who are more typically exposed to much lower concentrations. Finley *et al.* (1997) reported the urinary and blood plasma results of 5 adult human volunteers who ingested Cr(VI) in deionized water at concentrations ranging from 0.1 to 10.0 mg/L. Finley *et al.* (1997) found no increases of Cr in plasma or RBC at 0.1 mg/L. The study reported average absorption increased with elevated Cr(VI) exposure. Moreover, one of the three participants in Finley *et al*. (1997) absorbed considerably more when given Cr(VI) at 10 mg/L, with 8% absorption. These data suggest that in humans, similar to rodents, there is a saturation level for stomach reductive capacity.

In Kerger *et al.* (1996), Cr(III) as chromic chloride, Cr(VI) as potassium dichromate, and Cr(III) as Cr(VI) reduced in orange juice to Cr(III) prior to consumption, were ingested by adult volunteers to examine absorption and elimination kinetics. A single dose of 5 mg Cr (in 0.5 liters deionized water) was administered and blood and urine Cr levels were followed for 1-3 days prior to and up to 12 days after ingestion. The study found transient

increases in the levels of Cr in RBCs and plasma following administration and noted that the profile of Cr in the blood and urine in the study was more consistent with absorption of Cr(III) than Cr(VI). However, this conclusion is uncertain because the study includes only a few participants and these participants appeared to have substantially different patterns of absorption.

Collectively, these data raise questions as to whether the profile of total Cr in RBCs and plasma can be used as a measure of systemic absorption of Cr(VI). Furthermore, current speculation regarding differences in half-life and elimination does not allow for identification of which form of Cr was absorbed into tissues.

5. Interspecies differences in the expression and regulation of transporter genes across the gastrointestinal tract

Data indicate that Cr(VI) is readily taken up into cells, whereas Cr(III) enters only passively, thereby leading to levels of Cr in tissues that are orders of magnitude higher in Cr(VI)-treated animals as compared to Cr(III)-treated animals (Thompson *et al*., 2010). Cr(VI) uptake is generally believed to be mediated by the solute carrier (SLC) 4A anion transporter family, SLC4A1 in particular, based on findings that erythrocytes express high levels of the SLC4A1 anion transporter and readily take up Cr(VI) (Cohen *et al.,* 1993; Markovich, 2001). Studies in rats indicate that SCLC4A1 is abundantly expressed in erythrocytes and kidney, but comparatively limited elsewhere. However, within the rat GI tract, SLC4A1 expression was found to be highest in the duodenum (Kudrycki *et al*., 1990). The SLC4A2 family member is more abundantly expressed than SLC4A1 in the rat small intestines, with relative levels as follows: distal small intestine < proximal small intestine ≅ duodenum << large intestine < stomach (Kudrycki *et al*., 1990)..

Thus, the differences in Cr(VI) uptake across species might be due to the differences in expression and regulation (e.g. by intracellular pH) of the SLC4A genes in the GI tract. Moreover, as there are numerous SLC genes (and other anion transporters), a comprehensive review of their function [e.g. ability to transport sulfate and/or Cr(VI)] and expression is important for risk assessment given the differences in tumor site location but rodent species and is not addressed in the Draft Toxicological Review.

C. Refining the Existing Physiologically-Based Pharmacokinetic (PBPK) Models

PBPK modeling is essential for accurate extrapolation across species for purposes of developing a scientifically defensible risk assessment. The Draft Toxicological Profile states:

> *By employing chemical- and species-specific parameter values for tissue volumes, process rates, and reaction kinetics, PBPK models are used to extrapolate internal dosimetry of chemicals across routes of exposures, dose ranges and species. In risk assessment, the use of PBPK models quantitatively reduces uncertainties in*

these extrapolations, thus partially and completely obviating the need to apply uncertainty factors (UFs) in the derivation of exposure limits protective of cancer and noncancer effects (Clewell and Anderson, 1985)" (U.S. EPA, 2010d; p. 52).

We concur; PBPK models are essential for quantifying the extremely important interspecies differences in kinetics between rodents and humans, and for extrapolation from the high exposures of the NTP drinking water study (2008a) study to environmentally-relevant exposures. In addition, PBPK models can be used for evaluation of sensitive subgroups with reduced gastric acid production and/or conditions that result in a more neutral pH stomach. Correct assessment of risk in the low dose range and for sensitive human subpopulations requires use of PBPK modeling for Cr(VI).

1. Expanding upon existing PBPK models to quantify species-specific reduction rate and capacity measures

The default approaches used in the Draft Toxicological Review do not account for interspecies differences in GI anatomy and physiology, both of which specifically affect target tissue dose, nor do they account for potential differences across a range of concentrations. It is essential that these factors be addressed in the course of developing the RfD and oral cancer slope factor for ingested Cr(VI). As already discussed in other comments, PBPK modeling is the best approach for addressing such factors. However, there is no suitable PBPK model currently available. The models published by O'Flaherty *et al.* (1996, 2001) are not sufficiently detailed to accommodate the requirements of the current risk assessment because they do not have a small-intestine compartment and are limited to humans and rats. EPA acknowledges the limitation of the current models in the Draft Toxicological Review stating that "*neither the rat nor human version of the model in its present form has been subjected to formal computerized optimization of parameter values*" (U.S. EPA, 2010d; p. 62). It is clear from the Draft Toxicological Review that EPA concurs with the value of a PBPK model for risk assessment of Cr(VI). As noted previously, models to substantially improve the risk assessment of Cr(VI) are under development as part of the Cr(VI) MOA Research Project.

2. Quantifying internal dosimetry in target tissues for the cancer and noncancer risk assessments

Interspecies variability between rats and mice might be significant, and extrapolations to humans are not likely to be accurately described by simplistic scaling approaches. As discussed above, the tumors observed in the small intestine of NTP (2008a) mice likely represent a portal-of-entry effect associated with direct contact of Cr(VI) with epithelial cells lining the lumen of the small intestine. As such, both the rate of reduction and capacity for reduction directly affect target tissue dose and the potential for Cr(VI) to pose a cancer hazard. Simple $BW^{3/4}$ scaling is not likely to be the correct approach for scaling from animals to humans. Instead, as already discussed, scaling between species should be based on PBPK models. Determination of internal dose in the target tissue, will allow one to better assess the direct relationship between dose and response. More specifically, the

major advantage of evaluating dose-response relationships on the basis of internal or delivered dose is that doing so provides a stronger biological basis for extrapolating and comparing responses across studies, species, routes, and dose levels (U.S. EPA, 2006a).

The use of a PBPK model is the most appropriate way that target tissue dose in the small intestine of mice can be scaled to target tissue in humans. In fact, EPA states:

> *Among the compartmental pharmacokinetic models, PBPK models are the most appropriate and useful for conducting the extrapolations needed to derive reference values because they model the underlying physiological and chemical processes that determine chemical disposition, and they can be used to predict target organ concentrations for hypothetical exposures (Krishnan and Andersen, 2001; Andersen, 1995; Leung, 1991; Rowland, 1985; Himmelstein and Lutz, 1979). By simulating the kinetics and dose metric of chemicals, PBPK models can reduce the uncertainty related to interspecies, intraspecies, route-to-route, duration, and high-dose to low-dose extrapolations needed to derive RfC, RfD, and cancer unit risk estimates (U.S. EPA, 2006a; p. 2-9).*

EPA acknowledges that use of PBPK modeling would decrease the uncertainty in both the RfD and oral cancer slope factor and we concur. The Cr(VI) MOA Research Project will provide critical information to fill many of EPA-identified data gaps and, in so doing, will improve EPA's ability to extrapolate findings observed in laboratory animals to humans. EPA should include the findings of the toxicokinetic research and use PBPK models currently under development.

D. Deficiencies in EPA's MOA Analysis

Currently, there is insufficient data to allow for a definitive determination of the MOA underlying the development of tumors in the small intestine of mice exposed to Cr(VI) at the high concentrations administered in the NTP (2008a) study. Additionally, there is insufficient data to determine whether the MOA underlying the small intestinal tumors would be operational at environmentally relevant exposures. Despite this lack of sufficient data, in the course of developing the Draft Toxicological Review, EPA conclude that:

> T*he hypothesized mode of action for carcinogenicity induced by hexavalent chromium is via mutagenesis. The hypothesis is that carcinogenicity can be induced directly by reduced forms of chromium interacting with DNA to form adducts and crosslinks that can lead to DNA breaks and mutations, and indirectly by free radical species generated during the reduction process than can also lead to DNA breakage and mutagenesis* (U.S. EPA, 2010d; p.202).

This conclusion is perplexing given the strong evidence in the NTP drinking water study that proliferation is likely to be an early and necessary key event underlying Cr(VI)-induced carcinogenesis of the mouse small intestine. The NTP drinking water study findings also provide evidence for the temporal sequencing of subsequent key events. If Cr(VI) was acting as a mutagen, the early hyperplasia, evident by 90 days, should result in a short time-to-tumor. However, the time-to-tumor formation was extended, and treatment did not effect survival (i.e., animals were not dying early as would be expected if tumors developed early in life) in the NTP drinking water study.

Moreover, with a mutagenic MOA, one would expect neoplasms at sites of contact with the highest Cr(VI) concentrations (e.g., the glandular stomach and forestomach). The only logical argument for the absence of these tumors, and duodenal-to-ileal progression of tumors as a function of dose, is that Cr(VI) reduction in the more proximal portions of the alimentary canal lowered the dose to proximal tissues, and there was either greater uptake in the small intestine epithelium or greater sensitivity, as compared to the epithelium of the stomach or forestomach. Additionally, there is no evidence of systemic carcinogenesis despite the accumulation of tissues such as the liver and kidney.

Environmentally relevant doses of Cr(VI) would not likely provide a sufficient dose of Cr(VI) to the small intestine to induce regenerative hyperplasia and thus carcinogenesis. The temporal progression of responses observed in the NTP drinking water study indicates histiocytic infiltration, and perhaps an inflammatory response, in rats and mice by 90 days; hyperplasia in mice at non-tumorigenic[3] and tumorigenic doses at 90 days; and tumorigenesis in mice at the highest doses with at 2 years (NTP, 2007, 2008a). These data indicate a multi-step progression that is more consistent with the rarity of these tumors and their long latency. Thus, the assumption of a mutagenic MOA is premature and cannot be supported without evidence from additional studies designed to specifically differentiate the MOA.

1. Insufficient evidence to support EPA's assumption of Cr(VI)-induced is acting through a mutagenic MOA

In developing the Draft Toxicological Review, EPA cites DNA damage in various tissues *in vitro,* or by oral gavage exposure. The positive findings from drinking water exposures are primarily negative and the sparse positive findings do not provide evidence of a mutagenic change because they were reported in tissues that did not demonstrate a neoplastic response in the NTP (2008a) study. For example, Devi *et al.* (2001) reported increased DNA damage in leukocytes via the Comet assay within 24 hour of exposure. Wang *et al.* (2006) reported increased DNA damage in peripheral lymphocytes via the Comet assay at days 1 and 5 of administration. Coogan et al. (1991) reported DNA-protein crosslinks in rat liver and splenic lymphocytes. In the NTP (2008a) study, chronic exposure to doses comparable to those employed by Devi *et al.* and Wang *et al.* did not result in tumors in any tissue other than the small intestines of mice in the NTP drinking water study. Further, there were no apparent toxicities to leukocytes or lymphocytes in mice in the NTP drinking water study.

Additionally, it is important to note that Wang *et al*. (2006), Devi *et al*. (2001), and Sekihashi *et al*. (2001) examined DNA damage using Comet assays. However, Comet assays measure genotoxicity and not mutagenicity, and a "genotoxic" agent does not necessarily cause tumors by a mutagenic MOA. Mutagenicity refers to the induction of permanent transmissible changes in the structure of the genetic material of cells or organisms; these changes (mutations) may involve a single gene or several genes. Genotoxicity is a broader

[3] Using concurrent controls

term that refers to the ability to interact with DNA and or the cellular apparatus that regulates the fidelity of the genome (e.g. spindle apparatus and topoisomerase enzymes). EPA needs to recognize the distinction between genotoxicity and mutagenicity in test results. The nature, type and biological relevance of the test system used for the study of the activity of Cr(VI) must be considered carefully when assessing the relative weight of the evidence for or against mutagenic activity.

Furthermore, positive findings in the studies cited by EPA may reflect oxidative stress rather than direct Cr(VI) DNA damage. The gavage doses used by Devi *et al.* [i.e., 0.21-26.9 **mg**/kg Cr(VI)] and Wang *et al*. [i.e., 8.8-35.4 **mg**/kg Cr(VI)] were likely sufficient to exceed the reductive capacity of the GI tract, and thus may have resulted in increased absorption of Cr(VI) which in turn may have lead to oxidative stress as reported by Bagchi *et al*. The studies by Bagchi and colleagues demonstrated high levels of Cr(VI)-induced oxidative stress in the tissues where genotoxicity was observed (Bagchi *et al*., 1995, 1997, 2002). In fact, the authors ascribed the DNA damage to oxidative stress (Bagchi *et al*., 1995, 1997, 2002).

Although Cr(VI) has been shown to be genotoxic *in vitro*, *in vivo* genotoxicity has not been consistently demonstrated (Nickens *et al*., 2010). In fact, positive *in vivo* genotoxicity findings are typically only evident with irrelevant routes of administration, such as intraperitoneal (i.p.) dosing (Shindo *et al*., 1989; Mirsalis *et al*., 1996; De Flora *et al*., 2006). Studies have demonstrated that route of administration is clearly a critical factor: with i.p. administration (up to 80 mg/kg) of potassium dichromate giving positive results and oral gavage (up to 320 mg/kg) giving negative results in two strains (MS/Ae and CD-1) of male mice (Shindo et al., 1989). This is a critical observation for assessing the potential cancer risk posed by oral exposures of humans to Cr(VI), because i.p. administration bypasses the detoxification that occurs with the reduction of Cr(VI) to Cr(III) in the GI tract, prior to systemic absorption.

Further, while EPA used *Wang et al., 2006; Devi et al., 2001; Sekihashi et al., 2001; Coogan et al., 1991* to demonstrate Cr(VI)-induced mutagenicity, most *in vivo genotoxicity* studies involving drinking-water exposure of both humans and animals suggest otherwise. Genotoxicity results for Cr(VI) administered by drinking water are generally negative (Shindo *et al*., 1989; De Flora *et al*., 2006, 2008; Mirsalis *et al*., 1996; Kuykendall *et al*., 1996; NTP, 2007). For example, no effects were observed on DNA cross-linking in leukocytes in volunteers who ingested a bolus dose of 5 mg potassium dichromate in 0.5 L of water (10 mg/L) (Kuykendall *et al*., 1996). De Flora *et al*. (2006) evaluated the effect of Cr(VI) exposure on micronucleus frequency in adult mice exposed via drinking water, and offspring of dams exposed to sodium dichromate dihydrate at concentrations up to 500 mg/L for up to 210 days. No effects on micronucleus frequency were observed in bone marrow, liver, or peripheral blood (De Flora *et al*., 2006). Results were also negative for a micronucleus test in bone marrow of mice administered Cr(VI) in drinking water at a concentration of 20 mg/L (Mirsalis *et al*., 1996). Most importantly, the only available target tissue genotoxicity data (De Flora *et al*., 2008) demonstrate that, at exposures far higher than current drinking-water exposures in California, Cr(VI)-induced genotoxicity. was not present. De Flora *et al.* (2008) found no significant changes in either DNA-protein crosslink

(DPX) or 8-hydroxy-2´-deoxyguanosine (8-OH-dG) formation in the mouse forestomach, glandular stomach, or duodenum. This *in vivo* study by De Flora *et al*. (2008) exposed SKH-1 hairless mice to 5 or 20 mg/L Cr(VI) in drinking water for 9 months. These doses were not tumorigenic during the 9-month study, and no oxidative DNA damage was observed in the mouse intestine. The lack of evidence for oxidative DNA damage could indicate that these doses did not cause oxidative stress, or that or that these exposures were insufficient to induce DNA damage. De Flora *et al.* also obtained mucosal scrapings from the forestomach, glandular stomach, and duodenum of untreated animals and measured DPX and 8-OH-dG *in vitro* and found that in contrast to the *in vivo* findings, Cr(VI) significantly increased DPX and 8-OH-dG *in vitro* (De Flora *et al*., 2008).

With regards to the study by De Flora and colleagues (2008), EPA states the following in the draft Toxicological Review:

> *It is worth noting the absence of positive findings in De Flora et al. (2008) given that the highest dose evaluated in this study is slightly less than chronic dosages associated with neoplasms of the oral cavity in rats (5.9-7.0 mg hexavalent chromium/kg-day), and slightly greater than those associated with neoplasms of small intestine in mice (2.4-3.1 mg hexavalent chromium/kg-day (NTP, 2008)* (U.S. EPA, 2010d; p.177).

An important caveat regarding this statement is that De Flora et al (2008) used a different strain of mice (SKH-1hairless mice) than was used in the NTP drinking water study and, as such, additional studies are needed to characterize genotoxicity in the strain of mice used in the NTP study and at doses that caused tumors. These studies are currently underway as a part of the Cr(VI) MOA Research Project. However, the current weight of evidence dose not support that Cr(VI) acted by a mutagenic MOA as no DNA damage occurred in target tissue of mice following 9 months of exposure at doses that were consistent with those that caused cancer in NTP study mice. Genotoxicity data in non-target tissue—tissues that have never demonstrated tumorigenesis *in vivo*—by unnatural routes of exposure or *in vitro*, should not be considered as evidence that Cr(VI) induced tumors by a mutagenic MOA, when considering that the relevant genotoxicity data are negative and the only target tissue genotoxicity data are negative.

2. Errors in reporting of genotoxicity results

The Draft Toxicological Review contains errors with regards to the reporting of genotoxicity test results. Kirpnick-Sobol *et al.* (2006) exposed pregnant mice to either potassium dichromate (Cr VI; 62.5 or 125.0 mg/L) or chromium (III) chloride (1875 or 3750 mg/L) in drinking water during gestational days 10 to 20. Both Cr(VI) and Cr(III) resulted in significant increases in the frequencies of large-scale DNA deletions in pups examined at 20 days of age. Additionally, embryos exposed to Cr(III) had significant increases in DNA deletions at 3-fold lower chromium tissue concentrations than in embryos exposed to Cr(VI). Kirpnick-Sobol *et al.* (2006) concluded that, although only small amounts of Cr(III) were absorbed, Cr(III) was highly effective at inducing DNA damage. However, EPA inappropriately cited Kirpnick-Sobol *et al.* (2006) as evidence of Cr(VI)-induced chromosomal damage (U.S. EPA, 2010d, p. 167, 176). Table 4-23 of the Draft Toxicological Review (U.S. EPA, 2010d; p.167) must be revised to include results of Cr(III) administration for Kirpnick-Sobol *et al*. (2006), and the text pertaining to Cr(VI) in

the Draft Toxicological Review (U.S. EPA, 2010d, p. 176) should be removed because this study is not specific to Cr(VI)-induced DNA damage.

Additionally, the genotoxicity test results from the 2007 NTP 90-day study of sodium dihydrate dichromate administered to F344/N rats, $B6C3F_1$ mice, BALB/c mice, and am3-C57Bl/6 mice were not presented accurately in the Draft Toxicological Review. More specifically, in Table 4-23 of the Draft Toxicological Assessment (U.S. EPA, 2010d; p.168) the micronuclei test result for the NTP (2007) study is labeled as ± for $B6C3F_1$ mice at 87.2 mg/L Cr(VI) dose. However, the NTP study (2007) showed no significant increase in micronucleated normochromatic erythrocytes (NCE) in either male or female $B6C3F_1$ mice exposed to ~20-350 mg/L Cr(VI). The NTP (2007) also carried out a comparative study, where three strains of male mice were exposed 90-days to roughly 20, 45, and 90 mg/L Cr(VI). In the comparative study, $B6C3F_1$ mice showed some evidence of an increase in micronuclei formation, but the NTP judged this finding to be equivocal because no dose group showed a significant increase over the control. Following standard practice, an increase that is not statistically significant should be summarized as negative in Table 4-23.

3. EPA did not thoroughly consider alternative MOAs

EPA's Cancer Risk Assessment Guidance clearly indicates that in the framework for evaluating the MOA, possibility of other MOA also should be considered and discussed (U.S. EPA, 2005a; p.2-46).

<u>Evidence for a proliferative MOA</u>

On page 211 of the Draft Toxicological Review, there is a brief discussion regarding potential alternative MOAs. Therein, EPA briefly considers whether a non-mutagenic MOA involving tumor formation as a consequence of persistent intestinal tissue damage and proliferation might explain the tumors observed in mice. However, EPA dismisses this potential MOA, stating, *"the NTP noted that no evidence of tissue damage or necrosis was observed in these animals…"* (U.S. EPA, 2010d; p.211). However, this is not accurate. While the NTP studies did not characterize the intestinal tissue as necrotic, they clearly state in both the 90-day and 2-year bioassays that the diffuse hyperplasia appeared to be secondary to tissue injury. Text from those studies is provided below:

NTP (2008a):
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 numbers of epithelial cells with increased numbers of mitotic figures. Collectively, these lesions are considered consistent with regenerative hyperplasia secondary to previous epithelial cell injury.

NTP (2007):
There were also increased numbers of mitotic figures in the hyperplastic epithelium. In addition, the epithelial cells lining the tips of the villi of many of the exposed mice were swollen and had vacuolated cytoplasm. Collectively, these duodenal lesions suggest regenerative hyperplasia secondary to previous epithelial cell damage or degeneration.

Regenerative hyperplasia preceded tumor formation in both dose and time (Figure 5), suggesting that proliferation is a key event necessary for tumor formation.

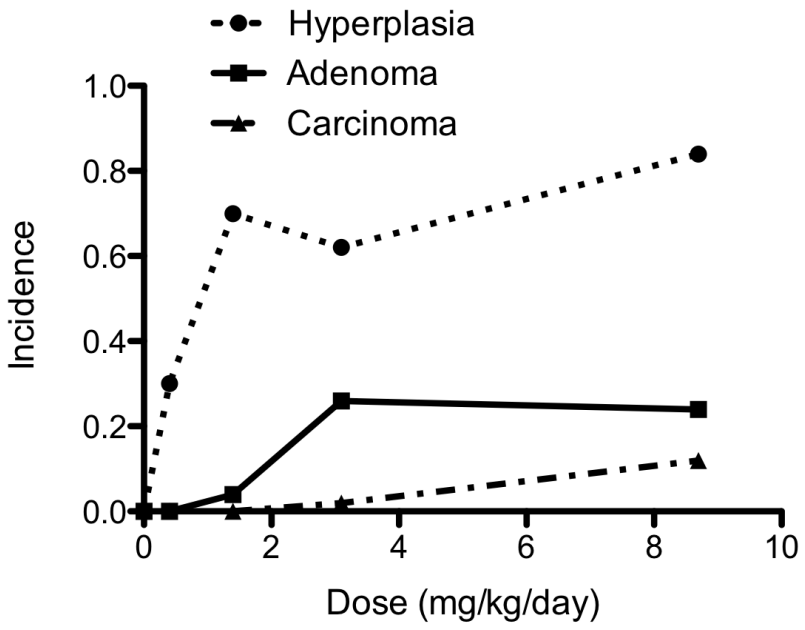

Figure 5. The incidences of hyperplasia, adenoma, and carcinoma observed in the NTP (2008a) mice

These findings indicate that the mouse intestinal lining was experiencing tissue damage and proliferation by as early as 90-days of initial exposure based on the NTP (2007) data. Notably, EPA does not provide rationale as to why necrosis is *the* form of cell death that is required for a proliferation-based MOA. Induction of apoptosis (see next comment) or premature terminal differentiation in villous cells could also lead to proliferative pressure in crypt cells.

Importantly, diffuse hyperplasia was not observed in the small intestine of rats, further supporting the hypothesis that it represents a causative or contributing factor in the mouse intestinal tumors. The absence of proliferation in the rat small intestine likely indicates a differences in toxicokinetics or toxicodynamics or both. These differences impart significant uncertainty as to whether the tumors in mice are relevant to humans. Given that there is very little information currently available regarding intestinal responses to Cr(VI), the MOA is unknown. Moreover, molecular investigations into the responses of the intestines in rats and mice could clarify the role of cytotoxicity, cell proliferation and perhaps DNA damage and mutation. In this regard, there is potential for such investigations to show that intestinal proliferation is a requisite key event in the intestinal carcinogenicity of Cr(VI). If so, it is possible that the cancer and noncancer effects of Cr(VI) in the intestines could be harmonized by the use of an RfD based on cell proliferation or some other precursor event such as cytotoxicity. Such studies are currently underway as a part of the Cr(VI) MOA Research Project.

Evidence that EPA's MOA is nonlinear

Discussions provided on pages 211-212 of the Draft Toxicological Review require clarity. EPA acknowledges that apoptosis might occur as a result of oxidative stress, altered signaling pathways, or genotoxic damage in the absence of visible pathology. Moreover, the Agency cites arguments made by Nickens *et al.* (2010) and others (e.g. Holmes *et al*., 2008; Thompson *et al.,* 2010), that chromium might confer phenotypes including a selection process for mutation prone cells. EPA then states:

> *Therefore, rather than an alternate mode of action per se, apoptosis induced by hexavalent chromium exposure is considered here to be a key event in the carcinogenic process* (U.S. EPA, 2010d; p. 212).

This implies that EPA's MOA requires that normal cells die in order to provide an advantage to initiated cells. It is not clear then whether this is truly a mutagenic MOA or rather a MOA involving killing of normal cells so that initiated cells, presumably with some resistance to the cytotoxic effects of chromium, can have a growth advantage. It also suggests that the cell proliferation observed is either irrelevant to the MOA or that the proliferation is a direct consequence of having selected for some population of altered or initiated cells. Considering that proliferation (but not tumors) was observed as early as 90 days, the possibility of a selective advantage due to proliferation seems unlikely. Furthermore, EPA's MOA undermines the rationale for linear low-dose extrapolation based on the "one-hit" hypothesis, because the Agency's MOA for Cr(VI) requires induction of apoptosis to provide for a selection process. In fact, if EPA's hypothesized MOA is correct, it is conceivable that the rat animal model proves that the MOA is in fact nonlinear. That is, data from the NTP studies indicate that Cr(VI) was likely delivered to the small intestine at some dose level, yet apoptosis or cell death was not induced and neither were tumors.

Epigenetics mechanisms not sufficiently considered in EPA's MOA Analysis

EPA cites several studies which conclude that epigenetic factors may be involved in the carcinogenicity of Cr(VI) (e.g. page 208-209). For example, there is evidence that chromium may suppress the expression of genes related to mismatch repair (MMR) pathways and thus confer a genomic instability phenotype. Such effects might then increase the risk for tumor formation without the direct induction of DNA lesions by chromium. As described in Thompson *et al*. (2010), molecular studies indicate the potential for chromium to alter DNA and histone methylation patterns, which can silence gene expression. For example, Sun *et al.* (2009) have shown that Cr(VI) exposure can increase cellular methylation of histone H3 lysine 9 (H3K9) in cultured cells, which was inhibited by pretreatment of cells with the antioxidant ascorbate (Sun *et al*., 2009). The treatment of cells with Cr(VI) produced time- and dose-dependent decreases in mRNA levels of the MMR gene MutL homolog 1 (MLH1). Interestingly, heritable changes in MMR genes such as MLH1 are associated with intestinal tumors; thus the rather rare tumors observed in mice might result from Cr(VI)-mediated changes in MMR genes without the involvement of a mutagenic MOA. At the very least, these data call into question a strictly mutagenic MOA. EPA included discussions related to MMR as evidence in support of the biological plausibility and coherence of the mutagenic MOA; but that is illogical. An alternative and equally plausible interpretation of these

findings is that chromium exposure potentiates mutagenesis by altering methylation patterns.

Lack or inconsistent evidence for EPA's hypothesized mutagenic MOA

Table 3 presents an evaluation of EPA's experimental support of the hypothesized mutagenic MOA using the Agency's MOA Framework Guidance in the Cancer Risk Assessment Guidance (U.S. EPA, 2005a).

Table 3. Evaluation of the Experimental Support for EPA's Hypothesized Mode of Action of Ingested Cr(VI)

MOA Framework for Analysis	EPA's Assessment in the Draft Toxicological Review	Inconsistent or Lack of Scientific Evidence
Strength, Consistency, and Specificity of Association	"A large database of experimental data exists on the mutagenic activity of hexavalent chromium compounds. In vitro, positive results were found in the majority of tests performed… in bacterial test systems… in yeast… in mammalian cell lines and primary cells. In vivo, most studies of the mutagenicity of hexavalent chromium yielded positive results" (U.S. EPA, 2010d; pp.203-204).	*1. In vivo* studies of drinking-water exposure to Cr(VI) in humans and animals were consistently negative (Shindo et al., 1989; De Flora et al., 2006, 2008; Mirsalis et al., 1996; Kuykendall et al., 1996; NTP, 2007).
		2. Only available target tissue genotoxicity data (De Flora et al. 2008) demonstrate that, at exposures far higher than current drinking-water exposures in California, Cr(VI)-induced mutagenicity was not present.
	"Several studies of occupational exposures via inhalation provide evidence of significant levels of chromium-induced DNA damage (Gambelunghe et al., 2003), and the formation of micronuclei (Benova et al., 2002; Vaglenov et al., 1999), chromosomal aberrations (Deng et al., 1988; Sarto et al., 1982), and sister chromatid exchange (Wu et al., 2001, 2000; Den et al., 1988; Sarto et al., 1982; Stella et al., 1982) in peripheral lymphocytes and/or buccal mucosal cells" (U.S. EPA, 2010d; p.205).	1. No studies were located regarding genotoxic effects in humans after oral exposure to Cr(VI). Kuykendall et al. (1996) is a negative genotoxicity study for oral exposure in humans. There are no positive studies that reported DNA-protein crosslinks in circulating blood lymphocytes at 10 mg/L.
		2. Recognized by EPA (U.S. EPA, 2010d; p. 178), the associations between Cr(VI) exposures and mutagenicity in workers are uncertain because of various confounding factors such as exposure levels that were often not quantified along with potential co-exposures to other compounds with mutagenic activity. These observations do not demonstrate mutagenicity as they occur in tissues for which tumors have not been observed.

Table 3. Evaluation of the Experimental Support for EPA's Hypothesized Mode of Action of Ingested Cr(VI)

MOA Framework for Analysis	EPA's Assessment in the Draft Toxicological Review	Inconsistent or Lack of Scientific Evidence
Dose-response Concordance and Temporal Relationship	"The available animal studies show that hexavalent chromium induces tumor in the tongue, oral mucosa, and intestine of rodents (NTP 2008)" (U.S. EPA, 2010d; p. 205).	1. NTP (2008a) study reported diffuse hyperplasia preceding tumor formation in both dose and time in the duodenum. Regenerative hyperplasia, secondary to previous epithelial cell injury may be suggestive of a non-mutagenic MOA. Moreover, the apparent absence of genotoxicity in the duodenum, and stomach at 5 and 20 mg Cr(VI)/L for 9 months reported by De Flora et al. (2008) argues against a mutagenic MOA.
		2. All of the drinking water concentrations used in the NTP study (as well as De Flora et al. 2008) were far in excess of typical environmental exposures and, in fact, were so large that normal protective mechanisms of gastric reductive capacity in the rodents were likely overwhelmed.
	"In three studies that used the comet assay to detect DNA following gavage exposures in mice, Devi et al. (2001) found evidence of DNA damage in leukocytes that peaked at 48 hours of postexposure, Wang et al. (2006) detected DNA damage in lymphocytes after 1- or 5-day consecutive exposures, and Sekihashi et al. (2001) detected DNA damage in stomach, colon, liver, kidney, bladder, lung, and brain within 8 hours of dosing that subsided by 24 hours post treatment " (U.S. EPA, 2010d; pp.205-206).	1. Sekihashi et al. (2001) administered potassium chromate in single gavage high dose of 85.7 mg/kg Cr(VI) which exceeded the highest doses tested in NTP (2008a) by more than 10-fold and are of questionable relevance given the dose and route of administration.
		2. The Comet assay used in the three studies measure DNA damage and not mutation, and these positive findings may relate to oxidative stress as opposed to direct Cr(VI) DNA damage.
		3. The gavage doses used by Devi et al. [i.e., 0.21-26.9 mg/kg Cr(VI)] and Wang et al. [i.e., 8.8-35.4 mg/kg Cr(VI)] were likely sufficient to exceed the reductive capacity of the GI tract, and thus may have resulted in increased absorption of Cr(VI) and resulting oxidative stress as reported by Bagchi et al. (Bagchi et al., 1995, 1997, 2002).

Table 3. Evaluation of the Experimental Support for EPA's Hypothesized Mode of Action of Ingested Cr(VI)

MOA Framework for Analysis	EPA's Assessment in the Draft Toxicological Review	Inconsistent or Lack of Scientific Evidence
Biological Plausibility and Coherence	"Based on the expected reduction capacity of an average 50 g mouse, it dose not appear that the reduction capacities were overwhelmed in the NTP bioassay. The average rate of hexavalent chromium exposure for all three strains of mice was estimated to have been 2.9 x 10-2 mg/hour at the highest dose (NTP, 2007). This rate is within the estimated reductive capacity of the mouse GI tract of 4.4 x10-2 mg/hour that is based on an estimated 0.33 mL/hour rate of drinking water consumption" (U.S. EPA, 2010d; p. 208).	1. Reductive capacity of the mouse is highly speculative and not based on any reliable measures. *Ex vivo* reduction capacity measures for humans cannot be reliably converted to reduction rate in mice using allometric scaling of bodyweight (i.e., $BW^{3/4}$). Estimates of mouse hourly drinking water consumption rate and stomach emptying time are not based on measures from the NTP study animals.
		2.Significant differences reported in gastrointestinal anatomy and physiology of rodents compared to humans have not been taken into account and mouse reductive capacity estimates are wholly unreliable.
		3.The NTP study data provides evidence of a portal of entry effect in the small intestine due to Cr(VI) escaping reduction in the stomach and being conveyed through gastric emptying to the small intestine. Clearly the evidence does not support the Agency's speculation that stomach reductive capacity was not exceeded.
	"…MMR, has recently been implicated in the genotoxic responses to hexavalent chromium exposure. It has been shown that the processing of chromium-DNA adducts by the MMR pathway is responsible for turning these lesions into frank DNA double-strand breaks (Peterson-Roth et al., 2005). This group found that cells deficient in MMR were not subject to the same toxic responses to hexavalent chromium as were cells with these repair processes intact" (U.S. EPA, 2010d; p. 208).	1. These "mutations" may be epimutations resulting from altered DNA methylation; turning off MMR genes by methylation is known to be an important mechanism in carcinogenesis. Using a cell line capable of detecting both mutations and epimutations, Klein et al. (2002) showed that about half of the apparent mutations in cells treated with 40 μM Cr(VI) were the result of DNA methylation.

Table 3. Evaluation of the Experimental Support for EPA's Hypothesized Mode of Action of Ingested Cr(VI)

MOA Framework for Analysis	EPA's Assessment in the Draft Toxicological Review	Inconsistent or Lack of Scientific Evidence
Biological Plausibility and Coherence (con't)	"In summary, DNA damage can occur following oral exposure to hexavalent chromium at doses that should be within the reductive capacity of the organism. This DNA damage may be repaired by error-prone mechanisms, resulting in DNA double-strand breaks and microsatellite instability, and further exacerbated by both hexavalent chromium-induced epigenetic effects that alter these DNA repair mechanisms and interference of DNA replication processes by hexavalent chromium" (U.S. EPA, 2010d; p.209).	1. Again, there is no definitive evidence that the doses in the NTP study were within the reductive capacity of the organism. This is based purely on hypothetical $BW^{3/4}$ scaling (see previous comment).
		2. As indicated, chromium-induced epigenetic effects may be involved in causing DNA damage from oral exposures to Cr(VI). This undermines EPA's assumption of a strictly mutagenic MOA and the Agency's low-dose linearity extrapolation.
		3. Mutations can result from DNA damage, but can also result from loss of mismatch repair and other types of genomic instability; however no data exist that can clearly elucidate the process by which Cr(VI) induces neoplastic progression. It is equally plausible that epigenetic changes (e.g., methylations) may be the potential driver that initiates the carcinogenesis process.

Table 3. Evaluation of the Experimental Support for EPA's Hypothesized Mode of Action of Ingested Cr(VI)

MOA Framework for Analysis	EPA's Assessment in the Draft Toxicological Review	Inconsistent or Lack of Scientific Evidence
Bioavailability	"Stout et al. (2009) postulated that if the threshold mechanisms proposed by De Flora et al. (2008) actually existed, then the doses that saturated the reductive capacity would likely represent an inflection point on a sublinear dose-response curve, with doses above the inflection point demonstrating an increasing rate of response per unit dose. To test this hypothesis, Stout et al. (2009) evaluated tissue concentrations and mouse small intestine neoplasm data for linearity and found that data that were statistically nonlinear were supralinear (i.e., exhibited a decreasing rate of response per unit dose), which does not support the presence of a reduction threshold" (U.S. EPA, 2010d; p. 211).	1. The lack of an inflection point in the NTP (2008a) toxicokinetic data suggests either that all of the doses or that none of the administered doses of the NTP study exceeded the rodent reductive capacity. NTP 21-day toxicokinetic study that included lower Cr(VI) concentrations (NTP, 2007) indicate no elevation of blood chromium concentrations in three rodent species exposed to ≤ 3 mg/L Cr(VI) for 21 days, suggesting that reductive capacity is lower than the lowest dose in the NTP study (5 mg/L).
		2. Sutherland et al. (2000) found significant tissue accumulation at the 3 and 10 mg/L Cr(VI) exposure levels, with the effect most pronounced at 10 mg/L Cr(VI). At 0.5 mg/L, no increase in tissue Cr accumulation occurred demonstrating reductive capacity is saturated at low doses but not high doses, which includes all the doses administered by NTP.
		3. The mouse reductive capacity was estimated using $BW^{3/4}$ scaling of estimated human reductive capacity. Bodyweight scaling is supported for metabolic reactions of systemically absorbed chemicals, not for chemically-mediated reactions and portal of entry effects.

Table 3. Evaluation of the Experimental Support for EPA's Hypothesized Mode of Action of Ingested Cr(VI)

MOA Framework for Analysis	EPA's Assessment in the Draft Toxicological Review	Inconsistent or Lack of Scientific Evidence
Other Possible MOAs	"Cells may also avoid targeted death due to changes in gene expression that lead to upregulation of pro-inflammatory and/or anti-apoptotic genes. These processes could be temporally similar to those of DNA damage and mutation, and may serve to lay the groundwork for the acquisition of other carcinogenic traits, including uncontrolled cell growth, leading to tumor formation. Therefore, rather than an alternate mode of action per se, apoptosis induced by hexavalent chromium exposure is considered here to be a key event in the carcinogenic process" (U.S. EPA, 2010d; p. 212).	1. A key event is defined as: "an empirically observable precursor step that is itself a necessary element of the mode of action or is a biologically based marker for such an element" (U.S. EPA, 2005a). Following EPA's argument for the role of apoptosis, Cr(VI) must induce apoptosis in order to result in tumorigenesis. We are unaware of any evidence that induction of apoptosis as a key event is consistent with a low-dose linear MOA. Moreover, the study upon which the entire oral assessment is based does not once refer to or otherwise imply that the tissue demonstrated apoptosis. As such, it this key even is not empirically observable, and it is therefore unclear how this can be supported as a key event in the MOA.

As described in Table 3, the current scientific evidence is for EPA's hypothesized mutagenic MOA is not consistent or absence. Further investigation of the MOA is warranted and currently underway as a part of the Cr(VI) MOA Research Project.

4. EPA's assumption that Cr(VI) acts by mutagenic MOA for all routes of exposure is not supported in the Draft Toxicological Review

EPA does not provide assessment of the MOA for cancer for any routes of exposure other than oral. In the Draft Toxicological Review, EPA presents background information and justification for hazard identification and dose-response assessments of **ingested Cr(VI) only** and not for dermal or inhalation exposures (U.S. EPA, 2010d; p.1). Without comprehensive reassessments of the carcinogenic response observed following inhalation and dermal exposures, the carcinogenic MOA for these routes of exposure cannot be determined.

5. EPA's MOA Analysis is not consistent with the Agency's Cancer Risk Assessment Guidance

The Draft Toxicological Review of Cr(VI does not adequately considered or incorporate all of the main concepts described in the Agency's 2005 Cancer Risk Assessment Guidance and the related *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA 2005b). The key features of EPA's Cancer Risk Assessment Guidance include:

1. Critical analysis of available information as the starting point for evaluation;
2. Understanding of the underlying mode of action;
3. Weight-of-evidence narrative to reach conclusions about the human carcinogenic potential of agents;
4. Two-step dose-response assessment that include (a) modeling the observed data to derive a point of departure (POD), followed by (b) extrapolation to lower exposures;
5. Identification of susceptible populations and lifestages;
6. Evaluation of risks from childhood exposures.

Because the Draft Toxicological Review does not include a complete evaluation of all available data for Cr(VI), the results are incomplete. Alternative MOAs have not been adequately addressed and the evidence to support a non-mutagenic MOA is arguably stronger than that for a mutagenic MOA. Further, because the MOA is unknown at this point in time, EPA is not justified in applying adjustments for early life stage exposures in accordance with the *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA 2005b).

6. Inconsistencies between EPA's Oral Cancer Assessment and New Jersey Department of Environmental Protection (NJDEP)'s Risk Assessment

It should be considered that although EPA used the modeling approach developed by NJDEP (NDEP 2009) as the quantitative basis for the cancer slope factor, conclusions regarding the MOA offered by NJDEP were not embraced by EPA. Specifically, NJDEP (2009) states, "*While the scientific literature provides ample data to support the conclusion that Cr 6+ can interact with DNA and can act as a mutagen, the NTP study provides evidence that additional modes of action (MOAs) may have functioned in the production of the mouse small intestine tumors*." NJDEP's conclusion is correct. EPA should reevaluate the evidence and conclude as NJDEP did that there is evidence of MOAs other than mutagenicity.

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7. EPA did not consider the pattern of responses in the mouse and rat small intestine in the context of other chemicals that elicit similar patterns.

The Hill criteria form part of the basis for the MOA Framework outlined in EPA's Cancer Risk Assessment Guidance (U.S. EPA, 2005a*)*. One of Hill criteria is analogy, which is typically referred to by risk assessors in the somewhat limited context of structural similarity or structure-activity relationships (SARs). Beyond structural similarity, similarities in biochemical properties (e.g. reactivity) and similarities in pathogenesis might also inform whether two chemicals share a given MOA. In NTP (2008a) and Stout (2009a) it is noted that the fungicide captan is the only other compound examined by NTP that resulted in both benign and malignant intestinal neoplasms of epithelial origin attributed to chemical exposure. The similarly structured fungicide folpet induces the same lesions as captan. Captan and folpet are clearly mutagenic *in vitro*, but evidence for *in vivo* mutagenicity is equivocal or negative (Arce *et al.,* 2010; Bernard and Gordon, 2000). Both captan and folpet react readily with thiols (e.g., GHS, cysteine, and proteins) and induce blunted villi and villous cytotoxicity, regenerative crypt cell proliferation, and adenomas/carcinomas in the mouse duodenum and jejunum (Cohen *et al*., 2010). However, neither chemical induces intestinal tumors in rats.

In 2004, EPA changed the Agency's cancer classification of captan from ''a probable human carcinogen'' (Category B) to ''not likely'' based, in part, on recommendations from an independent peer review of captan which concluded that captan acted through a non-mutagenic threshold MOA requiring "prolonged irritation of the duodenal villi as the initial key event'' (Gordon, 2007; U.S. EPA, 2004). As projections into the small intestinal lumen, the villi are covered predominantly with mature, absorptive enterocytes. These enterocytes are formed in the bottom of the crypts and move toward the top of the villi where they are shed. Blunting or hypertrophy of the villi and chronic inflammation, in rodents or in humans (Noffsinger and Waxman, 2007) is typical in duodenal disorders. Only the stem cell population in the crypt cells can actually evolve into malignant tumor (Potten and Loeffler,

1990; Potten *et al.,* 1990); expansion of this stem cell population in the crypts represents an increase in the number of potential cells that can develop the spontaneous genetic errors that are necessary for malignancy. Although Cr(VI) is not structurally similar to captan, it shares many of the aforementioned characteristics (i.e. reactivity, non-neoplastic lesions, neoplastic lesions, and species specificity). The similarities between Cr(VI) and captan make it plausible that they share a common MOA involving cytotoxicity in villous cells and sustained proliferative pressure on crypt cells that increase the risk of carcinogenesis. Notably, Cohen et al. (2010) concluded that the MOA in mice might be relevant to humans under chronic high dose exposures. EPA should consider these important data for captan and folpet in the Agency's evaluation of the MOA for Cr(VI). At the very least, the MOA for captan and folpet raise considerable doubt as to whether EPA can claim to know the MOA for the intestinal tumors in rodents exposed to Cr(VI) with the limited data that is currently available, and the fact that the agency very recently found that the only other chemical to produced these tumors acts by a non-mutagenic MOA.

E. Incomplete Assessment of the Weight-of-Evidence of Epidemiology Data and Questionable Human Relevance of NTP (2008a) Rodent Tumors

Epidemiological evidence does not support an association between ingestion of Cr(VI) and GI tract cancer. The epidemiological evidence involves hundreds of studies, conducted over the course of the last 60 years, including populations exposed to Cr(VI) at high levels of exposure relative to typical environmental exposures, but has not demonstrated that Cr(VI) causes cancer outside the respiratory system. Studies of environmentally-exposed populations have not demonstrated an increased risk of cancer from Cr(VI) exposure, and occupational epidemiology studies do not support an increased risk of GI tract cancer. Because Cr(VI) inhalation exposure is associated with lung cancer in certain industries, the potential for Cr(VI) to induce cancer in humans has been extensively studied, yet the findings are consistently negative for organs and tissues outside of the respiratory tract. EPA's weight of evidence conclusion that Cr(VI) is "likely to be carcinogenic to humans" is stated to be based, in part, on evidence of an association between Cr(VI) exposure and stomach cancer. However, such an association is not supported by the overall weight of evidence.

The epidemiologic literature in the Draft Toxicological Review is incomplete with respect to exposure to Cr(VI) and digestive-system cancers. As discussed here, specific existing and relevant studies appear to have been overlooked, and the omission of these studies could affect conclusions regarding human health risk associated with ingestion of Cr(VI).

1. EPA's conclusions regarding the weight of evidence for carcinogenicity in humans is not supported by the available epidemiology literature or its own analysis

Studies of human populations exposed to Cr(VI) in the environment over an extended time period are limited to those focused on populations residing in four geographic areas. EPA identified epidemiologic studies based in Liaoning Province, China (Kerger *et al.,* 2009, Beaumont *et al.,* 2008; Zhang and Li, 1997, 1987), Kings County/San Bernardino County, California (Fryzek *et al.,* 2001; Bick *et al.,* 1996), Nebraska (Bednar and Kies, 1991), and Glasgow, United Kingdom (Eizaguirre-Garcia *et al*., 2000, 1999). Exposure to Cr(VI) in these studies was presumably through ingestion of contaminated drinking water or incidental exposure to contaminated soil. Health outcomes evaluated in these studies were primarily, but not limited to, cancer incidence or mortality. EPA discussed the four studies of the human population exposed to Cr(VI) in drinking water in Liaoning, China (Zhang and Li 1997; Beaumont *et al.* 2008; Kerger *et al*. 2009) extensively in the Draft Toxicological Review and concluded that the data quality of these studies was not sufficient to support a conclusion regarding the presence or absence of a dose-response relationship among the observed cancer rates in the populations (U.S. EPA, 2010d; p. 80).

However, in the "Summary of Overall Weight of Evidence" EPA states that "*Cr(VI) is 'likely to be carcinogenic to humans' via the oral route of exposure based on…evidence of an association between oral exposure to Cr(VI) and stomach cancer in humans*" (U.S. EPA, 2010d; pp. 199-200). Although not referenced, this conclusion appears to be based solely on the epidemiologic studies of human populations in the Liaoning Province, China because neither Frysek *et al.* (2001), nor Armienta-Hernandez *et al.* (1995), nor Bednar and Kies (1991) nor Eizaguirre-Garcia *et al.* (1999) reported on risk of stomach cancer. Given the uncertainties (described below) regarding the original Zhang and Li study data which also thereby affect the studies by Beaumont *et al.* (2008) and Kerger *et al*. (2009) because they are based on the same original data, it is an overstatement to conclude that the overall weight of the evidence supports a conclusion that Cr(VI) is likely to be carcinogenic to humans via the oral exposure route. The quality of data from the Chinese villagers studies is poor and the interpretation of results is questionable. Moreover, it is contradictory for EPA to conclude that data from the Chinese studies are not sufficient to support a conclusion regarding the presence or absence of a dose-response relationship among the observed cancer rates in the human populations yet to conclude that these studies provide evidence that Cr(VI) is likely to be carcinogenic via the oral route of exposure (U.S. EPA 2010; p. 215).

2. No clear evidence that Cr(VI) caused cancer in populations exposed to Cr(VI) in the Liaoning Province of China

The original study by Zhang and Li (1987) was primarily descriptive in nature. The authors reported an increase in stomach cancer in rural villages near JinZhou, China, yet did not provide an analysis of the association between Cr(VI) exposure and health outcomes.

EPA should recognize that the Zhang and Li study data suffer from **significant** limitations, as detailed below, and these data are not reliable for assessing whether ingestion of Cr(VI) poses a cancer hazard. As both Kerger *et al*. (2009) and Beaumont *et al.* (2008) rely on the same data, both studies are subject to these limitations. These issues are not described in the Draft Toxicological Review but are very important considerations and clearly should be considered by EPA.

Issue 1:Cross-sectional study design, timing of exposure with disease

The cross-sectional study by Zhang and Li (1987) used estimates of Cr(VI) exposure based on information gathered at the time the study took place. A substantial weakness of this design is temporal ambiguity, or the lack of certainty that the exposure preceded development of the disease. Exposures reportedly started in mid-1964, and cancer mortality was ascertained from 1970 to 1978, only 6 to 14 years following initial exposure. This period of time is not sufficient for the development of stomach cancer and progression resulting in mortality. Depending on the type of exposure, a number of studies have identified the latency period for stomach cancer as being 20–50 years (Amandus, 1986; Correa, 2004). If Cr(VI) caused an increased in stomach cancer mortality, it would be expected to occur after the observation period. As the original authors were still working on papers related to this project in 1987 and 1997, one may presume that they did not observe an increase in stomach cancer subsequent to the observation period, or did not invest the time to study it. Thus, it is highly likely that the cancers observed during 1970–1978 began to develop *before* water contamination took place, thus greatly diminishing the role of the contaminated water as a causative factor in the etiology of the stomach cancers observed in this population.

Issue 2: Sampling and Analytical methods were not specified raising questions about the potential for errors due to lack of standardization

The authors did not provide a description of the methods/processes used to collect and analyze samples of well water purportedly containing Cr(VI). No standardized sampling protocol was followed. The authors do not specify when samples were analyzed in relation to when they were taken, what safeguards were taken to ensure correct measurements, whether multiple measurements were taken for each site, nor what standards were used or whether quality control samples were included. Further, the data were reported in a manner that exposure could not be assessed in either of the two extensive reassessments of these data (Beaumont *et al.,* 2008, Kerger *et al*. 2009). Maximum concentrations of 20 and 10 mg/L were reported for some villages, which equates to bright yellow water that would not likely be consumed, and is certainly not representative of current exposures to Cr(VI) in US drinking water supplies. Hence, it is questionable whether the concentrations reported were for water that was actually consumed.

Of note, the current limit of detection for Cr(VI) in water is approximately 1 μg/L. This limit has been achieved from recently developed methods that have been validated by both EPA and CDHS, using sophisticated equipment. It is highly questionable, therefore, that the researchers in China were able to measure Cr(VI) at 1 μg/L in 1965, as is reported in these studies (Beaumont *et al.* 2008).

Issue 3: Ecologic definition of exposure can lead to misclassification

The authors used an ecological measurement of exposure (i.e., community-level measurements of Cr(VI) concentrations in well water) to classify the exposures of individuals included in the study. This approach assumes that the subjects in the study consumed contaminated water, an assumption that can lead to misclassification of exposure if the assumption is not consistent with the actual circumstances. There is reason to question the assignment of exposure status. The authors state that villagers noticed that their drinking water in some areas had "turned yellowish (green while cooking) and was undrinkable." It is questionable whether residents continued to consume the contaminated water and thus, whether the assignment of exposure status is accurate. Concentrations of Cr(VI) among contaminated wells varied greatly within any village, such that a sizeable proportion of wells had minimal contamination and a significant proportion of wells had *no* contamination reported. Furthermore, contamination was restricted to certain geographic regions of the particular village and it was suggested that villagers may have been able to obtain drinking water from alternative sources and did not have to rely on contaminated well water. Thus, assignment of community-level exposure to Cr(VI) may have not accurately captured the true exposure of individuals in the study. Furthermore, no data were collected from individuals to determine their actual exposure (no questionnaire data were used to verify that individuals drank the water; no specimens were collected to analyze biomarkers).

Issue 4: Cancer mortality, not incidence, was used as the health outcome

Purported excesses in cancer were based on mortality rates—not incidence rates—for the regions studied. Mortality is a measure that is inherently dependent on prevention, detection and treatment. Comparing populations from two different areas based on mortality rates relies on the assumption that the two populations are comparable with respect to risk factors, access to health care and treatment. Villagers in rural regions may not have had the same access to medical facilities and treatment options as city dwellers, the population to whom they were compared. Furthermore, differences in gender, smoking, alcohol consumption, dietary factors, socioeconomic status (SES), and occupation, which are all relevant factors to stomach cancer risk could have existed between the study and comparison populations, but were not considered by the authors in their analyses as potential confounding factors. Thus, risk estimates of stomach cancer from consumption of Cr(VI) in drinking water reported by the authors are likely biased by the lack of adjustment for these confounding factors.

Issue 5: Use of Crude Cancer Rates

The original study authors only reported crude stomach cancer rates, and did not report rates adjusted for sex, age or any stomach cancer risk factors. Beaumont *et al.* attempted to correct these crude rates using age-adjustment ratios developed for all cancers. This adjustment is dependent on the assumption that the age-adjustment for all cancers would be consistent with that for stomach cancer. However, no correction for sex could be included. Although the ratio of men to women may be similar in each village and in the exposed and unexposed areas, it is also possible that it was not, and it is known that stomach cancer rates are far higher in men than women. In China as a whole from 1973-1975, the rates of stomach cancer mortality for men was 27.1 per 100,000, while that for women was only 13.0 per 100,000 (Yang *et al.,* 2006).

Issue 6: Other potential biases

Surveillance bias and reporting bias are possibilities in the original Zhang and Li (1987) study. Death reporting was handled by the police department, and there is no mention of how complete or accurate the reporting was. Given the fact that residents and government officials in the villages were aware of the Cr(VI) contamination in their water, it is possible that this knowledge influenced their motivation to ensure that cancer and other deaths were recorded. Trained interviewers were limited to gathering data from interviews with surrogates because the authors were studying deaths from cancer and the deceased subjects were not available for interview. Additionally, the interviewers were not blinded to the study hypothesis, nor were the surrogates who they were interviewing, which has the propensity to bias reporting of information.

In summary, there are serious problems with the original Zhang and Li (1987) data, and this in turn impacts the quality of subsequent studies that were based on these data (Beaumont *et al*., 2008; Kerger *et al.,* 2009). Study methods described in the original papers were poorly detailed, deviated from expected scientific protocol and lacked clearly defined scientific methodology. The characterizations of the study populations and chromium contamination (exposure) were inadequate. Thus, the reported associations between Cr(VI)-contaminated water and the purported excesses of cancer deaths cannot be accepted with any degree of confidence. In conclusion, these studies should not be used to assess the potential for Cr(VI) to pose a cancer hazard following ingestion.

3. Limitations of the original study data may have confounded conclusions of the reassessments by Beaumont *et al.*, (2008) and Kerger *et al.*, (2009)

The rate ratios associated with Cr(VI) exposure that were estimated by Beaumont *et al.* (2008) are adjusted for age only, which implies that other risk factors associated with stomach cancer (e.g. sex, family income, family history of stomach cancer, cigarette smoking and alcohol consumption) are not taken into account in the estimations. Thus, the

calculated risk estimates may be confounded by the effects of such other factors. Furthermore, because most of these other factors are themselves risk factors (increase the risk of stomach cancer), the Cr(VI) risk estimates calculated by Beaumont *et al*. can be expected to be positively biased away from the null (i.e., are overestimates of the true association between Cr(VI) exposure and stomach cancer).

In order to assess approximate magnitudes by which the Beaumont *et al*. (2008) risk estimates could be overestimating the true effect (if any), additional studies were identified that either a) calculated stomach cancer risk estimates from Cr(VI) exposure with adjustment for one or more of the above factors (Cole and Rodu 2005) or b) calculated stomach cancer risk estimates for other exposures with adjustment for one or more of the above factors (Yu and Hsieh 1991). In both instances, the studies also reported crude (unadjusted) risk estimates. We in turn used these crude risk estimates as a basis for comparison with the adjusted estimates. Numerical multipliers expressing the relationship between the adjusted and unadjusted risk estimates were then calculated. These multipliers provide an approximation of the proportion of the risk estimates that were attributed to confounding by other factors in these studies (Table 4).

Table 4. Influence of confounding on stomach cancer risk estimates

Study	Risk Factor	Crude Risk Estimate	Adjusted Risk Estimate	Absolute Difference (%)
Yu & Hsieh, 1991	Family History of Cancer	6.5	5.6 (2.7 – 11.4)†	0.9 (14%)
	Smoking	20.7	6.2 (2.2 – 17.0)†	14.5 (70%)
	Alcohol Use	2.9	0.8 (0.4 – 1.8)†	1.9 (72%)
	Strong Tea Consumption	0.9	0.3 (0.1 – 0.7)†	0.6 (67%)
Cole & Radu, 2005	Cr(VI)	1.37	0.8(0.69 –0.96) ‡	0.6 (40%)

† age, sex, family income, family history of stomach cancer, family history of other cancer, history of TB, blood type, cigarette smoking, alcohol, strong tea, fruit and milk consumption
‡ socioeconomic status

The Beaumont *et al.* (2008) age-adjusted rates using the study regions without Cr(VI) in drinking water and the population of the Liaoning Province as a whole were 1.82 and 1.69, respectively, suggesting an increase in stomach cancer rate of 82% and 69% due to Cr(VI) exposure. Using the observations of Yu and Hsieh (1991), study of stomach cancer risk factors in Chinese populations, controlling for smoking, alcohol use or strong tea consumption could have resulted in substantially lower adjusted rate ratios of 0.55 or 0.44 (Crude rate (1.82) x Percent of crude rate attributed to risk factor (70%) = absolute difference between crude and adjusted risk estimates (1.27). Theoretical adjusted rate is crude rate – difference between crude and adjusted (1.82-1.27 = 0.55). Socioeconomic status (SES) adjustment using the results of Cole and Radu (2005), could have resulted in

risk ratios of approximately 1.0 to 1.1 (using the 40% absolute difference between crude and adjusted rates from Table 3). It is clear from this example, that depending on the magnitude of the multiplier and comparison population, if one or more than one confounding factor was controlled for, the resulting adjusted risk estimates could vary substantially and the unadjusted rates used in all of the studies of the Chinese villager populations might quite easily be biased.

Kerger *et al.* (2009) follow a similar methodology as Beaumont *et al*. (2008) with comparisons based on the cancer rates reported in the original Zhang and Li study. The number of deaths upon which these rates were based was calculated using the reported cancer rates and estimated numbers of person-years at risk, which were estimated as the product of length of follow-up (9 years, 1970-1978) and the estimated size of the population in 1974, the midpoint of the follow-up period, based on decennial census data in China. This was a slightly different approach than applied by Beaumont *et al.* but did not have a significant different outcome than that used by Beaumont *et al.* Also, Kerger *et al.* did not include an age-adjustment approximation but commented that such an adjustment, as was included by Beaumont *et al.,* would not affect the results.

However, Kerger *et al*. (2009) differed in the grouping of study regions as compared to Beaumont et al., instead making comparisons between three groups: the industrial town of TangHeZi alone, three agricultural villages near TangHeZi on the north, west, and south that did not have Cr(VI) in groundwater, and the five agricultural villages to the east of TangHeZi that had Cr(VI) in groundwater throughout the observation period. The investigators reasoned that the industrial town of TangHeZi should not be grouped with the other, largely agricultural regions because of expected demographic differences between the agricultural community villages and the industrial town. Beaumont *et al*. included TangHeZi in the unexposed population comparison group without including a separate comparison with the unexposed villages. Kerger *et al*. also implemented a dose-response analysis across the exposed villages in which they used three surrogates for dose: 1) distance from the alloy plant, 2) average Cr(VI) well-water concentration in the 1965 survey, and 3) percentage of wells exceeding 0.05 ppm Cr(VI) in the 1965 survey.

Kerger *et al.* found that when comparing the Cr(VI)-exposed agricultural villages to the non-exposed agricultural villages, there were increases in relative risk of all cancers (RR = 1.10, 95% CI = 0.80, 1.51, age-adjusted), stomach cancer (1.22; 0.74, 2.01; crude) and lung cancer (1.76; 0.78, 3.98; crude), although the increases in risk were not statistically significant. Comparing the *non-exposed* agricultural regions to the industrial region, there was no elevation in the risk of all cancers (1.03; 0.77, 1.39; age-adjusted), a significant elevation in stomach cancer (1.70; 1.00, 2.89; crude) and a decrease in risk of lung cancer (0.45; 0.22, 0.94; crude) indicating that there may be different risk factors for stomach cancer in the villages as compared to the town. Comparing the exposed agricultural regions to the industrial region, there was a slight elevation in the risk of all cancers (1.14; 0.85, 1.52; age-adjusted), a significant elevation in stomach cancer (2.07; 1.25, 3.44; crude) and a decrease in risk of lung cancer (0.80; 0.44, 1.47; crude). Using the dose metrics, the authors concluded that all cancer or lung cancer risk were not associated with Cr(VI) in a dose-

dependent manner, although there was a suggestion of stomach cancer risk being inversely associated with the percent of wells with >0.05ppm Cr(VI).

While the Kerger *et al.* (2009) analysis relies on the same Zhang and Li original study data and thus suffers from the limitations noted above, the investigators attempted to improve on one of the limitations--specifically they attempted to address the confounding introduced by differences in the risk factors that might be introduced by differences in demographic makeup for populations residing in agricultural villages as compared to the industrialized town. It is important to note that cancer mortality was the endpoint included in the studies, not cancer incidence, and mortality measures depend on prevention and treatment. Comparing populations from two different areas based on mortality rates relies on the assumption that the two populations are comparable with respect to risk factors and treatments. Villagers in rural regions may not have had the same access to treatment as city dwellers, the population to whom they were compared, in part, in Beaumont *et al.* (2008). When making this correction, Kerger *et al*. found no significant increase in risk of stomach cancer mortality in exposed and unexposed villages.

Stomach cancer is and was at the time of the Chinese study, the most common form of cancer in China. Risk factors for stomach cancer include diet (nitrates in preserved food), smoking and alcohol consumption, poor refrigeration, and *H. pylori* infection. Gender is a risk factor for stomach cancer; more men than women develop stomach cancer, yet neither Beaumont *et al*. nor Kerger *et al*. was able to adjust for gender in their analyses. SES is also a risk factor for stomach cancer, because people of lower SES are less likely to have good refrigeration, receive quality health care and consume fresh fruits and vegetables, and are more likely to be exposed to other risk factors associated with certain occupations or areas of residence. JinZhou County included a central city and five suburbs, of which agriculture and industry were integral to the county. Residents of the cities may have been more likely to be of higher SES than residents of the villages. As demonstrated in the Kerger *et al.* (2009), villagers were at an increased risk of cancer independent of any exposure to Cr(VI). While Kerger *et al.* attempted to use an arguably more appropriate comparison population, that of unexposed villages, neither Beaumont *et al.* (2008) or Kerger *et al.* (2009) were able to control for the many other risk factors, including sex, socioeconomic status, diet, smoking or alcohol consumption, in their analyses, implying that the calculated risk measures were confounded by these and possibly other unmeasured factors. While both reassessments represent improvements over the original Zhang and Li analysis, both studies suffer from several weaknesses that relate to the quality and availability of the original data. As such, results from both studies should be interpreted with caution, and taken together, do not provide evidence supporting an increased risk of stomach cancer with Cr(VI) exposure.

4. No increased risk of gastrointestinal tract cancers found among chromite ore processing residue (COPR)-exposed New Jersey Residents

In December 2010, New Jersey Department of Health and Senior Services (NJDHSS) and New Jersey Department of Environment Protection (NJDEP) released a Health Consultation that evaluated the relationship between historic exposure to chromium from chromite ore processing residue (COPR) sites and the incidence of oral, esophageal, and stomach cancers in Jersey City, Hudson County (NJDHHS and NJDEP, 2010). Due to the date of its release, understandably the Health Consultation was not included in the Draft Toxicological Review. However, this study adds to the weight of evidence regarding the potential for Cr(VI) to pose a GI cancer hazard from environmentally-relevant exposures in the US, and should be considered by EPA in the final assessment. The findings of this assessment were null, adding to the overall weight of evidence that there is no causal relationship between human cancer and oral exposures to Cr(VI). The NJDHSS/NJDEP (2010) study is discussed in more detail below.

NJDHSS/NJDEP (2010) Study Background

COPR is a mixed mineral waste that contains both Cr(III) and Cr(VI). As a major center for processing and manufacturing chromium ore residue in the 1950s and 1960s, Hudson County has more than 160 COPR disposal sites, including 136 sites in Jersey City. COPR production began in Jersey City in 1901, and was used as fill material for many decades thereafter. Environmental investigation and remediation started in approximately 1985 and is continuing to date. To evaluate the incidence of oral and gastrointestinal tract cancers in relation to historic chromium exposure, the entire population residing in Jersey City from 1979 to 2006 was examined by NJDHSS/NJDEP.

NJDHSS/NJDEP (2010) Study Methods

Using the New Jersey State Cancer Registry (NJSCR)[4], a total of 739 oral cancer cases, 333 esophageal cancers, 651 stomach cancer cases, and 80 small intestine cancers were identified among Jersey City residents during the investigation period. A case was "*defined as an individual who was diagnosed with a new primary malignant cancer*" in the oral cavity and pharynx, esophagus, stomach, or small intestine. U.S. Census 1980, 1990, 2000 data were used for population count in each census block group, and cases for each cancer type were aggregated by census block group based on the case's residence at the time of diagnosis.

Using historic information on known COPR sites and their contaminant levels, NJDEP characterized the potential for residential Cr(VI) exposure in Jersey City. Each COPR Site was characterized as falling into one of three categories: 1) measured or estimated Cr(VI) concentration at 900 ppm or higher; 2) measured or estimated Cr(VI) concentration less

[4] Covering the entire state of New Jersey, NJSCR is a population-based incidence registry.

than 900 ppm; or 3) a known COPR site with no available total chromium or Cr(VI) concentration. Then, a 300-foot buffer was drawn around each COPR site boundaries, and the proportion of residential area for each census block group that fell within the buffer was calculated. Base on the proportion of the residential area within the buffer, census block groups were categorized into none, low, or high exposure intensity groups. "None" exposure group included census block groups that had no residential part intersected by a COPR site buffer. For the "high" exposure intensity, four classifications were created based the proportion of residential area in buffered areas of COPR sites. The exposure intensity groupings from NJDHSS/NJDEP 2010 study are provided in the table below from the Health Consultation.

Table 1. Exposure Intensity Group Definitions, and Number of Census Block Groups in Jersey City Classified by Four Exposure Grouping Methods.

Exposure Grouping Method	Exposure Groups	Exposure Group Definitions	Number of BGs[1]
1	• None • Any	• 0% of the area of the Residential Block Group (RBGA)[2] within 300 ft of any site • >0% of RBGA within 300 ft of any site	• 104 • 57
2	• None • Low • High	• 0% of RBGA within 300 ft of any site • All other BGs • ≥10% of RBGA within 300 ft of site with ≥900 ppm[3] Cr^{+6} ***or*** ≥25% of RBGA within 300 ft of any site	• 104 • 28 • 29
3	• None • Low • High	• 0% of RBGA within 300 ft of any site • All other BGs • ≥25% of RBGA within 300 ft of site with ≥900 ppm Cr^{+6} ***or*** ≥50% of RBGA within 300 ft of any site	• 104 • 42 • 15
4	• None • Low • High	• 0% of RBGA within 300 ft of any site • All other BGs • ≥50% of RBGA within 300 ft of site with ≥900 ppm Cr^{+6} ***or*** ≥75% of RBGA within 300 ft of any site	• 104 • 50 • 7

Note: [1] BG = Block group (U.S. Census 2000 boundaries)
[2] RBGA = Residential block group area
[3] ppm = parts per million (or milligrams per kilogram)

Source: NJDHSS and NJDEP2010
Census block groups not classified as "none" or "high" were classified as "low."

NJDHSS/NJDEP (2010) Study Results

The Health Consultation "*did not find* [that] *residential proximity to historic COPR sites is an increased risk of oral, esophageal, or stomach cancers in the population of Jersey City, or of combinations of these cancers with small intestine cancer*." Moreover, no dose-response relationship was found for oral, esophageal, and stomach cancers. During the investigation

period, oral cancer incidence was found to be similar across all groups with potential Cr(VI) exposure. Esophageal cancer incidence was elevated for males in areas near COPR sites, but the rate ratios were higher for the "low" exposure group than for the "high" exposure group. For female esophageal cancer incidence, no statistically significant elevation was found for all exposure groups with potential exposure. Stomach cancer incidence was not different across exposure categories. For combined esophageal, stomach, and small intestinal cancer incidence, the pattern was similar to that observed for male esophageal cancer, with no dose-response relationship.

As the Health Consultation is a descriptive analysis of cancer incidence, limitations include inability to assess personal exposure of individuals in the population and to account for population mobility. Cancers are likely to develop many years after exposure, but state cancer registry provides only an address at the time of diagnosis for each case. No information is available on length of time an individual may have lived at the address before diagnosis. Although further studies are needed, the null finding between Cr(VI) exposure and gastrointestinal cancer risk contribute to the weight of evidence that there is no association between environmental exposure to Cr(VI) and increased GI cancer risk.

5. The weight of evidence from occupational exposure studies does not support an association between exposures to Cr(VI) and GI tract cancer

Human exposure to chromium compounds by inhalation has been studied in a number of industries including the chromate production, chrome plating and chrome pigment, ferrochromium production, leather tanning, and chrome alloy production. In the Draft Toxicological Review, EPA listed numerous occupational epidemiologic studies of chromium-exposed workers that consistently demonstrated excess risks for lung cancer with chromium exposure (U.S. EPA, 2010d; p. 149).

Although occupational exposures mostly occur by inhalation, breathing Cr(VI) could expose tissues in the GI tract due to oral respiration and redistribution of inhaled particulates from the respiratory tract to the GI tract. Thus, the inhalation route of exposure would also expose tissues normally exposed through the ingestion route of exposure. Furthermore, GI effects including stomach ulcers and distress have been reported among Cr(VI) exposed workers (Mancuso and Hueper, 1951a; Macuso and Hueper, 1951b; Lucas and Kramkowski, 1975).

Gatto *et al.* (2010), which was not considered in the Draft Toxicological Review, is a systematic literature review and meta-analysis of oral cavity, esophageal, stomach, small intestine, colon, and rectal cancers among workers occupationally exposed to Cr(VI). Using PubMed, studies published from 1950 - 2009 that evaluated the relationship between Cr(VI) exposure and GI cancers were identified. Measures of effect and variability were extracted from 32 studies meeting specific inclusion criteria, and meta-analysis summary relative risk measures were calculated using random effects models and inverse variance weighting methods. Resulting meta- standardized mortality ratios (SMRs) for cancer were

as follows: oral cavity [1.02 (95% CI=0.77–1.34)]; esophagus [1.17 (95% CI=0.90–1.51)]; stomach [1.09 (95% CI=0.93–1.28)]; colon [0.89 (95% CI=0.70–1.12)]; and rectum [1.17 (95% CI=0.98–1.39)] (Table 4). Analyses of more highly exposed subgroups included in the studies or subgroups based on geographic region or by industry with recognized Cr(VI) exposures (welding, chrome plating, chromate production, and pigment production) did not result in elevated meta-SMRs except for esophageal cancer among US cohorts [meta-SMR =1.49 (95% CI=1.06–2.09)]. However, that finding was based on a subgroup of only four studies, one of which was a proportionate mortality ratio (PMR) study. PMR is a ratio of the number of deaths attributed to a specific cause to the total number deaths occurring in the population during a specified time period. PMR indicates the burden of a given cause of death relative to all deaths; it is not a measure of risk or of probability of dying from a specific cause. PMR has some limitations; if different populations have varying causes of disease that lead to death and if mortality rates are compared with PMR, findings could be distorted. Potential confounding by socioeconomic status (SES), diet and/or smoking, or limitations due to the healthy worker effect (HWE) were evaluated, and while smoking, diet and SES may be important factors that may have upwardly biased the meta-SMRs, HWE is not likely to have significantly affected the summary results. None of three studies reporting small intestine cancers observed a statistically significant increased risk. Gatto *et al*. (2010) concluded that these meta-analyses and literature review indicate that Cr(VI)-exposed workers are not at a greater risk of GI cancers than the general population.

Table 4. Meta-analysis results of epidemiologic studies of Cr(VI) exposed workers with any exposure, higher exposure, and subgroups within studies based on geographic region, significantly elevated lung cancer SMRs or occupational industry (from Gatto et al. 2010)

GI tract Cancer	Group or Subgroup	Number of studies[a]	Meta-SMR	95% CI	Hetero-geneity p-value[c]
Oral	Any exposure	18	1.02	0.77, 1.34	0.14
	Including more highly exposed subcohort	(2)	1.06	0.81, 1.40	0.16
Esophageal	Any exposure	15	1.17	0.90, 1.51	0.10
	Including more highly exposed subcohort 1[b]	(2)	1.15	0.86, 1.55	0.07
	Including more highly exposed subcohort 2[b]	(2)	1.20	0.89, 1.63	0.04
Geographical region	US	4	1.49	1.06, 2.09	0.26
	Europe	9	0.92	0.66, 1.28	0.27
	Removing PMR study[d]	8	0.97	0.58, 1.64	0.24
Occupational industry					
	Chromate pigment production/painting	4	1.17	0.72, 1.91	0.60
Stomach	Any exposure	29	1.09	0.93, 1.28	0.002
	Including more highly exposed subcohort 1[b]	(7)	1.05	0.88, 1.25	0.01
	Including more highly exposed subcohort 2[b]	(7)	1.06	0.89, 1.26	0.02
Geographical region	US	5	1.01	0.65, 1.57	0.25
	Europe	18	1.10	0.89, 1.37	0.002
	Japan	4	0.89	0.66, 1.19	0.42
Occupational industry	Plating	6	1.40	0.91, 2.15	0.02
	Chromate production	5	0.86	0.63, 1.18	0.30
	Chromate pigment production/painting	5	1.09	0.78, 1.53	0.49
Colon	Any exposure	13	0.89	0.70, 1.12	0.15
	Including more highly exposed subcohort 1[b]	(1)	0.94	0.76, 1.16	0.30
	Including more highly exposed subcohort 2[b]	(1)	0.92	0.75, 1.13	0.31
Rectal	Any exposure	20	1.17	0.98, 1.39	0.63
	Including more highly exposed subcohort 1[b]	(1)	1.17	0.98, 1.40	0.62
	Including more highly exposed subcohort 2[b]	(1)	1.16	0.97, 1.38	0.60

[a] Parentheses indicate the number of studies among those included in the main analysis of any Cr(VI) exposure that reported risk estimates for a more highly exposed subcohort of workers

[b] One study (Becker et al. 1999) reported risk estimates for two separate subcohorts of workers who were considered more highly exposed based on occupational tenure or type of welding which resulted in greater exposure to Cr(VI)

[c] p-value for heterogeneity

[d] removing Blair *et al.* (1995)

6. Potential for masking of GI cancer risk by lung cancer in the Gatto *et al*. (2010) meta-analysis is not supportable

Although some contend that the increased lung cancer risk observed in workers exposed to Cr(VI) masks an increase in GI cancer risk in the Gatto *et al*. (2010) meta analysis, review of the Gatto *et al*. findings does not support such a position. If this position was correct then one would assume that the meta-analysis would find null results for any other cancers that occur with a long latent period and a lower relative risk than lung cancer. However, that did not occur. Specifically, Gatto *et al.* (2010) reported a statistically significant increased bladder cancer risk in the meta-analysis, which indicates that if a significant risk exists, it may be measured in the meta-analysis regardless of increased risk of lung cancer in the cohorts included. As such, the null findings for oral, esophageal, stomach, small intestine, colon and rectal cancers were not expected to be masked by lung cancer, because the risk of bladder cancer was elevated despite the lung cancer risk, and bladder, lung and GI cancers all involve long latent periods (20 to 50 years).

Additionally, the bladder cancer risk estimate was developed in Gatto *et al.* (2010) to determine whether smoking rates were higher in the Cr(VI)-exposed occupational cohorts as compared to the general population. Current scientific evidence demonstrates that smoking is a risk factor for bladder cancer, while Cr(VI) exposure has not been associated with bladder cancer. Thus, if the Cr(VI) exposed worker cohorts had higher rates of smoking, they would likely have an increased risk of bladder cancer. In the meta-analysis, 14 of the 32 studies reported on bladder cancer risk with a statistically significant meta-SMR of 1.49 (95% CI = 1.20-1.85; p-heterogeneity = 0.23). Results were similar (meta-SMR =1.41; 95% CI = 1.15-1.73) with the exclusion of Montanaro *et al.* (1997)—a study of tanners that hypothesized a possible relationship between increased bladder cancer risk and occupational exposure to dyes. The statistically significant finding of elevated bladder cancer risk in Gatto *et al.* (2010) demonstrates that the occupational cohorts likely had higher rates of smoking than the reference population. This is an important finding to consider as smoking is believed to be a risk factor for several GI cancers as well (Seiz *et al.* 2009).

Furthermore, although the occupational workers evaluated in Gatto *et al*. (2010) had inhalation exposures to Cr(VI), their ingestion exposures were also likely substantial. As discussed in Gatto *et al.* (2010) oral exposures to Cr(VI) may be estimated from airborne concentrations using inhalation rate and an estimate of the fraction of airborne particles that are swallowed. The current occupational exposure limit in the U.S. is 5 $\mu g/m^3$, which was lowered from 50 $\mu g/m^3$ in 2006. Although human exposures to Cr(VI) is expected to have been highly variable with historical exposures in some industries far exceeding these limits (OSHA 2006), assuming the previous occupational exposure limit of 50 $\mu g/m^3$ reflects an exposure experienced by many chrome workers, the daily oral dose would be 0.25 mg/day (0.004 mg/kg-day for a 70 kg individual). This estimated dose is calculated using a workday inhalation rate of 10 m^3/day and an ingested fraction of 50% of inhaled Cr(VI), which represents the portion of inhaled particulates expected to be deposit in the oral region and/or to be swallowed (US EPA 1985).

To focus on worker cohorts that likely had higher oral exposures, potentially higher than inhalation exposures, in a separate analysis, we examined the risk of GI cancer in the aerospace industry painter cohorts exposed to Cr(VI). In the aerospace industry, chromate-based paints containing Cr(VI) have been applied as first-coat primer on metals for corrosion protection for many decades. Sabty-Daily *et al.* (2005) reported on two field investigations conducted at an aerospace facility. The purpose of this study by Sabty-Daily et al (2005) was to determine the size distribution and speciation of Cr(VI) in paint spray aerosol, as well as to determine where Cr(VI) particles from inhaled paint drift would impact in the respiratory tract. Sabty-Daily *et al.* found that the size distribution of total Cr particle in the paint aerosol had a Mass Median Aerodynamic Diameter (MMAD) of 7.5 μm. For Cr(VI) particle, the MMAD was 8.5 μm. Moreover, about 90% or more of the total Cr and Cr(VI) masses were particles greater than 2 μm MMAD, and on an average, 62% of total Cr and Cr(VI) mass in the paint aerosol consisted of particles greater than 10 μm MMAD. Most importantly, the study showed that about 72% of Cr(VI) mass in the paint spray aerosol inhaled by a spray painter was likely deposited in the airways of the head and nasal pharyngeal regions, indicating potentially significant ingestion exposure due to redistribution of Cr(VI) to the GI tract.

As such, it is reasonable that occupational studies on painters with exposure to chromate-containing paints are of particular interest for assessing the relationship between ingestion exposures of Cr(VI) and GI tract cancer risk given the potential for aerosolized Cr(VI) particles to redistribute to the GI tract. However, studies have consistently demonstrated no association between GI cancer risk and Cr(VI) exposure (Dalager *et al.,* 1980; Guberan *et al.,* 1989; Boice *et al.,* 1999). Specifically, among 997 male painters exposed to zinc chromate spray paints, significantly elevated lung cancer standardized mortality ratio (SMR) was observed but no excess risk for GI tract cancers were reported (Dalager *et al.*, 1980). In Guberan *et al*. (1989), painters who were exposed to metal protective primers containing zinc chromate pigments had significant excess risk for lung cancer, but not for GI tract cancers (Guberan *et al*., 1989). Boice *et al.* (1999) evaluated the risk of cancer and other diseases among a cohort of workers engaged in aircraft manufacturing. The cohort included a subgroup analysis of more than 3,000 workers exposed to chromate based on job titles such as electroplaters, etch tank operators, and aircraft production painters (Boice *et al.,* 1999; Marano *et al.,* 2000). Their exposure to chromate occurred primarily from operating process equipment tank lines used for plating or corrosion protection using chromate-based primers or paints (Marano *et al*., 2000). Similar to all of the other studies, Boice *et al.,* (1999) found no increase in lung cancer or cancer of the GI tract.

7. EPA's descriptor, "*likely to be carcinogenic to humans*" is not supported by the weight of evidence

In the synthesis of human, animal, and other supporting evidence, EPA states that "*human studies in which health outcomes (primarily cancer) were evaluated among populations that resided near sources of industrial waste containing Cr(VI) compounds and unknowingly consumed Cr(VI) in drinking water provide some evidence of possible associations between*

oral exposure to Cr(VI) and cancer". We disagree with this conclusion. As discussed here, the epidemiologic evidence does not support an association between Cr(VI) oral exposure and cancer of the GI tract or in other non-respiratory target organs or tissues. It is reasonable to conclude that the tissue dose which caused cancer in rodents is not achievable in target tissue of humans due to lower external exposures, even at the extreme upper bound of occupational and environmental exposures or due to enhanced ability to detoxify Cr(VI) through reduction prior to absorption. The lack of supporting human evidence, despite the large numbers of studies investigating relatively high-dose historical Cr(VI) exposures in humans, argues against the possibility that Cr(VI) is carcinogenic to humans by the oral route. EPA should give additional consideration to the full weight of evidence and revise the descriptor accordingly.

Because the concentrations of Cr(VI) that caused cancer in mice in the NTP study (20 mg/L and higher) are more than 200-fold higher than the federal MCL and ~4,000 fold higher than typical California drinking water exposures, it may be concluded that differences in toxicokinetics alone could explain the lack of consistency between theoretical risks and actual measures of incidence. To improve the risk assessment such that standards can be based on meaningful measures of risk, additional data and assessment are needed to extrapolate from the high doses that caused cancer in rodents to environmentally-relevant exposures in humans.

Finally, on this subject, one might question whether differences in dose alone explain the inconsistencies of the positive rodent cancer bioassay data and human epidemiology data. For example, measured concentrations of Cr(VI) in California drinking water are typically 1 to 10 μg/L. Assuming a drinking-water ingestion rate of 2 L/day, the drinking-water exposure would be 0.002 to 0.02 mg Cr(VI)/day (or 0.00003 to 0.0003 mg Cr(VI)/kg-day for a 70 kg individual). Thus, potential daily drinking-water exposure to Cr(VI) by humans is far lower (~10 to 100 times) than the daily exposures of historical worker cohorts—calculated above at ~0.004 mg/kg-day for a 70 kg individual (Gatto *et al*., 2010). Even though the duration of drinking water exposure is expected to be far longer than that of an occupational exposure, both the drinking water dose and the occupational dose from historical exposures are far lower than the dose that caused tumors in rodents (>1 mg/kg-day) (Stout *et al*., 2009a). These points of comparison suggest that the tumors observed in rodents of the NTP drinking water study are not quantitatively relevant to humans. As part of the MOA Framework, whether cancers in animals are quantitatively relevant to humans should be incorporated into the evaluation.

F. Need for EPA to Delineate Clear Inclusion Criteria of Studies Evaluated in Hazard Identification

Although EPA's effort to summarize all relevant literature is applauded, a more transparent review process is needed. EPA has not provided clear information as to how information was included for review and consideration. Page 2 of the Draft Toxicological Review states that the literature review is current through September 2010; however many recent papers that provide important information, which were published before September 2010, were not included (e.g., Gatto *et al.,* 2010; Chiu *et al.,* 2010), and many others were cited but not fully considered. Further, it is not clear how newly published data will be considered.

In addition, EPA did not release the results of the literature review in a timely manner. EPA's process for IRIS assessments includes posting of the literature review and data call in before the health risk assessment is initiated. EPA conducted the literature review in 2008, and it is dated 12/15/2008. However, the Agency did not make the literature review public or request additional public input until November 2, 2009. In response to the data call in, in January 2010, the Cr(VI) Panel of ACC submitted additional references of relevant peer-reviewed literature for EPA to review and consider in the development of information for use in its IRIS review of Cr(VI) (See Attachment F of ACC comments). However, most of these papers, which are critical to a comprehensive evaluation of Cr(VI), were not cited in the Draft Toxicological Review. Further, it is evident from the dates of the posted literature review (12/2008) and posting of the literature review (11/2009) that EPA began development of the Draft Toxicological Review prior to seeking public input. This is inconsistent with EPA's usual process to post the literature review and request comment prior to initiation of the draft review.

By not following the usual process for literature review, several key papers were not given adequate consideration, which is evidence by the fact that the hazard identification in the Draft Toxicological Review is incomplete, and in some cases not accurate. Further, EPA conducted the hazard identification to be specific for oral exposures, yet reached a conclusion that Cr(VI) acted by a mutagenic MOA by all routes of exposure. There is no basis or literature review provided to support this position. Cr(VI) does not cause cancer by dermal exposure and significant literature supports that Cr(VI) acts by non-mutagenic MOAs upon inhalation. As inclusion and exclusion of certain papers will significantly impact the conclusion of the IRIS review, specification of inclusion criteria and a more transparent process for the literature review would greatly advance the IRIS process and this Draft Toxicological Review and facilitated public input.

G. Uncertainties in Using NTP (2008a) Study to Derive the RfD

The NTP approach of testing dose levels far in excess of typical environmental exposures is not unique to Cr(VI), and poses challenges when attempting to use such data to predict adverse affects in humans exposed at relevant doses. NRC (2007) *Toxicity Testing in the 21st Century: A Vision and a Strategy* points to the growing realization that high-dose experiments in animals are unlikely to be predictive of low-dose effects in humans. The high dose levels of Cr(VI) used in the NTP (2008a) drinking water study are of questionable relevance to much lower environmental exposure levels that humans are typically exposed, especially in the absence of refined PBPK modeling to extrapolate across species and doses. Well recognized in the field of toxicology, responses may be qualitatively, as well as quantitatively, different at high vs. low dose levels. This is especially important given that the doses used in the NTP (2008a) drinking water study likely overwhelmed the animals' capacity to reduce Cr(VI) to Cr(III) in the gastrointestinal tract, resulting in tissue damage and subsequently tumors in the small intestine.

The Cr tissue accumulation data from the NTP (2008a) drinking water study, along with the findings of hyperplasia in the small intestine at all doses, indicate that all doses in the NTP study likely exceeded the reduction capacity of the mouse stomach. However, the reductive capacities of the mouse and rat stomach were not quantified in the NTP (2008a) resulting in a serious data gap for understanding the MOA underlying the tumorigenic response. Therefore, extrapolating from these doses to doses that are environmentally relevant likely overestimates the risk estimates for cancer, as well as the non-cancer effects used as the basis for the RfD. While the tissue Cr concentration data from the NTP (2008a) study do not demonstrate whether a threshold exists at lower doses, toxicokinetic data generated as a part of the Cr(VI) MOA Research Project will provide will provide information necessary to determine a threshold for absorption and should be considered in addition to the NTP (2008a) study for the derivation of the RfD. Findings from the Cr(VI) MOA Research Project will provide critical data to characterize the MOA and the toxicokinetics of Cr(VI) and, as such will significantly improve EPA's dose-response assessment for Cr(VI).

1. EPA's use of diffuse epithelial hyperplasia data in the female mice duodenum as the critical effect is questionable

Diffuse hyperplasia observed at all doses in the duodenum of female and male mice is likely a high-dose POE effect, and, therefore, it is questionable as to whether this effect can be extrapolated across does and species without consideration of differences in kinetics by dose. It is well recognized that Cr(VI) is detoxified, i.e., reduced to Cr(III) which is much less readily absorbed, following ingestion. There is certainly a dose at which the rate of

reduction is insufficient to convert all of an ingested dose of Cr(VI) to Cr(III) in the stomach, and as a result, Cr(VI) is passed to the small intestine.

The NTP (2008a) study authors stated that the diffuse hyperplasia observed in the mouse small intestines was "consistent with regenerative hyperplasia secondary to previous epithelial cell injury." Similar language was used to describe the observed intestinal hyperplasia in the 90-day NTP (2007) study. The Draft Toxicological Review states,

> *As indicated in Section 4, due to its morphological similarity to adenoma, focal epithelial hyperplasia was classified as a preneoplastic lesion by NTP (2008), and so the possibility exists that diffuse epithelial hyperplasia may also represent a preneoplastic lesion. However even though this possibility exists and thus this lesion may progress to cancer (i.e., adenoma) in some cases, the EPA considers the selection of this critical effect on which to base the derivation of the RfD (a noncancer endpoint) to be appropriate because definitive data on the progression of this particular lesion do not currently exist* (U.S. EPA, 2010d; p.221).

However, a different conclusion is equally plausible. Specifically, although histiocytic infiltration was noted in both rodent species in the 90-day and 2-year NTP bioassays, intestinal hyperplasia was not observed in rats in either study at any doses (NTP, 2007, 2008a). In contrast, diffuse hyperplasia occurred at similar incidence in the three highest doses, yet adenoma incidence was only significantly elevated in the two highest doses relative to controls. Presence of hyperplasia with and without tumor formation suggests that Cr(VI) induced cell proliferation independent of DNA damage. These data suggest that the proliferation observed in mice is a necessary precursor for the development of intestinal neoplasms. Use of diffuse epithelia hyperplasia data to derive an RfD applicable to humans is likely uncertain, indicating the need for PBPK models to extrapolate the findings in the NTP drinking water study across species and from the high doses tested to environmentally-relevant concentrations.

2. Methods of Analysis-Including Models (PBPK, BMD, etc.)

For improved model fit in the derivation of the RfD, the two highest dose groups were dropped. However, plateaus or other changes in the dose-response at the high-dose region may reflect changes in toxicokinetics such as saturation of metabolism. Other models, which include the high-dose region, such as the Hill model, should also be evaluated and the results presented in the Draft Toxicological Review.

Further, the cell proliferation incidence is 22% and 32% at the lowest doses for male and female mice, respectively, in the NTP drinking water study (2008a), with no effect observed in the controls. As a result the benchmark dose at a 10% increased response (BMD_{10}) and 95% confidence limit of the BMD_{10} ($BMDL_{10}$) are lower than the experimental range. This poses a problem as EPA's Benchmark Dose Technical Guidance states:

> *In general, studies with more dose groups and a graded monotonic response with dose will be more useful for BMD analysis…Studies in which responses are only at the same level as background or at or near the maximal response level are not considered adequate for BMD analysis. It is preferable to have studies with one or more doses near the level of the BMR to give a better estimate of the BMD, and thus, a shorter confidence interval. Studies in which all dose levels show changes compared with control values (i.e., no NOAEL) are readily useable in BMD analyses, unless the lowest response level is much higher than that at the BMR* (U.S. EPA, 2000; p. 14).

EPA should address this issue in the final assessment

H. Cr(VI) MOA Research Project Schedule Modified In Accordance with IRIS Schedule and Process

The IRIS track schedule of Cr(VI) has been modified over time without notification. Table 5 provides is a summary of several date changes that that we have noted when the IRIS track was checked between June, 2009 and October, 2010.

Table 5. Changes in Cr(VI) IRIS Assessment since June 2009

Approximate Date when IRIS Track was checked (Calendar Year)	Final Assessment Announcement Date for IRIS (Federal Fiscal Year)
June 2009	3rd Quarter of 2012
December 2009	3rd Quarter of 2010
June 2010	4th Quarter of 2010
October 2010	2nd Quarter of 2011

The Cr(VI) MOA Research Project was launched in 2009 and designed to meet the original final assessment date of 3rd federal fiscal quarter of 2012, originally set by EPA (Table 5). Hence, the project would have been complete in time for consideration if EPA had not accelerated its schedule. When the final assessment date was changed to the 2nd federal fiscal quarter of 2011, the schedule for Cr(VI) MOA Research Project was substantially accelerated to coincide with EPA's schedule to finalize the Toxicological Review and IRIS update (**Figure 6**). As such, findings from Cr(VI) MOA Research Project will be available at approximately the same time or before the EPA's Toxicological Review is finalized. Results of the Cr(VI) MOA Research Project generated to date, have been communicated to state and federal regulatory agencies, including EPA, and are being communicated to the scientific community through peer-review publications and a special session at the 2011 Society of Toxicology (SOT) annual conference.

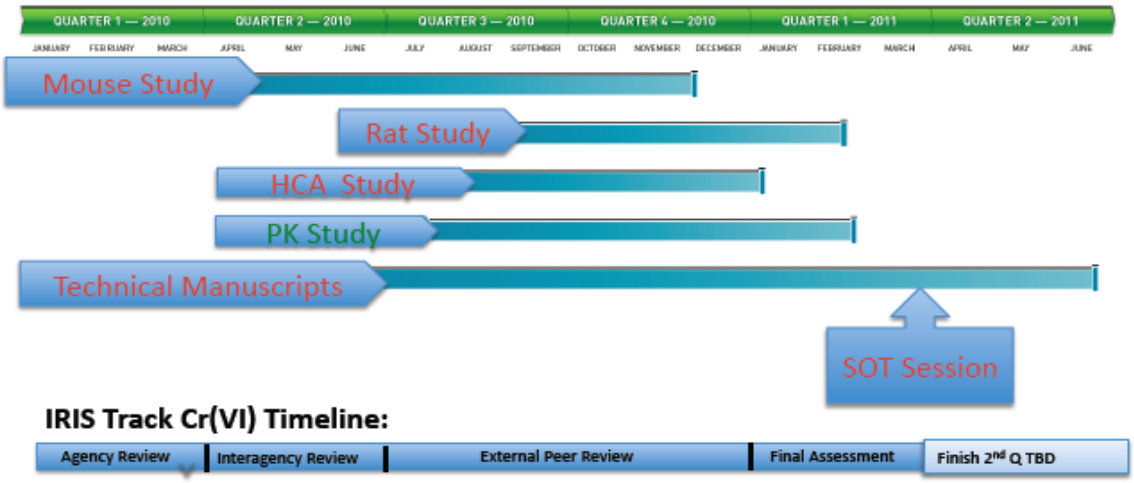

Figure 6. Schedule for Cr(VI) MOA Research Project and EPA IRIS update.

In the Draft Toxicological Review, EPA identified several critical data gaps in the information currently available for its quantitative risk assessment. The Cr(VI) MOA Research Project will provide the data and tools to fill many of the EPA-identified data gaps and, in so doing, will improve EPA's ability to extrapolate findings observed in laboratory animals to humans. Importantly, the Cr(VI) MOA Research Project was designed to build upon and extend NTP's findings and to provide the information that EPA prefers to use as the basis for cancer risk assessment as described in the Agency's Cancer Risk Assessment Guidance (U.S. EPA, 2005a). The data generated through the Cr(VI) MOA Research Project, will allow EPA to address some of the data gaps underlying the current Toxicological Review of Cr(VI) and should significantly improve extrapolation of the NTP study findings to humans.

I. Erratum

Although we did not review the Draft Toxicological Review specifically for editorial issues, we identified a few errors that should be corrected in the final assessment. They are summarized below, but should not be considered inclusive of all possible errors as we did not check facts on all data in the Draft Toxicological Review:

1. Page 6, Table 2-1, Barium oxide should be barium chromate.
2. Page 16, Table 2-4, Page 73, Table 4-2, The note "MISSING" appears as a reference.
3. Page 7, Header for 2.2. The section heading includes bioaccessibility but bioaccessibility is not discussed till section 3.1
4. Page 24, 4th line, "calorimetric" should be "colorimetric". We note that the same typographical error occurs in Zhitkovitch (2005), but it is technically incorrect and should not be reiterated.

5. Page 30, Table 3-7. The concentrations reported from the MacKenzie *et al.* (1958) study should be Cr(VI), not $K_2Cr_2O_4$. It is presented correctly in the text as chromate ion.
6. Page 35, line 3, chromium is misspelled

CONCLUSION

As identified throughout the Draft Toxicological Review, there are many data gaps in the information currently available for quantitative risk assessment. Current scientific knowledge on Cr(VI) toxicokinetics is limited in the low dose range, and disparate tumorigenic responses observed in mice and rats in the NTP (2008a) drinking water study suggest species-specific differences in gastric reduction and/or absorption of Cr(VI) in target tissues of the small intestine. Toxicokinetic and toxicodynamic data in the target tissue and in the low dose range are needed to address these data gaps. Moreover, the current data are insufficient for a definitive determination of MOA. EPA's assumption of a mutagenic MOA for ingested Cr(VI) is not supported by relevant *in vivo* drinking water studies with observation of diffuse hyperplasia, secondary to tissue damage, at all NTP (2008a) doses in the duodenum, late time to tumor, and no DNA damage in small intestine at 5 and 20 mg/L (De Flora *et al.*, 2008).

The Cr(VI) MOA Research Project was designed to build upon and extend NTP's findings and to provide the information (e.g., refined PBPK models capable of reasonably estimating internal dosimetry in rodents and predicting internal dosimetry in humans) that EPA prefers to use as the basis for cancer risk assessment as described in the Agency's Cancer Risk Assessment Guidance (U.S. EPA, 2005a). The data generated through this Research Project, will allow EPA to address data gaps inherent in the current Draft Toxicological Review of Cr(VI) and should significantly improve extrapolation of the NTP study findings to humans.

For decades, the risk assessment for ingested Cr(VI) has been based on a non-cancer approach due, in part, to the assumption that reduction of Cr(VI) in the stomach at doses consistent with the current drinking water exposure limits would be sufficient to prevent cancer (Proctor *et al.,* 2002b). However, with the evolution of risk assessment methods and need to describe the MOA for cancer risk assessment, the question of whether Cr(VI) reduction is saturated is no longer the most pertinent question; rather we seek to understand the key events in the MOA, and the internal dosimetry associated with these events. The uncertainties associated with the use of default risk assessment methods and the lack of a clearly defined MOA elucidate the need for improved pharmacokinetic and MOA data for Cr(VI)-induced tumors. Because studies to fill these data gaps are currently on-going, it will is not be necessary for EPA to rely on default approaches in the risk assessment of ingested Cr(VI). Toxicokinetic and MOA data generated as a part of the Cr(VI) MOA Research Project will provide a more scientific robust basis for developing a RfD and oral cancer slope factor for Cr(VI) and will enhance the scientific credibility of the EPA's assessment. As a result, this will ensure that subsequent regulations are truly based on the based available science in accordance with EPA guidelines.

REFERENCES

Agency for Toxic Substances and Disease Registry (ATSDR). (2008). Toxicological profile for Chromium (Draft for Public Comment). Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service.

Amandus, H.E. (1986). Mortality from stomach cancer in United States cement plant and quarry workers, 1950-80. *Brit. J. Ind. Med.* **43**, 526-28.

Arce, G.T., Gordon, E.B., Cohen, S.M., Singh, P. (2010). Genetic toxicology of folpet and captan. *Crit. Rev. Toxicol*. **40,** 546-74.

Armienta-Hernandez, MA., Rodriguez-Castillo,R. (1995). Environmental exposure to chromium compounds in the valley of Leon, Mexico. *Environ. Health. Perspect.* **103**, 47–51.

Bagchi, D., Hassoun, E.A., Bagchi, M., Stohs, S.J. (1995). Chromium-induced excretion of urinary lipid metabolites, DNA damage, nitric oxide production, and generation of reactive oxygen species in Sprague-Dawley rats. *Comp. Biochem. Physiol. C. Pharmacol. Toxicol. Endocrinol.* **110**, 177-87.

Bagchi, D., Vuchetich, P.J., Bagchi, M., Hassoun, E.A., Tran, M.X., Tang, L., Stohs, S.J (1997). Induction of oxidative stress by chronic administration of sodium dichromate and cadmium chloride [cadmium II] to rats. *Free Radic. Biol. Med*. **22**, 471-78.

Bagchi, D., Balmoori, J., Bagchi, M., Ye, X., Williams, C.B., Stohs, S.J. (2002). Comparative effects of TCDD, endrin, naphthalene and chromium (VI) on oxidative stress and tissue damage in the liver and brain tissues of mice. *Toxicology* **175**, 73-82.

Ball, J.W., Izbicki, J.A. (2004). Occurrence of hexavalent chromium in ground water in the western part of the Mojave Desert, California. *Appl. Geochem.* **19**,1123-35.

Beaumont, J.J., Sedman, R.M., Reynolds, S.D., Sherman, C.D., Li, L.H., Howd, R.A., Sandy, M.S., Zaise, L., Alexeeff, G.V. (2008). Cancer mortality in a Chinese population exposed to hexavalent chromium in drinking water. *Epidemiology* **19**, 12–23.

Beaver, L.M., Stemmy, E.J., Schwartz, A.M., Damsker, J.M., Constant, S.L., Ceryak, S.M., Patierno, S.R. (2009). Lung inflammation, injury, and proliferative response after repetitive particulate hexavalent chromium exposure. *Environ. Health. Perspect*. **117**, 1896-902.

Becker, N. (1999). Cancer mortality among arc welders exposed to fumes containing chromium and nickel. Results of a third follow-up: 1989–1995. *J. Occup. Environ. Med*. **41**, 294–303.

Bednar, C.M, Kies, C. (1991). Inorganic contaminants in drinking water correlated with disease occurrence in Nebraska. *J. Am. Works Assoc.* **27**, 631-35.

Bernard, B.K., Gordon, E.B. (2000). An evaluation of the common mechanism approach to the Food Quality Protection Act: Captan and four related fungicides, a practical example. *Int. J. Toxicol*. **19**, 43–61.

Bick, R.L., Girardi, T.V., Lack, W.J., Costa, M., Titelbaum, D. (1996). Hodgkin's disease in association with hexavalent chromium exposure. *Int J Hematol.* **64**, 257–262.

Blair, A., Burg, J., Foran, J., Gibb, H., Greenland, S., Morris, R., Raabe, G., Savitz, D., Teta, J., Wartenberg D., et al. (1995). Guidelines for application of meta-analysis in environmental epidemiology. ISLI Risk Science Institute. *Regul. Toxicol. Pharmacol.* **22,** 189–97.

Boufounos, C., Bertolo, R., Almodovar, M., Hirata, R. (2009). Natural occurrence of hexavalent chromium in a sedimentary aquifer in Urânia, State of São Paulo, Brazil. *Anais da Academia Brasileira de Ciências* **81**, 227-42.

Boice Jr., J.D., Marano, D.E., Fryzek, J.P., Sadler, C.J., McLaughlin, J.K. (1999). Mortality among aircraft manufacturing workers. *Occup. Environ. Med*. **56**, 581–97.

Broadway, A., Cave, M.R., Wragg, J., Fordyce, F.M., Bewley, R.J.F., Graham, M.C., Ngwenya, B.T., Farmer, J.G. (2010). Determination of the bioaccessibility of chromium in Glasgow soil and the implications for human health risk assessment. *Sci. Total Environ.* Doi: 10.1016/j.scitotenv.2010.09.007.

California Department of Public Health (CPDH). (2009). Chromium 6 in drinking water sources: Sampling results. Available at: http://www.cdph.ca.gov/certlic/drinkingwater/Pages/Chromium6sampling.aspx.

Campbell, J.L., Tan, Y., Clewell, H.J. (2009) Development of a PBPK model for hexavalent chromium in rats and mice to estimate exposure to oral mucosa and small intestine. *Toxicologist* 108, 98 (Abstract) Poster ID # 108.

Chiu, A., Shi, X.L. Lee, W.K., Hill, R., Wakeman, T.P. Katz, A., Xu, B., Dalal, N.S., Robertson, J.D., Chen, C., Chiu, N., Donehower, L. (2010). Review of chromium (VI) apoptosis, cell-cycle arrest, and carcinogenesis. *J. Environ. Sci. Health C Environ. Carcinog. Ecotoxicol. Rev.* **28**, 188-230.

Cohen, M.D., Kargacin, B., Klein, C.B., Costa, M. (1993). Mechanisms of chromium carcinogenicity and toxicity. *Crit. Rev. Toxicol*. **23**, 255-81.

Cohen, S.M., Gordon, E.B., Singh, P., Arce, G.T., Nyska, (2010). A. Carcinogenic mode of action of folpet in mice and evaluation of its relevance to humans. *Crit. Rev. Toxicol.* **40**, 531-45.

Cole, P. and Rodu, B. (2005). Epidemiologic studies of chrome and cancer mortality: a series of meta-analysis. *Regul. Toxicol. Pharmacol*. **43**, 225-31.

Collins, B.J., Stout, M.D., Levine, K.E., Kissling, G.E., Melnick, R.L., Fennell, T.R., Walden, R., Abdo, K., Pritchard, J.B., Fernando, R.A., Bruka, L.T., Hooth, MJ. (2010). Exposure to hexavalent chromium resulted in significantly higher tissue chromium burden compared to trivalent chromium following similar oral doses to male F344/N and female B6C3F1 mice. *Toxicol. Sci.* DOI: 10.1093/toxsci/kfq263.

Coogan, T.P., Motz, J., Snyder, C.A., Squibb, K.S., Costa, M. (1991). Differential DNA-protein crosslinking in lymphocytes and liver following chronic drinking water exposure of rats to potassium chromate. *Toxicol. Appl. Pharmacol.* **109**, 60–72.

Correa, P. (2004). Is gastric cancer preventable? *Gut* **53**, 1217-19.

Dalager, N.A., Mason, T.J., Fraumeni Jr., J.F., Hoover, R., Payne, W.W. (1980). Cancer mortality among workers exposed to zinc chromate paints. *J. Occup. Med.* **22**, 25–29.

De Flora, S., Camoirano, A., Bagnasco, M., Bennicelli, C., Corbett, G.E., Kerger, B.D. (1997). Estimates of the chromium(VI) reducing capacity in human body compartments as a mechanism for attenuating its potential toxicity and carcinogenicity. *Carcinogenesis* **18**, 531-37.

De Flora, S. (2000). Threshold mechanisms and site specificity in chromium(VI) carcinogenesis. *Carcinogenesis* **21**, 533-41.

De Flora, S., Iltcheva, M., Balansky, R. (2006). Oral chromium(VI) does not affect the frequency of micronuclei in hematopoietic cells of adult mice and of transplacentally exposed fetuses. *Mutat. Res.* **610**, 38-47.

De Flora, S., D'Agostini, F., Balansky, R., Micale, R., Baluce, B., Izzoti, A. (2008). Lack of genotoxic effecgs in hematopoietic and gastrointestinal cells of mice receiving chromium (VI) with the drinking water. *Mutat. Res.* **659**, 61-66.

Devi, K.D., Rozati, R., Saleha Banu, B., Jamil, K., Grover, P. In vivo genotoxic effect of potassium dichromate in mice leukocytes using comet assay. *Food Chem. Toxicol.* **39**, 859-865.

Donaldson, R.M. Jr., Barreras, R.F. (1966). Intestinal absorption of trace quantities of chromium. *J. Lab. Clin. Med*. **68**, 484-93.

Eizaguirre-Garcia, D., Rodriguez-Andres, C., Watt, G.C, Hole, D. (1999). A study of leukemia in Glasgow in connection with chromium-contaminated land. *J. Public Health Med*. **21**, 435-38.

Eizaguirre-Garcia, D., Rodriguez-Andres, C., Watt, G.C. (2000). Congenital anomalies in Glasgow between 1982 and 1989 and chromium waste. *J. Public Health Med.* **22**, 54–58.

Edel, J., Sabbioni, E. (1985). Pathways of Cr (III) and Cr (VI) in the rat after intratracheal administration. *Hum. Toxicol*. **4**, 409-16.

Finley, B.L., Kerger, B.D., Katona, M.W., Gargas, M.L., Corbett, G.C., Paustenbach, D.J. (1997). Human ingestion of chromium (VI) in drinking water: Pharmacokinetics following repeated exposure. *Toxicol. Appl. Pharmacol.* **142**, 151-59.

Fryzek, J.P., Mumma, M.T., McLaughlin, J.K., Henderson, B.E., Blot, W.J. (2001) Cancer mortality in relation to environmental chromium exposure. *J. Occup. Environ. Med*. **43**, 635-40.

Gao, M., Levy, L.S., Braithwaite, R.A., Brown, S.S. (1993). Monitoring of total chromium in rat fluids and lymphocytes following intratracheal administration of soluble trivalent or hexavalent chromium compounds. *Hum. Exp. Toxicol*. **12**, 377-82.

Gatto, N.M., Kelsh, M.A., HaMai, D., Suh, M., Proctor, D.M. (2010). Occupational exposure to hexavalent chromium and cancers of the gastrointestinal tract: A meta-analysis. *Cancer Epidemiol*. **34**, 388-99.

Gonzalez, A.R., Ndundu, K., Flegal, A.R. (2005). Natural occurrence of hexavalent chromium in the Aromas Red Sands Aquifer, California. *Environ. Sci. Technol*. 39, 5505–11.

Gordon, E. (2007). Captan:Transition from 'B2' to 'not likely'. How pesticide registrants affected the EPA Cancer Classification Update. J*. App. Toxicol.* **27**, 519–526.

Guberan, E., Usel, M., Raymond, L., Tissot, R., Sweetnam, P.M. (1989). Disability, mortality, and incidence of cancer among Geneva painters and electricians: a historical prospective study. *Br. J. Ind. Med*. **46**, 16–23.

Holmes, A.L., Wise, S.S. , Wise, J.P., Sr. (2008). Carcinogenicity of hexavalent chromium. *Indian. J. Med. Res.* **128**, 353-72.

Kargacin, B., Squibb, K.S., Cosentino, S., Zhitkovich A., Costa M. (1993). Comparison of the uptake and distribution of chromium in rats and mice. *Biol. Trace Element Res.* **36**, 307-18.

Kerger, B.D., Paustenbach, D.J., Corbett, G.E., Finley, B.L. (1996). Absorption and elimination of trivalent and hexavalent chromium in humans following ingestion of a bolus dose in drinking water. *Toxicol. Appl. Pharmacol*. **141**, 145-58.

Kerger, B.D., Butler, W.J., Paustenbach, D.J., Zhang, J., Li, S. (2009). Cancer mortality in chinese populations surrounding an alloy plant with chromium smelting operations. *J. Toxicol. Environ. Health A* **72**, 329–44.

Kirpnick-Sobol, Z., Reliene, R., Schiestl, R.H. (2006). Carcinogenic Cr(VI) and the nutritional supplement Cr(III) induce DNA deletions in yeast and mice. *Cancer Res.* **66**, 3480-84.

Klein, C. B., Su, L., Bowser, D., Leszczynska, J. (2002). Chromate-induced epimutations in mammalian cells. *Environ. Health Perspect.* **110 Suppl 5**, 739-43.

Kudrycki, K.E., Newman, P.R., Shull, G.E. (1990). cDNA cloning and tissue distribution of mRNAs for two proteins that are related to the band 3 Cl-/HCO3- exchanger. *J. Biol. Chem.* **265**, 462-71.

Kulongoski, J.T., Belitz, K. (2005). Ground-water quality data in the Monterey Bay and Salinas Valley Basins, California. 2005—Results from the California GAMA Program. USGS Data Series 258. Prepared in cooperation with the CSWRCB.

Kuykendall, J.R., Kerger, B.D., Jarvi, E.J., Corbett, G.E., Paustenbach, D.J. (1996). Measurement of DNA-protein cross-links in human leukocytes following acute ingestion of chromium in drinking water. *Carcinogenesis* **17**, 1971-77.

Long, J.T. (2010) State pushes costly new water standard. CalWatchdog. February. 2010. Available at: http://www.calwatchdog.com/2010/02/10/new-state-pushes-costly-new-water-standard/

Lucas, J.B., Kramkowski, R.S. (1975). Health hazard evaluation determination report number 74-87-221. Cincinnati, OH: US Department of Health Education and Welfare Center for Disease Control National Institute for Occupational Safety and Health.

Mackenzie RD, Byerrum RU, Decker CF, Hoppert CA, Langham RF. 1958. Chronic toxicity studies. II. Hexavalent and trivalent chromium administered in drinking water to rats. AMA Arch Ind Health 18(3):232-234.

Mancuso, T.F., Hueper, W.C. (1951a) Occupational cancer and other health hazards in a chromate plant: a medical appraisal. I. Lung Cancers in chromate workers. *Ind. Med. Surg*. **20**, 358–363.

Mancuso, T.F., Hueper, W.C. (1951b) Occupational cancer and other health hazards in a chromate plant: a medical appraisal. II. Clinical and toxicologic aspects. *Ind. Med. Surg*. **20**, 393–407

Marano, D.E., Boice Jr., J.D., Fryzek, J.P., Morrison, J.A., Sadler, C.J., McLaughlin, J.K. (2000). Exposure assessment for a large epidemiological study of aircraft manufacturing workers. *Appl. Occup. Environ. Hyg*. **15**, 644–56.

Markovich, D. (2001). Physiological roles and regulation of mammalian sulfate transporters. *Physiol. Rev.* **81**, 1499-1533.

McCarroll, N., Keshava, N., Chen, J., Akerman, G., Kligerman, A., Rinde, E. (2010). An evaluation of the mode of action framework for mutagenic carcinogens case study II: chromium (VI). *Environ. Mol. Mutagen.* **51**, 89-111.

Mirsalis, J.C., Hamilton, C.M., O'Loughlin, K.G., Paustenbach, D.J., Kerger, B.D., Patierno, S. (1996). Chromium (VI) at plausible drinking water concentrations is not genotoxic in the in vivo bone marrow micronucleus or liver unscheduled DNA synthesis assays. *Environ. Mol. Mutagen*. **28**, 60-63.

Montanaro, F., Ceppi, M., Demers, P.A., Puntoni, R., Bonassi, S. (1997) Mortality in a cohort of tannery workers. *Occup. Environ. Med*. **54**, 588–91.

Nickens, K.P., Patierno, S.R., Ceryak, S. (2010). Chromium genotoxicity: A double-edged sword. *Chem. Biol. Interact*. **188**, 276-88.

National Researc Council (NRC). (2009). Science and Decisions: Advancing risk assessment. Toward a unified approach to dose-response assessment: the need for an improved dose-response framework. pp. 127-87. The National Academis Press, Washington DC.

NRC (2007). Toxicity testing in the 21st Century: A vision and strategy. . The National Academis Press, Washington DC.

National Toxicology Program (NTP). (2007). NTP technical report on the toxicity studies of sodium dichromate dihydrate administered in drinking water to male and female F344/N rats and B6C3F1 mice and male BALB/c and am3-C57BL/6 mice. January 2007 National Institutes of Health Public Health Service U.S. Department of Health and Human Services. Accessed at: /http:// ntp.niehs.nih.gov/ntp/htdocs/ST_rpts/TOX72.pdfbm94934.

NTP. (2008a). Final technical report on the toxicology and carcinogenesis studies of sodium dichromate dihydrate in F344/N rats and B6C3F1 mice. Accessed at: /http://ntp.niehs.nih.gov/files/546_web_FINAL.pdfS.

NTP (2008b). NTP technical report on the toxicology and carcinogenesis studies of chromium picolinate monohydrate (CAS NO. 27882-76-4) in F344/N rats and B6C3F1 mice (feed studies). *NIH Publication No. 08-5897*.

New Jersey Department of Environmental Protection (NJDEP). (2009). Derivation of ingestion-based soil remediation criterion for Cr6+ based on the NTP chronic bioassay data for sodium dichromate dihydrate. Available at http://www.state.nj.us/dep/dsr/chromium/soil-cleanup-derivation.pdf

New Jersey Department of Health and Senior Services (NJDHSS) and New Jersey Department of Environment Protection (NJDEP). (2010). Health Consultation: Analysis of oral, esophageal, and stomach cancer incidence near chromium-contaminated sites in Jersey City (a/k/a Hudson County Chromium Sites), Hudson County, New Jersey. http://www.state.nj.us/health/eoh/cehsweb/documents/hudson_co_chromium_hc.pdf

Nickens, K.P., Patierno, S.R., Ceryak, S. (2010). Chromium genotoxicity: A double-edged sword. Chem. Biol. Interact. 188, 276-88.

Noffsinger, A., Waxman, I. (2007). Preinvasive neoplasia in the stomach: Diagnosis and treatment. *Clin. Gastroenterol. Hepatol*. **5**, 1018–1023.

O'Flaherty, E.J. (1996). A physiologically based model of chromium kinetics in the rat. *Toxicol. Appl. Pharmacol.* **138**, 54-64.

O'Flaherty, E.J, Kerger, B.D, Hayes, S.M., Paustenbach, D.J. (2001). A physiologically based model for the ingestion of trivalent chromium and hexavalent chromium by humans. *Toxicol. Sci*. **60**, 196-213.

Occupational Safety and Health Administration (OSHA). (2006). Occupational exposure to hexavalent chromium; final rule. US Department of Labor.

Office of Management and Budget (OMB). (2004). Final Information Quality Bulletin for Peer Review. M-05-03.

Oze, C., Bird, D.K., Fendorf, S. (2007). Genesis of hexavalent chromium from natural sources in soil and groundwater. *Proc. Nat. Acad. Sci.* **104**, 6544–49.

Potten, C.S., Loeffler, M. (1990). Stem cells: Attributes, cycles, spirals, pit- falls and uncertainties. Lessons for and from the crypt. *Development* **110**, 1001–1020.

Potten, C.S., Owen, G., Roberts, S.A. (1990). The temporal and spatial changes in cell proliferation within the irradiated crypts of the murine small intestine. *Int. J. Radiat. Biol.* **57**, 185–199.

Proctor, D., Hays, S., et al. (2002a) Rate of hexavalent chromium reduction by human gastric fluid. Abstract 1700. Society of Toxicology, Nashville, TN.

Proctor DM, Otani JM, Finley BL, Paustenbach DJ, Bland JA, Speizer N, et al. (2002b). Is hexavalent chromium carcinogenic via ingestion? A weight-of-evidence review. J Toxicol Environ Health A 65(10):701-46.

Proctor D.M., Panko, J.P., Liebig, E.W., Scott, P.K., Mundt, K.A., Buczynski, M.A., Barnhart, R.J., Harris, M.A., Morgan, R.J., Paustenbach, D.J. (2003). Workplace airborne hexavalent chromium concentrations for the Painesville, Ohio chromate production plant (1943–1971). *Appl. Occupl. Environ. Hyg*. **18**, 430–449.

Sabty-Daily, R.A., Harris, P.A., Hinds, W.C., Froines, J.R. (2005). Size distribution and speciation of chromium in paint spray aerosol at an aerospace facility. *Ann. Occup. Hyg*. **49**, 47–59.

Salnikow, K., Zhitkovich, A. (2008). Genetic and epigenetic mechanisms in metal carcinogenesis and cocarcinogenesis: nickel, arsenic, and chromium. *Chem. Res. Toxicol.* **21**, 28-44.

Sekihashi, K., Sasaki, T., Yamamoto, A., Kawamura, K., Ikka, T., Tsuda, S., Sasaki, Y.F. (2001). A comparison of intraperitoneal and oral gavage administration in comet assay in mouse eight organs. *Muta. Res*. **27**, 39-54.

Seiz, H.K., Cho, C.H. (2009). Contribution of alcohol and tobacco use in gastrointestinal cancer development. *Methods Mol. Biol.* **472**, 217-41.

Shindo, Y., Toyoda, Y., Kawamura, K., Kurebe, M., Shimada, H., Hattori, C., Satake, S. (1989). Micronucleus test with potassium chromate(VI) administered intraperitoneally and orally to mice. *Mutat. Res.* **223**, 403-406.

Soares, M.E. Veira, E., Bastos Mde, L. (2010). Chromium speciation analysis in bread samples. *J. Agric. Food Chem.* **58**, 1366–70.

Stern, A.H. (2010). A quantitative assessment of the carcinogenicity of hexavalent chromium by the oral route and its relevance to human exposure. *Environ. Res.* Doi:10.1016/j.envres.2010.08.002.

Stern, A.H., Yu, C.H., Black, K., Lin, L, Lioy, P.J., Gochfeld, M., Fan, Z.H. (2010) Hexavalent chromium in house dust—a comparison between an area with historic contamination from chromate production and background locations. *Sci. Total Environ*. **408,** 4993-98.

Stout, M.D., Herbert, R.A., Kissling, G.E., Collins, B.J., Travlos, G.S., Witt, K.L., Melnick, R.L., Abdo, K.M., Malarkey, D.E., Hooth, M.J. (2009a). Hexavalent chromium is carcinogenic to F344/N rats and B6C3F1 mice after chronic oral exposure. *Environ. Health Perspect.* **117**, 716-22.

Stout, M.D., Nyska, A., Collins, B.J., Witt, K.L., Kissling, G.E., Malarkey, D.E., Hooth, M.J. (2009b). Chronic toxicity and carcinogenicity studies of chromium picolinate monohydrate administered in feed to F344/N rats and B6C3F1 mice for 2 years. *Food Chem. Toxicol.* **47**, 729-33.

Sun, H., Zhou, X., Chen, H., Li, Q., Costa, M. (2009). Modulation of histone methylation and MLH1 gene silencing by hexavalent chromium. *Toxicol. Appl. Pharmacol.* **237**, 258-66.

Sutherland JE, Zhitkovich A, Kluz T, Costa M. (2000). Rats retain chromium in tissues following chronic ingestion of drinking water containing hexavalent chromium. *Biol. Trace. Elem. Res.* **74**, 41-53.

Takahashi, Y., Kondo, K., Hirose, T., Nakagawa, H., Tsuyuguchi, M., Hashimoto, M., Sano, T., Ochiai, A. and Monden, Y. (2005). Microsatellite instability and protein expression of the

DNA mismatch repair gene, hMLH1, of lung cancer in chromate-exposed workers. *Mol. Carcinog.* **42**, 150-58.

Thompson, C., Haws, L, Harris, M. Gatto, M., Proctor, D. (2010). Application of the U.S. EPA mode of action framework for purposes of guiding future research: A case study involving the oral carcinogenicity of hexavalent chromium. *Toxicol. Sci.* DOI:10.1093/toxsci/kfq320.

United States Environmental Protection Agency (U.S. EPA) (1985). Rapid assessment of exposure to particulate emissions from surface contamination sites. EPA/600-8-85002. Office of Health and Environmental Assessement. Washington D.C., United States Environmental Protection Agency.

U.S. EPA. (1991). Methods 218.6 for dissolved hexavalent chromium in drinking water, ground water, and industrial water effluents by ion chromatography. Available at: http://water.epa.gov/scitech/swguidance/methods/bioindicators/upload/2007_07_10_methods_method_218_6.pdf.

U.S. EPA. (1998). Toxicological review of hexavalent chromium in support of summary information on the integrated risk information system (IRIS). *U.S. Environmental Protection Agency, Washinton, D.C.*

U.S. EPA. (2000). Benchmark Dose Technical Guidance (External Review Draft). EPA/630/R-00/001. *Risk Assessment Forum: U.S. Environmental Protection Agency, Washington, D.C.*

U.S.EPA (2002). Supplemental Guidance for Developing Soil Screening Levels for Superfund Sites. US Environmental Protection Agency, Washington, DC. Available online at http://www.epa.gov/superfund/health/conmedia/soil/

U.S. EPA. (2004). Captan: Cancer Reclassification; Amendment of Reregistration Eligibility Decision; Notice of Availability. Federal Register69:68357–68360.

U.S. EPA. (2005a). Guidelines for carcinogen risk assessment, EPA/630/P-03/001F. Risk Assessment Forum: U.S. Environmental Protection Agency, Washington, D.C.

U.S. EPA. (2005b). Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens, EPA/630/R-03/003F. Risk Assessment Forum: U.S. Environmental Protection Agency, Washington, D.C.

U.S. EPA. (2006a). Approaches for the Application of Physiologically Based Pharmacokinetic (PBPK) Models and Supporting Data in Risk Assessment. National Center for Environmental Assessment, Washington, DC; EPA/600/R-05/043F.

U.S.EPA. (2006b). Harmonization in Interspecies Extrapolation: Use of BW 3/4 as Default Method in Derivation of the Oral RfD (External Review Draft). EPA/630/R-06/001. *Risk Assessment Forum: U.S. Environmental Protection Agency, Washington, D.C.*

U.S. EPA. (2007). Framework for Determining a Mutagenic Mode of Action for Carcinogenicity: Using EPA's 2005 Cancer Guidelines and Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens. *U.S. Environmental Protection Agency, Washinton, D.C.*

U.S. EPA. (2008). Annotated Reference Outline for the Toxicological Review of Hexavalent Chromium (Chromium VI). December 15, 2008. In Support of Summary Information for Integrated Risk Information System. NCEA, Washington, D.C. Available at http://www.epa.gov/IRIS/pdfs/Litsearch_chromium.pdf

U.S.EPA (2009). NCEA Policies and Procedures for Conducting IRIS Reviews. Attachment 2. July 30, 2009. NCEA, Washington, D.C. available at: http://www.epa.gov/iris/pdfs/Policy_IRIS_Peer_Reviews.pdf

U.S.EPA (2010a). USEPA Regional Screening Tables. May. Available online at http://www.epa.gov/reg3hwmd/risk/human/rb-concentration_table/index.htm

U.S.EPA (2010b) http://cfpub.epa.gov/ncea/iris_drafts/recordisplay.cfm?deid=200316

U.S. EPA (2010c) http://cfpub.epa.gov/ncea/iris_drafts/recordisplay.cfm?deid=221433

U.S. EPA. (2010d). Toxicological Review of Hexavalent Chromium. (CAS No.18540-29-9). In Support of Summary Information on the Integrated Risk Information System (IRIS) (External Review Draft). *U.S. Environmental Protection Agency, Washinton, D.C.*

Vitale, R., Mussoline, G., Rinehimer, K. (1997). Environmental monitoring of chromium in air, soil, and water. *Regul. Toxicol. Pharmacol*. **26**, S80-S85.

Wang, X.F., Xing, M.L., Shen, Y., Zhu, X., Xu, L.H. (2006). Oral administration of Cr(VI) induced oxidative stress, DNA damage and apoptotic cell death in mice. *Toxicology* **228**, 16-23.

Yang, L. (2006). Incidence and mortality of gastric cancer in China. *World J. Gastroenterol*. **12**, 17-20.

Yu, G.P., Hsieh, C.C. (1991). Risk factors for stomach cancer: A population-based case-control study in Shanghai. *Cancer Causes Control* **2**, 169-74.

Zhang, J., Li, X. (1987). Chromium pollution of soil and water in Jinzhou (Chinese language with English abstract). Zhonghua Yu Fang Yi Xue Za Zhi (Chinese Journal of Preventive Medicine) 21:262-264.

Zhang, J.D., Li., S. (1997). Cancer mortality in a Chinese population exposed to hexavalent chromium in water. *J. Occup. Environ. Med*. **39**, 315-19.

Zhitkovich, A. (2005). Importance of chromium-DNA adducts in mutagenicity and toxicity of chromium(VI). *Chem. Res. Toxicol.* **18**, 3-11.

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Attachment C:

The U.S. EPA determined that the MOA for intestinal tumors involves a mutagenic MOA. Molecular data in this target tissue are lacking and are needed to discern whether the MOA is more likely related to mutagenicity or observed species differences in intestinal hyperplasia.

Microarray technology enables the simultaneous profiling of genome expression elicited by hexavalent chromium (Cr(VI)), and facilitates the elucidation of intestinal and oral mucosa differential gene expression as a function of dose and time. Changes in gene expression have been (or will be) phenotypically anchored to complementary assessments of physiological and biochemical endpoints, including gross physiology, histopathology, total Cr tissue levels, cytokine levels, DNA-Cr adducts and reduced/oxidized glutathione (GSH/GSSG) measurements. Briefly described are preliminary toxicogenomic analyses pertinent to the MOA [1] and that are phenotypically anchored to histopathological and biochemical analyses of the mouse small intestine.

Animal study design

Southern Research Institute (Birmingham, Al) obtained 5-7 week old female B6C3F1 mice (16-24 g) and Fischer rats (70-110 g) from Charles Rivers Laboratories International, Inc. Animals were acclimated for 7 days and fed *ad libitum* with NTP-200 wafers (Zeigler Bros, Gardners, PA). Rodents were continuously exposed to Cr(VI)-containing sodium dichromate dihydrate (SDD) dissolved in drinking water at 0, 0.3, 4, 14, 60, 170 and 520 mg/L. The dose of 14 mg/L SDD was removed from the rat exposure study. Animals were euthanized using CO_2 on days 8 and 91, and samples of oral mucosa, duodenal, and jejunal epithelia were collected in vials containing TRIzol (Invitrogen, Carlsbad, CA) and snap-frozen in liquid nitrogen. The samples were stored at -80°C until further analysis until shipped on dry ice to Michigan State University for gene expression analysis.

RNA isolation

Frozen oral mucosa and intestinal samples were homogenized using a Mixer Mill 300 tissue homogenizer (Retsch, Germany). Total RNA was isolated according to the manufacturer's protocol with an additional acid phenol:chloroform extraction. Isolated RNA was resuspended in RNA storage solution (Ambion

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Inc., Austin, TX), quantified (A_{260}), and quality was assessed by determining the A_{260}/A_{280} ratio and by visual inspection of 1 μg on a denaturing gel.

Microarray analysis

Dose-dependent changes in gene expression were examined using mouse and rat 4 x 44 K Agilent whole genome oligonucleotide microarrays (Agilent Technologies, Inc., Santa Clara, CA). Treated samples were co-hybridized with vehicle controls to individual arrays according to manufacturer's protocol (Agilent Manual: G4140-90050 v. 5.0.1). All experiments were performed with three biological replicates with two independent labelings (Cy3 and Cy5) of each sample (dye swap) for every dose group within each time point and species. Microarray slides were scanned at 532 nm (Cy3) and 635 nm (Cy5) on a GenePix 4000B scanner (Molecular Devices, Union City, CA). Images were analyzed for feature and background intensities using GenePix Pro 6.0 (Molecular Devices). All data passed our laboratory quality assurance protocol [2] and deposited in TIMS dbZach data management system [3]. Microarray data were normalized using a semi-parametric approach [4] and the posterior probabilities were calculated using an empirical Bayes method based on a per gene and dose basis using model-based *t* values [5]. Gene expression data were initially ranked and prioritized using a |fold change|>1.5 and P1(*t*) value >0.999 cut-off to identify differentially expressed genes. Table 1 provides a summary of samples that have been processed for gene expression.

Table 1. Summary of toxicogenomic sample analysis.

Tissue	Mouse		Rat	
	Day 8	Day 91	Day 8	Day 91
Duodenum	✓	✓	✓	IP
Jejunum	✓	IP	IP	IP
Oral mucosa	✓	✓	✓	IP

IP - in progress.

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Quantitative real-time PCR (QRTPCR)

QRTPCR was used to verify expression of select genes identified as differentially regulated in the microarray analysis. Total RNA (1 µg) was reverse transcribed by SuperScript II (Invitrogen) using an anchored oligo-dT primer as described by the manufacturer. The cDNA (1 µl) was used as a template in a 30 µl PCR reaction containing 0.1 µM of forward and reverse gene-specific primers, 3 mM $MgCl_2$, 1 mM dNTPs, 0.025 IU AmpliTaq Gold, and 1× SYBR Green PCR buffer (Applied Biosystems, Foster City, CA). PCR amplification was conducted on an Applied Biosystems PRISM 7500 Sequence Detection System. cDNAs were quantified using a standard curve approach and the copy number of each sample was standardized to 3 housekeeping genes (Actb, Gapdh, Hprt for mouse and ActB, Hprt, Rpl13a for rat) to control for the differences in RNA loading, quality, and cDNA synthesis [6].

Preliminary Results and Discussion

Mouse duodenal gene expression was evaluated using whole genome 4x44K Agilent oligonucleotide microarrays containing 21,307 unique annotated genes. Statistical analysis (|fold change|>1.5, P1(*t*)>0.999) identified a dose-dependent increase in the number of differentially regulated transcripts. A total of 6542 (day 8) and 4630 (day 91) duodenal genes were differentially expressed at one or more doses. Using the same filtering criteria (|fold change|>1.5, P1(*t*)>0.999), 4448 differentially expressed genes were identified in the jejunal dataset at day 8. Comparative analysis identified 3432 genes commonly expressed in duodenum (day 8 vs. 91) with 3785 overlapping genes between the duodenum and jejunum datasets (day 8). Relaxing the filtering criteria (|fold change|>1.2, P1(*t*)>0.9) to avoid exclusion of genes that may fall short of the initial cut-offs (|fold change|>1.5, P1(*t*)>0.999) approximately doubled the number of overlapping differentially expressed genes, indicating the differential expression of duodenal genes at day 91 was a subset of the genes differentially expressed at day 8. Similarly, the differential expression of jejunal genes was a subset of the duodenal genes differentially expressed. In addition to more genes being differentially expressed in the duodenum at day 8 as compared to the jejunum, duodenal day 8 gene expression also exhibited larger fold changes. These findings are consistent with the proposed MOA, where Cr(VI) is reduced as it passes through the intestinal tract, and therefore less Cr(VI) is delivered to the jejunum resulting in relatively less dynamic gene expression.

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Database for Annotation, Visualization and Integrated Discovery (DAVID) was used to associate functions to the differentially expressed genes [7]. Over represented functions included oxidoreductase activity, antigen processing and presentation, cell cycle and DNA replication, lipid metabolism and transport, cell death, and cellular assembly and organization. In contrast, other functions such as chromatin modification, cell to cell signaling, and cell differentiation were not over represented in the duodenum differential gene expression dataset. Several of these over represented functions can be phenotypically anchored to complementary changes in biochemical measures of oxidative stress (e.g. GSH/GSSG ratio) as well as histopathological evidence of hyperplasia. Table 2 summarizes the significant biochemical and histopathology changes relative to the proposed MOA key events.

Table 2. Summary of relevant biochemical and histopathology results.

Endpoint	Proposed MOA key event	Tissue	Mouse		Rat	
			Day 8	Day 91	Day 8	Day 91
Reduced GSH/GSSG ratio	Oxidative stress	Duodenum	✓	✓	IP	IP
		Jejunum	NC	✓	IP	IP
Crypt hyperplasia	Cell proliferation	Duodenum	✓	✓	IP	IP
		Jejunum	NC	✓	IP	IP
Histiocytic infiltration	Inflammation	Duodenum	NC	✓	IP	IP
		Jejunum	NC	✓	IP	IP

NC, no change; IP, in progress

For example, several genes involved in oxidative stress exhibited dose-dependent expression at 8 and 91 days. SDD significantly induced (~1.6- to 6-fold) members of the redox-sensitive, Nrf2 transcription factor family, including Gpx2, Gsr, Prdx1, Ub2r1, Usp14, Hip2, Atf4 and Mrp1 (Figure 1). Nrf2 target genes are involved in antioxidant, detoxification, and cytoprotective functions. Induction of members of the Nrf2 pathway suggests activation of intestinal defense mechanisms in response to xenobiotic stress, consistent with reduced

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GSH/GSSG ratio. Figure 1 includes dose-dependent changes in Nrf2, Atf4, and Gpx2 expression. Taken together, these data suggest significant nonlinearities in oxidative stress responses.

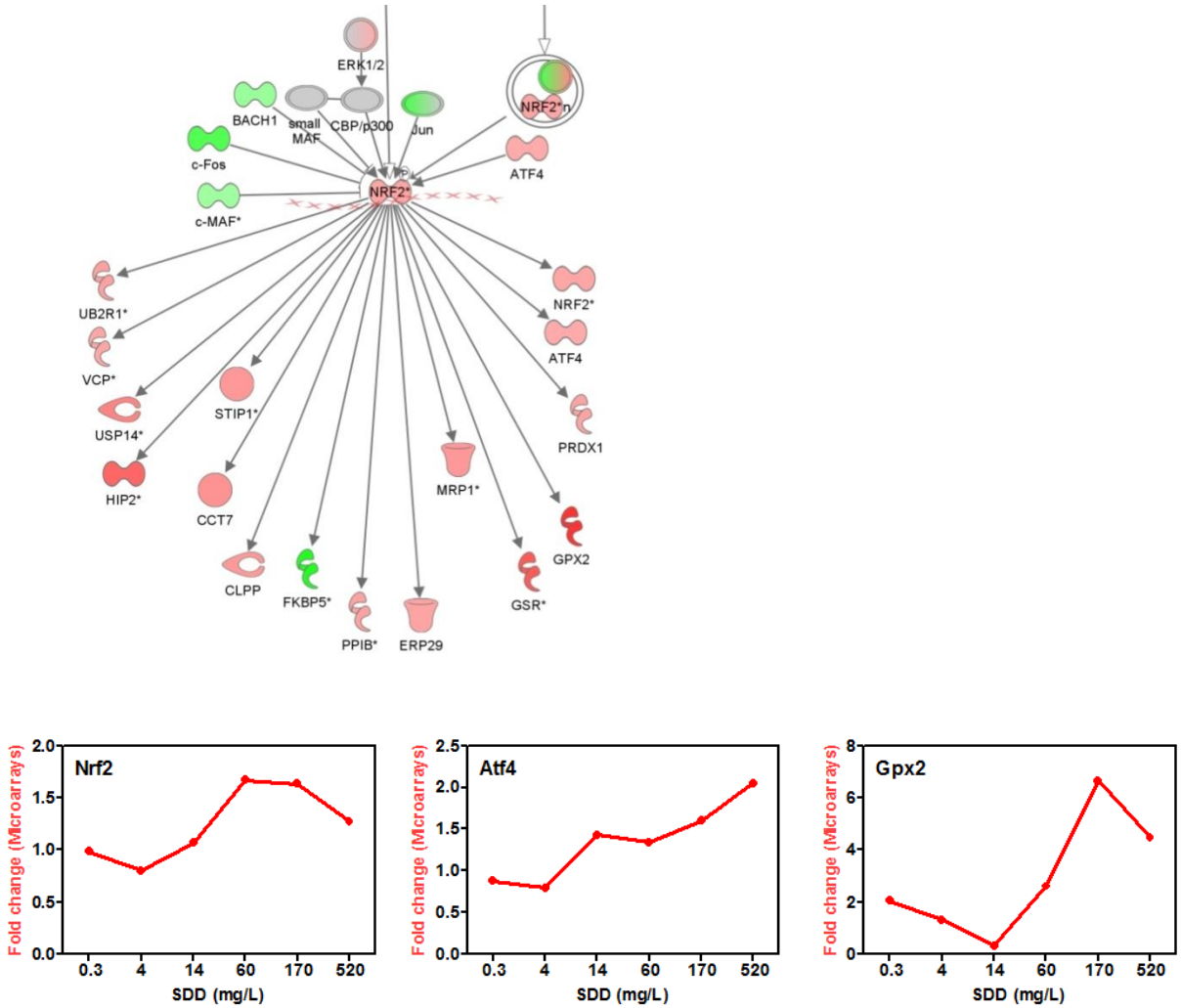

Figure 1. Nrf2-mediated oxidative stress response pathway (Ingenuity Pathway Analysis). Red and green colors represent induced and repressed genes, respectively, while different shapes correspond to different functional families. Dose-dependent induction of Nrf2 and two target genes, Atf4 and Gpx2, in duodenal epithelial scrapings at day 8.

SDD induced cell proliferation as indicated by intestinal crypt hyperplasia and the dose-dependent induction of Myc. In addition, there was induction of several down-stream Myc target genes, including Rcl, Grpel1, Cdca7, Heatr1, Ttc27, Nop56, Mrps22, Timm9/10/22, Adk, and Mina (Figure 2). These genes exhibited similar dose-dependent induction with ~2-fold greater efficacy at day 8 compared to day 91 and when compared to jejunum at day 8. Myc regulates gene expression associated with cell proliferation, apoptosis, growth and metabolism [8]. For example, Mina is directly regulated by Myc, and inhibition by RNAi severely suppresses

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cell proliferation [9]. Figure 2 includes the dose-dependent induction in Myc and its target genes, Mina and Nop56.

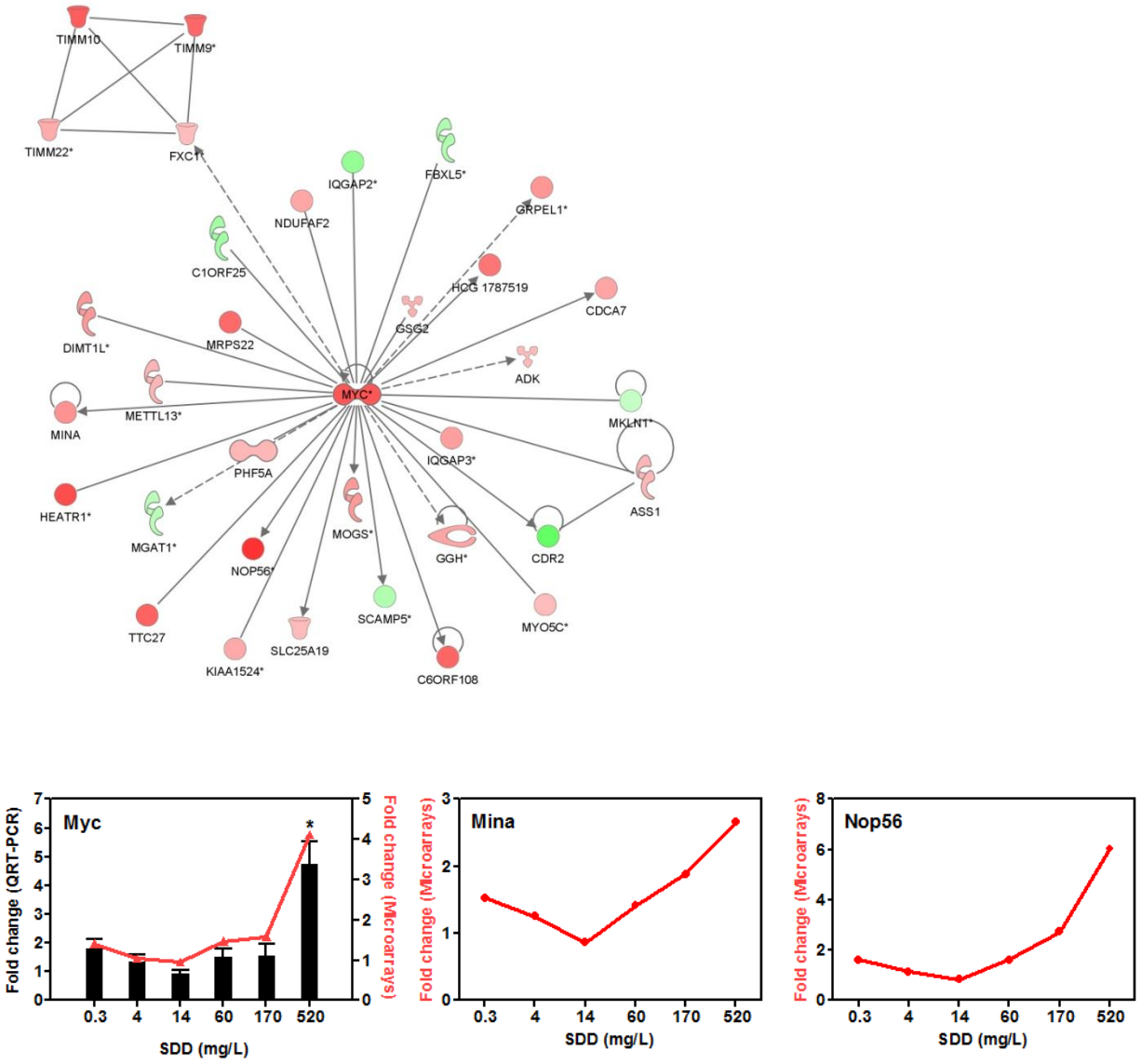

Figure 2.
Myc-mediated proliferation pathway (Ingenuity Pathway Analysis). Red and green colors represent induced and suppressed genes, respectively, while different shapes correspond to different functional families. Dose-dependent induction of Myc and its two target genes, Mina and Nop56, in duodenal epithelial scrapings at day 8 are shown below. Myc induction on the microarray (red line) was confirmed using QRTPCR (black bars: mean ± standard error of 5 independent replicates). Asterisk (*) indicates $p<0.05$ vs. 0 mg/L SDD.

In summary, Cr(VI)-elicited dose-dependent intestinal gene expression changes in the mouse are consistent with the measured changes in histopathology and biochemistry. Moreover, the changes are consistent with proposed key events in our hypothesized MOA [1]. Importantly, changes in transcripts related to DNA

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repair pathways will be linked to data on Cr(VI) present in DNA when these analyses are completed. It should also be noted that these gene changes can also be expressed as a function of tissue dose following development of suitable PBPK models.

Additional data analyses are ongoing. To date, our functional analysis focused on establishing evidence for the key events proposed within our hypothesized MOA. Analysis of other key events that may also contribute to the MOA of Cr(VI) warrant further investigation. As always, complete elucidation of the role of each differentially regulated gene is not feasible due to incomplete gene annotation, as well as the lack of fully elucidated pathways. In addition, proteomic (e.g. expression levels and post-translational modifications) and metabolomic changes may not be reflected by changes in gene expression.

References

1. Thompson, C.M., et al., *Application of the U.S. EPA Mode of Action Framework for Purposes of Guiding Future Research: A Case Study Involving the Oral Carcinogenicity of Hexavalent Chromium.* Toxicol Sci, 2010.
2. Burgoon, L.D., et al., *Protocols for the assurance of microarray data quality and process control.* Nucleic Acids Res, 2005. **33**(19): p. e172.
3. Burgoon, L.D. and T.R. Zacharewski, *dbZach toxicogenomic information management system.* Pharmacogenomics, 2007. **8**(3): p. 287-91.
4. Eckel, J.E., et al., *Normalization of two-channel microarray experiments: a semiparametric approach.* Bioinformatics, 2005. **21**(7): p. 1078-83.
5. Eckel, J.E., et al., *Empirical bayes gene screening tool for time-course or dose-response microarray data.* J Biopharm Stat, 2004. **14**(3): p. 647-70.
6. Vandesompele, J., et al., *Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes.* Genome Biol, 2002. **3**(7): p. RESEARCH0034.
7. Dennis, G., Jr., et al., *DAVID: Database for Annotation, Visualization, and Integrated Discovery.* Genome Biol, 2003. **4**(5): p. P3.
8. Dang, C.V., *c-Myc target genes involved in cell growth, apoptosis, and metabolism.* Mol Cell Biol, 1999. **19**(1): p. 1-11.
9. Tsuneoka, M., et al., *A novel myc target gene, mina53, that is involved in cell proliferation.* J Biol Chem, 2002. **277**(38): p. 35450-9.

Attachment D:

Comments on USEPA's Draft IRIS Report for Hexavalent Chromium

December 21, 2010

Prepared for:

American Chemistry Council
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Prepared by:

SUMMIT TOXICOLOGY

1.0 Introduction

Overall, USEPA's draft T*oxicological Review of Hexavalent Chromium* (USEPA, 2010) appears to be fairly comprehensive and well written. We have prepared comments, focusing on the dose-response assessment sections of the document for consideration. Our comments are organized into two sections: comments on the dose response assessment (**Section 2.0**), and a summary of ongoing research (**Section 3.0**)

2.0 Dose-Response Comments

Comments on USEPA's oral RfD and cancer potency estimates for hexavalent chromium are summarized below.

2.1 Comments on Oral Reference Dose

<u>Dose Measure Selection</u>
The critical endpoint on which the oral RfD was based (diffuse epithelial hyperplasia in small intestine of male and female mice) was assessed in terms of administered dose (unscaled mg/kg-day). Because these effects occur at the portal-of-entry, and therefore occur prior to absorption into blood, the response is not related to systemic dose. EPA should include consideration/discussion of alternative dose measures such as tissue (duodenal) dose using some form of modeling. It is recognized that existing PBPK models for chromium do not provide a description of tissue dose in the small intestines. However, a refined PBPK model is currently under development (see Section 3). Once complete, EPA should consider using the model to assess the dose-response relationship for chromium and duodenum hyperplasia in terms of target tissue dose.

<u>Data Set Selection</u>
In USEPA's dose-response assessment for the oral RfD, the data sets for male and female animals were assessed separately. The dose-response data for duodenal hyperplasia in male and female do not appear to be significantly different from one another, and the response in both sexes increases in a non-monotonic manner (**Figure 1A**). Furthermore, there are no mechanistic data to support a potential sex difference for the critical endpoint, and the resulting RfD value will be applied equally to populations of men and women in subsequent risk assessments. Rather than dropping the two highest dose groups from the female data set (relying on only three dose groups and excluding seven dose groups [two dose groups from female mice and all five dose groups from make mice] to derive the RfD), USEPA should consider using the male and female data sets pooled together to increase dose coverage and statistical power of the data set. Based upon visual inspection of the pooled data set, consideration of potential outliers should include dose-response modeling of: (1) all ten dose groups; (2) dropping the three highest dose groups (two female groups and one male group [circled in green in Figure 1A], leaving seven dose groups for modeling); and (3) dropping the female dose group at 57.3

mg/L and the male dose group at 85.7 mg/L [circled in red in Figure 1A], leaving eight dose groups modeled). The points of departure from the three approaches can then be compared prior to selection of one for use in RfD derivation.

2.2 Comments on Cancer Potency Estimates

Dose Measure Selection

The critical endpoint (adenomas/carcinomas in small intestine) was assessed in terms of administered dose, allometrically scaled to the ¾ power. Although it is recognized that allometric scaling reflects the default dose measure for cancer assessment as a matter of USEPA policy (USEPA, 1992, 2005), this approach is not appropriate for this endpoint. Because these effects occur at the portal-of-entry, the response is not related to systemic dose, and thus should not be subject to dividing by body weight or allometric scaling. Furthermore, by scaling dose to the ¾ power, the dose measure used for the cancer assessment is internally inconsistent with the dose measure used for the oral RfD (unscaled mg/kg-day), despite the fact that they share common steps in their respective modes of action. At minimum, USEPA should consider harmonizing the noncancer and cancer dose-response assessments for chromium with respect to dosimetry, using a dose measure such as mass per epithelial surface area (mg/cm^2). It is recognized that existing PBPK models for chromium (O'Flaherty, 1996; O'Flaherty et al., 2001) do not provide a description of tissue dose in the small intestines. However, a refined PBPK model is currently under development (see Section 3). Once complete, EPA should consider using the model to assess the dose-response relationship for chromium and tumors of the small intestines in terms of target tissue dose.

Data Set Selection

In deriving cancer potency estimates for chromium, data sets for male and female animals were assessed separately. The dose-response data for small intestinal tumors in male and female mice do not appear to be significantly different from one another as suggested by visual inspection of **Figure 1B** and by the nearly identical potency estimates derived by USPEA (0.5 per mg/kg-day for males, 0.6 per mg/kg-day for females). Furthermore, there are no mechanistic data to support a potential sex difference for the critical endpoint, and the resulting cancer potency factor will be applied equally to populations of men and women in subsequent risk assessments. To improve the statistical power of the dose-response modeling, USEPA should consider pooling the data from both sexes prior to dose-response modeling.

In deriving cancer potency estimates for chromium, USEPA summed the tumor incidence across the duodenum and jejunum. Inspection of **Figure 1B** indicates a clear response gradient for tumor incidence (duodenum>jejunum>ileum), which likely mirrors a tissue dose gradient for hexavalent chromium. By combining tumors across intestinal sections and using a crude dose measure of administered dose, important information on this gradient is lost. As noted above, a refined PBPK model is currently under development (see **Section 3.0**). Once complete, EPA

should consider using the model to assess duodenum and jejunum tumor response with respect to the gradient of tissue doses.

3.0 Ongoing Research and Toxicokinetic Data Collection

To support risk assessment activities, the existing PBPK model for chromium in rats and humans (**Figure 2A**; O'Flaherty 1996; O'Flaherty et al., 2001) will be revised to include an expanded description of the gastrointestinal tract, including separate compartments for target tissues (oral cavity, duodenum, jejunum; **Figure 2B**). To provide a description of tissue dose in the small intestines, the model will include parameters for competing rates for hexavalent and trivalent chromium (**Figure 3**). The revised PBPK model will be able to: (1) provide a description of tissue dose in tumor target tissues for use in dose-response assessment; (2) assess variation in human model parameters for consideration of potentially sensitive subpopulations. Ongoing research to help collect the parameters and data need to support PBPK model development is summarized below.

<u>Ex Vivo Reduction Studies</u>

In addition to the studies discussed in Section 3.1.1 of the draft IRIS document, an *ex vivo* study has been conducted using human gastric fluid to examine the effects of real vs. simulated fluid, pH (1.5 vs. 4.5), dilution, antacids, and food on the reduction of hexavalent chromium. These results were presented in 2002 at the Society of Toxicology Conference (Proctor et al. 2002). The results of these studies are summarized below.

- *Real vs. Simulated* - Real human stomach fluid was found to have a much greater capacity to reduce Cr(VI) than synthetic stomach fluid (**Figure 4A**). It reduces approximately 2,000 μg Cr(VI)/L of stomach fluid over approximately 60 minutes and between 300μg - 1000μg Cr(VI)/L within the first two minutes. In comparison, synthetic stomach fluid reduces approximately 150 μg Cr(VI)/L over approximately 60 minutes and 70μg Cr(VI)/L within the first two minutes. However, the first-order rates of reduction were similar between synthetic and real stomach fluid. Therefore, synthetic stomach fluid is a reasonable surrogate for testing factors that might effect the rate of Cr(VI) reduction in real human stomach fluid, but is likely to underestimate reduction capacity by approximately a factor of 10.
- *pH* – pH can vary across individuals and within individuals over time. It is also known that the pH environment can impact the reduction of Cr(VI) to Cr(III). Within 60 minutes, pH 1.5 synthetic stomach fluid reduced approximately 150 µg Cr(VI)/L of stomach fluid, whereas pH 4.5 stomach fluid reduced approximately 50 µg Cr(VI)/L of synthetic stomach fluid (**Figure 4B**). Within the first two minutes, pH 1.5 reduced approximately 60 µg Cr(VI)/L of synthetic stomach fluid, whereas pH 4.5 reduces approximately 40 µg Cr(VI)/L of synthetic stomach fluid. The shapes of the

two curves are similar, especially at early time points. However, at later time points, the pH 1.5 stomach fluid has a sustained capacity to reduce Cr(VI). The pH 4.5 synthetic stomach fluid reduced the Cr(VI) much more rapidly (half-life = 0.75 minutes) than the pH 1.5 solution (half-life 4.7 and 9.9 minutes for two tests). Therefore, while the overall capacity to reduce Cr(VI) is decreased by raising the pH of the synthetic stomach fluid, the rate of reduction was accelerated.

- *Dilution* – A ten-fold dilution of synthetic stomach fluid reduced approximately 20 percent (30 µg Cr(VI)/L) of the amount of Cr(VI) as full-strength stomach fluid (approximately 150 µg Cr(VI)/L) over a 60-minute period. Thus, diluted stomach fluid reduces approximately the same amount of Cr(VI), if not more, as full-strength stomach fluid when put in terms of actual gastric fluid and enzymes. After approximately 2 minutes, 10 µg Cr(VI) was reduced per liter of 10% stomach fluid. The first-order rate of reduction for the diluted synthetic stomach fluid had a longer half-life than full strength synthetic stomach fluid (4 minutes versus 0.75 minutes, respectively).
- *Antacids* - Synthetic stomach fluid with the presence of Rolaids (pH 8.2) was able to reduce as much Cr(VI) as synthetic stomach fluid at pH 4.5. Therefore, besides the effect of drastically raising the pH of the synthetic stomach fluid, the presence of Rolaids® did not affect the reduction capacity of the synthetic stomach fluid. After approximately 60 minutes, synthetic stomach fluid with Rolaids® reduced approximately 50 µg Cr(VI)/L of stomach fluid. After approximately 2 minutes, the synthetic stomach fluid with Rolaids® reduced approximately 40 µg Cr(VI)/L of synthetic stomach fluid. The half-life of Cr(VI) reduction in the presence of Rolaids was longer than without (2.37 minutes versus 0.75 minutes, respectively).
- *Food* - After approximately 60 minutes, the synthetic stomach fluid with food reduced approximately 30,000 µg of Cr(VI) per liter of stomach fluid (**Figure 4C**). After approximately 4 minutes, 10,000 µg/L of Cr(VI) were reduced by synthetic stomach fluid with food. Coffee alone has a very high capacity to reduce Cr(VI), and therefore if a meal were consumed with coffee, the resulting amount of Cr(VI) reduction would be even greater than observed with food alone.

A manuscript that describes the methods and results of the *ex vivo* study for human gastric fluid is in preparation (Hays et al., 2011, in preparation). Additional studies with real human, rat and mouse stomach fluid have been designed and will be conducted within the next two months. These studies will include fed and fasted conditions in rodents and humans. Together, these studies will be used to estimate Cr(VI) reduction rates and capacities for use in the revised PBPK model for chromium.

In vivo pharmacokinetic studies

Although the NTP (2008) bioassay includes excellent data for describing chromium behavior in rats and mice, a number of data gaps were identified, including tissue levels in the small intestinal sections and the oral cavity. For this reason, a supplementary 90-day drinking water study for hexavalent chromium in rats and mice is ongoing. The study design includes a test concentration range that mirrors the NTP test range, with the addition of two low dose groups, for a total of seven treatment groups (0, 0.3, 4, 14, 60, 170, 520 mg/L). Tissue chromium will be determined in the following tissues: oral mucosa, duodenum, jejunum, ileum, glandular stomach, plasma, red blood cells, and liver.

4. References

Hays S, Aylward L, Kirman C and D Proctor. 2011. Reduction rate and capacity of real and simulated stomach fluid. (In preparation)

O'Flaherty E. 1996. A physiologically based model of chromium kinetics in the rat. Toxicol Appl Pharmacol 138:54-64.

O'Flaherty E, Kerger B, Hays S, Paustenbach D. 2001. A physiologically based model for the ingestion of chromium(III) and chromium (VI) by humans. Toxicol Sci 60:196-213.

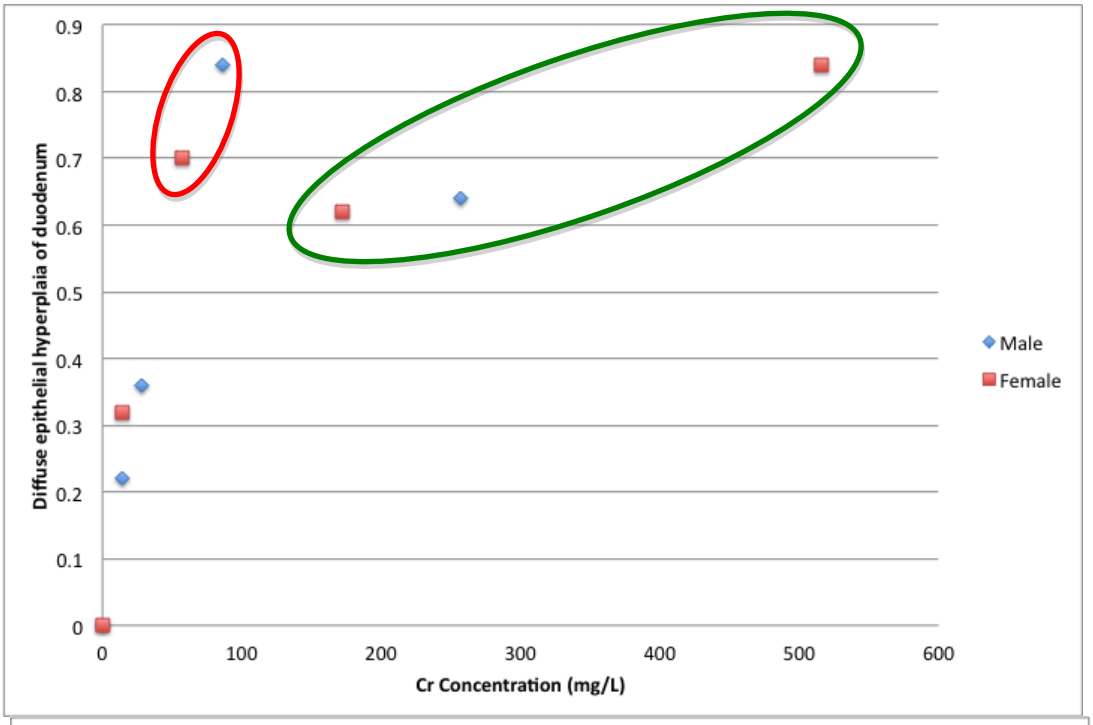
NTP. 2008. Toxicology and carcinogenesis studies of sodium dichromate dehydrate in F344/N rats and B6C3F1 mice. National Toxicology Program. TR 546.
Proctor D, Hays S, Ruby M, Liu S, Sjong A, Goodman M, Paustenbach D. 2002. Rate of hexavalent chromium reduction by human gastric fluid. Toxicol Sci 66(1-s):347.

USEPA. 1992. Draft report: a cross-species scaling factor for carcinogen risk assessment based on equivalence of mg/kg3/4/day. Federal Register 57(109):24152-24173.

USEPA. 2005. Guidelines for carcinogen risk assessment. U.S. Environmental Protection Agency. EPA/630/P-03/001/B

Figure 1. Dose-Response Data for Small Intestines Lesions in Male and Female Mice (NTP, 2009): (A) Diffuse epithelial hyperplasia of the duodenum; (B) Tumors in the duodenum, jejunum, and ileum. (see text for discussion of circled data points)

(A)



(B)

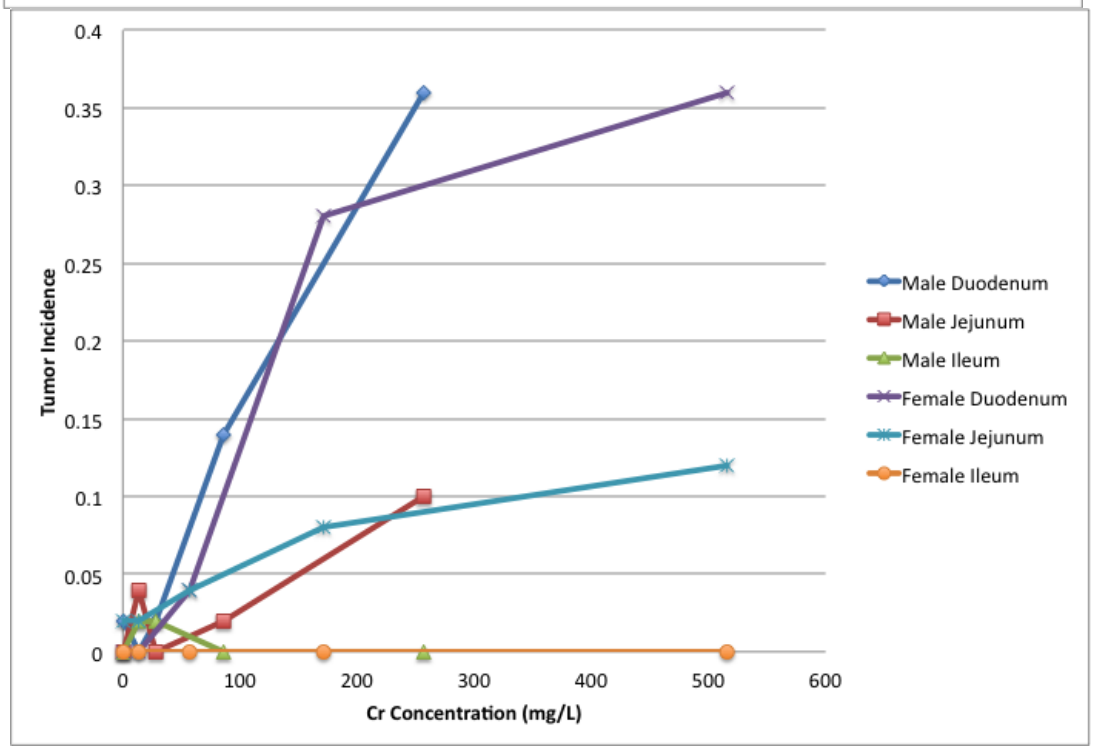
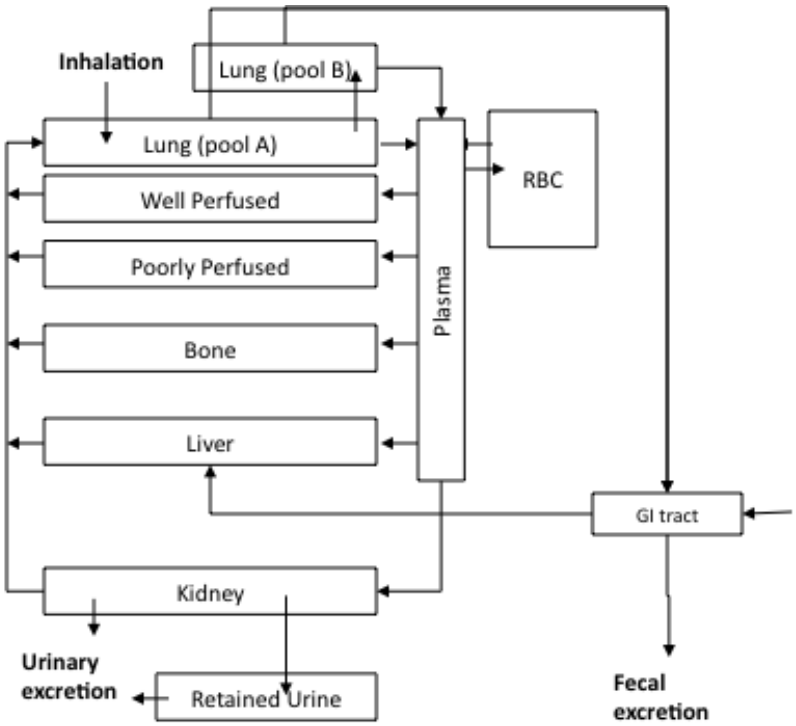


Figure 2. Existing (A) and Proposed (B) PBPK models for Chromium

(A)



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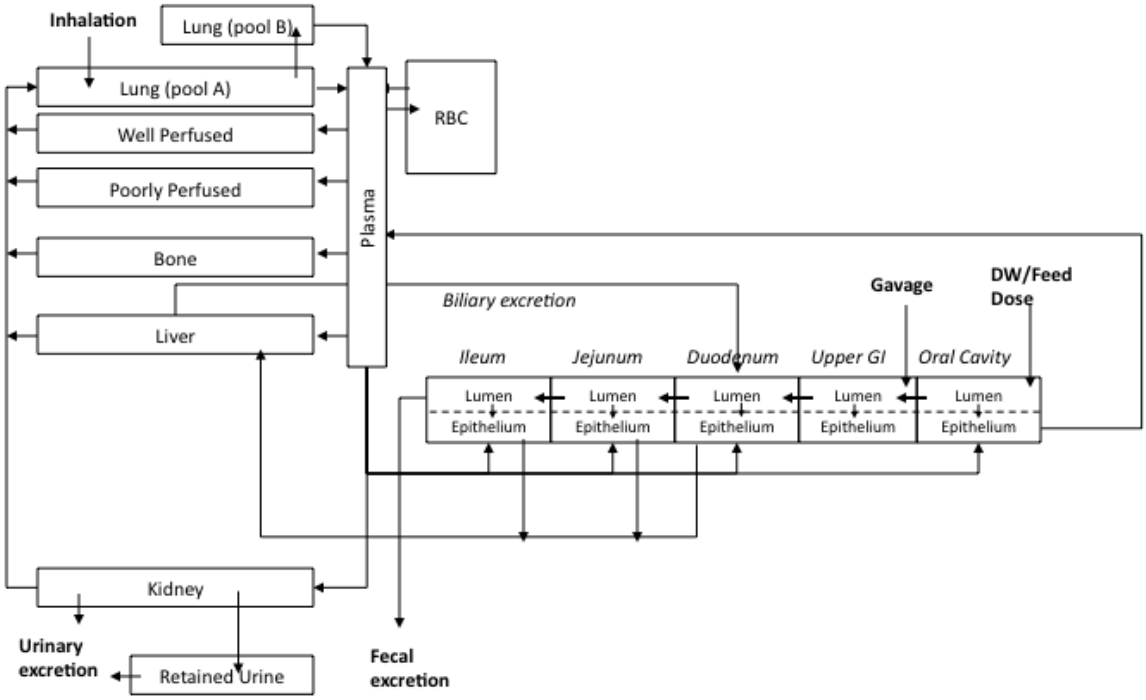


Figure 3. Competing Rates for Hexavalent Chromium During Absorption

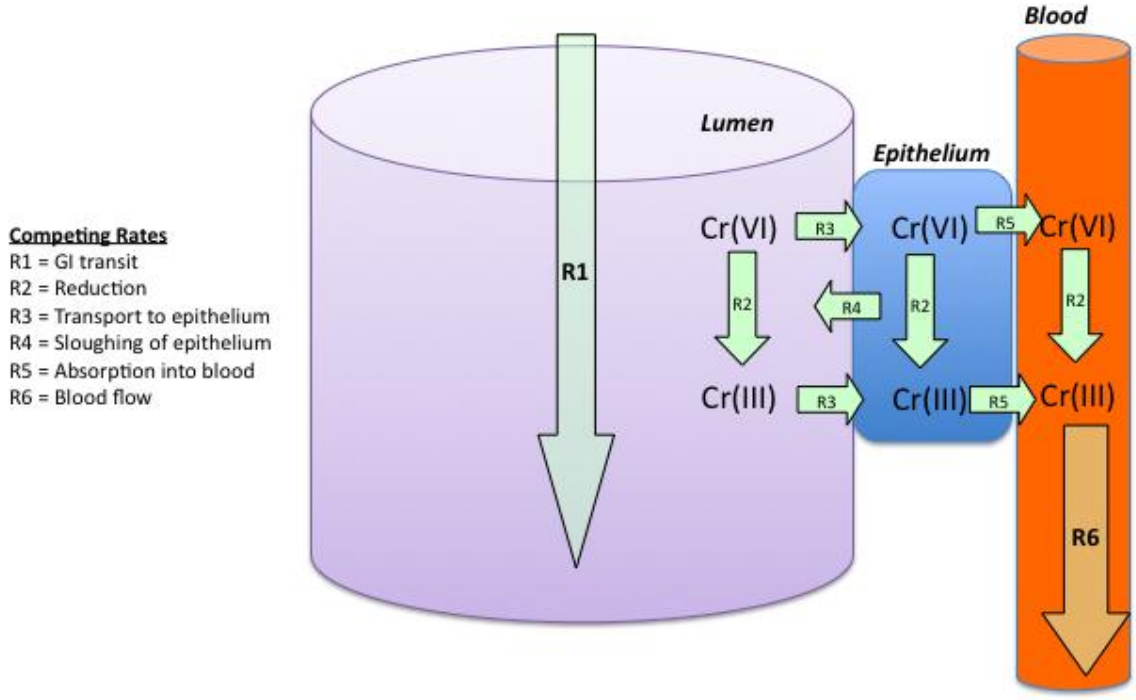
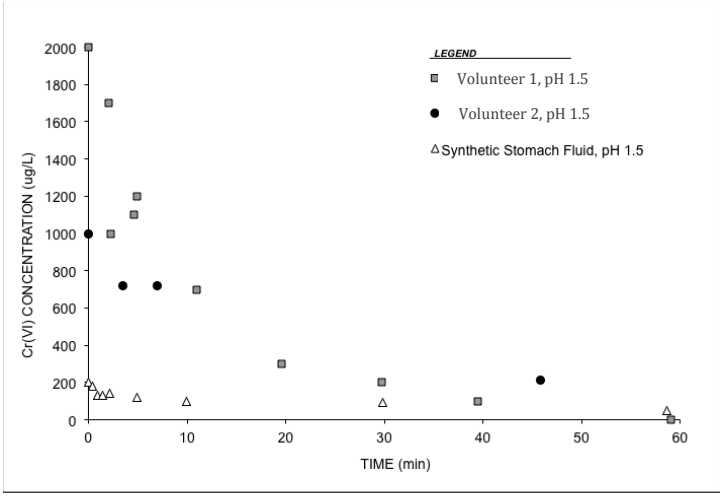
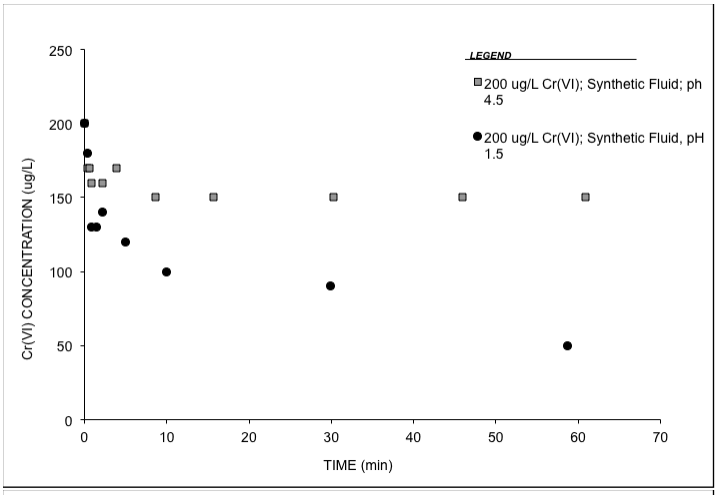
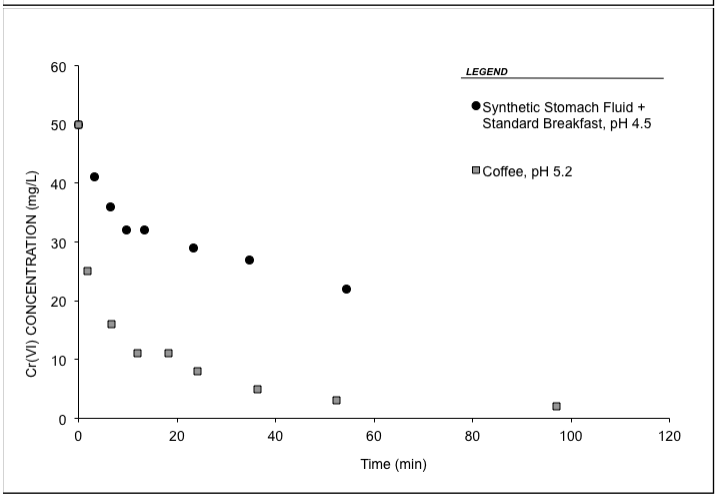


Figure 4. Ex Vivo Data for Cr Reduction: (A) Real vs. synthetic fluid; (B) pH 1.5 vs. pH 4.5; [C] presence of food.







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October 19, 2009

Comments to the draft document entitled "Public Health Goal for Hexavalent Chromium in Drinking Water", prepared by the California Environmental Protection Agency (Office of Environmental Health Hazard Assessment)

Although I find that several parts of the OEHHA Document are written appropriately and with competence, I have some important reasons of concern, as follows.

Comment #1. Detoxification of Cr(VI) in the organism

I started working on this subject more than 30 years ago, by investigating Cr(VI) reduction in blood and liver (e.g., S. De Flora, *Nature* 271, 455-6, 1978; F.L. Petrilli and S. De Flora, *Mutat. Res.* 54, 139-147, 1978), in the respiratory tract (e.g., F.L. Petrilli *et al.*, *J. Clin. Invest.* 77, 1917-24, 1986; S. De Flora *et al.*, *Cancer Res.* 47, 4740-5, 1987), in the gastric environment (e.g., S. De Flora *et al.*, *Mutat. Res.* 192, 159-174, 1987), and in other body compartments (reviewed in S. De Flora *et al.*, *Carcinogenesis*, 18, 531-7, 1997). Some of my papers are correctly reported in the OEHHA Document.

These data, generated in *ex vivo* studies in both humans and animal models, led me to develop the theory that Cr(VI) genotoxicity and potential carcinogenicity tend to be attenuated or suppressed in the body. This theory is widely accepted in the international literature. For instance, when commenting my data, the IARC Working Group "interpreted these findings as indicating mechanisms that limit the activity of Cr(VI) compounds *in vivo*" (IARC Monographs, Vol. 49, 1990). In the ATSDR Document, the US Department of Health and Human Services indicated that these "mechanisms limit the bioavailability and attenuate the potential effects of Cr(VI) compounds *in vivo*. Thus, the oral toxicity of chromium is low" (US Dept. of Health and Human Sciences,

1993). The USEPA concluded that “the body’s normal physiology provides detoxification for Cr(VI)” (USEPA, 1991).

Although it is evident that Cr(VI) detoxification mechanisms represent formidable barriers against Cr(VI) toxicity, genotoxicity, and carcinogenicity, I do not pretend that they are infinite and cannot be saturated. Under certain conditions, especially in animal models, they may be overwhelmed as a function of the dose and of the administration route (see Comment #9). Therefore, the statement, reported on page 17 of the OEHHA Document, that according to my studies the Cr(VI) detoxifying mechanisms in the organism are “essentially inhexhaustive” does neither reflect my opinion nor what is written in my papers.

Comment #2. Genotoxicity of oral Cr(VI) in the intestinal tract

On page 37 is stated that no study to date has looked for DNA damage in the oral cavity or gastrointestinal tract following oral administration of Cr(VI). It is also stated that these studies are needed. The authors of the document overlooked our *ad hoc* study (S. De Flora *et al.*, *Mutat. Res.* 659, 60-69, 2008), in which we demonstrated that the daily administration of sodium dichromate to SKH-1 mice, at the doses of 5 or 20 mg/L for 9 consecutive months, failed to enhance the frequency of DNA-protein crosslinks and did not cause oxidative DNA damage, measured in terms of 8-oxo-dGuo, in mouse forestomach, glandular stomach, and duodenum.

Comment #3. Genotoxicity studies of oral Cr(VI) in cells outside the GI tract

Table 2 and pages 37-41 of the OEHHA Document summarize studies on the genotoxicity of Cr(VI) administered by the oral route.

Again, relevant literature data were overlooked. In a study of mine (S. De Flora *et al*., *Mutat. Res*. 610, 38-47, 2006), potassium dichromate and sodium dichromate failed to affect the frequency of micronucleated erythrocytes in bone marrow and peripheral blood of BDF1 and Swiss mice of both genders when administered with the drinking water, up to a concentration of as much as 500 mg Cr(VI)/L for up to 210 consecutive days. Even a single intragastric dose of 17.7 mg/kg body weight was negative. In addition, the same Cr(VI) salts, administered to pregnant Swiss albino mice, up to a concentration of 10 mg/L drinking water, did not cause any toxic or genotoxic effect in fetus liver or peripheral blood.

Surprisingly, unless I missed them somewhere else in the document, even the NTP studies evaluating the frequency of micronucleated erythrocytes in peripheral blood were not cited. These studies (J. Bucher, *Toxic. Rep. Ser.* 72, 1-G4, 2007) showed that sodium dichromate was negative in both male and female B6C3F1 mice, in which the compound was administered for 3 consecutive months at concentrations ranging between 21.8 and 349.0 mg Cr(VI)/L. Sodium dichromate was also negative in male BALB/c mice, and gave results which were classified as equivocal in a further experiment in male B6C3F1 mice, in which the increase of micronucleated erythrocytes frequency did not reach the statistical significance threshold. In male C57BL/6 mice transgenic for *PhiX174am3*, the compound was positive in one experiment but negative in another one.

Thus, on the whole, when introduced with the drinking water at doses exceeding up to 10,000 times drinking water standards for total chromium, or even following massive intragastric administration, the bulk of evidence is that Cr(VI) compounds do not increase the frequency of micronucleated erythrocytes in mice of both genders and various age, belonging to a variety of strains (BDF1, Swiss albino, Swiss Webster, B6C3F1, and BALB/c).

Comment #4. The NTP carcinogenicity study in mice and rats

I believe that the NTP carcinogenicity study with sodium dichromate dihydrate was quite important, timely, and well executed. What I disagree with is the interpretation of the results obtained.

When I had the opportunity, years ago, to see the design of this study, I expected that forestomach tumors and perhaps glandular stomach would have developed at the highest doses of sodium dichromate tested, which were extremely high. The reason for this expectation is that, as specified in Comment #1, the detoxifying capacity of the gastric environment is not infinite. Moreover, as also explained in the OEHHA Document, the rodent forestomach is a vulnerable tissue, particularly to irritants such as high-dose Cr(VI). Indeed, evaluation of Cr(VI) carcinogenicity in the stomach was the major focus of the NTP study and the major premise for performance of this study. Therefore, it is particularly important that, even at the extremely high Cr(VI) doses tested, no stomach tumor was induced by sodium dichromate in either rodent species.

The conclusion of the NTP study, as reported in the only paper published in a peer-reviewed journal (Stout *et al*., *Environ. Hlth Perspect.*, 117, 716-22, 2009), was that "Cr(VI) exposure resulted in

increased incidences of rare neoplasms of the squamous epithelium that lines the oral cavity (oral mucosa and tongue) in male and female rats, and of the epithelium lining the small intestine in male and female mice". As noted in the OEHHA Document, a statistically significant increase of oral cancers only occurred at the highest dose tested (516 mg/L sodium dichromate) in both male and female rats. A statistically significant increase of small intestine tumors only occurred at the highest dose tested in male mice (257.4 mg/L) and at the two highest doses tested in female mice (172 and 516 mg/l). These are huge doses! One should go to the lab and see the color and appearance of water containing hundreds or even tens mg/L Cr(VI). Nobody would drink this water unless for suicidal purposes (which probably would be unsuccessful, see Comment #8). No effect was observed at the lowest doses tested in the NTP study, corresponding to 5-30 mg Cr(VI)/L water (which still are quite high doses), which is in agreement with the conclusions of our genotoxicity study (S. De Flora *et al*., *Mutat. Res.* 659, 60-67, 2008), ruling out that DNA damage may occur not only in the forestomach and glandular stomach but also in the duodenum of mice receiving sodium dichromate with the drinking water, at the doses of 5 and 20 mg Cr(VI)/L (see Comment #2).

It should be noted that in the NTP study there were significant decreases of certain tumors in Cr(VI)-treated rodents, such as a decrease of total benign tumors in both rats (females only) and mice (males only), which by the way was the only concomitant change in the two rodent species, a decrease of pituitary gland tumors in both male and female mice, and a decrease of liver adenomas in both male and female mice, which was the only effect observed at 2 or 3 Cr(VI) concentrations. Clearly, although these decreases are statistically significant, they do not mean that Cr(VI) is protective but highlight the fact that, likewise, significant increases at high doses are not biologically significant and do not bear relevance to the human situation.

Comment #5. Carcinogenic potency of Cr(VI) in humans

On page 58, last paragraph, it is stated that IARC (1990) concluded that Cr(VI) is a "strong" carcinogen for the respiratory system. This statement is not correct. As quoted on page 42 of the OEHHA Document, the IARC concluded that "there is sufficient evidence in humans for the carcinogenicity of Cr(VI) compounds as encountered in the chromate production, chromate pigment industry and chromium plating industries", i.e., only in 3 occupational settings (out of hundreds) that in the past involved the inhalation of very high Cr(VI) doses, often leading to ulcers and perforations of the nasal septum. The need for high Cr(VI) doses to induce lung cancer is confirmed by more recent study, such as the Gibb *et al.* (2000) study, which is extensively reported and

discussed in the OEHHA Document. As everybody knows, the Mancuso's data, that U.S. EPA used for the potency estimate, are highly biased.

Comment #6. Carcinogenicity of Cr(VI) in animal models

Apart from the NTP study, this subject is discussed on pages 56-57, where it is concluded that "rodents are relatively insensitive to Cr(VI) when it is administered by inhalation". It may be added that the large majority of the studies reviewed in detail by IARC (see pages 115-142 of the Monograph) were negative, and most positive data were generated at implant sites only and at a single, high doses, i.e., under conditions that could by-pass or overwhelm the body defense mechanisms.

Comment #7. Link between inhalation exposure to Cr(VI) and cancer of digestive organs

In 1988, the WHO concluded that "there is insufficient evidence to implicate chromium as a causative agent of cancer in any organ other than the lung" (WHO, *Environmental Health Criteria*, Vol. 61, 1988). In 1990, the IARC concluded that "for cancers other than of the lung and sinonasal cavity, no consistent pattern of cancer risk has been shown among workers exposed to chromium compounds" (IARC Monograph, Vol. 49, 1990). This conclusion was reiterated, almost *verbatim*, in a review article published 3 years later (Cohen *et al.*, *Crit. Rev. Toxicol.* 23, 255-81, 1993). My further analysis of recent studies confirmed these conclusions (S. De Flora, *Carcinogenesis*, 21, 533-541, 2000).

In the last paragraph of page 72, the OEHHA Document concludes that "a summary of the findings of multiple studies where workers were exposed to Cr(VI) by the inhalation route (conducted by OEHHA) was suggestive of a link between inhalation exposure to Cr(VI) and cancer of the digestive organs". This conclusion is surprising and contrasts with the actual results of the OEHHA study, which are reported in Tables 7 and 8 on pages 62-69. In fact, taking into account statistically significant variations, the analysis of 30 studies led to the following results for cancers of the digestive system:

– Oral cavity and pharynx: no significant change in 9 studies. *Note that the results in humans do not agree with the results of the NTP carcinogenicity study in the oral cavity of mice*.
– All digestive: significant increase in 2/10 (20%).
– Esophagus: no significant change in 10 studies.

– Stomach: significant increase in 3/25 (12%). Note that, at least in two studies, exposure to compounds other than Cr(VI) may have occurred.
– Colon: no significant increase in 16 studies. Interestingly, in 4 studies (Axelsson, 1980; Deschamps, 1995; Moulin, 1990; Sorahan, 2000) the cancer data for colon also included data for cancer of the small intestine. *Again, note that the results in humans do not agree with the results of the NTP carcinogenicity study in the small intestine of rats.*
– Rectum: no significant change in 11 studies.
– Liver and gall bladder: no significant increase in 9 studies and, on the contrary, a significant decrease in 1 study (11.1%).
– Pancreas: no significant change in 12 studies.

The analysis of the same epidemiological studies led to the following results for tumors of the respiratory tract:
– Lung cancer: significant increase in 18/29 studies (62.1%).
– Nonmalignant respiratory diseases: no increase in 18 studies but, on the contrary, a significant decreases in 4 (22.2%) studies (Birk, 2006; Hayes, 1989: Korallus, 1993; Moulin, 1990).

It is well known that, to reach a conclusion, epidemiological data have to be consistent in different studies, a requirement that was taken into account by the IARC Working Group. In this light, the conclusion of the OEHHA Document does not appear to be supported by the results of the OEHHA study.

Comment #8. Examination of evidence for chromium carcinogenicity

This Section of the OEHHA Document (pages 72-74) tries to summarize the data reported in the previous pages. In my comment, I will follow the same subtitles used in the Document.

Human studies. In addition to the considerations on the carcinogenic potency (see Comment #5) and on the link between inhalation exposure to Cr(VI) and cancer of digestive organs (see Comment #7), the OEHHA Document relies on the Chinese study, whose limitations are extensively discussed on pages 69-71. Note that this controversial study was further examined in a recent article (B.D. Kerger *et al., J. Toxicol. Environ. Hlth*, 72, 329-44, 2009), which is not quoted in the Document.

Animal studies. I already forwarded my considerations on the interpretation of the NTP study (see Comment #4). As to the Borneff *et al.* (1968) study, which is extensively reported and discussed both in the text and in Appendix D of the OEHHA Document, this study was so obsolete, inadequate and full of problems that the IARC Working Group (including myself and other 20 scientists) decided not even to cite it in the 1990 Monograph. Incidentally, it is noteworthy that the Borneff *et al.*, study suggested an increase of forestomach tumors in mice (that even the author interpreted with a great caution) while the NTP study suggested an increase of small intestine tumors in mice. Who is right?

Genotoxicity. As previously discussed (Comments #2 and #3), the data reported in the OEHHA Document are largely incomplete.

Toxicokinetics. As previously discussed (Comment #1), I do not pretend that detoxification mechanisms are infinite. In any case, they are formidable barriers that imprint a threshold character to Cr(VI) carcinogenesis (see Comment #9).

Toxicity. It should be noted that the oral toxicity of Cr(VI) is rather low. In rodents, the LD_{50} by the oral route is >50 mg Cr(VI)/kg body weight (S.C. Gad *et al.*, 1986, which is cited elsewhere in the Document). Furthermore, the NTP carcinogenicity study provided evidence for the great tolerability of high-dose Cr(VI). In humans, the OEHHA Document provides on page 57 two examples of fatal acute ingestion of Cr(VI), in both cases at doses of hundreds milligrams. There are several other reports of episodes of accidental ingestion or tentatives of suicides with Cr(VI), most of which luckily failed. For instance, following ingestion of Cr(VI) compounds in the 8-20 g range, 8 subjects survived and 3 subjects died (S. De Flora *et al.*, in Berthon, G. (ed.) *Handbook of Metal Ligand Interactions in Biological Fluids. Bioinorganic Medicine*, Marcel Dekker, NY, Vol. 2, pp. 716-25).

Mechanism. This section of the Document summarizes some mechanisms of Cr(VI). Regarding the meaning of the intracellular Cr(VI) reduction, when in 1989 I prepared a review (cited in the Document) together with the late Karen Wetterhahn, the best researcher on Cr(VI) biochemical toxicology ever, we agreed on the interpretation that when Cr(VI) reduction occurs close to DNA target molecules, it is an activation mechanism (uptake-activation theory). However, when Cr(VI) reduction occurs in the cell cytoplasm or in any case far away from DNA, it is a detoxification (uptake-detoxification theory), due to the myriad of intracellular ligands that block Cr(VI) or its

derivatives before reacting with DNA. Here is a further mechanism responsible for the occurrence of thresholds in Cr(VI) toxicology.

Conclusion. It is surprising that this chapter reaches the conclusion that "the findings of available human, animal, genotoxic, and toxicokinetics studies all indicate that Cr(VI) is a possible human carcinogen by the oral route". It is intriguing that all data that were evaluated to be either incomplete or heavily criticized in the Document itself now become the starting point to reach the above conclusion and to develop a proposal of PHG.

Comment #9. Thresholds

I started developing the firm belief that the carcinogenicity of certain compounds is governed by threshold mechanisms in 1978. In that year I published a study in which I demonstrated that genotoxic compounds that tend to be detoxified in the body, among which sodium dichromate, are either not carcinogenic or carcinogenic only at certain conditions (S. De Flora, *Nature*, 271, 455-6, 1978). Thereafter, I validated this theory in a number of studies. Some of my papers have the keyword "threshold" in the title (e.g., S. De Flora, *Toxicol. Pathol.*, 12, 337-43, 1984; S. De Flora, *Ann. Am. Conf. Ind. Hyg.*, 12, 145-55, 1988; F.L. Petrilli and S. De Flora, *Sci. Total Environ.*, 71, 357-64, 1988; S. De Flora, *Carcinogenesis*, 21, 533-41, 2000) or in subtitles (e.g., S. De Flora and K. Wetterhahn, *Life Chem. Rep.*, 7, 169-244, 1989).

All the patterns that characterize Cr(VI) toxicity and genotoxicity *in vivo* and carcinogenicity in both humans and animal models, along with mechanistic considerations, as previously discussed, point to the existence of threshold mechanisms in Cr(VI) toxicity and carcinogenicity. As previously mentioned (Comment #1), the existence of thresholds for Cr(VI) is widely accepted in the international literature and by major Agencies, such as IARC, U.S. EPA, and U.S. Dept. of Health and Human Sciences.

The lack of thresholds, as claimed in APPENDIX A of the OEHHA Document, would imply that even a single Cr(VI) molecule, introduced in the organism, would be able to reach the DNA of target cells, which is unbelievable. It should be added that threshold mechanisms occur not only at toxicokinetic and metabolic levels but also after DNA damage, e.g., due to DNA repair and apoptosis. My lab investigated these processes by analyzing *in vivo* both transcriptome (A. Izzotti *et al.*, *Mol. Carcinogenesis*, 35, 75-84, 2002) and proteome (A. Izzotti *et al.*, *Int. J. Oncol.*, 24, 1513-22, 2004).

Comment #10. Potency calculation and evaluation of PHG

Having worked for 49 years in an Institute of Hygiene and Preventive Medicine and a Department of Health Sciences at the University, I appreciate any effort to guard public health. However, starting from inconsistent epidemiological and experimental data and denying the occurrence of threshold mechanisms in Cr(VI) toxicity and carcinogenicity lead to unrealistic figures.

The proposed PHG for Cr(VI) of 0.06 ppb (µg/L) in drinking water means that it is believed that concentrations higher than that, of course with a safety margin, are not detoxified in the body after oral intake. The results of the NTP carcinogenicity study in mice and rats that, as noted in Comment #7, were not consistent with the results of epidemiological studies, were used as a major conceptual base for claiming that Cr(VI) is carcinogenic also by the oral route and for calculating the proposed PHG. The concentrations of Cr(VI) in water that produced significant variations of tumor incidence in the NTP study were in the range of hundreds mg/L, i.e., millions of times higher than the proposed 0.06 µg/L PHG.

Texas Commission on Environmental Quality
Comments Regarding the U.S. Environmental Protection Agency
Draft Toxicological Review of Hexavalent Chromium in Support of
Summary Information on the Integrated Risk Information System (IRIS)
Notice of Public Comment Period and Listening Session
75 FR 60454, September 30, 2010
Docket ID No. EPA-HQ-ORD-2010-0540

On September 30, 2010, the U.S. Environmental Protection Agency (EPA) published a Federal Register notice (Federal Register/Vol. 75, No. 189/Thursday, September 30, 2010/Notices) of a 60-day public comment period (ending November 29, 2010) for the, "Draft Toxicological Review of Hexavalent Chromium in Support of Summary Information on the Integrated Risk Information System (IRIS)," hereafter referred to as the draft assessment (EPA/635/R-10/004A). On November 10, EPA extended the comment deadline 30 days to December 29, 2010 (Federal Register/Vol. 75, No. 217/Wednesday, November 10, 2010/Notices). The draft IRIS assessment provides a draft oral slope factor (SFo of 0.5 per mg/kg-day) based on small intestine tumors in male mice in the National Toxicology Program (NTP 2008) drinking water study. The Texas Commission on Environmental Quality (TCEQ) has developed comments on the draft assessment to the extent practicable in the time allotted by EPA, focusing on the draft SFo, and provides the following comments for EPA consideration.

General Comments:

The assessment of the carcinogenic potential of hexavalent chromium (CrVI) has great implications in a regulatory context. Given their important role in the protection of public health, EPA regulatory risk assessors have a duty to perform the most scientifically-defensible assessments possible while giving careful and due consideration to comments and recommendations from other regulatory agencies, the public, external experts, stakeholders, etc. Although regulatory risk assessors have a penchant for erring on the side of health-protectiveness and conservative defaults, if erring on the side of conservatism significantly overestimates risk or hazard and is not fully justified, then harm to public health may result from diverting public, industry, and government attention and resources away from chemicals that may represent more of a public health risk at environmental levels. Therefore, TCEQ encourages EPA to give full, thoughtful, and careful consideration and evaluation to comments and recommendations from TCEQ, other regulatory agencies, the public, and external experts.

TCEQ is concerned that recent draft EPA assessments (e.g., dioxin, arsenic, formaldehyde) along with the draft CrVI assessment seem to demonstrate a pattern where the EPA timeline is sufficient for a less-than-desirable level of initial EPA analysis but insufficient: (1) for the public to be able to provide fully detailed comments on the many shortcomings of the draft assessments; (2) for EPA to seriously and meaningfully evaluate the scientific merit of public comments; (3) for EPA to conduct the additional analyses required to fully respond to public comments and appropriately revise the draft assessment based on the scientific merit of comments; and (4) for EPA to conduct the fully credible, balanced, and transparent assessment the public deserves where the effects of the significant uncertainties associated with certain key decisions and procedures are fully examined qualitatively and quantitatively. Such shortcomings undermine the confidence of States and other parties who often rely on EPA toxicity

factors and over time, will tend to marginalize EPA in terms of a reliable source for scientifically objective, defensible, and predictive toxicity factors. This may be one reason States are progressively deriving more toxicity factors as opposed to relying on EPA assessments, which often rely heavily on a penchant for default procedures representing a seemingly nonobjective and insurmountable hurdle for alternative analyses strongly supported by data (e.g., nonlinear dioxin carcinogenicity assessment, cytotoxicity-induced regenerative cell proliferation carcinogenic mode-of-action (MOA) for formaldehyde-induced respiratory tract cancer).

90-Day Comment Period:

The 90-day comment period is insufficient for regulatory agencies and others to provide the most thorough and meaningful comments possible based on an in-depth review and analysis of the draft IRIS assessment. There is great complexity associated with multiple issues relevant to the assessment of CrVI risk and hazard. The draft IRIS assessment alone is 300 pages, and there are hundreds of pages (at a bare minimum) of other documents and studies relevant to the assessment of CrVI risk and hazard. Given the complexity and volume of relevant materials, it is impracticable for EPA to expect detailed specific comments from external experts given the short period allowed for a critical review of the draft assessment and more specifically, the procedures, calculations, and supporting arguments employed by EPA therein. Given that external experts cannot devote all their time to review and comment, the 90-day comment period only allows a superficial review of the draft assessment at best, leads to a less-than-desirable level of transparency and peer review, and undermines confidence in the process. Consequently, TCEQ is only able to provide comments based on a cursory review. If EPA seeks more detailed and meaningful public input and technical comments, at a minimum EPA should extend the comment period at least 30 days past the December 29 deadline to allow stakeholders to: (1) perform a more detailed review of the volumes of relevant information; (2) more fully examine statistical procedures and the rationale and scientific support for key EPA decisions and analyses; and (3) provide more detailed specific comments on all problematic issues associated with the draft IRIS assessment.

Toxicology-Based Comments:

Biological Plausibility of a Mutagenic Carcinogenic MOA and Exceedance of the Mouse Gastrointestinal (GI) Tract Reductive Capacity

EPA's conclusion that mutagenicity (and consequently carcinogenicity) can occur at doses within GI reductive capacity relied on an entirely speculative mouse reductive capacity, flawed arguments, and is not scientifically sound. When discussing data supportive of the hypothesized mutagenic MOA for CrVI (and default linear, low-dose extrapolation by corollary), EPA admits that overwhelming the GI reductive capacity of the mouse is a plausible explanation for CrVI-induced genotoxicity following sufficiently high mouse oral exposure. By corollary, overwhelming the mouse's GI reductive capacity is a plausible explanation for CrVI-induced carcinogenicity in the NTP (2008) drinking water study. However, EPA wholly rejects this "plausible explanation" (p. 207) since, "there are inconsistencies."

Firstly, all studies are rarely (if ever) 100% consistent, and lack of 100% consistency does not preclude sound conclusions based on best scientific judgment and consideration of all relevant data in a weight of evidence approach. For example, there are inconsistencies with CrVI being genotoxic *in vivo* and *in vitro* since not all results are positive (see Tables 4-23 and 4-21 of draft assessment), but this certainly does not (and should not) preclude EPA from concluding that CrVI is genotoxic (see Section 4.7.3.4).
Secondly, as evidence that exceedance of the mouse GI reductive capacity is not required for genotoxicity and carcinogenicity, EPA indicates that: (1) the average rate of CrVI exposure at even the highest dose in the NTP (2008) study was within the "estimated" reductive capacity of the mouse GI tract; (2) Devi et al. (2001) found positive genotoxicity results in leukocytes at doses > 10-fold lower than those used in the NTP study and within the "estimated" reductive capacity of the mouse; and (3) Stout et al. (2009) did not find an upward inflection (threshold) point in nonlinear data (tissue concentration and/or mouse small intestine neoplasm data) as evidence of where dose may have saturated reductive capacity. However, regarding (1) above, *the "estimated" mouse GI reductive capacity is entirely speculative* (scaling from humans to mice with body weight ($BW^{3/4}$)). In fact, EPA elsewhere (p. 211) states, "data are not available for the reductive capacity of the mouse." Regarding (2), the Devi et al. (2001) study was an oral gavage study while the speculative GI reductive capacity was calculated on an hourly basis. Thus, *a direct comparison of the speculative hourly mouse reductive capacity and the bolus doses in the Devi et al. gavage study is not appropriate.* Additionally, the positive results for leukocytes examined in Devi et al. (2001) are of questionable relevance for the carcinogenic MOA compared to the entirely relevant negative genotoxicity findings in the cancer target tissues examined in De Flora et al. (2008). *The DNA damage demonstrated by Devi et al. (2001) in mouse leukocytes does not result in cancer-causing mutations in that tissue, much less demonstrate how CrVI causes cancer in actual target tissues where De Flora et al. (2008) did not find DNA damage,* even at drinking water concentrations 50 and 200 times the federal maximum contaminant level (MCL) (i.e., "brightly yellow" levels). Regarding (3) above, Stout et al. (2009) also relied upon the speculative mouse GI reductive capacity to conclude that the absence of an upward inflection point in nonlinear data did not support a threshold. However, as the "estimated" mouse reductive capacity is entirely speculative, no scientifically sound conclusions can be made by Stout et al. (2009) or EPA based on it. *It is more plausible that all doses exceeded actual mouse GI reductive capacity (see TCEQ comments below).* Therefore, all data used by Stout et al. are from points on the dose-response curve higher than the inflection point, making the observance of an inflection point impossible. *Contrary to the draft assessment, EPA cannot make sound scientific conclusions concerning the relationship between GI reductive capacity and the potential for genotoxicity and/or carcinogenicity in the absence of actual mouse GI reductive capacity data or similarly informative data. Overwhelming the reductive capacities of the mouse and rat GI tracts remains a plausible explanation for the carcinogenicity observed in NTP (2008).* There are data which are informative concerning whether or not mouse GI reductive capacity was exceeded at the NTP (2008) study doses. More specifically, NTP (2007) provides evidence of CrVI absorption in mice at around 10 mg/L and higher in drinking water (see blood results in Table G1), but not at lower doses. This evidence strongly suggests that GI reductive capacity was exceeded by all mouse doses (14.3-267 mg/L) in the NTP (2008) study. In regard to rat reductive capacity and the oral carcinogenicity observed in NTP (2008), NTP (2007) (see blood results in Table G1) and Sutherland et al. (2000) provide evidence of CrVI absorption in rats at around 10 mg/L and higher in drinking water, but not at lower doses. Similar to

the mouse, these rat results strongly suggest that reductive capacity was exceeded by all rat doses (14.3-516 mg/L) in the NTP (2008) study. *Thus, for both mice and rats, EPA had data strongly suggesting that NTP (2008) doses exceeded GI reductive capacity.* Had the NTP (2008) doses associated with 14.3-516 mg/L truly been within actual GI reductive capacity, CrVI would have been effectively reduced to CrIII and significant absorption into the bloodstream would not have occurred in NTP (2007) at water levels around ≥ 10 mg/L. Instead of relying on these actual data, EPA relied on a speculative mouse reductive capacity estimate to make a key decision and conclude that mutagenicity (and consequently carcinogenicity) can occur at doses within the GI reductive capacity. *For EPA to admit that overwhelming the reductive capacity of the mouse GI tract was likely responsible for the carcinogenicity observed in NTP (2008) would inconveniently put EPA off the linear, low-dose extrapolation pathway with issues EPA is ill-prepared to address quantitatively within this carcinogenic assessment* (e.g., doses at which the mouse and human GI reductive capacities are exceeded (thresholds for carcinogenicity), human relevance of the mouse tumors given exceedance of the mouse GI reductive capacity and given truly environmentally relevant lifetime human doses), *especially given the lack of data necessary to address some of these issues* (e.g., lack of species-specific GI reductive capacity data).

The above comments highlight serious shortcomings in EPA's story about exceedance of the mouse GI reductive capacity not remaining a plausible explanation for CrVI-induced genotoxicity and subsequent carcinogenicity. EPA's discussion fails to adequately support their conclusions concerning study doses not exceeding mouse GI reductive capacity. *TCEQ notes that for EPA to acknowledge this explanation would be contrary to their use of default linear, low-dose extrapolation (i.e., no biological threshold for CrVI mutagenicity based on stomach/GI reductive capacity) and call into question the human relevance of the mouse tumors observed.*

Human Relevance of the Mouse Tumors

The small intestine neoplasms in mice (and oral cancers in rats) observed in NTP (2008) are of questionable relevance to humans. Reasons include: (1) mouse GI reductive capacity may have certainly been exceeded (e.g., there are no actual mouse GI reductive capacity data, blood data from NTP (2007) suggest that NTP (2008) doses exceeded GI reductive capacity); (2) epidemiological worker data are not supportive; and (3) the NTP (2008) study doses are not relevant to the truly low, typical environmental doses. The issue in (1) was discussed in TCEQ comments above.

Regarding (2), *epidemiological worker data do not support elevated GI cancer risk.* A meta-analysis of thirty-two CrVI worker studies (Gatto et al. 2010) showed no significant increase in GI tract cancers (although a much smaller highly-exposed subgroup had slightly elevated esophageal cancer). Additionally, none of the studies reported statistically elevated oral cavity or small intestine risk. For example, the meta-analysis included GI tract cancer data obtained from the study authors of Luippold et al. (2003) and Gibb et al. (2000), which did not show excess cancers of the GI tract (e.g., stomach, oral). This information is relevant since workers can be exposed to air concentrations sufficiently high that ingestion is significant. For example, 48% and 39% of the chromate workers in Public Health Service (PHS 1953) had yellow tongues and teeth, respectively. Yellow tongues and teeth were not attributable to smoking and yellow tongue scrapings contained chromium (see pp. 76-77 and Figures 10 and 11 of PHS 1953). While this

discoloration was due to the ingestion of relatively high oral doses of CrVI by these workers, no excess GI cancers were found in PHS (1953) or in Luippold et al. (2003) or Gibb et al. (2000), which evaluated some of the same workers. Regarding a comparison between worker and NTP (2008) study doses, Gatto et al. (2010) estimated a daily worker oral dose of 0.004 mg/kg-day, which could vary by an order of magnitude in either direction depending on cohort-specific air concentrations and particulate size/solubility. The doses that produced small intestine cancers in mice (and oral cancers in rats) in NTP (2008) are orders of magnitude higher than this estimated occupational oral dose (whether +/- an order of magnitude). The difference in GI cancer outcome between NTP (2008) and Gatto et al. (2010) and these other worker studies could be that although workers were exposed to estimated oral doses significantly higher than typical environmentally relevant doses, exposure was within the GI reductive capacity of the workers as opposed to the laboratory mice/rats in NTP (2008) exposed to significantly higher doses beyond their GI reductive capacity. *The bottom line is that even in occupational workers exposed to sufficiently high air levels of CrVI as to produce (via ingestion) yellow tongues and teeth in 39-48% of the workers, PHS (1953) looked for but did not find excess GI cancers or any cancer excesses outside the respiratory tract (see p. 56 of PHS 1953), and these study results are supported by other studies as well (e.g., Gatto et al. 2010, Luippold et al. 2003, Gibb et al. 2000).*

In regard to (3), *the NTP (2008) study drinking water doses are not relevant to humans.* For example, the mouse doses (0.38-8.7 mg/kg-day) are 130-3000 times higher than the human adult dose ((0.1 mg/L x 2 L/day)/70 kg = 0.0029 mg/kg-day) at the federal MCL. CrVI drinking water concentration data from Midland, Texas, have been used recently to suggest that the NTP (2008) doses are relevant to human exposures since the lowest cancer-producing dose from the NTP study scaled to humans using $BW^{3/4}$ (0.166 mg/kg-day) is comparable to the estimated human dose at the maximum detected concentration (5.41 mg/L) in Midland (0.155 mg/kg-day) (Collins et al. 2010). However, this comparison is erroneous for several reasons. The NTP (2008) study is a lifetime exposure study where laboratory animals were exposed to a constant concentration in drinking water. By contrast, based on community input to TCEQ, some people in the affected area in Midland were already drinking bottled water due to generally poor water quality (e.g., high total dissolved solids). Additionally, others stopped drinking the water as CrVI concentrations began to rise and the water began to turn yellow around ≥ 1 ppm, which was indicated in the source (TDSHS 2009) cited by Collins et al. (2010) but which the authors for some reason failed to mention. Consequently, public exposure was for far less than a lifetime. Also, although exposure concentrations changed over time, they were significantly lower than the maximum concentration assumed by Collins et al. (2010). Thus, this comparison by Collins et al. (2010) is based on erroneous assumptions in a failed attempt to demonstrate the human relevance of the NTP study doses. Although there is significant uncertainty in how water concentrations changed over time, a more reasonable worst-case scenario might be: 0.7 mg CrVI/L (average) x 2 L/day x 5 years/70 years = 0.0014 mg/kg-day, which is over 110 times less than the lifetime average mouse dose cited by Collins et al. (2010). *The doses on NTP (2008) are hundreds or thousands of times higher than typical environmentally relevant doses. Therefore, for this and other reasons discussed, study results and the draft SFo are of questionable utility and predictive ability for use in risk assessment.*

Disparate EPA Scientific Standards

EPA appears to hold a higher standard for the scientific defensibility of data that do not support a default or pre-determined EPA assessment pathway. For example, in discussing the hypothesized mutagenic carcinogenic MOA, EPA did not consider the De Flora et al. (2008) drinking water study data to be informative about genotoxicity in the cancer target tissues because it was only for 9 months, although it is still a chronic study and genotoxicity/mutagenicity would be expected early in the carcinogenic process if a CrVI produces cancer though a mutagenic MOA. These data would lend weight against a mutagenic MOA and subsequent linear, low-dose extrapolation. Conversely, EPA judged comparisons of entirely speculative estimates of mouse GI reductive capacity to various study doses (e.g., Devi et al. 2001, Stout et al. 2009) as sufficient to conclude that genotoxicity/mutagenicity can occur at doses within GI reductive capacity, which is needed to justify the absence of a threshold and to assert use of linear, low-dose extrapolation. EPA's selection of relevant study data reflects a bias, where data supporting EPA's default linear, low-dose extrapolation are considered sufficiently conclusive and any data not supporting that approach are dismissed.

In addition to the comments above pertaining to an example of apparent disparate standards applied to data within the CrVI assessment, *there appears to be inconsistency across assessments regarding the data deemed by EPA to be sufficient to support the direction of an assessment.* For example, using EPA's apparent standard of "inconsistency" as applied to data concerning exceedance of GI reductive capacity in the current assessment as sufficient to discount a certain hypothesis as unsupportable (i.e., existence of a biological threshold for CrVI mutagenicity/carcinogenicity based on GI reductive capacity), it is abundantly clear that EPA should have never derived a unit risk factor (URF) for Hodgkin lymphoma and leukemia for formaldehyde in the 2010 draft assessment. Only a minority of epidemiological data support a link, the hypothesized MOA is highly speculative and biologically implausible (e.g., Lu et al. 2010), EPA indicates that there is no way to derive a meaningful URF for environmental exposure where risk is determined by environmentally irrelevant peak exposures, there is no dose-response relationship between cumulative exposure and risk that might have produced a meaningful URF, and yet EPA derived a formaldehyde URF for non-Hodgkins lymphoma and leukemia not only in the midst of inconsistency but of scientific indefensibility. *Disparate standards are even applied by EPA to the same data depending upon whether they support default assessment procedures.* For example, in the 2010 draft dioxin reanalysis, EPA judged AhR-mediated MOA data to sufficiently support the biological plausibility of dioxin being a known human carcinogen, but judged the same MOA data as insufficient to justify the corollary nonlinear carcinogenic assessment. *Overall, this appears to lend support to the existence of a double standard where a high standard is applied to data contrary to a pre-determined path* (e.g., EPA's treatment of De Flora et al. 2008 in the CrVI genotoxicity discussion), *requiring only the interjection of some level of ever-present uncertainty for rejection, while a lower standard is used to judge data that justify the default or desired path* (e.g., EPA's treatment of Devi et al. 2001 and Stout et al. 2009 in the discussion of CrVI GI reductive capacity, EPA's hypothesized MOA and derivation of formaldehyde URFs for Hodgkin lymphoma and leukemia, EPA's treatment of the formaldehyde BBDR model).

In effect, the unequal treatment of data results in "cherry-picking" data, an unbalanced and biased approach towards risk assessment, and undermines user and public

confidence. *The same standard should be applied to data regardless of whether or not they support a EPA default procedure or preferred assessment pathway (e.g., linear, low-dose extrapolation based on an assumption of no threshold).*

Bioavailability

Serious issues exist regarding the predictiveness of the draft SFo given likely differences between the bioavailability in mice (and rats) at the doses used in NTP (2008) and in humans at typical environmentally relevant doses. In regard to the bioavailability of CrVI, TCEQ notes that the human study cited by EPA where as high as 10% of CrVI was absorbed (Kuykendall et al. 1996) involved a bolus dose 25 times higher than the dose associated with consuming 2 liters of drinking water all at once at the current MCL. The limited human bioavailability at the high bolus dose used raises serious questions about the bioavailability at much lower, environmentally relevant doses (e.g., lower, non-bolus doses). Additionally, the rodent data cited by EPA are of little relevance for proving bioavailability in humans at environmentally relevant doses as the rodent doses cited (p. 210) were very high on a body weight basis and human GI reductive capacity is expected to be different. Humans and mice are likely to differ in GI reductive capacity (a likely important determinant of risk) due to several factors such as varying stomach pH, fluid production rates, food content, and emptying and Cr reduction rates. For example, the human fasted stomach pH is around 2-3 times less than that of the mouse and rat (McConnell et al. 2008, Ruby et al. 1996), which would be expected to be associated with a greater human CrVI reductive capacity. *The differences between the bioavailability in mice (and rats) at the doses used in NTP (2008) and in humans at typical environmentally relevant doses would have to be quantitatively accounted for to derive a scientifically defensible and predictive SFo for regulatory decision making.* This is especially critical considering that the NTP (2008) doses likely exceeded the mouse (and rat) GI reductive capacity (see TCEQ comments above).

Genotoxicity versus Mutagenicity

EPA appears to inappropriately automatically equate and discuss genotoxicity data as direct evidence of mutagenicity. While evidence of genotoxicity certainly has bearing on potential mutagenicity and is important supportive information under EPA guidelines (EPA 1986, 2007), it is not direct evidence of the generation of mutations as seemingly characterized by EPA in the draft CrVI assessment. EPA guidelines on mutagenicity risk assessment (EPA 1986) concern *heritable* mutagenic changes, and not all carcinogenic chemicals that are capable of interacting with DNA will have a mutagenic MOA for cancer (EPA 2007). EPA discusses no positive *in vivo* data for mutagencity in cancer target tissues in oral animal studies, only genotoxicity data (e.g., DNA-protein crosslinks, DNA strand breaks) in non-target tissues of unknown relevance to the tumors observed in NTP (2008) which EPA inappropriately automatically equates and discusses as direct evidence of mutagenicity (see first paragraph p. 204). This *in vivo* genotoxicity discussed by EPA does not result in cancer-causing mutations in those tissues (e.g., liver, leukocytes), much less explain how CrVI causes cancer in actual target tissues for which existing genotoxicity data (De Flora et al. 2008) are negative.

Interspecies Scaling

The interspecies scaling used by EPA should be fully justified. The draft SFo was calculated using $BW^{3/4}$ scaling from mice to humans (p. 229). The tumors observed in mice (small intestine tumors) were portal-of-entry (POE) and not systemic in nature. EPA (2005) is unclear as to whether the data which support this adjustment include POE tumor data. *EPA should fully justify use of $BW^{3/4}$ scaling for this purpose or conduct no such adjustment, especially given that humans and mice are likely to differ in GI reductive capacity (see TCEQ comments above).*

Imminent Generation of Data Critical to the Carcinogenic MOA Analysis

TCEQ strongly urges EPA to postpone finalizing the draft CrVI assessment as the generation of new data critical to understanding the carcinogenic MOA is imminent. Unlike the typical situation where regulatory agencies are asked to delay an assessment for years pending results of a study which might be informative, study data are currently being generated that are directly relevant and critical to a scientifically defensible carcinogenic MOA analysis by EPA. The overall goal of the CrVI MOA Research Program is to understand the contribution of different potential carcinogenic MOAs for CrVI (e.g., genotoxicity, cytotoxicity, inflammation, oxidative stress) across a broad range of doses in order to provide both statistical and biological understanding of potential thresholds for CrVI carcinogenicity. The contributions of various MOAs over a range of doses will be determined by a combination of genome-wide microarray analyses in intact animals, high data content imaging of activation of key DNA-damage pathways, and consideration of dose dependencies in dosimetry. These data may elucidate the shape of the rodent dose-response curve and the human relevance of these responses prior to development of the final SFo. Detailed information may be found at http://www.tera.org/Peer/Chromium/Chromium.htm. All technical manuscripts are expected to be completed no later than the end of the 2nd quarter, 2011, before the final assessment is due in the 3rd quarter (http://cfpub.epa.gov/ncea/iristrac/index.cfm?fuseaction=viewChemical.showChemical&sw_id=1107). *The data to be generated by the CrVI MOA Research Program will address many important MOA data gaps (see the Appendix) and are of paramount importance to a scientifically rigorous CrVI carcinogenic assessment.* These data may help explain such issues as why the mutagenic MOA hypothesized in the draft assessment (even at exposures below the GI reductive capacity) would predict GI tumors in highly orally-exposed workers (PHS 1953) and in multiple tissues in the NTP (2008) study but in fact such tumors did not occur. Additionally, they may explain more convincingly than the draft assessment (Section 4.7.3.3) why intestinal tumors only occurred in animals with prolonged hyperplasia, or may support an alternative carcinogenic MOA as much more plausible (e.g., Thompson et al. 2010). *TCEQ strongly encourages EPA to utilize these data to inform the carcinogenic MOA analysis and revise the draft assessment as justified (even if the EPA timeline is pushed farther out) as opposed to viewing these important data as an inconvenient late development in the assessment process and simply interjecting some level of uncertainty and proceeding down the previously prescribed path.*

Implication-Based Comments:

While significant implications themselves do not speak to the scientific defensibility of the draft SFo for CrVI, they emphasize the critical importance of deriving the most scientifically defensible, biologically relevant, and predictive toxicity factors possible.

Health-Protective Environmental Media Levels

Because the draft SFo for CRVI is relatively high, there are important implications for the calculation of health-protective environmental media levels such as EPA surface soil preliminary remediation goals (PRGs) and the MCL. Soil PRGs for CrVI may decrease by a factor of 10 or more even without the use of age-dependent adjustment factors (ADAFs). Soil PRGs will be at the low end of the range of background chromium soil levels (US mean of 37 mg/kg, ATSDR 2008), with a residential PRG of 0.29 mg/kg and a commercial/industrial PRG of 5.6 mg/kg. *Soil CrVI PRGs within background chromium levels will require costly remediation site-specific soil studies to differentiate between CrVI and other forms (e.g., CrIII) at all sites where it is a chemical of potential concern (COPC).*

The draft SFo also has significant implications for the federal drinking water MCL. *Using the EPA acceptable risk range (1E-06 to 1E-04) and draft SFo, the MCL would need to be from 0.07 to 7 ppb (without use of ADAFs) for adequate protection of public health. Compared to the current MCL of 100 ppb, this represents approximately a 14-1,400 fold decrease.* With typical US drinking water supplies containing total chromium levels within a range of 0.2 to 35 ppb (most supplies < 5 ppb, ATSDR 2008), a new MCL for total chromium of 0.07-7 ppb conservatively based on the draft SFo could be exceeded on a wide basis depending upon the target risk level used. If a CrVI-specific MCL is promulgated, water suppliers would have to begin analyzing for chromium using a method that can speciate forms and one sensitive enough to detect chromium at concentrations much lower than now required to demonstrate compliance with the current MCL. Available analytical methods do not appear to be capable of detecting CrVI at the lower end of the potential new MCL range (ATSDR 2008). *A new MCL may be problematic for many public drinking water supplies.* For example, a recent California drinking water survey showed that 14% of drinking water sources had concentrations of ≥ 10 ppb CrVI (ATSDR 2008), which is above the potential new MCL range of 0.07-7 ppb based on the EPA acceptable risk range and the draft CrVI SFo. Additionally, based on a review of treatment removal technologies, process-efficient and cost-effective methods for CrVI removal from drinking water supply sources appear to be lacking (Sharma et al. 2008).

Closing Remarks:

TCEQ acknowledges the significant agency effort and resources required to produce draft toxicological assessments, review public comments, and make scientifically justified revisions and additions. The public deserves regulatory agencies to be able to make good risk management decisions using realistic risk estimates based on the most scientifically defensible and predictive toxicity factors possible, not based on toxicity factors of uncertain predictive ability that are just conservative by default. Consequently, for this and other draft assessments, TCEQ urges EPA to give thoughtful scientific and common-sense consideration to these and other comments and the weight of scientific evidence

which supports or contradicts key decisions and procedures employed in the draft EPA assessment. Agreement with the ultimate final SFo value necessarily implies agreement with its ability to reasonably predict risk at commonly encountered, environmentally relevant doses, and agreement with the unavoidable conclusions about public health that will naturally follow from risk estimates based on the SFo. Additionally, TCEQ encourages EPA to postpone finalizing the draft assessment as necessary since the generation of new data which will address important MOA data gaps is imminent through the CrVI MOA Research Program. Appropriate consideration and incorporation of these data would result in a more scientifically rigorous CrVI carcinogenic assessment.

References

Agency for Toxic Substances and Disease Registry (ATSDR). (2008) Draft Toxicological Profile for Chromium. Available online at http://www.atsdr.cdc.gov/ToxProfiles/tp7.pdf.

Collins, BJ; Stout, MD; Levine, KE; et al. (2010) Exposure to Hexavalent Chromium Resulted in Significantly Higher Tissue Chromium Burden Compared to Trivalent Chromium Following Similar Oral Doses to Male F344/N Rats and Female B6C3F1 Mice. Toxicol Sci Available online September 15, 2010 doi:10.1093/toxsci/kfq263.

De Flora, S; D'Agostini, F; Balansky, R; et al. (2008) Lack of genotoxic effects in hematopoietic and gastrointestinal cells of mice receiving chromium (VI) with the drinking water. Mutat Res 659:60–67.

Devi, KD; Rozati, R; Saleha Banu, B; et al. (2001) In vivo genotoxic effect of potassium dichromate in mice leukocytes using comet assay. Food Chem Toxicol 39(8):859–865.

Environmental Protection Agency (EPA). (2007) Framework for Determining a Mutagenic Mode of Action for Carcinogenicity: Using EPA's 2005 Cancer Guidelines and Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens. Washington, DC. EPA 120/R-07/002-A.

Environmental Protection Agency (EPA). (2005) Guidelines for Carcinogen Risk Assessment. Washington, DC: Risk Assessment Forum. EPA/630/P-03/001B.

Environmental Protection Agency (EPA). (1986) Guidelines for Mutagenicity Risk Assessment. Risk Assessment Forum. Washington, DC. EPA/630/R-98/003.

Gatto, NM; Kelsh, MA; Mai, DH; et al. (2010) Occupational exposure to hexavalent chromium and cancers of the gastrointestinal tract: a meta-analysis. Cancer Epidemiology 34(4)388-399.

Gibb, HJ; Lees, PS; Pinsky, PF; et al. (2000) Lung cancer among workers in chromium chemical production. Am J Ind Med 38(5):606.

Kuykendall, JR; Kerger, BD; Jarvi, EJ; et al. (1996) Measurement of DNA-protein cross-links in human leukocytes following acute ingestion of chromium in drinking water. Carcinogenesis 17(9):1971–1977.

Lu, K; Collins, LB; Ru, H; et al. (2010) Distribution of DNA adducts caused by inhaled formaldehyde is consistent with induction of nasal carcinoma but not leukemia. Toxicol Sci 116:441-451.

Luippold, RS; Mundt, KA; Austin, RP; et al. (2003) Lung cancer mortality among chromate production workers. Occup Environ Med 60(6):451–457.
McConnell, EL; Basit, AW; Murdan; S. (2008) Measurements of rat and mouse gastrointestinal pH, fluid and lymphoid tissue, and implications for in-vivo experiments. J Pharm Pharmacol 60:63-70.

National Toxicology Program (NTP). (2007). Toxicity Studies of Sodium Dichromate Dihydrate (CAS No. 7789-12-0) Administered in Drinking Water to Male and Female F344/N Rats and B6C3F1 Mice and Male BALB/c and am3-C57BL/6 Mice. Washington, DC: National Toxicology Program; NTP TR 72. Available online at http://ntp.niehs.nih.gov/ntp/htdocs/ST_rpts/tox072.pdf

National Toxicology Program (NTP). (2008) NTP technical report on the toxicology and carcinogenesis studies of sodium dichromate dehydrate (CAS No. 7789-12-0) in F344/N rats and B6C3F1 mice (drinking water studies). Washington, DC: National Toxicology Program; NTP TR 546. Available online at http://ntp.niehs.nih.gov/files/546_web_FINAL.pdf.

Public Health Service (PHS). (1953) Health of workers in the chromate producing industry. Washington, DC: Division of Occupational Health, Public Health Service, Publication No. 192.

Ruby, MV; Davis, A; Schoof, R: et al. (1996) Estimation of lead and arsenic bioavailability using a physiologically based extraction test. Environ Sci Technol 30:422-430.

Sharma, SK; Petrusevski, B; Amy, G. (2008) chromium removal from water: a review. J Water Supply Res T-Aqua 57:541-553.

Stout, MD; Herbert, RA; Kissling, GE; et al. (2009) Hexavalent chromium is carcinogenic to F344/N rats and B6C3F1 mice after chronic exposure. Environ Health Perspect 117(5):716–722.

Sutherland, JE; Zhitkovich, A; Kluz, T, et al. (2000) Rats retain chromium in tissues following chronic ingestion of drinking water containing hexavalent chromium. Biol Trace Element Res 74:41-53.

Texas Department of State Health Services (TDSHS). (2009) Letter health consultation: Evaluation of chromium in private wells, West CR 112, Midland, Midland county, Texas. Texas Department of State Health Services, Austin, TX. Available online at http://www.atsdr.cdc.gov/HAC/pha/WestCountyRoad112/WestCountyRoad112LHC06-03-2009.pdf.

Thompson, CM; Haws, LC; Harris, MA; et al. (2010) Application of the US EPA Mode of Action Framework for Purposes of Guiding Future Research: A Case Study Involving the Oral Carcinogenicity of Hexavalent Chromium Toxicol Sci Available online October 14, 2010 doi:10.1093/toxsci/kfq320.

Appendix: Carcinogenic MOA Data Gaps Addressed by the CrVI MOA Research Program

Key Event	Data Gap	Aspect of the MOA Study to Fill Data Gap
1. Saturation of stomach reductive capacity	1. Interspecies differences in the reductive capacity. 2. Dose at which Cr(VI) will not reach the duodenum in mouse	Toxicokinetic data (see next step) will be used to develop and refine species-specific PBPK models for quantifying reduction capacity. PBPK models may be used to extrapolate between species and from higher to lower doses
2. Uptake of Cr(VI) by the intestinal mucosa	1. Measures of Cr absorption into epithelial tissues in rats and mice. 2. Basal and Cr(VI) induced changes in SLCs 3. Differences between intestinal mucosa and that of upper alimentary tract.	Toxicokinetic study 1. measure Cr absorption and accumulation 2. Ex vivo reduction rate and capacity 3. Toxicogenomic analyses 4. Inter-species variability
3. Oxidative stress leading to tissue damage and inflammation	1. Measure of oxidative stress and/or inflammation in the intestines	1. GSH/GSSG ratios in intestinal epithelial tissue and blood 2. Cytokines (22-plex) in intestinal epithelial tissue and blood 3. Gene Expression in intestinal epithelial tissue
4. Intestinal cell proliferation	1. NOEL for intestinal diffuse hyperplasia 2. Mechanistic basis for interspecies differences 3. Driver of proliferation (cytotoxicity, mitogenesis, mutation, etc.)	1. Histopathology in new lower doses 2. Gene expression in intestinal epithelial tissue anchored to pathology 3. Compare all data to determine the cause of proliferation
5. DNA Damage or Epigenetic Changes	1. Measures of DNA damage in target tissue. 2. Are the tumors in the NTP study the result of Cr mediated DNA damage, oxidative DNA damage, proliferative pressure, or some combination?	1. Cr bound to DNA 2. 8-OH-dG 3. Gene expression for changes in DNA repair 4. Genotoxicity in crypts
6. Conversion of DNA damage to mutation	1. Measures of DNA mutations in target tissue 2. Hot spots for Cr(VI) induced mutation? 3. Do mutations occur early or late?	1. In vivo mutation analysis of select codons (e.g., ras codons 12, 13, and 61) or exon sequencing (e.g., p53 exons 5-8).